COLLEGE of AMERICAN PATHOLOGISTS

Template for Reporting Results of Biomarker Testing of Specimens From Patients With Diffuse Large B-Cell Lymphoma, Not Otherwise Specified (NOS)

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For the Members of the Cancer Biomarker Reporting Committee, College of American Pathologists

CAP Diffuse Large B-Cell Lymphoma Biomarker Template Revision History

Version Code
The definition of the version code can be found at www.cap.org/cancerprotocols.

Version: DLBCL_Biomarkers 1.0.0.0

Summary of Changes
This is a new template.

Diffuse Large B-Cell Lymphoma Biomarker Reporting Template

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Completion of the template is the responsibility of the laboratory performing the biomarker testing and/or providing the interpretation. When both testing and interpretation are performed elsewhere (eg, a reference laboratory), synoptic reporting of the results by the laboratory submitting the tissue for testing is also encouraged to ensure that all information is included in the patient's medical record and thus readily available to the treating clinical team.

DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL), NOT OTHERWISE SPECIFIED (NOS)

Select a single response unless otherwise indicated.

Note: Use of this template is optional.

+ SPECIMEN TYPE
+ ___ Peripheral blood
+ ___ Bone marrow
+ ___ Lymph Node (specify site): _____
+ ___ Other (specify): _____

RESULTS

+ Protein Expression (by immunohistochemistry [IHC] or flow cytometry) (Notes A and B)
+ ___ BCL2
    + ___ Not detected
    + ___ Detected
+ ___ CD5
    + ___ Not detected
    + ___ Detected
+ ___ CD20
    + ___ Not detected
    + ___ Detected
+ ___ CD30
    + ___ Not detected
    + ___ Detected
+ ___ Ki-67
    + ___ Not detected
    + ___ Detected
+ ___ MYC
    + ___ Not detected
    + ___ Detected
+ ___ Other marker(s) tested (specify): _____
    + ___ Not detected
    + ___ Detected

+ Subtype Classification (Note C)
+ ___ Germinal center-like
+ ___ Non-germinal center-like

+ Chromosomal Abnormalities (by fluorescence in situ hybridization [FISH]) (Note B)
+ ___ *MYC* rearrangement
    + ___ Not detected
    + ___ Detected
    + ___ Other (specify): _____
+ ___ *BCL2* rearrangement
    + ___ Not detected
    + ___ Detected
    + ___ Other (specify): _____
+ ___ *BCL6* rearrangement
    + ___ Not detected
    + ___ Detected
    + ___ Other (specify): _____
+ Other probes tested (if applicable)
    + Specify probe: _____
    + Specify results: _____

+ Cytogenetic testing complete karyotype (specify): _____

+ Somatic Gene Mutations (by sequencing) (Note D)
+ ___ Not detected
+ ___ Detected (specify variant): _____
+ ___ Other (specify): _____

+ Other Markers Tested (if applicable)
+ Specify marker: _____
+ Specify results: _____

+ METHODS

+ Protein Expression (select if tested) (Notes A and B)
+ ___ IHC
    + ___ BCL2 (specify clone): _____
    + ___ CD5 (specify clone): _____
    + ___ CD20 (specify clone): _____
    + ___ CD30 (specify clone): _____
    + ___ Ki-67 (specify clone): _____
    + ___ MYC (specify clone): _____
    + ___ Other(s) (specify clone): _____

+ ___ Flow cytometry
    + ___ BCL2 (specify clone): _____
    + ___ CD5 (specify clone): _____
    + ___ CD20 (specify clone): _____
    + ___ CD30 (specify clone): _____
    + ___ Other(s) (specify clone): _____

+ ___ FISH
    + *BCL2* probe
    + ___ Break apart
    + ___ Fusion
    + *BCL6* probe
    + ___ _Break apart
    + ___ Fusion
    + *MYC* probe
    + ___ Break apart
    + ___ Fusion

+ Subtype Classification (Note C)
+ ___ Hans (CD10, BCL-6, MUM1)
+ ___ Choi (GCET1, CD10, MUM1, BCL6, FOXP1)
+ ___ Tally (CD10, GCET1, MUM1, FOXP1, LMO2)
+ ___ Gene expression profiling (specify platform/method): _____
+ ___ Other (specify): _____

+ Gene Sequencing (Note D)
+ Gene sequencing platform (specify): _____
+ Maximum sensitivity: _____ (variant allele frequency)
+ Genes/exons sequenced (specify): _____

Explanatory Notes

A. Protein Expression
The antibodies listed are included in the template because they have therapeutic or prognostic significance.

CD20 assessment is mandatory for therapeutic planning because the standard therapy for diffuse large B-cell lymphoma (DLBCL) patients is R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone).[1] Knowledge of CD20 expression is therefore recommended as a justification for using rituximab.

CD30 assessment is recommended because of the potential utility of the anti-CD30 antibody drug conjugate, brentuximab vedotin. Approximately 10% to 15% of DLBCL cases express CD30, and these patients may be eligible for this agent if they fail standard therapy.[2]

CD5 assessment is thought to be of prognostic value because a small subset (5%-10%) of DLBCL cases. Patients with CD5+ DLBCL have a more aggressive clinical course. These patients tend to be older and have elevated serum LDH levels, poorer performance status, and a higher frequency of central nervous system involvement.[3]

MYC assessment by immunohistochemistry (IHC) is of prognostic value and needs to be evaluated in conjunction with conventional cytogenetic analysis or FISH to assess chromosome locus 8q24/MYC rearrangements. Approximately 10% of DLBCL cases carry *MYC* translocations, and up to 30% to 40% of cases overexpress MYC by IHC, with positivity defined in various studies as >40% or >50% positive cells. Therefore, MYC can be overexpressed via mechanisms other than translocation.[4,5]

The combination of *MYC* and *BCL2* and/or *BCL6* gene rearrangements as shown by conventional cytogenetic or FISH analysis is known as double (or triple) hit lymphoma. Patients with this combination of abnormalities have a poor prognosis.[6]

MYC positivity by IHC may be useful as a screen for MYC translocations as it is rare for a translocation positive case to be negative for MYC by IHC. MYC expression combined with BCL2 overexpression is also associated with a poorer prognosis (so-called IHC double hit lymphoma).

BCL2 assessment also has prognostic value and needs to be evaluated in conjunction with conventional cytogenetic analysis or FISH to assess chromosome locus 8q24/*MYC* rearrangements. In patients treated with CHOP, BCL2 overexpression correlates with poorer prognosis in the germinal center type of DLBCL.[7] BCL2 overexpression combined with MYC overexpression correlates with a poorer prognosis (IHC double hit lymphoma).

B. Fluorescence In Situ Hybridization (FISH)
Recent studies have demonstrated that DLBCL with rearrangements of *MYC* and *BCL2* or *BCL6* comprise a distinct subgroup of cases, often termed double hit lymphomas, characterized by overlapping morphologic features with Burkitt lymphoma and a more aggressive clinical course.[8-11]

C. Subtyping
Studies have shown there are prognostic differences in DLBCL that are germinal center derived (GC) versus non-germinal center derived (NGC). Several methodologies have been proposed for predicting GC versus NGC derivation.[12] In general, DLBCL of GC type is associated with a better prognosis. The most commonly applied immunohistochemical methodologies, which serve as a substitute for gene expression arrays (a gold standard for GC versus NGC), are Hans classifier,[13] Choi classifier,[14] and Tally classifier.[15]

D. Sequencing

Somatic variants in *TP53*, *MYD88*, *PAX5*, *TNFRSF14,* and other genes have been shown to correlate with cell of origin, patient outcome, and diagnosis in some studies. When such variants are identified they should be reported according to the Human Genome Variant Society (HCVS) guidelines nomenclature.

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