



Alpha-Fetoprotein (AFP) and AFP-L3

Test code: 19529X

Clinical Use

- Determine risk of hepatocellular carcinoma (HCC)
- Diagnose HCC
- Determine prognosis for individuals with HCC
- Monitor HCC therapy

Clinical Background

HCC is the fifth most common cancer in the world, with an incidence of >20 per 100,000 in China and eastern Asia and 8 to 10 per 100,000 in the United States.^{1,2} The most significant risk factors for HCC are chronic infection with hepatitis B or C virus (HBV and HCV, respectively) and hepatic cirrhosis. Individuals at risk for HCC are typically monitored with serial hepatobiliary ultrasounds, as well as other imaging techniques (eg, CT scans), and serial measurements of alpha-fetoprotein (AFP).

AFP is a glycoprotein synthesized by the fetal yolk sac, fetal liver, testicular non-seminomatous germ cell cancers, and malignant hepatic cells. It is the most established marker of HCC. Reliance on AFP levels to detect HCC, however, is confounded by the fact that AFP may be elevated in individuals with chronic HBV or HCV infection and hepatic cirrhosis. The sensitivity and specificity of AFP for diagnosing HCC vary with the population studied and the cut-off value above which AFP is considered positive. They range from 52% to 80% and 90% to 98%, respectively.³

Measurement of an AFP glycoform may prove to be clinically superior to measuring AFP. Three glycoforms, determined by the degree of fucosylation of the N-acetylglucosamine-linked sugar chain, have been identified. The glycoforms are separated in vitro by their ability to bind the lectin *Lens culinaris* agglutinin (LCA), a carbohydrate binding protein isolated from lentil seeds. AFP-L1 is non-LCA binding and the major glycoform found in individuals with nonmalignant hepatopathy (eg, cirrhosis or chronic HBV infection). AFP-L2 has an intermediate LCA binding capacity and is primarily produced by yolk sac tumors. AFP-L3 is produced by malignant liver cells, binds to LCA with high affinity, and is the major glycoform found in individuals with HCC.^{4,5}

AFP-L3 levels $\geq 10\%$ are associated with a 7-fold increased risk of developing HCC within the next 21 months and can be elevated 3 to 21 months before HCC is detected by standard imaging techniques.^{6,7} The diagnostic sensitivity

and specificity ranges from 36% to 66% and 77% to 95%, respectively.^{8,9} Because AFP-L3 is produced by malignant hepatocytes, its measurement helps distinguish non-malignant hepatic disease from HCC.^{6,8-12} Malignant liver cells that produce AFP-L3 have an increased tendency for rapid growth, early invasion, and intra-hepatic metastasis, thus making AFP-L3 an indicator of poor prognosis in affected individuals.^{6,8-12}

Individuals Suitable for Testing

- Individuals at risk for HCC
- Individuals with primary or recurrent HCC

Specimen Requirements

1.5 mL frozen serum; 0.5 mL minimum

Method

- Immunofluorescent liquid-phase binding assay
 - Simultaneous measurement of AFP-L3 and non-AFP-L3 forms of AFP
 - Ion exchange chromatography to separate AFP-L3 and non-AFP-L3 glycoforms
 - Fluorescence detection of the 2 chromatography fractions
- Results reported: total AFP and percentage of AFP-L3
- Analytical sensitivity: 0.8 ng/mL AFP; 0.5% AFP-L3
- Analytical specificity: no interference observed for vitamins B1, B6, and B12; alpha, beta, and gamma interferon; ibuprofen; acetaminophen; and acetylsalicylic acid⁶
- CPT code*: 82105

Reference Range

AFP: 1.6 – 4.5 ng/mL

AFP-L3: 0.5 – 9.9%

These ranges apply to non-pregnant adults only.

Interpretive Information

Individuals with an increased percentage of AFP-L3 (AFP-L3%), relative to total AFP present in serum, are at increased risk of developing HCC. AFP-L3-secreting hepatocytes have an increased tendency for rapid growth, early invasion, and intra-hepatic metastasis, thus leading to a poorer prognosis in affected individuals. AFP and AFP-L3 levels decline to normal levels with effective therapy; rising levels suggest disease progression or recurrence.⁵

Not all hepatic cancers secrete AFP or AFP-L3. AFP and AFP-L3% may be elevated in individuals with germ cell tumors and other cancers including those of the gastric and biliary tract, lung, pancreas, and testes.⁸ AFP may also be elevated in individuals with viral infections, liver diseases other than HCC, women who are pregnant, and children less than 18 months of age. Decreased AFP-L3% may occur if free and conjugated bilirubin are >20 mg/dL.⁶

Results should be interpreted in conjunction with other laboratory and clinical findings.

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