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Laboratory Workup of Lymphoma in Adults

Guideline From the American Society for Clinical Pathology and the College of American Pathologists

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• ***Context.***—**The diagnostic workup of lymphoma continues to evolve rapidly as experience and discovery led to the addition of new clinicopathologic entities and techniques to differentiate them. The optimal clinically effective, efficient, and cost-effective approach to diagnosis that is safe for patients can be elusive, in both community-based and academic practice. Studies suggest that there is variation in practice in both settings.**

Objective.—**To develop an evidence-based guideline for the preanalytic phase of testing, focusing on specimen requirements for the diagnostic evaluation of lymphoma.**

Design.—**The American Society for Clinical Pathology, the College of American Pathologists, and the American Society of Hematology convened a panel of experts in the laboratory workup of lymphoma to develop evidence-based recommendations. The panel conducted a systematic review of literature to address key questions. Using the Grading of Recommendations Assessment, Development, and Evaluation approach, recommendations were derived based on the available evidence, strength of that evidence, and key judgements as defined in the Grading of Recommendations Assessment, Development, and Evaluation Evidence to Decision framework.**

Results.—**Thirteen guideline statements were established to optimize specimen selection, ancillary diagnostic testing, and appropriate follow-up for safe and accurate diagnosis of indolent and aggressive lymphoma.**

Conclusions.—**Primary diagnosis and classification of lymphoma can be achieved with a variety of specimens. Application of the recommendations can guide decisions on specimen suitability, diagnostic capabilities, and correct use of ancillary testing. Disease prevalence in patient populations, availability of ancillary testing, and diagnostic goals should be incorporated into algorithms tailored to each practice environment.**

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The diagnosis and classification of lymphoma has evolved into a complex, multimodal process that requires rigorous attention to and quality assurance of numerous preanalytical, analytical, and postanalytical details. After exclusion of purely leukemic disorders and plasma cell neoplasms, the 2016 revised 4th edition of the World Health Organization (WHO) *Classification of Tumours of Haematopoietic and Lymphoid Tissues*[1] details more than 60 distinct mature lymphoid neoplasms. Although accurate prediction of outcome has long been a primary goal of lymphoma classification, direction of management has been in some respects a secondary goal, historically, because of the limited

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Kroft, Sever, and Cheung served as cochairs of the ASCP, CAP, and ASH Expert Panel.

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available therapeutic options. This has changed dramatically in recent years given the proliferation of disease-specific regimens and the explosion of novel agents that show targeted activity in distinct subtypes of lymphoma.

Consequently, accurate diagnosis and classification have become more important than ever. Yet, even as the diagnostic process for lymphoma has grown more complex and requires application of an ever-growing array of ancillary diagnostic techniques, clinical practice has shifted progressively toward less-invasive procedures. Effectively, pathologists are being asked to do more with less. Given these trends, it is logical to suspect that a tipping point will eventually be reached at which the quality of the diagnostic process will begin to degrade. Overlaid on this is the increasing need to act as responsible stewards of limited health care resources. Yet little guidance exists regarding the appropriate procurement techniques, specimen requirements, triage processes, and algorithms for deployment of specialized ancillary studies in the diagnosis of lymphomas. Perhaps as a consequence of this lack of guidance, practice patterns vary considerably from center to center. Understanding the limitations and advantages as demonstrated by the available evidence will help health care teams and patients choose a diagnostic testing strategy that is best suited for their goals and resources.

To address the uncertainty related to the workup of suspected lymphomas, the American Society for Clinical Pathology (ASCP), the College of American Pathologists (CAP), and the American Society of Hematology (ASH) convened a joint workgroup to develop an evidence-based guideline to address appropriate evaluative processes. The primary goal of this guideline was to develop recommendations for the preanalytic phase of testing, with a focus on specimen requirements. The overarching key question that drove the activity of this expert panel (EP) was:

> What are the specimen requirements for an accurate diagnosis in all adult patients with clinical features raising consideration of lymphoma?

To address this question, the EP derived a set of key questions, as detailed in the Objectives section of this article. These questions were crafted to query issues related to relative effectiveness of various procedures, specimen requirements for effective and accurate diagnosis, how to triage tissue for ancillary techniques, and the performance characteristics of those ancillary techniques.

PANEL COMPOSITION

The ASCP, CAP, and ASH convened an EP consisting of practicing pathologists and hematologists with expertise and experience in the laboratory workup of lymphoma. The ASCP, CAP, and ASH jointly approved the appointment of the project, cochairs, and EP members. In addition, a methodologist experienced in systematic reviews and guideline development consulted with the panel throughout the project.

An advisory panel of pathologists and hematologists was convened to provide feedback on the key questions for the literature search, vet the draft guideline statements prior to the open comment period, and review and provide feedback for the manuscript and supplemental digital content (SDC).

METHODS

This evidence-based guideline was developed following the standards endorsed by the National Academy of Medicine.[2] A detailed description of the methods and the systematic review used to create this guideline can be found in the supplemental digital content.

OBJECTIVES

The EP addressed the overarching question, "What are the specimen requirements for an accurate diagnosis in all adult patients with clinical features raising consideration of lymphoma?" This led to the following key questions:

1. To what degree do specimen types allow for accurate primary diagnosis of indolent non-Hodgkin lymphoma (NHL), aggressive NHL, and Hodgkin lymphoma (HL) (hereafter, when all 3 subsets are considered together, referred to as lymphoma)?
2. For each specimen type, what are the optimum and minimum requirements for accurate primary diagnosis or exclusion of lymphoma?
3. What are the appropriate analytical triage processes by which fresh tissue can be distributed for lymphoma?
4. What are the diagnostic test characteristics of the available ancillary assays, and how does additional testing of the primary specimen influence diagnostic accuracy to enable actionable therapy for lymphoma?

Outcomes of Interest

The primary outcomes of interest included laboratory performance, clinical outcomes, and complication rates. Laboratory data and test performance characteristics included diagnostic test characteristics, sensitivity and specificity of testing methods, concordance rates between the intervention and the gold standard, tumor percentage rates, tissue adequacy and viability for testing, and tissue heterogeneity. Clinical outcomes included overall survival, disease-free survival, progression-free survival, recurrence-free survival, time to recurrence, and response to therapy (eg, complete or partial response). Complication rates included number and nature of adverse events, repeat procedures, rebiopsy rates, misdiagnoses, and misclassification rates. See the SDC for a detailed description of outcomes of interest.

Literature Search and Collection

Literature search strategies were developed for the concepts of lymphoma and specimen procurement. In consultation with the EP, a comprehensive literature search for relevant evidence was completed by the medical librarian using Ovid MEDLINE and Elsevier Embase on July 28, 2017, encompassing the publication dates of January 1, 2007 through July 28, 2017. The search strategy used controlled vocabulary (ie, Medical Subject Headings [MeSH], Embase Subject Headings [Emtree]) and keywords derived from the key questions. Database searches were supplemented with a search for unindexed literature, including a review of clinical trials and pertinent organizations' websites. Guidelines were included if they had been published in English since 2012. Members of the EP were also polled for relevant unpublished data at the onset of the project. The literature searches were rerun on September 15, 2018, to capture recent literature, and EP members were polled for relevant unpublished data. In addition, EP members were surveyed for any relevant new data that might affect the recommen-

Table 1. Strength of Evidence	
Designation	**Description**
High	There is high confidence that available evidence reflects true effect. Further research is very unlikely to change the confidence in the estimate of effect. Included studies will be of high or intermediate quality
Moderate	There is moderate confidence that available evidence reflects true effect. Further research is likely to have an important impact on the confidence in estimate of effect and may change the estimate. Included studies will be of intermediate or low quality
Low	There is limited confidence in the estimate of effect. The true effect may be substantially different from the estimate of the effect. Included studies will be of low quality
Very low	There is very little confidence in the estimate of effect. The true effect is likely to be substantially different from the estimate of effect. Any estimate of effect is very uncertain. Included studies will be of low or very low quality

dation on October 11, 2019. Detailed information regarding the literature search, including the search terms used, and inclusion criteria is available in the SDC.

Inclusion Criteria

Studies were selected for inclusion in the systematic review of evidence if they met the following criteria: (1) the study population consisted of adult patients with clinical features raising consideration for primary indolent lymphoma, aggressive lymphoma, or HL; (2) the study evaluated either the use of large- or small-volume incisional or excisional biopsies for accurate diagnosis of lymphoma, optimum or minimum lymphoma biopsy specimen collection and handling requirements, analytical triage processes for fresh tissue, or diagnostic accuracy or diagnostic specificity of ancillary testing when using initial diagnostic specimens; (3) the study reported on diagnostic specificity or accuracy, patient survival outcomes, adequacy of tissue for analysis or diagnosis, appropriate use of tissue or testing, concordance between intervention and the standard of care, or rate of tissue artifact introduction as a primary outcome; and (4) the study was a peer-reviewed full-text article in the English language. Detailed information about the inclusion criteria is available in the SDC.

Exclusion Criteria

Search results and articles suggested by the EP were excluded from the systematic review if they were meeting abstracts; noncomparative or qualitative studies, including editorials, commentaries, and letters; narrative reviews; consensus documents; case reports; studies conducted in cell lines; animal studies; full-text articles not available in English; studies with fewer than 30 patients per study arm; and/or non–peer-reviewed studies. Because of a diagnosis pathway requiring bone marrow analysis, studies evaluating the diagnostic workup of chronic lymphocytic leukemia, small lymphocytic lymphoma, and hairy cell leukemia were excluded. Detailed information about the exclusion criteria is available in the SDC.

Quality Assessment

Each study received a risk of bias assessment and each recommendation an aggregate assessment of the strength of evidence (Table 1). Refer to the SDC for definitions of the strength of evidence.

Assessing the Strength of Recommendations

Development of recommendations required that the EP review the identified evidence and make a series of key judgements using the Grading of Recommendations Assessment, Development, and Evaluation[3] approach. See Table 2 for the definitions of strength of recommendation. A summary of the key judgements the EP considered, including the benefits and harms of each guideline statement using the Grading of Recommendations Assessment, Development, and Evaluation Evidence to Decision (EtD) framework,[4] can be found in the SDC.

RESULTS

A total of 6783 unique studies met the search term requirements. Based on review of abstracts from these studies, 724 articles met the inclusion criteria and continued to full-text review. Of these, a total of 224 articles were included for qualitative analysis and potential data extraction, and 42 studies provided data that informed the recommendations. Data were not extracted from excluded articles, but these were available as discussion or background references. Additional information about the systematic review is available in the SDC, including a Preferred Reporting Items for Systematic Reviews and Meta-Analyses diagram outlining details of the review.

The panel convened multiple times to develop the scope, draft recommendations, review and respond to solicited feedback, and assess the quality of evidence that supported the final recommendations. An open comment period was posted on the ASCP Web site (www.ascp.org) from September 27, 2018 to October 29, 2018, during which the draft recommendation statements were posted for public feedback. The EP approved the final recommendations with a supermajority vote after review of the feedback from the advisory panel and patient representatives. Refer to the SDC for more details.

Each organization instituted a review process to approve the guideline. The ASCP assigned the review of the guideline to a special review panel representing the ASCP Executive Committee. For the CAP, an independent review panel representing the CAP Council on Scientific Affairs was assembled to review and approve the guideline. The independent review panel was masked to the EP and vetted through the disclosure of interest process. The ASH Guideline Oversight Committee and the Committee on Quality voted to affirm the value of the guideline recommendations for hematologists. The ASH Committee on Quality determined that despite the value to the hematologist, the guideline did not meet ASH methodologic criteria for organizational approval of evidence-based guidelines and requested that ASH's name be withdrawn from the title. The final recommendations are summarized in Table 3.

Table 2. Strength of Recommendations		
Designation	**Recommendation**	**Evidence-to-Decision Judgement**
Strong recommendation	Recommend for or against a particular practice (can include "must" or "should")	Supported by assessment with the GRADE EtD framework showing EP consensus of judgements directed to the far right or far left poles of the framework
Conditional recommendation	Recommend for or against a particular practice (can include "should" or "may")	Supported by assessment with the GRADE EtD framework showing EP consensus of judgements directed towards the center of the framework or with a dispersed pattern

Abbreviations: EtD, Evidence to Decision; EP, expert panel; GRADE, Grading of Recommendations Assessment, Development, and Evaluation.

GUIDELINE STATEMENTS

1. **Strong Recommendation.**—Clinical care providers should use surgical biopsy when feasible in a clinical setting where HL is highly suspected.

The strength of evidence to support this guideline statement is *low*.

This recommendation is based on a limited body of evidence contained in 2 retrospective studies[5,6] of both HL and NHL. These studies compared core needle biopsy (CNB) with surgical biopsy (where available) and indicated lower diagnostic sensitivity of CNB for HL than for NHL. The study of Groneck et al[5] observed diagnostic sensitivity of CNB for HL of only 50% for cases in which surgical biopsy was eventually performed. The positive predictive

Table 3. Summary of Guideline Statements	
Guideline Statement	**Strength of Recommendation**
1. Clinical care providers should use surgical biopsy when feasible in a clinical setting where Hodgkin lymphoma is highly suspected	Strong recommendation
2. Clinical care providers should obtain excisional or core needle biopsy specimens in patients with high suspicion of lymphoma	Strong recommendation
3. Clinical care providers should *not* use FNA cytomorphology alone without ancillary testing to achieve a definitive diagnosis of lymphoma. *Note:* Cytomorphology alone without ancillary studies has low sensitivity and low predictive value. *Note:* A defined subset of lymphoma requires architectural assessment and cannot be reliably diagnosed and subclassified by FNA	Strong recommendation
4. Clinical care providers should follow up with patients with negative results for persistent signs and symptoms of lymphoma and pursue larger-volume biopsy when clinical suspicion for lymphoma persists	Strong recommendation
5. Clinical care providers may use PET with FDG to identify sites for biopsy in patients with suspected transformed/aggressive-histology lymphoma. As feasible, biopsies should be directed to the site of greatest FDG avidity	Conditional recommendation
6. Clinical care providers may obtain bone marrow biopsies for the primary diagnosis in select patients with suspected lymphomas. *Note*: For certain lymphoma types (eg, splenic low-grade lymphomas, lymphoplasmacytic lymphomas) bone marrow biopsy may be preferred to more invasive surgical methods	Conditional recommendation
7. Clinical care providers may use CSF for the evaluation of primary or secondary CNS lymphoma in select patients	Conditional recommendation
8. Clinical care providers should use a combined morphologic and flow cytometric evaluation of CSF in the investigation of possible primary or secondary CNS lymphoma in select patients	Strong recommendation
9. Based on low negative predictive values, clinical care providers should follow up with patients with negative results for persistent signs and symptoms of CNS lymphoma and pursue repeat CSF examination or biopsy when clinical suspicion for lymphoma persists	Strong recommendation
10. Clinical care providers should use immunophenotyping by flow cytometry and/or IHC in addition to morphology for the evaluation of specimens for the diagnosis and subclassification of lymphomas	Strong recommendation
11. Clinical care providers may use FISH analysis when evaluating specimens in patients with suspected or confirmed lymphoma, or in the subclassification of lymphoma. FISH analysis is feasible on specimens obtained by FNA and may increase diagnostic yield. *Note:* Demonstration of the appropriate rearrangements is required for a diagnosis of high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements	Conditional recommendation
12. Clinical care providers should not routinely use up-front PCR-based clonality studies of antigen receptor genes (ie, T-cell receptor and immunoglobulin) in the initial investigation of lymphoma. There may be a confirmatory role in certain settings for these studies	Conditional recommendation
13. Clinical care providers may use molecular tests to aid in classification of lymphomas. For example, pathologists may use *MYD88* L265P to aid in the classification of indolent B-cell lymphoma. *Note:* This recommendation statement refers to non-FISH molecular tests	Conditional recommendation

Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; FDG, 2-deoxy-2-[fluorine-18]fluoro-D-glucose; FISH, fluorescence in situ hybridization; FNA, fine-needle aspiration; IHC, immunohistochemistry; PCR, polymerase chain reaction; PET, positron emission tomography.

value (PPV) of CNB for HL is relatively high (again, based on limited data), as it is in most studies involving NHL, but the need for eventual open biopsy in half or more of patients with high pretest suspicion for HL decreases the potential utility of minimally invasive procedures for the diagnosis of HL to a greater degree than for the diagnosis of NHL.

Potential sources of bias in the studies used to help derive this recommendation include the lack of surgical biopsy correlation in every case and the reliance on CNB alone for HL cases considered initially diagnostic—an aspect of retrospective studies that harbors at least the potential to overestimate diagnostic specificity and PPV of CNB in the diagnosis of HL. Nonetheless, a side-by-side comparison of performance characteristics of CNB in the setting of HL versus NHL appears to indicate poorer diagnostic efficiency of CNB for HL than for NHL.

The evidence base supporting this recommendation comprised 2 studies that compared CNB specimens with the gold standard surgical biopsies.[5,6] Both studies are retrospective cohort designs and were assessed as low quality with a very serious aggregate risk of bias. Although many other identified studies used surgical biopsy as the reference standard,[7–15] studies reporting on the diagnostic test characteristics of surgical biopsies were lacking. The EP rendered a strong recommendation despite its reliance on relatively low-quality evidence. Surgical biopsy has evolved as usual practice, likely based on accumulated anecdotal experience and the relative lack of utility of flow cytometry and other ancillary methods in the routine diagnosis of HL, but also based on older studies (outside of the date range for literature review of this guideline) indicating that false negatives are more common in HL than in NHL when limited sampling (either fine-needle aspiration [FNA] or CNB) is used for diagnosis.[16–20] All EP members agreed that the benefits of using surgical biopsies are moderate to large, whereas the harms of its use are moderate to small. Additionally, the EP discussed the potential for misdiagnosis when a large-volume biopsy is not feasible. Taken together, all EP members agreed that the benefits of using surgical biopsy outweighed any potential adverse events from the relatively more invasive procedure and that this statement would be acceptable to key stakeholders and feasible to implement. Refer to the SDC for the strength-of-evidence assessment for this statement and a complete summary of the EtD framework. The open comment period largely yielded agreement with this recommendation, with more than 95% of respondents either agreeing with the recommendation as written or agreeing with minor modifications. Disagreement (or recommendation for modification) largely centered around 2 themes: (1) many respondents commented that HL can be diagnosed on limited material in some cases, and (2) the recommendation should distinguish between primary HL diagnosis and diagnosis of HL recurrence, which may require a lower burden of proof and therefore may be more amenable to the use of limited material. With respect to the first concern, the ability of CNB to yield diagnosis in "some cases" of HL is not disputed, but there is no way to predict which cases those will be, and the data indicate a high likelihood of rebiopsy for any given patient with high suspicion for HL on clinical grounds. The EP was sympathetic to the second concern and agrees that there is likely a greater role for CNB in the diagnosis of recurrent disease than in primary diagnosis; however, the fact remains that data on the diagnostic efficiency of CNB in the setting of recurrent HL are limited.

2. **Strong Recommendation.**—Clinical care providers should obtain excisional biopsy or CNB specimens in patients with high suspicion of lymphoma.

The strength of evidence to support this guideline statement is *moderate*.

The utility of CNB for primary diagnosis of lymphoma was supported by numerous studies from different countries, study populations, and practice settings. The study designs were highly diverse and as such were not directly comparable. Most were retrospective studies with inherent selection bias examining only lymphomas, whereas others were unselected, including nonlymphomatous malignancies, which constitute a much larger percentage of tumors in clinical practice. The 2 highest-quality studies[21,22] (both graded as intermediate quality) demonstrated sensitivity, specificity, and PPV approaching that of excisional (surgical) biopsy to diagnose lymphoma versus other malignancy or benign reactive lymphadenopathies. In the meta-analysis, which pooled data from 16 cohort studies evaluating ultrasound-guided CNB for detection of malignancy in patients with head and neck lesions,[21] the sensitivity of CNB for malignant lymphoma was 92%, with a PPV of 97%, a negative predictive value (NPV) of 85%, and no statistical difference when compared to excisional biopsy. A randomized controlled trial[22] reported superior sensitivity of 98.7% and NPV of 84.6% with ultrasound-guided CNB to the most abnormal appearing node compared with open surgical biopsy, which carried a sensitivity of 88.7% and NPV of 54.3%. The authors offered an explanation that surgical biopsy sites in this study were likely selected as the most accessible nodes or those with the lowest complication risk, leading to frequent sampling of reactive nodes rather than those involved with lymphoma. The fact that surgical biopsies also have a false-negative rate was possibly underreported because of the study designs, impacting selection and reporting bias. Other retrospective studies reported more variability, with diagnostic accuracy of CNB ranging from 64% to 98%.[5,6,11,23,24]

Beyond detection of lymphoma, the rates of lymphoma subclassification with CNB also showed variability. Methods for subclassification include immunophenotyping (eg, immunohistochemistry [IHC], flow cytometry), which will be further addressed in statement 10. The rate of correct WHO classification ranged from 68% to 96%.[7,11,24–26] Diagnosis of 2 particular subtypes was least robust: HL and follicular lymphoma. Sensitivity for HL was significantly lower,[6,27] as addressed in statement 1. Follicular lymphoma also showed inferior classification accuracy of 68% for all grades and 8% for grade 3.[28] The requirements for adequate sampling, immunophenotyping, and architectural evaluation of follicular lymphoma were also extensively discussed in the open comment period as a potential pitfall of CNB. The EP further recommends caution in the diagnosis of peripheral T-cell lymphoma, which can be challenging even in excisional biopsies. None of the series had sufficient numbers of T-cell lymphomas to derive any statistically valid statement on accuracy of CNB in these lymphomas.

A number of variables contribute to the accuracy and safety of CNB diagnosis of lymphoma. Most studies used image-guided techniques including computerized tomography (CT)–guided[8,24] and ultrasound-guided[5–7,11,22,23] modal-

ities, contributing to the favorably low complication rate of CNB; complications predominantly included minor hemorrhage (ranging from 0% to 17% with splenic biopsies),[22,24,27] pain (9%), and splenic rupture (1%). This compared with an overall 15.8% complication rate in surgical biopsies attributable to pain and hemorrhage.[10] Although important for the individual interventionist, types and gauges of needles (range, 14–20 gauge) and number of passes/cores (range, 2–6) were highly variable and not significantly associated with diagnostic accuracy when reported.[23] These characteristics were determined by operator preference, anatomic location and proximity to large blood vessels, diagnostic approach, and institutional capabilities such as on-site interventional radiology. The EP recognizes that for many pathologists, it is intuitively obvious that samples obtained with larger-gauge needles are diagnostically superior to those acquired with narrower gauges. Nevertheless, appropriate studies have not been performed to inform a recommendation on this point. As absence of evidence is not equivalent to evidence of absence, the EP encourages the performance of well-designed studies to test this hypothesis. Formalin was the fixative of choice for histology in the referenced studies.[5,11,22] Formalin is also the expert opinion preference, because IHC and molecular diagnostics are optimized for this fixative in most institutions.

A frequent topic of investigation is the application of CNB techniques in anatomic locations difficult to access, such as mediastinal, intrathoracic, retroperitoneal, or intra-abdominal masses or spleen. Most of the individual studies did not meet selection criteria for evidence-based recommendations. Nevertheless, the benefit of low complication rates of CNB with clinically useful rates of accurate diagnosis outweighs the potential harm of surgical interventions in those situations and had broad support from the EP, open comment period, and patient representatives.

Considerations that were less frequently investigated included the possibility of tissue exhaustion before appropriate ancillary testing could be completed and the diminished availability or absence of residual tissue for research. This would affect academic environments in particular. The lack of residual tissue for subsequent biomarker testing may limit the patient's access to therapies and clinical trials. Other factors outside of technical specifications favoring CNB included cost and wait time for the diagnostic procedure.[11,22] These are important in resource-constrained environments and require individual approaches tailored to patient preference and clinical urgency to achieve optimal outcomes.

The evidence base for this statement comprised 11 studies, 3 reporting on the use of excisional biopsies and CNB specimens for diagnosing lymphoma versus nonlymphoma[21–23] and 8 studies[5–8,11,24,27,28] reporting on biopsy use for subclassification of lymphoma. The aggregate risk of bias for studies reporting on diagnosis of lymphoma versus nonlymphoma was serious, and the aggregate risk of bias for the 2 studies reporting on subclassification was very serious. As the evidence was not downgraded for any other factors, the strength of evidence for the entire statement was defined as moderate. Based on the available evidence, all EP members agreed that the moderate to large benefits of using excisional biopsies or CNBs outweighed the moderate to trivial potential harms of using a more invasive procedure than FNA. Although the EP was divided on the magnitude of costs associated with performing the biopsies, all EP members felt this recommendation would be acceptable to key stakeholders and feasible to implement. Refer to the SDC for the strength-of-evidence assessment for this statement and a complete summary of the EtD framework.

In summary, primary diagnosis can be safely accomplished with CNB if patient selection, technique, and diagnostic methods are optimized and awareness of diagnostic pitfalls prevents false-negative diagnosis.

3. **Strong Recommendation.**—Clinical care providers should *not* use FNA cytomorphology alone without ancillary testing to achieve a definitive diagnosis of lymphoma.

Note: Cytomorphology alone without ancillary studies has low sensitivity and low predictive value.

Note: A defined subset of lymphoma requires architectural assessment and cannot be reliably diagnosed and subclassified by FNA.

The strength of evidence to support this guideline statement is *low*.

It should be emphasized that this recommendation is specifically for using FNA cytomorphology alone without further ancillary testing for the primary diagnosis of lymphoma. It does not categorically exclude the FNA approach if ancillary testing including flow cytometry and IHC is applied concurrently, as outlined in recommendation statement 10. Only 5 studies meeting selection criteria for this guideline statement that compared FNA cytomorphology and a gold standard comparator, including excisional tissue biopsy or other diverse studies (eg, repeat procedure, addition of ancillary testing, tissue biopsy) and clinical follow-up, were identified. The scenarios were typical for FNA sampling: evaluation of head and neck suspected lymphoma,[29] palpable cervical lymph nodes,[30] difficult-to-access nodes or masses,[31] defined patient populations such as bone marrow transplant patients with a more limited number of differential diagnostic questions,[32] or a specific organ where most neoplasms were other tumors and lymphomas were infrequent incidental findings, as exemplified by the study of thyroid FNAs.[33]

In a cervical lymph node study[30] on FNAs from 851 patients in Tunisia, the incidence of lymphoma was 6.9% of total neoplasms. The authors did not attempt classification beyond broad categories of HL, aggressive lymphoma, indolent lymphoma, or diagnoses suggestive of lymphoma. Within this framework and study population the overall sensitivity was determined to be 95.5%, and specificity, PPV, and NPV for lymphoma or suggestive of lymphoma all exceeded 97%. The study demonstrated the utility of FNA in recognizing the presence of lymphoma versus other tumors as a diagnostic triage tool prior to more invasive biopsy or surgery but did not claim definitive diagnostic capability. A study of head and neck lymphomas by Roh et al[29] retrospectively examined 173 patients who had a high clinical suspicion of lymphoma with 109 receiving FNAs prior to tissue biopsy. Compared with tissue biopsy, there was a high rate of incorrect diagnoses of benign or nondiagnostic conditions, with the incorrect rates lowest for B-cell lymphomas (7 of 61), intermediate for T-cell lymphomas (10 of 33), and highest for HL (8 of 15). Of concern to the authors was the delay in diagnosis from the time of clinical suspicion to final tissue confirmation: when FNA was performed first and incorrectly diagnosed as benign, the time to correct final

diagnosis was significantly prolonged ($P < .05$). A multi-institutional study[33] on the diagnosis of primary thyroid lymphomas directly compared the diagnostic accuracy of FNA cytomorphology alone versus FNA plus flow cytometry or polymerase chain reaction (PCR) clonality testing, demonstrating much higher diagnostic accuracy if ancillary studies were performed ($P < .05$). Of the 29 cases diagnosed as lymphoma, only 14 (48%) received a specific diagnosis of a specific subtype of lymphoma. The magnitude of diagnostic improvement with ancillary testing was also demonstrated in a study of deep-seated lesions sampled with endoscopic ultrasound-guided FNA. Whereas cytology assessment alone had a sensitivity of 53% for lymphoma diagnosis, the addition of cell blocks and complementary flow cytometry improved sensitivity to 93.4%. Also examining diagnostic capability in difficult-to-reach locations was a study on transthoracic FNA of peripheral pulmonary lesions in patients with a known hematologic malignancy or after bone marrow transplantation.[32] The yield of the first FNA for a positive diagnosis was 68% for a diagnosis of lymphoma, compared with 67% for infection and 90% for lung cancer. The study reported a 25% complication rate, including a high rate of pneumothorax (20%) and bleeding (8%), including one death in a patient with abnormal hematologic parameters; these results temper the assumption that FNA lacks significant patient risk by showing it is subject to potential harm in high-risk organs (eg, lung).

The evidence base supporting this recommendation comprised 5 studies that reported low sensitivities and predictive values when FNA cytomorphology alone was used.[29–33] All 5 studies were of a retrospective cohort study (RCS) design and were assessed as low quality with an aggregate very serious risk of bias. Based on the available evidence, all EP members agreed that the moderate to large benefits of avoiding FNA cytomorphology alone outweighed any potential small to trivial harms. The decision to create a strong recommendation statement was further based on the harms to patients misdiagnosed using FNA cytomorphology without further ancillary testing. The limitations of FNA cytomorphology alone exemplified in these studies were also recognized in the open comment period, with many comments that patient selection for FNA approach to diagnosis is often based on the much higher probability of nonhematopoietic malignancy with high diagnostic yields, difficult-to-access lesions with limited options for low procedural risk to the patient, or as an assessment tool to evaluate for relapsed lymphoma. In these situations, FNA cytomorphology alone has a role but provides insufficient accuracy without ancillary studies for the primary diagnosis and classification of lymphoma. The effectiveness of combining cytomorphology with ancillary studies is further discussed in statements 10 through 13. Although most EP members felt this recommendation would be acceptable to key stakeholders, a small minority believed that some stakeholders would probably not find the guidance acceptable. However, all EP members still agreed that this recommendation would be feasible to implement. Refer to the SDC for the strength-of-evidence assessment for this statement and a complete summary of the EtD framework.

4. **Strong Recommendation.**—Clinical care providers should follow up patients with negative results for persistent signs and symptoms of lymphoma and pursue larger-volume biopsy when clinical suspicion for lymphoma persists.

The strength of evidence to support this guideline statement is *moderate*.

This recommendation relates to the NPV of a tissue biopsy for excluding lymphoma. The study of Pugliese et al[22] is a prospective cohort study comparing open surgical biopsy with power Doppler ultrasonography–assisted CNB using a 16-gauge needle. Core needle biopsy yielded inadequate samples in 2.1% of procedures, which were excluded from analysis. The NPV was 54.3% for open surgical biopsy and 84.5% for CNB, with the gold standard being subsequent biopsy demonstrating lymphoma. These seemingly counterintuitive results almost certainly relate to bias in the determination of which lymph node to biopsy. Whereas specific Doppler-determined angioarchitecture criteria guided the lymph node selection in the CNB arm, the choice of lymph node for open surgical biopsy was not based on any specific criteria, but rather was left to the "surgeon's discretion." The meta-analysis by Novoa et al[21] found an NPV of 85% for excluding lymphoma for CNBs in lymph nodes from the head and neck region. In this analysis, 7.4% of lymph node CNBs were deemed inadequate. In their retrospective study, Balestreri et al[9] reported CNB results in 137 patients with a final diagnosis of lymphoma (Hodgkin or non-Hodgkin), and determined that the original CNB was "inconclusive" in 18 (13%). Finally, Han et al[23] studied the performance characteristics of CNBs of cervical lymph nodes using various gauge needles, guided by routine ultrasound directed at the "most suspicious" lymph node. In this study, 7.8% of biopsies were considered inadequate. Repeat biopsy was performed in 54% of inadequate cases, and 51% of these repeat biopsies revealed lymphoma. For biopsies with adequate material, these authors report an NPV of 99.3% for benign versus malignant discrimination. The EP recognizes that this recommendation statement may seem self-evident. However, it is not clear that ordering providers uniformly appreciate the potential magnitude of the NPV for various biopsy procedures, as enumerated above. Given the inability of an initial negative or nondiagnostic test result to definitively rule out the presence of lymphoma, continued clinical vigilance is required to ensure that additional biopsy is considered when suspicion for lymphoma persists.

Four studies that evaluated the benefits of following patients with negative results comprised the evidence base for this statement.[9,21–23] Three studies reported on diagnostic test characteristics and carried an aggregate moderate strength of evidence,[21–23] whereas the remaining study was of low quality and reported on specimen adequacy.[9] As the evidence was not further downgraded for any factor, the strength of evidence for the entire statement was defined as moderate. Based on the available evidence, all EP members agreed that the moderate to large benefits of following patients with negative results outweighed the small to trivial potential harms of follow-up. Although the EP was divided on the magnitude of costs associated with follow-up and the impact on health equity, all members felt this recommendation would be acceptable to key stakeholders and feasible to implement. Refer to the SDC for the strength-of-evidence assessment for this statement and a complete summary of the EtD framework. The open comment period yielded overwhelming agreement with this recommendation as written (91.6%) or with modification (6.2%). Several

comments related to the ambiguity of the word *negative* in this recommendation. The EP recognized this ambiguity and elected to retain this wording for the purpose of concision. However, the EP notes that the word *negative* in this context should be construed as inclusive of diagnostic terms such as *reactive*, *negative for malignancy*, *inconclusive*, and *nondiagnostic*. Such terms are not applied uniformly by interpreting pathologists, and consequently are likely interpreted in different ways by ordering providers with respect to the level of certainty they imply as to the absence of malignancy. Diagnostic terms such as *inadequate* or *insufficient*, in contrast, are likely generally understood to require an additional diagnostic procedure, but the criteria for the use of these terms are not standardized for CNBs. A number of comments related to the importance of choosing an appropriate lymph node to biopsy (either initially or at follow-up). The EP agrees with this sentiment; as illustrated in the Pugliese et al[22] study described above, a lack of robust criteria for selection of lymph nodes to sample will affect the NPV of tissue biopsies. Multiple comments were posted reflecting the general sentiment that "more is better," including suggestions that small-volume biopsies will always carry a substantial false-negative rate, and that a gold standard excisional biopsy should be the initial diagnostic procedure whenever possible. Although this is an intuitively attractive concept, the data do not necessarily support this approach (at least in the context of NPV for lymphoma versus benign lymph node discrimination). The Pugliese et al[22] study suggests that the choice of which lymph node to sample is a much more important criterion than the volume of the biopsy. Finally, the EP notes that technical issues related to biopsy procedure (eg, type and gauge of needle, number of passes, total volume of tissue obtained, experience of operator) also likely affect NPV, but these have not been adequately studied in a controlled fashion.

5. **Conditional Recommendation.**—Clinical care providers may use positron emission tomography (PET) with 2-deoxy-2-[fluorine-18]fluoro-D-glucose (FDG) to identify sites for biopsy in patients with suspected transformed/aggressive-histology lymphoma. As feasible, biopsies should be directed to the site of greatest FDG avidity.

The strength of evidence to support this guideline statement is *low*.

Positron emission tomography using FDG is now widely adopted in the initial evaluation and treatment assessment of lymphoma.[34] Based on studies that documented reliable FDG avidity in the majority of lymphomas—in particular, diffuse large B-cell lymphoma (DLBCL), HL, and follicular lymphoma—current consensus guidelines recommend the use of PET in combination with CT for staging and end-of-treatment response assessment.[34] Prior studies also suggested that FDG uptake, generally quantified by measuring the standardized uptake value (SUV), trended higher in patients with aggressive histology lymphomas.[35] These data highlighted a potential application of PET or PET with CT: to guide the initial biopsy site for patients with suspected lymphoma, or to investigate the potential for histologic transformation in patients with suspected or known indolent lymphoma.

In the single study identified by the systematic review, Noy et al[36] reported on a retrospective cohort of 40 patients with biopsy-confirmed transformed lymphoma. Regardless of the site of biopsy, the highest SUV within each patient's PET scan ranged from 3.2 to 40, with a mean value of 15. The majority of patients (63%) with transformed lymphomas had at least 1 site with an SUV higher than 10 (and 50% had an SUV >13).[36] These data built on previous retrospective work from the same group published prior to this systematic review's search date. Investigators reported that FDG uptake was generally lower in patients with indolent lymphoma compared with those with aggressive disease (excluding transformed lymphoma).[37] Although an overlap between indolent and aggressive histologies was noted in the low-SUV range, all patients with indolent lymphomas had PET scans that were reliably associated with SUV 13 or lower.[37] The EP was aware of a prospective cohort study published as a letter to the editor (and thus excluded from the systematic review). Bodet-Milin et al[38] reported a single-institution study of 38 patients with indolent lymphoma and clinical suspicion for transformation, in whom biopsy was targeted to the site of maximal SUV on PET imaging. Transformation was biopsy confirmed in 17 cases; the remaining 21 cases did not demonstrate histologic evidence of transformation or evidence of transformation on subsequent follow-up. Patients with documented transformation had a higher median maximal SUV (18.5) compared with patients without (maximal SUV 8.6). With a threshold maximal SUV of 14, sensitivity and PPV of PET-based biopsy were each 94% and specificity and NPV were each 95%. These results paralleled the previously discussed retrospective reports that support the ability of PET to distinguish between indolent and aggressive histologies.

The evidence base supporting this recommendation comprised 1 low-quality retrospective study.[36] The EP felt that the identified study highlighted the utility of PET to guide biopsy when clinical or laboratory findings prompt concern for an aggressive histology lymphoma (including transformation). In particular, high FDG uptake should raise suspicion for aggressive-histology lymphoma (or histologic transformation in a patient with known or suspected indolent lymphoma). Biopsy remains the gold standard for disease confirmation and should be targeted to the site of greatest FDG avidity when feasible. The EP emphasizes the importance of clinical judgement in determining whether the site of greatest FDG avidity is accessible and safe to biopsy. Although all EP members agreed there were substantial benefits of using PET in this situation, the harms of its use were considered to range from large to trivial, with the majority of the EP deeming the harms to be moderate. When the benefits were compared with the harms, a majority of the EP members believed the benefits to outweigh the harms, with a very small minority feeling that there was a balance. Although many of the EP members supposed that using PET to identify biopsy sites would result in moderate to large costs, all EP members felt this conditional recommendation would be acceptable to key stakeholders and feasible to implement. Refer to the SDC for the strength-of-evidence assessment for this statement and a complete summary of the EtD framework.

Feedback from stakeholders during the open comment period emphasized the lack of prospective evidence supporting the use of PET in patients with *suspected* lymphoma. The available study, however, did include patients undergoing their initial diagnostic biopsy for lymphoma; as such, the EP felt that the evidence supported the use of PET in patients in whom an initial biopsy was

required to investigate suspected lymphoma. The EP further felt that the potential benefit to patients in facilitating a more accurate and timely diagnosis outweighed the potential harms (including increased radiation exposure) and costs associated with PET imaging.

6. **Conditional Recommendation.**—Clinical care providers may obtain bone marrow biopsies for the primary diagnosis in select patients with suspected lymphomas.

Note: For certain lymphoma types (eg, splenic low-grade lymphomas, lymphoplasmacytic lymphomas [LPLs]), bone marrow biopsy may be preferred to more invasive surgical methods.

The strength of evidence to support this guideline statement is *very low*.

Most studies addressing the role of bone marrow examination in the diagnosis of lymphoma, including the literature extracted for this guideline, did not directly address the primary diagnosis of lymphoma in general. The 3 studies[39–41] outlined exemplify the most common study objectives: incidence of bone marrow involvement at primary diagnosis in the context of staging procedures of 1215 patients,[40] comparison of diagnostic yield of aspirate versus biopsy in 143 lymphoma patients,[39] and the diagnostic accuracy of bone marrow examination versus tissue sampling for the diagnosis of splenic marginal zone lymphoma and splenic diffuse red pulp small B-cell lymphoma, comparing immunophenotype and classification on bone marrow biopsies and splenectomy specimens.[41]

It is self-evident that bone marrow examination is the diagnostic modality of choice in lymphomas that may only involve bone marrow and no other sites, such as LPL. Initiating the diagnostic workup of suspected splenic lymphomas that involve bone marrow can most often be done more safely with bone marrow aspiration and biopsy, with a very low reported complication rate of 0.08%, mostly hemorrhagic events.[42] Other diagnostic modalities are associated with a higher risk to the patient, with a complication rate of up to 16.7% reported for splenic FNA or biopsy, including serious adverse events such as splenic rupture[27] (see also statement 2). Although architectural features of white pulp versus red pulp involvement can only be assessed on splenectomy or splenic biopsy specimens, the data obtained in bone marrow sampling and immunophenotyping with concordance of immunophenotype in bone marrow and involved spleen may be sufficient for determining treatment options.[41] In addition, the bone marrow results can inform discussions with the patient weighing the potential benefits versus the additional risk associated with splenectomy or other splenic sampling.

Successful primary diagnosis of lymphoma is highly dependent on patient population and specific lymphoma subtype. Therefore, no evidence-based recommendation can be rendered as to when a primary diagnosis of lymphoma may be attempted with bone marrow examination only. Two extracted studies exemplified the high variation of rate of involvement and diagnostic yield. The study conducted in South Africa[40] demonstrated the dramatic difference of lymphoma subtypes in HIV-positive versus HIV-negative patients, with statistically significant higher rates of bone marrow involvement for Burkitt lymphoma (53% versus 9.1%) and HL (53.5% versus 22.6%) in HIV-positive versus HIV-negative patients. A primary diagnosis by bone marrow examination only was rendered in 10.3% of cases, aided by IHC, flow cytometry, conventional karyotyping, and fluorescence in situ hybridization (FISH). A study from Egypt was conducted on staging bone marrows and elaborated on the varying incidences of lymphoma subtypes and bone marrow involvement in different countries.[39] Discordant bone marrow involvement by low-grade lymphoma and other tissue involvement by high-grade lymphoma was infrequently reported at 4.8%, and discordant high-grade bone marrow transformation with low-grade tissue involvement was reported at 4.5%.[40]

The evidence base supporting this recommendation comprised 3 studies.[39–41] All 3 studies had an RCS design and were assessed as low[39,41] or very low[40] quality. The aggregate risk of bias across the 3 studies was very serious and strength of evidence was further downgraded for inconsistency and indirectness. Based on the available evidence, the EP members considered the benefits of bone marrow biopsy use to range from small to large, with the majority assessing the benefits to be moderate. Similarly, the EP members considered the harms to range from moderate to trivial, with the majority believing the harms to be small. The EP, with agreement by a large majority (93%) of open comment period respondents, concluded that in select patients, benefits of bone marrow biopsy and aspiration with the potential for a definitive primary diagnosis can outweigh harm. Because the procedural complication rate is very low, potential harms to the patient are primarily misclassification of benign versus malignant, incorrect subclassification, incorrect grading if there is bone marrow discordance of lymphoma grade, and false-negative results. Diagnostic pitfalls are similar to those of other small tissue biopsies as outlined in statements 1 and 2, with the additional challenge of decreased sensitivity of ancillary studies in decalcified tissue.[42] The EP members were divided when discussing resource use and health equity in recommending bone marrow biopsies in this setting. A majority of the EP members felt that use of bone marrow biopsies would entail a negligible cost, whereas a minority felt that their use could result in either moderate additional costs or moderate savings. All EP members felt that bone marrow biopsy use either could have no impact on health equity or would result in likely increased health equity. Taken together, all EP members agreed this recommendation would be acceptable to key stakeholders and feasible to implement. Refer to the SDC for the strength-of-evidence assessment for this statement and a complete summary of the EtD framework.

7. **Conditional Recommendation.**—Clinical care providers may use cerebrospinal fluid (CSF) for the evaluation of primary or secondary central nervous system (CNS) lymphoma in select patients.

The strength of evidence to support this guideline statement is *very low*.

This statement is based upon the findings reported in 1 study[43] that concluded that flow cytometric immunophenotyping, together with cytomorphology, is useful in the appropriate clinical setting in the diagnosis of CNS lymphoma. Clinical follow-up data were available for 306 patients with CSF samples that underwent both cytologic examination and flow cytometry. Based on a positive brain biopsy or positive clinical follow-up, the PPV and NPV of combination cytology and flow cytometry were 79% and

89%, respectively. For patients at high risk, based on a history of lymphoma and/or abnormal imaging studies, the PPV of the combined approach was 89% and the NPV was 86%. In the 65 unselected patients who underwent brain biopsy, when compared with cytology alone, combination morphologic and flow cytometric evaluation of CSF increased the PPV from 50% to 92% for detection of CNS lymphoma. The authors proposed an algorithm for the directed use of flow cytometry in the diagnosis of CNS lymphoma based upon clinical findings, history of lymphoma and/or abnormal imaging, clinical index of suspicion, and CSF cytomorphology.

The evidence base supporting this recommendation comprised one very low-quality RCS.[43] Based on this limited evidence, the EP members were divided on multiple domains on the EtD framework. Although all EP members agreed the benefits of using CSF in the diagnosis of CNS lymphoma were moderate to large, the harms of its use were considered to range from large to trivial, with a majority of the EP deeming the harms to be moderate. When the benefits were compared with the harms, a majority of EP members concluded that the benefits outweighed the harms, with a very small minority concluding that there was only a balance. In terms of resource use, the costs of using CSF ranged from moderate costs to large savings, with the majority of EP members understanding the costs to be moderate or negligible. Most of the EP members agreed that the use of CSF would result in no impact on health equity, whereas a minority of members thought its use could result in either reduced or increased equity. However, when all domains were considered, all EP members agreed that this conditional recommendation would be acceptable to key stakeholders and feasible to implement. Refer to the SDC for the strength-of-evidence assessment for this statement and a complete summary of the EtD framework.

The open comment period produced comments that supported the recommendation as written (74.11%) or indicated agreement with suggested modifications (16.89%). A general concern over the adequacy of CSF specimens was expressed, especially from patients with suspected primary CNS lymphoma in whom tissue involvement may not cross into the CSF for detection by cytology and flow cytometry. The EP recognized this concern. However, despite the relatively low sensitivity of CSF examination for new CNS lymphoma diagnosis, the EP concluded that CSF examination was warranted as an initial diagnostic maneuver, given the much greater invasiveness of a brain biopsy. Several comments emphasized that negative findings from cytology and flow cytometry should prompt tissue biopsy in patients with a high index of clinical suspicion for CSF involvement. These concerns are discussed further in statement 8.

8. **Strong Recommendation.**—Clinical care providers should use a combined morphologic and flow cytometric evaluation of CSF in the investigation of possible primary or secondary CNS lymphoma in select patients.

The strength of evidence to support this guideline statement is *very low*.

Several studies[43,44] have found that flow cytometric evaluation of CSF improves diagnostic accuracy in the diagnosis of primary or secondary CNS lymphoma when compared with morphologic examination alone. In one study of CSF from 65 patients who underwent a subsequent brain biopsy, combined morphologic and flow cytometric evaluation of CSF increased the PPV from 50% (morphology only) to 92% (combined morphology and flow cytometry) for CNS lymphoma.[43] In that study, the NPV for combined morphologic and flow cytometric evaluation was 52% in unselected patients; however, in higher-risk patients (history of lymphoma and/or suspicious findings on brain imaging) the NPV of combined analysis was 89%.[43] Another study, of 128 CSF samples from patients with B- or T-cell neoplasms involving the CNS with clinical follow-up, found that combined morphologic and flow cytometric evaluation had increased sensitivity and comparable specificity to the use of either modality alone. In addition, flow cytometric assessment was found to be 100% sensitive if at least 220 leukocytes were examined.[44]

Multiple additional published studies on the diagnostic evaluation of CNS lymphoma also found that flow cytometric evaluation of CSF improved diagnostic accuracy compared with morphologic examination alone. For example, in a study of 51 patients with newly diagnosed aggressive B-cell lymphomas, 11 patients (22%) were positive by flow cytometric assessment, but only 1 of the 11 patients had positive morphologic findings.[45] Similarly, flow cytometric analysis was more sensitive than cytology for the detection of lymphoma in patients with relapsed/treated disease.[46] In addition, morphologic examination can be very helpful for the identification of aggressive lymphomas in paucicellular CSF specimens, which may be falsely negative by flow cytometric analysis. A combined morphologic and flow cytometric approach maximizes the ability to detect lymphomatous involvement of CSF.

Two studies that demonstrated improved CNS lymphoma diagnostic accuracy with cytomorphology plus flow cytometry when compared with cytomorphology alone[43,44] comprised the evidence base for this statement. Included studies were of an RCS design and assessed as low[44] and very low[43] quality with an aggregate very serious risk of bias. All EP members agreed that addition of flow cytometry to cytomorphology improves accuracy and that the benefits of its use are moderate to large, whereas the perceived harms of adding flow cytometry ranged widely from trivial to large. Despite the divide in perceived harms, all EP members agreed that providing this guidance is a priority and that the benefits of recommending flow cytometry paired with cytomorphology of CSF outweigh the harms. Because of the low predictive value of CSF cytomorphology alone, the decision to create a strong recommendation statement was further based on potential harms to patients if CSF cytomorphology alone were used. A majority of the EP members concluded that flow cytometry paired with cytomorphology could lead to moderate cost increase, whereas a minority of the members deemed the added cost to be negligible. When considering the available evidence combined with the knowledge that combined cytomorphologic and flow cytometric analyses for CSF samples is standard of care in some institutions, all EP members felt this recommendation would be acceptable to key stakeholders and feasible to implement. Refer to the SDC for the strength-of-evidence assessment for this statement and a complete summary of the EtD framework.

The open comment period produced comments that supported the recommendation as written (80.3%) or indicated agreement with suggested modifications (11.2%). Many of the comments emphasized the known limitations of flow cytometric immunophenotyping in the absence of

adequate cellularity and abnormal cellular morphology on Cytospin (Thermo Fisher Scientific, Waltham, Massachusetts) preparations of CSF, particularly when the clinical index of suspicion is high. In addition, imaging studies were recommended for establishing high levels of clinical suspicion. In settings with high clinical suspicion and inadequate CSF cellularity for flow cytometry, some comments recommended tissue biopsy with the addition of flow cytometric and/or IHC immunophenotyping for diagnosis of lymphoma involvement. Several comments, however, stressed that CSF examination alone should not be used for the diagnosis of primary CNS lymphoma. Finally, there were comments that supported the use of molecular studies, including clonality, in selected cases.

9. **Strong Recommendation.**—Based on low NPVs, clinical care providers should follow up patients with negative results for persistent signs and symptoms of CNS lymphoma and pursue repeat CSF examination or biopsy when clinical suspicion for lymphoma persists.

The strength of evidence to support this guideline statement is *very low*.

The basis for this recommendation is the finding of moderate NPV for CSF evaluation to exclude CNS involvement with lymphoma documented by the 2 studies.[43,44] The study by Pittman et al[43] was an RCS of 373 patients with CSF samples submitted for flow cytometry (with or without cytology) for evaluation of lymphoma. The population was heterogeneous; 31% of patients had a known history of lymphoma, whereas 27% had no such history and no suspicious radiographic features. In higher-risk patients (known lymphoma or suspicious radiology), the NPV for combined flow cytometry and cytology CSF evaluations was 89%, when compared with positive open brain biopsy. However, when lower-risk patients were included, the NPV decreased to 52% compared with open brain biopsy; in other words, for those who underwent open brain biopsy in this study, almost half of patients with initial negative or nondiagnostic CSF results subsequently had evidence of CNS lymphoma. Cesana et al[44] assessed the value of flow cytometry and cytomorphology results on 227 CSF samples from patients investigated for hematologic malignancies. In patients investigated for mature B- or T-cell lymphomas (comprising 128 CSF samples), the sensitivity for cytology and flow cytometry were 53% and 77%, respectively, whereas the corresponding NPVs compared with retrospective clinical assessment for cytology and flow cytometry were 84% and 91%, respectively. However, this study is challenging to interpret, as the majority of patients (>97%) already had a known hematologic malignancy and were undergoing CSF evaluation as part of staging, treatment (intrathecal chemotherapy), or assessment for relapse. It is unclear whether the 6 patients being investigated for suspected lymphoma ultimately had evidence of CNS involvement based on retrospective assessment.

These data build on the historical documentation of high false-negative rates for CSF evaluation of lymphoma.[45,47] False negatives may arise because of low cellularity of CSF samples, inadequate sample volumes, challenges in differentiating lymphoma cells from reactive cells, sites of CNS involvement distant from the leptomeninges, or exposure to corticosteroids prior to sampling.[43,46,48] Given the inability of an initial negative or nondiagnostic test result to definitively rule out the presence of lymphoma, continued vigilance is required to determine if open brain biopsy (in patients with parenchymal brain lesions) or further CSF sampling is warranted. Repeated sampling of CSF (more than once), sending larger sample volumes,[48] and the addition of ancillary testing[43,44,46,47] may be considered to reduce false-negative CSF evaluations. However, clinical judgement is required to determine whether further sampling or pursuit of tissue (brain) biopsy is preferred when clinical or radiographic suspicion for lymphoma remains. Considerations may include surgical accessibility and safety of tissue biopsy, pretest probability for lymphoma or alternative diagnoses, potential harm from delayed diagnosis, and patient preference.

The evidence base in support of this recommendation comprised 2 retrospective studies that addressed CSF evaluation for individuals with suspected CNS lymphoma.[43,44] Included studies were of an RCS design and assessed as low[44] and very low[43] quality. Based on the available evidence, all EP members concluded that the significant benefits of following patients with negative initial results outweighed the moderate potential harms of follow-up and reinvestigation. In particular, the EP highlighted the inability of initial negative or nondiagnostic results to definitively rule out lymphoma. The decision to create a strong recommendation statement was further based on the concern of considerable harm in missing a diagnosis of CNS/CSF lymphoma in patients who are not followed closely and reinvestigated when suspicion for lymphoma remains. Unfortunately, identified literature did not provide definitive evidence on whether repeat sampling reduces false negatives. The EP discussed the need for clinical judgement in these situations and drafted a list of considerations, which have been included in the discussion above. Although the EP was divided on the magnitude of costs associated with follow-up and the impact on health equity, all EP members agreed this recommendation would be acceptable to key stakeholders and feasible to implement. Refer to the SDC for the strength-of-evidence assessment for this statement and a complete summary of the EtD framework. The open comment period yielded overwhelming agreement (97%) with this recommendation as written or with modification.

10. **Strong Recommendation.**—Clinical care providers should use immunophenotyping by flow cytometry and/or IHC in addition to morphology for the evaluation of specimens for the diagnosis and subclassification of lymphomas.

The strength of evidence to support this guideline statement is *moderate*.

Immunophenotyping by IHC staining and/or flow cytometry, in addition to morphology, is well established as critical for lymphoma diagnosis and subtyping. Numerous studies have found that flow cytometry of fresh, unfixed tissue can be used to identify clonal B-cell populations in a variety of specimens and lymphoma subtypes. One study found that flow cytometric analysis identified clonal B-cell populations in biopsies from 382 of 471 patients (81%) with B-cell NHLs, including low- and high-grade B-cell lymphomas.[49] Another study found that flow cytometric analysis identified clonal B-cell populations in 147 of 169 lymph node biopsy or FNA specimens (87%) involved by B-cell lymphoma.[50] Two studies on FNA specimens involved by B-cell NHL

compared with reactive lymphoid tissue found that flow cytometric analysis identified clonal B cells, based on restricted immunoglobulin light-chain expression, with a sensitivity of 82% to 88%, specificity of 72% to 100%,[51,52] PPV of 93%, and NPV of 48%.[51] In FNA specimens from B-cell lymphomas that were negative or inconclusive for surface immunoglobulin light chain, flow cytometric assessment of cytoplasmic immunoglobulin light-chain staining and/or Bcl-2 expression was helpful for establishing a diagnosis of B-cell lymphoma in 83% of cases.[53]

Flow cytometry can also be used to identify clonal T-cell populations,[54] based on aberrant expression of pan–T-cell markers. A study of 50 peripheral T-cell lymphomas found aberrant overexpression, underexpression, or absence of expression of 1 or more pan–T-cell markers (CD3, CD7, CD5, CD2) in 46 of 50 cases (92%).[55] Another study detected aberrant T-cell marker expression by flow cytometric analysis in 97 of 155 samples (63%) from 38 patients with angioimmunoblastic T-cell lymphoma.[56] Multiple studies have found that flow cytometric analysis can also identify clonal T-cell populations based on overexpression or restricted expression of T-cell receptor (TCR) V beta-chain epitopes using proprietary antibodies and technology. One study[57] found 27 of 30 T-cell NHL biopsy specimens (90%) exhibited overexpression of a V beta-chain epitope. This technique had a sensitivity of 90% and specificity of 98% for the detection of clonal T-cell populations,[57] but has not been widely used because of its relatively high cost and complexity. A promising new approach to identify clonal T-cell populations by flow cytometric analysis is based on restricted expression of the TCR beta-chain constant region 1 (*TRBC1*). In a study[54] of 51 specimens with suspected T-cell lymphomas, this approach had a sensitivity of 97%, specificity of 91%, and correlated with PCR-based T-cell clonality testing.

Beyond establishing clonal B- and T-cell populations, flow cytometry findings can also be used to establish a specific lymphoma diagnosis in a variety of specimens. Flow cytometric analysis achieved diagnosis and categorization of NHL in 89 of 92 cases (97%) according to the 2001 WHO classification of hematolymphoid neoplasms,[58] in 63 of 73 cases (86.3%) of B-cell NHLs, and 5 of 7 cases (71.4%) of T-cell NHLs with diagnostic agreement between flow cytometry findings and morphology/IHC findings.[59] For FNA-based studies, combined cytologic and flow cytometric analysis had a sensitivity of 98% and specificity of 100% for a final B-cell lymphoma diagnosis, including subtyping.[60] It should be noted that cases of pure lymphoid populations in this study were chosen to undergo flow cytometry if the patient was older than 50 years or had a history of NHL, multiple enlarged lymph nodes, or persistent lymphadenopathy of unknown cause. For endoscopic ultrasound-guided FNA specimens, flow cytometry enabled a diagnosis of a specific B-cell or T-cell lymphoma in 121 of 152 lymphoma cases (79.6%), including 114 of 126 B-cell lymphoma cases (90.5%); for 5 cases (3.3%), the lymphoma diagnosis was based solely on flow cytometric findings.[31]

Virtually all classification schemes including the WHO lymphoma classification rely on a panel of immunophenotypic markers for the diagnosis and subtyping of B-cell and T-cell neoplasms.[1] Three studies[22,24,31] examining small-volume biopsies guided with various imaging techniques demonstrated the utility of IHC. In one recent study of 152 lymphoma cases diagnosed based on endoscopic ultrasound-guided FNA, IHC staining, in conjunction with histologic examination, was able to establish an NHL diagnosis in 93.4% of patients, and a WHO classification–based specific diagnosis in 88.8% of patients.[31] Another study of 185 power Doppler ultrasonography–guided lymph node CNBs found that histologic examination combined with IHC staining had a sensitivity of 98.7% and NPV of 84.6% for a specific lymphoma diagnosis.[22] Another study of 74 CT-guided needle biopsies that underwent histologic examination, including IHC staining with a panel of markers for possible lymphoma, was able to establish a specific lymphoma diagnosis in 43 of 45 cases (96%), and had overall sensitivity of 93% and specificity of 100%.[24] In a study by Hsi et al,[61] peripheral T- and NK-cell lymphomas were evaluated and demonstrated increased accuracy of T-cell lymphoma diagnosis by IHC.

The evidence base for this statement comprises 19 studies.* Twelve studies reported strong diagnostic test characteristics using flow cytometry on CNB and surgical biopsies[49,50,57–59,62] or on FNA specimens,[51–53,56,60,63] and 1 study reported strong diagnostic test characteristics when using IHC on FNA specimens.[31] An additional 6 studies[6,7,21,22,24,27] reported strong diagnostic test characteristics when using IHC as part of a routine diagnostic workup on CNB specimens and were used by the EP as indirect evidence to inform the statement. The 19 studies included 1 intermediate-quality systematic review with meta-analysis,[21] 1 intermediate-quality randomized controlled trial,[22] 5 intermediate- to low-quality prospective cohort studies,[49,52,57,58,63] and 12 RCSs of low[6,7,24,31,50,51,59] or very low quality.[27,53,56,60,62] Based on the identified studies, all EP members agreed that immunophenotyping by flow cytometry and/or IHC in addition to morphology was accurate and provided large benefits.[61] Although the EP members considered the harms to range from moderate to trivial, all EP members agreed that the benefits outweighed the potential harms. A majority of the EP members concluded that combination flow cytometry and/or IHC with morphologic assessment would result in moderate costs and probably no impact on health equity. All EP members agreed that guidance in this area is a priority at this time and feel that the recommendation statement is acceptable to key stakeholders and feasible to implement. Refer to the SDC for the strength-of-evidence assessment for this statement and a complete summary of the EtD framework. The open comment period produced comments that overwhelmingly supported the recommendation (99.2%) as written or with suggested modifications. Many of the comments pointed out the need for cytogenetic analysis, including FISH, and for molecular testing, in selected cases, for diagnosis and classification.

11. **Conditional Recommendation.**—Clinical care providers may use FISH analysis when evaluating specimens in patients with suspected or confirmed lymphoma, or in the subclassification of lymphoma. Fluorescence in situ hybridization analysis is feasible on specimens obtained by FNA and may increase diagnostic yield.

Note: Demonstration of the appropriate rearrangements is required for a diagnosis of high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements.

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* *References 6, 7, 21, 22, 24, 27, 31, 49–53, 56–60, 62–64.*

The strength of evidence to support this guideline statement is *low*.

Diagnosis and subclassification of low-grade B-cell lymphomas on FNA is hampered by limited intact tissue architecture to provide support for a diagnosis of lymphoma and to accurately subclassify the lymphoma. The 2 FISH-related studies[65,66] in the evidence base reported directed use of particular FISH probes to aid in diagnosis of lymphoma in FNA specimens. The FISH probes were chosen based on clinical history, morphologic features, and/or immunophenotype. Importantly, as noted during the open comment period, neither study tested the up-front use of panels of FISH probes, which would be expected to significantly increase costs and risk of false-positive results. In one study,[66] FISH evaluation was requested most commonly for subclassification, and was added to cases of DLBCL, Burkitt lymphoma, high-grade B-cell lymphoma, follicular lymphoma, and mantle cell lymphoma. Fluorescence in situ hybridization was positive in 61% of cases, negative in 26%, and indeterminate in 12% (including 2% that failed to hybridize). In the second study,[65] successful results were documented in 95% of the 298 cases in which FISH was deployed. In a number of cases, FISH results contributed information that resulted in a change from the initial diagnosis based on morphologic and IHC evaluation. Notably, this included a revised diagnosis of dual- or triple-hit lymphoma in 13 cases in which the initial diagnoses were reported as DLBCL not otherwise specified (n = 6), B-cell lymphoma unclassifiable with features intermediate between large B-cell lymphoma and Burkitt lymphoma (n = 4), and follicular lymphoma (n = 3).

A number of new or provisional entities in the revised *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues* are defined by rearrangements that require detection by cytogenetic or molecular methods such as FISH.[1] The so-called double- or triple-hit lymphomas, highlighted above, are now designated as high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements.[67] *MYC* translocations are a sine qua non for this diagnosis, with either *BCL2* or *BCL6* translocations accompanying this in double-hit lymphomas and all 3 translocations present in triple-hit lymphomas. Because metaphase analysis is rarely routinely performed on lymphoma specimens, FISH plays a crucial role in rendering this diagnosis. Because there are no robust tools to definitively predict which DLBCLs may be double- or triple-hit lymphomas, it is reasonable to perform FISH for *MYC* translocations on all samples with large B-cell morphology and, if positive, to proceed with *BCL2* and/or *BCL6* FISH.[68,69] Another rare subtype of large B-cell lymphoma newly recognized by WHO is one associated with an *IRF4* rearrangement[67]; immunoglobulin H is the most frequent partner and FISH is required for its detection because this translocation is cytogenetically cryptic.

Chromosomal rearrangements detectable by FISH underlie a large portion of the current classification system for lymphoma, as laid out in the current *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*.[1] Furthermore, there is strong evidence of the prognostic impact of particular chromosomal rearrangements in the setting of lymphoma. Perhaps because FISH studies already represent the standard of care, studies addressing the specific contribution of FISH to lymphoma diagnosis were scarce within the parameters of the literature review. The EP considerations in formulating this recommendation were strengthened by the wide range of comments provided during the open comment period. Comments stressed the need for comprehensive clinicopathologic diagnosis not based solely on the demonstration of chromosomal rearrangements by FISH, the need for particular FISH studies not just for diagnosis but for prognosis and treatment planning, the suitability or lack thereof of a variety of specimen types for FISH testing, and the lack of evidence to support the use of broad or nontargeted FISH panels in the workup of lymphoma. The recommendation statement was drafted based on the limited evidence available in the form of 2 studies[65,66] focusing on the role of FISH in the diagnosis of lymphoma in FNA specimens in the context of our collective experience in the routine standard-of-care use of FISH in diagnosis and subclassification of lymphoma in specimens of all types.

There is a lack of peer-reviewed studies focused on FISH analysis for the evaluation of suspected or confirmed lymphoma in non-FNA specimens, such as excisional biopsy specimens, likely because this is the gold standard and represents routine clinical practice. Both included studies were of an RCS design and were assessed as low quality with an aggregate very serious risk of bias.[65,66] Based on the available evidence, a majority of EP members agreed that the benefits of using FISH when evaluating specimens were moderate to large, whereas the harms of its use were moderate to small, and thus benefits of use outweighed the potential harms. Although use of FISH may carry a moderate cost, the majority of the EP members concluded that this recommendation would be acceptable to key stakeholders and feasible to implement. Refer to the SDC for the strength-of-evidence assessment for this statement and a complete summary of the EtD framework. The open comment period produced comments that supported the recommendation as written (75.3%) or indicated agreement with suggested modifications (20.2%), with 95.5% total agreement. As noted by respondents during the open comment period, the presence of particular FISH rearrangements in isolation is not sufficient to provide a specific diagnosis, nor to confirm a diagnosis of overt lymphoma. However, respondents agree that there is a role for FISH analysis, especially in the stratification of high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements.

12. **Conditional Recommendation.**—Clinical care providers should not routinely use up-front PCR-based clonality studies of antigen receptor genes (ie, TCR and immunoglobulin) in the initial investigation of lymphoma. There may be a confirmatory role in certain settings for these studies.

The strength of evidence to support this guideline statement is *low*.

The evidence base shows high but imperfect sensitivity and specificity of molecular testing for immunoglobulin gene rearrangements. There is a paucity of data on the performance characteristics of up-front molecular testing for TCR gene rearrangements in formulating the recommendation. The EP was concerned about the potential harms associated with up-front molecular testing that could expose a large population of patients to possible false-positive or false-negative diagnoses, unnecessary medical costs, or use of limited biopsy material for unnecessary testing. The current laboratory standard for multiplex PCR clonality

testing is based on the EuroClonality/BIOMED-2 protocol.[70,71] Roepman et al[72] reported the results of EuroClonality/BIOMED-2 protocol–based B-cell clonality testing in 183 patients, 29% of them with B-cell lymphoma. The reported sensitivity was 93% (95% CI, 87%–97%) and specificity 92% (95% CI, 88%–95%). A study by Brozic et al[53] performed both B- and T-cell clonality testing on specimens from 98 patients, 31% with B-cell lymphoma and the remainder with reactive lymphadenopathy. The reported sensitivity and specificity of B-cell clonality testing for B-cell lymphoma were 77% (95% CI, 58%–90%) and 88% (95% CI, 78%–95%), respectively. The other 3 studies in the evidence base did not use the EuroClonality/BIOMED-2 protocol[50] or were based on smaller patient cohorts,[73,74] but were in general agreement with these reported sensitivity and specificity ranges.

B-cell lymphomas often undergo flow cytometry immunophenotyping. B-cell clonality can be determined by light-chain restriction on flow cytometry immunophenotyping; up-front molecular B-cell clonality testing may be superfluous in such cases. One of the studies[50] in the evidence base reported the results of both flow cytometry immunophenotyping and molecular clonality studies. Of 149 B-cell lymphomas, 131 (88%) showed light-chain restriction by flow immunophenotyping, which would presumably have obviated the need for molecular studies. Of the 18 cases without light-chain restriction, 14 (78%) were DLBCLs, which generally can be diagnosed based on histologic features without the need for clonality studies of any sort. Thus, in the case of B-cell lymphomas overall, the added value of up-front immunoglobulin molecular clonality studies in the initial diagnosis setting combined with routine flow cytometry immunophenotyping appears to be quite limited.

Data on T-cell clonality testing were scant in the evidence base; neither sensitivity nor the added value of T-cell clonality testing for detection of T-cell lymphoma could be assessed based on the 5 identified studies in the evidence base. The study by Brozic et al[53] reported T-cell clonality testing on 30 B-cell lymphomas and 68 reactive lymphadenopathies; false-positive T-cell clonality results were reported in 10% of B-cell lymphomas and 13% of reactive lymphadenopathies. The literature indicated a high degree of variability in the specificity of T-cell clonality studies depending on test characteristics.[75] The incidence of T-cell lymphoma is low, with about 1 to 2 new cases of T-cell lymphoma diagnosed per 100 000 population per year in the United States,[76] and T-cell lymphoma represents a minority of lymphomas worldwide.[77] Given the low incidence of T-cell lymphoma, even relatively high specificity could still lead to significant numbers of false-positive TCR clonality results in unselected patients. Note that *false-positive* in this context refers to biological false positives, that is, the detection of nonmalignant clonal T-cell expansions. These are false-positive with respect to the question of "Is a T-cell lymphoma present?" but are true positives for the question of "Is a clonal T-cell expansion present?" The latter question encompasses a different set of clinical circumstances.

The nature of molecular clonality testing is currently expanding to the use of next-generation sequencing–based assays capable of identifying, and following from specimen to specimen, specific small clonal T-cell,[78] B-cell, or plasma cell populations.[64] By their nature, next-generation sequencing–based studies are more sensitive than multiplex PCR-based techniques, both in identification of rearrangements that might have been previously missed because of somatic hypermutation interfering with multiplex PCR and in the presence of a minority of clonal lymphoma cells in a majority reactive background. They also inherently raise questions of the meaningfulness of small clonal populations and their role in primary lymphoma diagnosis. Insufficient data were present in the literature to allow the EP to make recommendations regarding newer high-sensitivity molecular clonality testing platforms.

The evidence base for this statement comprised 5 studies.[50,53,72–74] All studies were RCSs and were assessed as low[50,72–74] or very low quality.[53] Based on the identified evidence, the EP members were divided on multiple domains of the EtD framework. When considering the accuracy of up-front PCR-based clonality and antigen receptor gene rearrangement assays, only half of the members deemed the assays to be accurate. This led to EP members variably assessing the benefits as small to large. However, the harms were still believed to be small or trivial. When benefits were weighed against the harms of performing the assays, the EP members were divided, with the majority concluding the benefits to not outweigh the harms and a minority stating that there was a balance between benefits and harms. Based on the lack of evidence supporting the accuracy of these assays and the weight of benefits versus harms, the EP recommends against the routine use of molecular clonality tests as up-front assays. All EP members believe this statement to be acceptable to key stakeholders and feasible to implement. Refer to the SDC for the strength-of-evidence assessment for this statement and a complete summary of the EtD framework. During the open comment period, there were numerous comments supportive of the recommendation, with rare respondents advocating for routine up-front molecular clonality testing to avoid delays in patient care. Several respondents noted the need for clarity in what is meant by "up-front" clonality testing and the utility of molecular clonality testing in specific clinical and diagnostic situations. The EP is not recommending against the use of molecular clonality testing in general; rather, the EP does not recommend routine up-front testing in the absence of a specific clinical or diagnostic need. The lack of support for routine up-front molecular clonality testing does not exclude a role for situation-specific molecular clonality testing in lymphoma diagnosis.

13. **Conditional Recommendation.**—Clinical care providers may use molecular tests to aid in classification of lymphomas. For example, pathologists may use *MYD88* L265P to aid in the classification of indolent B-cell lymphoma.

Note: This recommendation statement refers to non-FISH molecular tests.

The strength of evidence to support this guideline statement is *low*.

Both targeted candidate gene and genome-wide approaches have led to the discovery of numerous mutations across the spectrum of lymphomas. Although rare examples currently play a role in diagnosis, others are best designated as prognostic markers, and yet others (albeit sometimes overlapping with the first 2 groups) have therapeutic implications. Currently, the *MYD88* L265P mutation is the only example of a mutation that can be used to facilitate the diagnosis of a specific lymphoma, namely LPL. In one

study,[79] Sanger sequencing and allele-specific PCR were able to detect the L265P mutation in formalin-fixed and decalcified bone marrow samples; PCR in particular was able to detect the mutation in bone marrow infiltrations below 1% of lymphoma cells and clearly distinguish patients with confirmed Waldenström macroglobulinemia/LPL and other indolent lymphomas, including chronic lymphocytic leukemia/small lymphocytic lymphoma and splenic marginal zone lymphoma. These results build on prior data that demonstrate the utility of *MYD88* L265P testing; the mutation is detectable in nearly all individuals with Waldenström macroglobulinemia/LPL, approximately 50% of those with immunoglobulin M monoclonal gammopathy of undetermined significance, in some forms of large B-cell lymphoma, and rarely in other indolent B-cell lymphoproliferative disorders.

Overall, 5 studies[50,53,74,79,80] evaluated the use of PCR/mutational analysis to aid in classifying lymphoma subtypes; 3 studies[50,53,74] are discussed in detail in the previous statement on up-front use of PCR-based clonality studies of antigen receptor genes. The current recommendation focused on 2 RCSs[79,80] that were assessed as low[80] and very low[79] quality. Based on the identified evidence, all EP members agreed that mutational analysis may be valuable when classifying lymphoma subtypes, and that benefits of its use outweigh small to trivial harms. In terms of resource use, the EP members were divided, with the majority believing addition of mutational analysis would result in a moderate cost and the minority concluding that the costs would range from large to negligible. Despite disagreement on resource use, all EP members believe this statement to be acceptable to key stakeholders and feasible to implement. Refer to the SDC for the strength-of-evidence assessment for this statement and a complete summary of the EtD framework. The open comment period produced comments that supported the recommendation as written (88.4%) or indicated agreement with suggested modifications (11.6%), with all respondents in total agreement. The open comment period respondents mentioned the importance of the sensitivity and specificity of the assay and suggested that panel testing may be more efficient in the subclassification of lymphoma. The section on emerging assays in this guideline will provide a more comprehensive list of up-and-coming markers that may have clinical utility in the diagnosis and subclassification of lymphoma in the near future.

Good Practice Statements

According to the Grading of Recommendations Assessment, Development, and Evaluation approach, good practice statements are recommendations that panels may consider important but are not appropriate to be formally rated for quality of evidence.[4] In addition to the set of key questions formulated a priori, the EP decided to write the good practice statements listed in Table 4, which reflect expert consensus opinions supported by a limited number of studies and data that were not formally included in the evidence base or systematically rated. The EP wanted to address the following questions:

- Under what circumstances does second review by an expert in hematopathology improve the accuracy of diagnosis?
- To what extent do pathologists use clinical characteristics and radiographic data in the formation of pretest probability and what is the role of this information in determining the diagnosis?
- To achieve efficient patient management, what elements related to specimen handling should be included in the pathology report, and if a biopsy specimen is deemed suboptimal for diagnosis, what elements should be included in the report to explain why the specimen is suboptimal?

A targeted literature search was performed based on these questions. The EP cochairs reviewed the available literature and incorporated the data collected into a preguideline development practice survey to arrive at the good practice statements. A detailed process for the literature review for the good practice statements is included in the SDC.

Secondary Review

1. For the diagnosis of difficult-to-classify lymphomas, laboratories should have a robust peer review process. Peer review may include a second review by a more experienced pathologist or a consensus review by a group of pathologists.

Table 5 lists lymphoma subtypes by the degree of difficulty in their classification.[81–90] Multiple studies show that secondary reviews in pathology increase the diagnostic accuracy. The EP recommends that institutions incorporate their own peer review processes into their quality assurance programs.

Clinical Information for Pretest Probability

2. Pathologists should use appropriate clinical information in the workup and classification of lymphoma and lymphoma subtypes. Clinical information may include:
   - Clinician consultation (eg, direct communication with the hematologist/oncologist, tumor board discussions)
   - Review of clinical records (eg, radiographic imaging, clinical staging, and patient/disease characteristics including age, comorbidities, and disease aggressiveness)
   - Laboratory investigations (eg, complete blood count, lactate dehydrogenase, serum protein electrophoresis, viral studies)

During the development of the guideline, the EP sent out a practice survey to collect data on what clinical information influences pathology workup for lymphoma and its subtypes. Most of the survey respondents agreed that clinical information review appropriately influences the workup of lymphoma. In addition, the EP discussed the importance of direct communication with the patient's treating physicians, especially in difficult-to-classify cases.

Specimen Handling

3. Laboratorians should include the following specimen-handling elements in the final surgical pathology report:
   - Anatomic site
   - Specimen condition (eg, fresh, in fixative, in RPMI)
   - Procedure type (eg, excisional or incisional biopsy, CNB, FNA)
   - Gross description of the specimen (eg, dimensions, color, texture, shape, number of cores)
   - Any fixative used
   - Availability of unfixed specimens for ancillary testing

In addition to the elements listed above, laboratorians

Table 4. Good Practice Statements
1. For the diagnosis of difficult-to-classify lymphomas, laboratories should have a robust peer review process. Peer review may include a second review by a more experienced pathologist or a consensus review by a group of pathologists
2. Pathologists should use clinical information in the workup and classification of lymphoma and lymphoma subtypes
3. Laboratorians should include specimen-handling elements in the final pathology report
4. Laboratories should provide appropriate turnaround times for lymphoma test results to inform clinical decision-making
5. Laboratories should establish policies to ensure efficient allocation and use of tissue for lymphoma testing
6. Laboratories that send out tests for lymphoma diagnosis should have a process in place to ensure that specimens are sent and reviewed by outside reference laboratories in a timely manner

should also include comments on specimen adequacy for histology and ancillary testing in the final pathology report.

Turnaround Time

4. Laboratories should provide appropriate turnaround times for lymphoma test results to inform clinical decision-making.
5. Laboratories should establish policies to ensure efficient allocation and use of tissue for lymphoma testing.
6. Laboratories that send out tests for lymphoma diagnosis should have a process in place to ensure that specimens are sent and reviewed by outside reference laboratories in a timely manner.

Laboratory testing and diagnosis in lymphoma vary as tests used can be different for each lymphoma subtype. The EP surveyed pathologists and clinicians for information on turnaround time for each of these tests; Table 6 summarizes the suggested turnaround times. The efficient management and accurate diagnosis of lymphoma is essential to clinical decision-making. Laboratories must strive to provide the most efficient turnaround times to ensure that patients are provided the most complete information about their diagnosis in time to optimize treatment with their clinician.

Limitations

Readers will note that the guideline makes no recommendations regarding key questions 2 and 3, related to minimum and optimal specimen requirements and appropriate triage process of tissue for various studies, respectively. No recommendations were generated because evidence bases to inform recommendations around these issues effectively do not exist. Similarly, although key question 1a includes peripheral blood, pleural fluid, pericardial fluid, serous body fluids, and CSF, only sufficient evidence to inform recommendations concerning CSF were identified. The results of the practice survey conducted during the development of this guideline hopefully begin to address these issues in the absence of formal evidence.

Table 5. Secondary Reviews for Different Lymphomas

Highly Reproducible Lymphoma Types[a]	Lymphoma Types for Which Secondary Review is Suggested
Nodular sclerosis classic HL	Rare HL subtypes
DLBCL	DLBCL with suspicion for Burkitt lymphoma
CLL/SLL[b]	High-grade B-cell lymphoma
MCL	FL with suspicion for transformation
Low-grade FL	All T-cell and NK-cell lymphomas
	CLL with suspicion for Richter transformation
	Marginal zone lymphoma

Abbreviations: CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; HL, Hodgkin lymphoma; MCL, mantle cell lymphoma; NK, natural killer; SLL, small lymphocytic lymphoma.

[a] Highly reproducible lymphoma types are defined as lymphoma types with high interobserver agreement between reviewing pathologists.[81–84,87–89]

[b] CLL/SLL was considered out of scope for this guideline.

Emerging Assays

The EP members appreciate that evolving applications for ancillary testing continue to arise for the diagnosis, subclassification, prognostication, and guidance of therapy in lymphoma; at present, these indications are beyond the scope of our guideline. Herein, we highlight future avenues of development.

Flow Cytometry

Flow cytometric analysis, although not routinely used to identify cases of classic HL, when used in a study of 53 cases was found to have a sensitivity of 88.7% and specificity of 100%.[91] A study of nonneoplastic T cells in reactive lymphoid tissue and lymphoid tissue involved by classic HL found an increased CD4:CD8 ratio in HL compared with that in reactive lymph nodes and overexpression of CD7 in the CD4-positive T cells in HL compared with reactive lymph nodes; using an optimized mean fluorescence intensity cutoff for CD7 expression in CD4-positive T cells, this approach had a sensitivity of 69% and specificity of 90% for the diagnosis of classic HL.[62]

Fluorescence In Situ Hybridization

The use of FISH probes in the diagnosis of less common lymphoma subtypes was not covered by our literature review. For example, detection of *MALT1* rearrangements,[65,66] and less frequently rearrangements involving *BCL10* and *FOXP1*, may aid in the diagnosis of some extranodal marginal zone lymphomas, although collectively these rearrangements are detected in less than 30% of cases.[92] Other specific FISH studies not discussed in our evidence base can be very helpful in the diagnosis of specific entities; for example, FISH for *TCL1* gene rearrangements is superior to conventional cytogenetic studies for the diagnosis of T-cell prolymphocytic leukemia.[93] Fluorescence in situ hybridization may play a role in prognostication of anaplastic large cell lymphomas that are negative for anaplastic lymphoma kinase (ALK); those with *DUSP22* translocations have a better prognosis, approximating that of anaplastic large cell lymphoma, ALK positive, whereas those with *TP63* translocations are more aggressive.[93] Other tailored FISH studies may provide additional prognostic or otherwise therapeutically relevant information in specific clinical settings beyond the scope of this guideline.

Table 6. Routine Turnaround Time (TAT) Targets for Lymphoma Tests[a]	
Test	**TAT, Business Days**
FISH for unique translocations	5–7
Flow cytometric analysis	1–2
Immunohistochemistry	1–2
Morphologic assessment	1–2
PCR for antigen receptor gene rearrangements	5–7

Abbreviations: FISH, fluorescence in situ hybridization; PCR, polymerase chain reaction.

[a] Data derived from practice survey sent to pathologists and hematologists.

Molecular Studies

Although we reviewed the role of the *MYD88* L265P mutation in the diagnosis of LPL, our literature review did not cover the role of this mutation or of *CXCR4* mutations in predicting response to therapy. Although LP patients with the *MYD88* L265P mutation respond well to therapy with inhibitors of Bruton tyrosine kinase (BTK), those with *CXCR4* mutations, seen in about 35% of LPLs, generally experience a diminished response to BTK inhibitors,[94] and analysis for the latter mutation may be warranted in a subset of patients.

An abundance of different mutations has been described in DLBCL. None are currently required to render a diagnosis of DLBCL not otherwise specified, but their presence has prognostic and potential therapeutic connotations**.** Among peripheral T-cell lymphomas, the currently best recognized subtype–mutational profile association is in the context of lymphoma derived from follicular helper T cells, in particular angioimmunoblastic T-cell lymphoma. *RHOA*, *IDH2*, and *TET2* mutations occur more often in angioimmunoblastic T-cell lymphoma than in any others, and though not currently required to render a diagnosis, their detection may help facilitate diagnosis.[95,96] Furthermore, *IDH2* mutations can be targeted by specific inhibitors. T-cell large granular lymphocytic leukemia does not require mutational testing to render a diagnosis, but the presence of *STAT3* mutations may help guide therapy.[97]

In summary, although the detection of only 1 or 2 mutations is currently of value in some lymphoid neoplasms, others are associated with a larger number of mutations that may play a role in prognosis and therapeutic decision-making. This, together with the escalating number of mutations described across the spectrum of lymphomas, suggests that single-mutation analysis or limited sequencing panels may not suffice and that larger panels using high-throughput sequencing will be used more frequently in future daily practice.

Guideline Revision

This guideline will be reviewed every 4 years, or earlier in the event of publication of substantive and high-quality evidence that could potentially alter the original guideline recommendations. If necessary, the EP will reconvene to discuss potential changes. When appropriate, the panel will recommend revision of the guideline to the ASCP and CAP for review and approval.

CONCLUSIONS

The primary diagnosis and classification of lymphoma can be achieved through analysis of a variety of specimen types. The application of the evidence-based recommendations presented here may guide decision-making regarding appropriate specimens, diagnostic capabilities, and correct use of ancillary testing. Disease prevalence in patient populations, availability of ancillary testing, and diagnostic goals should be incorporated into algorithms tailored to each practice environment. In addition, to fully inform decision-making, it is important not only to examine the advantages of the available approaches, but also to assess their limitations. An overarching feature of the evidence base was that it was uniformly of weak quality. Readers may note that several strong recommendations have been made based on this weak evidence. In addition to the strength of evidence, the EP used the Grading of Recommendations Assessment, Development, and Evaluation EtD framework to provide a sequence of key judgements to consider, including weighing the benefits against the harms of each statement. For most of the strong recommendations based on low strength of evidence, it was determined that providing a recommendation for the opposite action could result in substantial harms to patients.

Understanding the limitations and advantages as demonstrated by the available evidence will help health care providers and patients manage expectations and choose a diagnostic testing strategy that is best suited to their goals and resources. Each patient must be fully informed about the different diagnostic strategies, as well as other factors, to efficiently achieve a lymphoma diagnosis.

Guideline Funding and Management of Conflict of Interest

The ASCP, CAP, and ASH provided funding for the administration of the project. Direct funding from for-profit companies was not accepted. All EP members volunteered their time. They received travel support from their organizations to attend project meetings. In accordance with a joint disclosure of interest policy by ASCP, CAP, and ASH, members of the EP and advisory panel disclosed all financial relationships with and interests in for-profit companies from 24 months prior to appointment as well as during the guideline development process. They also disclosed nonfinancial interests relevant to the guideline topic. Disclosures were reviewed by a Disclosure of Interest Review Committee composed of members and staff of the 3 organizations. On appointment, the majority of the EP, including the 3 cochairs, had no direct financial conflicts of interest with any for-profit company that could be affected by the guidelines. During the guideline development process, members of the EP reported new interests and relationships, but this majority balance was maintained. The complete disclosures of the EP members are provided in the Appendix. Please see the SDC for full details on the disclosure of interest policy.

Disclaimer

Clinical practice guidelines reflect the best available evidence supported in practice. They are intended to assist physicians and patients in clinical decision-making and to identify questions and settings for further research. With the rapid flow of scientific information, new evidence may emerge between the time a clinical practice guideline is developed and when it is published or read. Clinical practice guidelines are not continually updated and may not reflect

the most recent evidence. Clinical practice guidelines address only the topics specifically identified therein and are not applicable to other interventions, diseases, or stages of diseases. Furthermore, guidelines cannot account for individual variation among patients and cannot be considered inclusive of all proper methods of care or exclusive of other treatments. It is the responsibility of the treating physician or other health care provider, relying on independent experience and knowledge, to determine the best course of treatment for the patient. Accordingly, adherence to any CPG is voluntary, with the ultimate determination regarding its application to be made by the physician considering each patient's individual circumstances and preferences. The ASCP and CAP organizations make no warranty, express or implied, regarding clinical practice guidelines and specifically exclude any warranties of merchantability and fitness for a particular use or purpose. The ASCP and CAP organizations assume no responsibility for any injury or damage to persons or property arising out of or related to any use of this statement or for any errors or omissions.

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APPENDIX. Disclosed Interests and Activities From November 2017 to May 2020[a]		
Adam Bagg, MD	Consulting	Amgen
	Lecture fees (honoraria)	Cornell University
		American Society for Clinical Pathology
		College of American Pathologists
		Loma Linda University
		American Registry of Pathology
		City of Hope Cancer Center
		International Society for Laboratory Hematology
		West Penn Allegheny Health System
		United States and Canadian Academy of Pathology
		Brigham and Women's University
		Yale University
	Position of influence	Associate Editor, *Journal of Molecular Diagnostics*, American Society for Investigative Pathology
		American Medical Foundation
	Research funding	Novartis-Penn Alliance
	Advisory board	Beckman Coulter
		Targeted Oncology
		Jazz Pharmaceuticals
		Blueprint Pharmaceuticals
		Acceleron Pharmaceuticals
Matthew C. Cheung, MD	Research funding	Gilead
		Immunovaccine
		GlaxoSmithKline
		Celgene
		Janssen
		Allos
		Acerta
		Abbvie
		Hoffman-LaRoche
		IMV and Merck
Catherine Diefenbach, MD	Stock options/bonds	Gilead
	Advisory board	Genentech
		Seattle Genetics
		Bristol Myers Squibb
		Merck
		Celgene
		Janssen
		MorphoSys
	Research funding	Genentech
		Seattle Genetics
		Bristol Myers Squibb
		Incyte
		Millennium
		Merck
		Lymphangioleiomyomatosis (LAM) Therapeutics
		MEI Pharma
David M. Dorfman, MD, PhD	Royalties	Genentech
William G. Finn, MD	Stock options/bonds	Laboratory Associates of Michigan
	Patent	US patent No. 7,853,432
Dita A. Gratzinger, MD, PhD	Research funding	KaloBios
Patricia A. Gregg, MD	Consulting fees	NeoGenomics
Steven H. Kroft, MD	Position of influence	Editor-in-chief, *American Journal of Clinical Pathology*

APPENDIX. Continued		
John P. Leonard, MD	Consulting fees	Hospira
		Bayer
		Juno Therapeutics
		Teva
		Celgene
		Kite Pharma
		Genmab
		Nanostring
		Regeneron
		AbbVie
		Sutro Biopharma
		Sunesis
		Genentech
		Biotest
		Bristol Meyers Squibb
		Gilead
	Research funding	Genentech
		Celgene
		Abbvie
		Gilead
		Pharmacyclics
		Regeneron
		Karyopharm
		Bristol Meyers Squibb
		National Institutes of Health
		Leukemia and Lymphoma Society
		Leukemia Research Foundation
Cordelia E. Sever, MD	Research funding	Horiba
		Sight Diagnostics
		Techcyte Inc
Sonali Smith, MD	Consulting fees	Gilead
		Juno
		Pharmacyclics
		TG Therapeutics
		Genentech
		Nanostring
		Portola
		AbbVie
		Celgene
	Research funding	Celgene
		TG Therapeutics
		Acerta
		Pharmacyclics
		Janssen
		National Cancer Institute/Southwest Oncology Group (SWOG)
	Position of influence	Lymphoma Research Foundation
	Lecture fees (honoraria)	Genentech
		Kite
Ronald L. Weiss, MD, MBA	Stock options/bonds	AvanSciBio

[a] The following individuals have reported no relevant disclosures: Brooke Billman, MLIS, AHIP; Lesley Souter, PhD; and Christina B. Ventura, MPH, MT(ASCP).