

ZAP-70 Analysis by Flow Cytometry

Clinical Background

- Clinically, chronic lymphocytic leukemia (CLL) is a heterogeneous disease.
- With the more aggressive type, the CLL cells typically express unmutated immunoglobulin variable genes and the 70-KD zeta chain-associated protein (ZAP-70).
- In more indolent disease, the CLL cells express mutated variable genes but lack expression of ZAP-70. Expression of CD38 by CLL cells has also been correlated with disease aggressiveness, but does not appear to be as good of a predictor as ZAP-70 or immunoglobulin gene mutational status.

Indications for Ordering

This test can be ordered to help with the clinical management of patients with established diagnoses of CLL. It is not appropriate to order this test to screen for CLL, help establish a diagnosis of CLL, or for patients with other types of lymphoproliferative disorders or leukemias besides CLL.

Additional Ordering Notes

- If CLL is suspected, Leukemia/Lymphoma Phenotyping (Comprehensive-Whole Blood) (0096299) should be ordered to help establish or confirm a diagnosis of CLL.
- If a definitive diagnosis of CLL is established after correlating the phenotype with morphologic and clinical information, an additional peripheral whole blood sample should then be obtained for ZAP-70 analysis. The time interval between initial diagnosis of CLL and obtaining a specimen for ZAP-70 analysis is not critical since CLL ZAP-70 levels appear to be stable.
- In general, the initial peripheral blood specimen used for Leukemia/Lymphoma Phenotyping (Comprehensive-Whole Blood) (0096299) will be suboptimal for ZAP-70 analysis due to time delays often encountered before establishing a diagnosis of CLL, which in turn can result in lower cell viability and false-negative ZAP-70 staining relative to a fresh specimen. However, if there is concern a delay could adversely affect patient care or with possible logistical problems obtaining a second specimen, ZAP-70 analysis and Leukemia/Lymphoma Phenotyping (Comprehensive-Whole Blood) (0096299) can be ordered on a single whole blood specimen that will be used to perform both tests at the same time.

Interpretation

- CLL cells are identified from inspection of histograms as lymphocytes showing coexpression of CD5 and CD19. If CLL cells are not present in the submitted specimen, a comment

will be made to this effect and the reported ZAP-70 results will reflect the CD19 positive CD5 negative B-cells. The standard test results include:

- ZAP-70
 - Positive: Greater than 30 percent of CLL cells stain above control levels.
 - Negative: Less than 20 percent of CLL cells stain above control levels.
 - Indeterminate: Between 20 percent - 30 percent of CLL cells stained above control levels and are not clearly positive or negative. Recommend repeat testing after six months if clinically indicated.
 - CD19+CD5+ (CLL cells) percentage of lymphocytes
 - CD19+ (B-cells) percentage of lymphocytes
 - CD5+ (T-cells) percentage of lymphocytes
- Comparisons will also be made to previous flow cytometry studies and the current specimen results.

Limitations

- Results of this test should always be correlated with morphologic and clinical information.
- Not all patients with positive ZAP-70 CLL cells have aggressive disease, and not all patients with ZAP-70 negative cells have indolent disease.
- Any possible clinical significance of ZAP-70 expression in lymphoproliferative diseases other than CLL is unknown.

Methodology

Staining for intracellular ZAP-70 is performed on a control normal peripheral blood specimen as well as the submitted sample. Percentages of ZAP-70 positive CLL cells are determined with negative threshold cutoff values set using ZAP-70 stained normal B-cells, as well as isotype control stained CLL cells.

References

1. Rassenti LZ, et al. ZAP-70 compared with immunoglobulin heavy-chain gene mutation status as a predictor of disease progression in chronic lymphocytic leukemia. *N Engl J Med* 2004;351:893-901.
2. Orchard JA, et al. ZAP-70 expression and prognosis in chronic lymphocytic leukemia. *Lancet* 2004;363:105-11. Shanafelt TD, et al. Prognosis at diagnosis: integrating molecular biologic insights into clinical practice for patients with CLL. *Blood* 2004;103:1202-1210.
3. Crespo M, et al. ZAP-70 expression as a surrogate for immunoglobulin-variable-region mutations in chronic lymphocytic leukemia. *N Engl J Med* 2003;348:1764-75.

Test Information

0092392

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For specific collection, transport, and testing information, refer to the ARUP Web site at www.aruplab.com.