Diffuse Large B-Cell Lymphoma Molecular Subtyping

Background Information

Microarray gene expression profiling (GEP) studies have identified two molecular subtypes of diffuse large B-cell lymphoma (DLBCL) based on the similarity of their expression signatures to normal B-cell subsets: activated B-cell-like (ABC) DLBCL and germinal center-like (GCB) DLBCL.[1] The distinction between these two subsets has prognostic and biologic significance, with the ABC subtype having a worse outcome and distinct pathobiology that includes activation of the B-cell receptor and nuclear factor (NF)-kB pathways.

Because global GEP assays, which require fresh or frozen tissue, are generally not practical for routine clinical care, many investigators have attempted to further simplify this subclassification system. Immunohistochemical algorithms that correlate with GEP subclassification have been utilized, but are fraught with technical and interpretive issues that may limit their use.[3-5] Molecular assays using more limited gene sets, including a 14-gene model to assign ABC and GCB DLBCL subtype designed by Wright et al, have also been reported.[6,7]

Cleveland Clinic Laboratories offers a novel multiplex, single-tube, gene expression assay on the ICEPlex® system (Primer Dx, Mansfield, MA, USA) that allows differentiation between GCB and ABC DLBCL subtypes in formalin-fixed, paraffin-embedded (FFPE) specimens. This RT-PCR assay employs the Wright 14-gene signature, together with house-keeping genes, to provide a linear predictor score that classifies cases into GCB, ABC or unclassifiable categories. Results of FFPE testing with this assay show excellent concordance with global GEP studies from matched fresh or frozen tissue (96.7% agreement for assignment of ABC cases, and 90.0% agreement for all subtypes).

Clinical Indications

This assay is intended for molecular subtyping of DLBCL.

Interpretation

Results are reported as “germinal center expression signature identified” or “activated B-cell expression signature identified.” Cases with borderline linear prediction scores are reported as “unclassifiable expression signature identified.”

Methodology

RNA is extracted from FFPE tissue and cDNA prepared by reverse transcription. Quantitative, multiplex RT-PCR is performed for 14 genes and 2 housekeeping control genes. Normalized copy numbers of the 14 genes are used to calculate a linear predictor score to identify germinal center vs. activated B-cell expression signature.

Limitations of the Assay

This assay is intended for subtyping of diffuse large B-cell lymphoma only. This test is not intended for analysis of other forms of non-Hodgkin lymphoma.

References

1. Alizadeh AA, Eisen BM, Davis RE, *et al*. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature*. 2000;403:503-511.
2. Lenz G, Wright G, Dave SS, *et al*. Stromal gene signatures in large B-cell lymphomas. *New Engl J Med*. 2008;359:2313-2323.
3. Hans CP, Weisenburger DD, Greiner TC, *et al*. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood*. 2004;103:275-282.

4. de Jong D, Rosenwald A, Chhanabha M, *et al*. Immunohistochemical prognostic markers in diffuse large B-cell lymphoma: validation of tissue microarray as a prerequisite for broad clinical applications - a study from the Lunenburg Lymphoma Biomarker Consortium. *J Clin Oncol*. 2007;25:805-812.
5. Coutinho R, Clear AJ, Owen A, *et al*. Poor concordance among nine immunohistochemistry classifiers of cell-of-origin for diffuse large B-cell lymphoma: implications for therapeutic strategies. *Clin Cancer Res*. 2013;19:6686-6695.
6. Wright G, Tan B, Rosenwald A, *et al*. A gene expression-based method to diagnose clinically distinct subgroups of diffuse large B-cell lymphoma. *PNAS*. 2003;100:9991-9996.
7. Scott DW, Wright GW, Williams PM, *et al*. Determining cell-of-origin subtypes of diffuse large B-cell lymphoma using gene expression in formalin-fixed paraffin-embedded tissue. *Blood*. 2014;123:1214-1217.

Test Overview

Test Name	Diffuse large B-cell lymphoma molecular subtyping
Specimen Requirements	Formalin-fixed, paraffin embedded tissue block
Billing Code	90198
CPT Codes	81599, G0452

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