Protocol for the Examination of Specimens from Patients with Hodgkin Lymphoma

Protocol applies to Hodgkin lymphoma involving any site.[#]

Based on AJCC/UICC TNM, 7th Edition
Protocol web posting date: October 2009

Procedures
- Biopsy
- Resection of Lymph Node(s) or Other Organ(s)

Authors
Jerry W. Hussong, MD, DDS, FCAP*
Cedars-Sinai Medical Center, Los Angeles, California
Daniel A. Arber, MD
Stanford University School of Medicine, Stanford, California
Kyle T. Bradley MD, MS, FCAP
Emory University Hospital, Atlanta, Georgia
Michael S. Brown, MD, FCAP
Yellowstone Pathology Institute Inc, Billings, Montana
Chung-Che Chang, MD, PhD, FCAP
The Methodist Hospital, Houston, Texas
Monica E. de Baca, MD, FCAP
Physicians Laboratory Ltd, Sioux Falls, South Dakota
David W. Ellis, MBBS, FRCPA
Flinders Medical Centre, Bedford Park, South Australia
Kathryn Foucar, MD, FCAP
University of New Mexico, Albuquerque, New Mexico
Eric D. Hsi, MD, FCAP
Cleveland Clinic Foundation, Cleveland, Ohio
Elaine S. Jaffe, MD
National Cancer Institute, Bethesda, Maryland
Michael Lill, MB, BS, FRACP, FRCPA
Cedars-Sinai Medical Center, Los Angeles, California
Stephen P. McClure, MD
Presbyterian Pathology Group, Charlotte, North Carolina
L. Jeffrey Medeiros, MD, FCAP
MD Anderson Cancer Center, Houston, Texas
Sherrie L. Perkins, MD, PhD, FCAP
University of Utah Health Sciences Center, Salt Lake City, Utah
For the Members of the Cancer Committee, College of American Pathologists

*denotes the primary and senior author. All other contributing authors are listed alphabetically.

[#] The bone marrow or ocular adnexal protocols can also be used for Hodgkin lymphoma involving these sites.

The College of American Pathologists offers these protocols to assist pathologists in providing clinically useful and relevant information when reporting results of surgical specimen examinations of surgical specimens. The College regards the reporting elements in the “Surgical Pathology Cancer Case Summary (Checklist)” portion of the protocols as essential elements of the pathology report. However, the manner in which these elements are reported is at the discretion of each specific pathologist, taking into account clinician preferences, institutional policies, and individual practice.

The College developed these protocols as an educational tool to assist pathologists in the useful reporting of relevant information. It did not issue the protocols for use in litigation, reimbursement, or other contexts. Nevertheless, the College recognizes that the protocols might be used by hospitals, attorneys, payers, and others. Indeed, effective January 1, 2004, the Commission on Cancer of the American College of Surgeons mandated the use of the checklist elements of the protocols as part of its Cancer Program Standards for Approved Cancer Programs. Therefore, it becomes even more important for pathologists to familiarize themselves with these documents. At the same time, the College cautions that use of the protocols other than for their intended educational purpose may involve additional considerations that are beyond the scope of this document.

The inclusion of a product name or service in a CAP publication should not be construed as an endorsement of such product or service, nor is failure to include the name of a product of service to be construed as disapproval.

Surgical Pathology Cancer Case Summary (Checklist)

Protocol web posting date: October 2009

HODGKIN LYMPHOMA: Biopsy, Resection

Select a single response unless otherwise indicated.

Specimen (select all that apply) (Note A)
___ Lymph node(s)
___ Other (specify): _____
___ Not specified

Procedure
___ Biopsy
___ Resection
___ Other (specify): _____
___ Not specified

Tumor Site (select all that apply) (Note B)
___ Lymph node(s), site not specified
___ Lymph node(s)
Specify site(s): _____
___ Other tissue(s) or organ(s) (specify): _____
___ Not specified

Histologic Type (based on the 2008 WHO classification) (Note C)
___ Hodgkin lymphoma, histologic subtype cannot be determined
___ Classical Hodgkin lymphoma, histologic subtype cannot be determined
___ Nodular lymphocyte predominant Hodgkin lymphoma
___ Nodular sclerosis classical Hodgkin lymphoma
___ Mixed cellularity classical Hodgkin lymphoma
___ Lymphocyte-rich classical Hodgkin lymphoma
___ Lymphocyte-depleted classical Hodgkin lymphoma

***Pathologic Extent of Tumor (select all that apply) (Note D)**
*___ Involvement of a single lymph node region
*Specify site: _____
*___ Involvement of 2 or more lymph node regions on the same side of the diaphragm
*Specify sites: _____
*___ Involvement of lymph node regions on both sides of the diaphragm
*Specify sites: _____
*___ Spleen involvement
*___ Liver involvement
*___ Bone marrow involvement
*___ Other site involvement
*Specify site(s): _____

* Data elements with asterisks are not required. However, these elements may be clinically important but are not yet validated or regularly used in patient management.

***Additional Pathologic Findings**
*Specify: _____

Immunophenotyping (Immunohistochemistry) (Note E)
___ Performed, see separate report: _____
___ Performed
Specify method(s) and results: _____
___ Not performed

***Clinical Prognostic Factors and Indices (select all that apply) (Note F)**
*___ International Prognostic Score (IPS) (specify): _____
*___ B symptoms present
*___ Other (specify): _____

***Comment(s)**

Explanatory Notes

A. Specimen

Any number of specimen types may be submitted in the evaluation of Hodgkin lymphoma. Lymph nodes, mediastinal masses, bone marrow, spleen, lung, and liver are among the most common. Specimens submitted with a suspected diagnosis of Hodgkin lymphoma require special handling in order to optimize the diagnosis. Often, lymph node specimens are submitted where the differential diagnosis includes both Hodgkin and non-Hodgkin lymphomas, and, if possible, tissue should be obtained for possible molecular and other ancillary studies, which are often necessary for the diagnosis of non-Hodgkin lymphomas.[1,2] Most flow cytometry, molecular, and cytogenetic studies will not aid in the diagnosis of Hodgkin lymphoma. Immunophenotyping by immunohistochemical staining is necessary in the initial diagnosis of nearly all cases of Hodgkin lymphoma. Because of this, well-fixed sections are of paramount importance. The guidelines detailed below are suggested for specimen handling in cases of suspected Hodgkin lymphoma.

- Tissue should be received fresh. Unsectioned lymph nodes should not be immersed in fixative, and care should be taken to make thin (2 mm) slices perpendicular to the long axis of the node to ensure optimal penetration of fixative.
- The fresh specimen size, color, and consistency should be recorded, as should the presence or absence of any visible nodularity, hemorrhage, or necrosis.
- Touch imprints may be made from the freshly cut surface, and the imprints fixed in alcohol or air dried. Unstained air-dried imprints can be used for fluorescence in situ hybridization (FISH) or other studies if necessary.
- For microbiology studies: submit a fresh portion of the lymph node (or other specimen type) sterilely in appropriate medium.
- Flow cytometry immunophenotyping is not routinely used in the diagnosis of Hodgkin lymphoma, but if the differential diagnosis includes non-Hodgkin lymphoma, a fresh portion of the specimen should be submitted in appropriate transport medium such as RPMI.
- Fixation (record fixative[s] used for individual slices of the specimen):
    - Estimated time from excision to fixation should be noted, if possible, as this may impact preservation or recovery of certain analytes such as RNA and phosphoproteins in fixed tissues.
    - Zinc formalin or B5 produces superior cytologic detail but is not suitable for DNA extraction and may impair some immunostains (eg, CD30). B5 also has the additional limitation of requiring proper hazardous materials disposal.
    - Formalin fixation is preferable when the tissue sample is limited, as it is most suitable for immunohistochemistry as well as many other ancillary tests such as molecular/genetic studies and in-situ hybridization.
    - Over-fixation (ie, more than 24 hours in formalin, more than 4 hours in zinc formalin or B5) should be avoided for optimal immunophenotypic reactivity.

B. Tumor Site

Hodgkin lymphomas are nearly always nodal based with cervical lymph nodes more commonly involved. It can also frequently be seen involving mediastinal, axillary, and paraaortic lymph nodes. Extranodal Hodgkin lymphoma can rarely be seen. The anatomic distribution of Hodgkin lymphoma, however, varies depending on the histologic type.[3]

C. Histologic Type

This protocol recommends assigning histologic type based on the World Health Organization (WHO) classification of lymphoid neoplasms.[4] It was originally published in 2001 and more recently revised and updated in 2008.[4,5] This classification encompasses both Hodgkin and non-Hodgkin lymphomas and allows distinction of individual lymphoid neoplasms based upon morphologic, immunophenotypic, cytogenetic, and clinical features. While histologic examination typically is thought to be the gold standard, the majority of Hodgkin lymphomas will require immunohistochemical staining, especially at the time of initial diagnoses.[4-9] In addition, while Hodgkin lymphomas are currently divided into nodular lymphocyte predominant Hodgkin lymphoma and classical Hodgkin lymphomas (including nodular sclerosis, mixed cellularity, lymphocyte-rich, and lymphocyte-depleted subtypes), it should be recognized that classical Hodgkin lymphomas may not represent a single disease. In addition, there is overlap between some cases of Hodgkin lymphoma and non-Hodgkin lymphoma, particularly diffuse large B-cell lymphomas (so-called gray zone lymphomas).[4,10]

D. Pathologic Extent of Tumor (Stage)

The TNM classification is not used for staging Hodgkin lymphomas because the site of origin of the tumor is often unclear and there is no way to differentiate among T, N, and M. The Cotswold revision of the Ann Arbor staging classification is used for Hodgkin lymphoma.[11,12] It was originally published over 30 years ago.

Pathologic staging depends on the biopsy of multiple lymph nodes on both sides of the diaphragm, splenectomy, wedge liver biopsy, and bone marrow biopsy to assess distribution of disease.

Currently, staging for Hodgkin lymphoma is more commonly clinical than pathologic. Clinical staging generally involves a combination of clinical, radiologic, and surgical data. Physical examination, laboratory tests, imaging studies (eg, computed tomography [CT] scans, magnetic resonance imaging [MRI] studies, and positron emission tomography [PET]), biopsy (to determine diagnosis, histologic type, and extent of disease), and bone marrow examination are often required. Correct diagnosis and staging are the key factors in providing appropriate treatment.[13-15]

Cotswold Revision of the Ann Arbor Staging Classification of Hodgkin Lymphomas[13,14]

Stage I	Involvement of a single lymph node region (I), or lymphoid structure (eg, spleen, thymus, Waldeyer's ring).[#]
Stage II	Involvement of 2 or more lymph node regions on the same side of the diaphragm (II) (the mediastinum is considered a single site).[##]
Stage III	Involvement of lymph node regions on both sides of the diaphragm (III) which may be accompanied by extralymphatic extension in association with lymph node involvement (IIIE) or splenic involvement (IIIS).
Stage IV	Involvement of extranodal site(s) beyond those designated E.

[#] Multifocal involvement of a single extralymphatic organ is classified as stage IE and not stage IV.

[##] The number of lymph node regions involved may be indicated by a subscript: eg, II_3.

E designates involvement of a single extranodal site or contiguous or proximal known nodal site of disease.

E. Immunophenotyping

Immunophenotyping by flow cytometry and molecular testing by polymerase chain reaction (PCR) are currently not typically used or are not necessary for the diagnosis of Hodgkin lymphoma. Immunophenotyping using immunohistochemistry is necessary for the initial diagnosis of nearly all cases of Hodgkin lymphoma. It requires well-fixed tissue sections for optimal immunohistochemical staining and interpretation.

Immunophenotypes[1,4-8]

The following is to be used as a guideline for the more common immunophenotype for each subtype of Hodgkin lymphoma. It is however, not entirely comprehensive and individual cases may vary somewhat in their immunophenotypic profile.

Nodular lymphocyte predominant Hodgkin lymphoma*:* Lymphocyte predominant cells (LP cells; previously called L&H cells) are CD20+, CD79a+, PAX5+, CD45+, BCL6+, OCT-2+, BOB.1+, EMA +/-, CD15-, CD30-, CD43-, EBER-.

Nodular sclerosis classical Hodgkin lymphoma*:* Classical Hodgkin/Reed-Sternberg cells are CD30+, CD15+/-, CD45-, PAX5+/-, CD20-/+, CD79a-/+, EBER-/+, OCT-2-/+, BOB.1-/+, EMA-

Mixed cellularity classical Hodgkin lymphoma*:* Classical Hodgkin/Reed-Sternberg cells are CD30+, CD15+/-, CD45-, PAX5+/-, CD20-/+, CD79a-/+, EBER+/-, OCT-2-/+, BOB.1-/+, EMA-

Lymphocyte-rich classical Hodgkin lymphoma*:* Classical Hodgkin/Reed-Sternberg cells are CD30+, CD15+/-, CD45-, PAX5+/-, CD20-/+, CD79a-/+, EBER-/+, OCT-2-/+, BOB.1-/+, EMA-

Lymphocyte-depleted classical Hodgkin lymphoma*:* Classical Hodgkin/Reed-Sternberg cells are CD30+, CD15+/-, CD45-, PAX5+/-, CD20-/+, CD79a-/+, EBER+/-, OCT-2-/+, BOB.1-/+, EMA-

F. Clinical Prognostic Factors and Indices

The International Prognostic Score (IPS) was developed for Hodgkin lymphoma to predict outcome based on the following adverse factors: serum albumin <4g/dL, hemoglobin concentration <10.5 g/dL, male sex, age ≥45 years, stage IV disease, white blood cell count ≥15,000/mm^3, and lymphopenia <600/mm^3 or <8%. The rate of freedom from progression by risk category is: 0 factors 84%, 1 factor 77%, 2 factors 67%, 3 factors 60%, 4 factors 51%, and 5 or more factors 42%.[13]

Although not always provided to the pathologist by the physician submitting the specimen, certain clinical findings are known to be of prognostic value in all stages of Hodgkin and non-Hodgkin lymphoma. In particular, systemic symptoms of fever (greater than 38°C), unexplained weight loss (more than 10% body weight) in the 6 months before diagnosis, and drenching night sweats are used to define 2 categories for each stage of lymphoma: A (symptoms absent) and B (symptoms present). The presence of B symptoms is known to correlate with extent of disease (stage and tumor bulk), but symptoms also have been shown to have prognostic significance for cause-specific survival that is independent of stage.[13] In addition to the IPS, other prognostic factors,

including HIV status, Bcl-2 expression, and pretreatment interleukin-10 serum levels, may be important. [18-21]

References

1. Knowles D, ed. *Neoplastic Hematopathology*. Philadelphia, PA: Lippincott Williams and Wilkins; 2001.
2. Mills S, ed. *Histology for Pathologists*. Philadelphia, PA: Lippincott Williams and Wilkins; 2007.
3. Shimabukuro-Vornhagen A, Haverkamp H, Engert A, et al. Lymphocyte-rich classical Hodgkin's lymphoma: clinical presentation and treatment outcome in 100 patients treated within German Hodgkin's Study Group trials. *J Clin Oncol.* 2005;23(24):5739-5745.
4. Swerdlow S, Campo E, Harris N, Jaffe E, Pilero S, Stein H, Thiele J, Vardiman J, eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.* Geneva, Switzerland: WHO Press; 2008.
5. Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues.* Lyon, France: IARC Press; 2001. World Health Organization Classification of Tumours, Vol. 3.
6. Hsi E, Goldblum J, eds. *Hematopathology*. Philadelphia, PA: Churchill Livingstone Elsevier; 2007.
7. Zukerberg L, Collins AB, Ferry JA, Harris NL. Coexpression of CD15 and CD20 by Reed-Sternberg cells in Hodgkin's disease*. Am J Pathol.* 1991;139(3):475-483.
8. Jaffe E, Banks P, Nathwani B, et al. Recommendations for the reporting of lymphoid neoplasms: a report from the Association of Directors of Anatomic and Surgical Pathology. *Mod Pathol*. 2004;17(1):131-135.
9. Stein H, Marafioti T, Foss H, et al. Down-regulation of BOB.1/OBF.1 and Oct2 in classical Hodgkin disease but not in lymphocyte predominant Hodgkin disease correlates with immunoglobulin transcription. *Blood*. 2001;97(2):496-501.
10. Mani H, Jaffe E. Hodgkin lymphoma: an update on its biology with new insights into classification. *Clin Lymphoma Myeloma*. 2009;9(3):206-216.
11. Carbone P, Kaplan H, Musshoff K, et al. Report of the Committee on Hodgkin's Disease Staging Classification. *Cancer Res*. 1971;31(11):1860-1861.
12. Lister T, Crowther D, Sutcliffe S, et al. Report of a committee convened to discuss the evaluation and staging of patient's with Hodgkin's disease: Cotswolds meeting. *J Clin Oncol.* 1989;7(11):1630-1636.
13. Lymphoid neoplasms. In: Edge SB, Byrd DR, Carducci MA, Compton CC, eds. *AJCC Cancer Staging Manual.* 7th ed. New York, NY: Springer; 2009.
14. Sobin LH, Gospodarowicz M, Wittekind Ch, eds. *UICC TNM Classification of Malignant Tumours.* 7th ed. New York, NY: Wiley-Liss; in press.
15. Kwee T, Kwee R, Nievelstein R. Imaging in staging malignant lymphoma: a systematic review. *Blood*. 2008;111(2):504-516.
16. Hasenclever D, Diehl V. A prognostic score for advanced Hodgkin's disease: International Prognostic Factors Project on Advanced Hodgkin's Disease. *N Engl J Med.* 1998;339(21):1506-1514.
17. Allemani C, Sant M, De Angelis R, et al. Hodgkin disease survival in Europe and the U.S.: prognostic significance of morphologic groups. *Cancer*. 2006;107(2):352-360.
18. Vassilakopoulos T, Angelopoulou M, Siakantaris M, et al. Prognostic factors in advanced stage Hodgkin's lymphoma: the significance of the number of involved anatomic sites. *Eur J Haematol*. 2001;67(5-6):279-288.

19. Sup J, Alemany C, Pohlman B, et al. Expression of bcl-2 in classical Hodgkin's lymphoma: an independent predictor of poor outcome. *J Clin Oncol.* 2005;23(16):3773-3779.
20. Rautert R, Schinkothe T, Franklin J, et al. Elevated pretreatment interleukin-10 serum level is an International Prognostic Score (IPS)-independent risk factor for early treatment failure in advanced stage Hodgkin lymphoma. *Leuk Lymphoma.* 2008;49(11):2091-2098.
21. Rassidakis G, Medeiros LJ, Vassilakopoulos T, et al. Bcl-2 expression in Hodgkin and Reed-Sternberg cells of classical Hodgkin lymphoma predicts a poorer prognosis in patients treated with AVBD or equivalent regimens. *Blood.* 2002;100(12):3935-3941.