Alpha-fetoprotein

1 Name and description of analyte

1.1 Name of analyte
Alpha$_1$-fetoprotein (AFP)

1.2 Alternative names
None

1.3 Heading not used

1.4. Function(s) of analyte
AFP is a 70kDa glycoprotein consisting of a single polypeptide chain with approximately 4% carbohydrate content. It is produced by the fetal liver and yolk sac and acts as the main oncotic protein in the fetus; after birth, plasma [AFP] declines steadily until 18 months post-partum by which time AFP has been replaced by albumin. Yolk sac and liver derived AFP have different carbohydrate content. The half life of AFP is 4-5 days.

2 Sample requirements and precautions

2.1 Medium in which measured
AFP can be measured in serum, plasma or amniotic fluid.

2.2 Precautions re sampling, handling etc.
No special precautions are necessary for measuring AFP accurately and precisely; general precautions apply. However, if frozen and thawed the serum/plasma sample should be centrifuged before analysis.

3 Summary of clinical uses and limitations of measurements

3.1 Uses

Tumour marker

AFP is measured commonly to screen for, monitor and indicate prognosis in certain cancers; plasma [AFP] directly correlates with tumour burden. It is used to screen high risk groups for primary liver cancer (hepatocellular carcinoma – HCC) and is associated with germ cell tumours (non-seminomas of the ovary and testes). AFP and human chorionic gonadotrophin (hCG) are useful prognostic markers for patients with advanced nonseminomatous germ cell testicular tumours. Tumour-derived AFP will have differing carbohydrate content which is dependent on the saccharide transferase activity within tumour cells, but this does not appear to affect assay performance. High concentrations of AFP are also weakly associated with gastrointestinal tumours.

Prenatal screening

Maternal and amniotic fluid [AFP] are clinically relevant in antenatal screening. AFP is part of the triad of tests used to calculate the chances of the foetus having trisomy 18, 21 or neural tube defects. This test is offered to all pregnant women, especially those with fetuses displaying an increased nuchal translucency (NT) of >1.7±0.1mm. The screening test consists of measuring AFP, free beta-human chorionic gonadotrophin (β-hCG) and pregnancy

associated plasma protein A (PAPP-A) in the first trimester. Increased [AFP] is associated with neural tube defects while low concentrations are associated with trisomy 18 or 21.

Other

Benign conditions associated with raised [AFP] include normal pregnancy, hepatitis (acute or chronic/viral or autoimmune), ataxia telangiectasia, hereditary tyrosinemia, cirrhosis and inflammatory bowel disease.

Patients with cirrhosis are at a risk of developing HCC at a rate of ≥1.5%/year. Therefore, surveillance of HCC using AFP is offered as a cost-effective strategy (Bruix *et* al, 2011). A systematic review found low levels of AFP before liver transplantation showed a better overall outcome and increased disease free survival (Hakeem *et al*, 2012).

3.2 Limitations

- An [AFP] within the reference limits does not exclude malignancy.
- AFP results must be interpreted with reference to liver function tests in patients with liver disease.
- AFP is not recommended as a screening procedure for cancer detection in the general population; only those considered 'high risk' should be screened.
- Higher values will be found in newborns and pregnant women.
- AFP is not a useful tumour marker in patients with pure seminoma or dysgerminoma tumours.

4 Analytical considerations

4.1 Analytical methods

AFP is measured by a two-step sandwich immunoassay.

4.2 Reference method

None reported.

4.3 Reference materials

WHO 1st IRP #72/225 (Sizaret *et al* , 1976): This material was prepared from a pool of several hundred cord sera characterised by the Statens Serum Institut (SSI), Copenhagen, Denmark and it was established as the first WHO IS for AFP in 1975 (Sizaret *et al*, 1975).

4.4 Interfering substances

Specimens from patients who have received preparations of mouse monoclonal antibodies may contain human anti-mouse antibodies (HAMA) which may give falsely high results. Results must always be considered in relation to the clinical situation and to previous results. This is particularly important when serial results are being used for monitoring a patient's response to treatment.

4.5 Sources of error

- Measurement of AFP may be subject to high dose hook effect giving false negative results.
- Carryover may be seen if a sample >100,000 kU/L was analysed previously.

- When measuring AFP for antenatal screening purposes, amniocentesis should be performed after maternal blood has been drawn for measurement of AFP.
- Amniotic fluid specimens contaminated with fetal blood may exhibit abnormally high AFP values, which may lead to misinterpretation of test results.

5 Reference intervals and variance

5.1.1 Reference interval (adults)

The upper reference limit of normal is 6 kU/L.

The reference limit is not relevant if there are serial results; an increase or fall in AFP should be interpreted relative to the last AFP measurement available.

5.1.2 Reference intervals (others)

Neonate reference ranges have been established according to Figure 1.

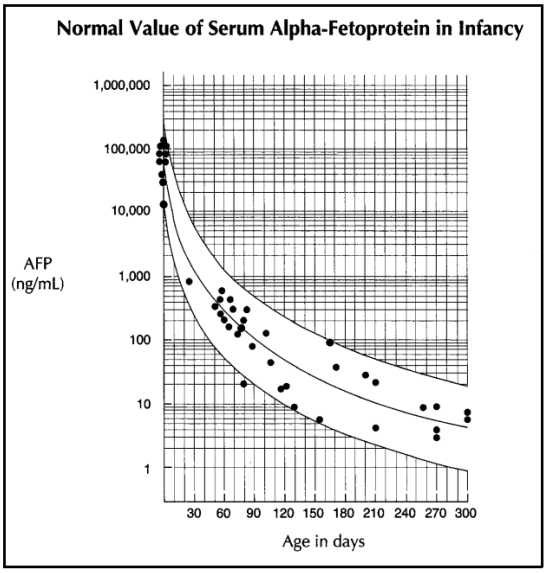

Figure 1 Graph showing changes in [AFP] early in infancy. The curves are upper border of the 95% prediction band (top), regression line in equation 1 (centre), and lower border of the 95% band (bottom) (Tsuchida *et al*, 1978).

5.1.3 Extent of variation

In patients with liver cirrhosis, fluctuating concentrations of AFP may reflect flare-ups of acute hepatitis, exacerbation of underlying liver disease or development of HCC

5.1.3.1 Inter-individual CV

This is not a useful discriminator for variance when used as a tumour marker. However, it has been reported as 12.2%.

5.1.3.2 Intra-individual variability

45.6%

5.1.3.3 CV of method

Desirable imprecision (within-laboratory) <7.5%

Actual imprecision is 5.5% (measured on Abbott ARCHITECT automated platform)

5.1.4 Sources of variation

None

6 Clinical uses of measurement and interpretation of results

6.1 Indications and interpretation

Indication	Result	Interpretation
Liver Disease	Increase >3 kU/L	Significant increase in AFP. Interpret with liver function profile. Very rapidly rising AFP is more consistent with acute liver damage than malignancy.
	Increase >15 kU/L on 2 or more instances	Suggest consideration of underlying malignancy
Neonates	Should fall with increasing age	Refer to Figure 1 before commenting on rate of fall
Antenatal screening	MoM >2	Increased risk of foetus with open spina bifida
	MoM >5	Increased risk of foetus having anencephaly
+hCG in germ cell tumours		Follow up after surgery requires calculation of the half life
	>10,000 kU/L before chemotherapy	This will alter chemotherapeutic approach and clinician should be informed
Follow-up for patients with germ-cell tumours	Increases of 1 kU/L	Not considered a significant change
	Increase of 2-3 kU/L	Should be passed to a specialist
	Increases of ≥4 kU/L	Analysis should be repeated in 2-4 weeks; tumour cell lysis, after initiation of treatment, may cause a transient increase in AFP

6.2 Confounding factors

Antenatal screening

The reliability of AFP evaluation in prenatal testing is dependent upon the accurate determination of gestational age. While elevated maternal [AFP] suggests an increased risk of neural tube defects, this is not a diagnostic test. Maternal AFP is also associated with premature deliveries and low birth weights.

7 Causes of abnormal results

7.1 High values

7.1.1 Causes

Concentrations >6 kU/L may be associated with liver pathology. However, value of >40 kU/L with abnormal LFTs strongly indicate HCC. Values of ≥400 kU/L are considered diagnostic of cancer in adults.

7.1.2 Investigation

Further investigations for abnormal AFP results include liver ultrasound or other imaging e.g. MRI or CT, and liver biopsy.

7.2 The lower limit of the reference range in adults is zero.

8 Performance

8.1 The sensitivity and specificity of AFP varied widely in studies (14 US studies) and it could not be attributed to the threshold effect of the different cut-off concentrations (Colli *et al*, 2006). Systemic reviews on identifying HCC development in high-risk patients showed that the sensitivity of AFP was 41–65% and specificity was 80–94% (Geebo *et al*, *2002*).

9 Systematic reviews and guidelines

9.1 Reviews

1. Bruix J, Sherman M, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. Hepatol Baltim Md. 2011 Mar;53(3):1020–2.

2. Hakeem AR, Young RS, Marangoni G, Lodge JPA, Prasad KR. Systematic review: the prognostic role of alpha-fetoprotein following liver transplantation for hepatocellular carcinoma. Aliment Pharmacol Ther. 2012 May 1;35(9):987–99.

3. Sizaret P, Anderson SG. The International Reference Preparation for alpha-foetoprotein. J Biol Stand. 1976 Apr;4(2):149.

4. Sizaret P, Breslow N, Anderson SG. Collaborative study of a preparation of human cord serum for its use as a reference in the assay of alphafoetoprotein. J Biol Stand. 1975;3(2):201–23.

5. Tsuchida Y, Endo Y, Saito S, Kaneko M, Shiraki K, Ohmi K. Evaluation of alpha-fetoprotein in early infancy. J Pediatr Surg. 1978 Apr;13(2):155–62.

6. Colli A, Fraquelli M, Casazza G, Massironi S, Colucci A, Conte D, et al. Accuracy of Ultrasonography, Spiral CT, Magnetic Resonance, and Alpha-Fetoprotein in Diagnosing Hepatocellular Carcinoma: A Systematic Review. Am J Gastroenterol. 2006 Mar;101(3):513–23.

7. Gebo KA, Chander G, Jenckes MW, Ghanem KG, Herlong HF, Torbenson MS, et al. Screening tests for hepatocellular carcinoma in patients with chronic hepatitis C: A systematic review. Hepatology. 2002 Nov 1;36(5B):s84–92.

9.2 Guidelines

1. Bruix J, Sherman M, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. Hepatol Baltim Md. 2011;53(3):1020–2.

9.3 Recommendations

The British society of Gastroenterology (BSG) has published guidance for the diagnosis and treatment of hepatocellular carcinoma (HCC) in adults. It states that a rising AFP over time, even if the level does not reach 400 kU/L, is virtually diagnostic of HCC.

BSG guidelines for the diagnosis and treatment of hepatocellular carcinoma (HCC) in adults (Gut 2003;52(Suppl III):iii1–iii8).
Available from: *https://www.bsg.org.uk/resource/bsg-guidelines-for-the-diagnosis-and-treatment-of-hepatocellular-carcinoma-hcc-in-adults.html*

10 Links

10.1 Related analytes
β-hCG, transaminases and LFT profile, PAPP-A.

10.2 Related tests
None

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