Serum Alpha-fetoprotein: I. Evaluation of Quantitative Assays Adapted to Automated Immunoassay Systems

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ABSTRACT

Serum alpha-fetoprotein (s.AFP) has been established as a useful tool in monitoring of high-risk pregnancies, as an indicator of fetal neural tube defects, and has been used as an adjunct tumor marker and for monitoring therapeutic efficacy in the treatment of certain tumors. To date, the methods for measuring s.AFP are based upon the immunologic principle and are manual methods. The purpose here is to relate the evaluation of two automated systems for the assay of s.AFP. The automated systems are based upon the following immunoassay methods: a microparticle capture enzyme separation and final quantitation by reflectance fluorescence, and a solid phase ‘sandwich’ separation coupled with enzyme activity measurement (EIA). The reference method is a competitive binding radioimmunoassay. It has been found by us that the automated methods directly transfer analytically with the manual assay. All methods are referenced to the same standard (WHO 1st Intl. Std. for AFP 72/225).

Introduction

Alpha-fetoprotein (AFP) is the major fetal serum protein produced by the yolk sac, liver, and, to a lesser amount, by gastrointestinal tract and kidney. In 1944, Pedersen identified its being present in newborn calf serum, and, in 1956, it was identified as a constituent of the human fetus. Alpha-fetoprotein is a single-chain glycoprotein with a molecular weight of approximately 70,000 daltons. Its physical and chemical properties are similar to human albumin, but it is immunologically distinct. Alpha-fetoprotein does appear to be ancestrally related with the AFP gene residing on the long arm of chromosome 4 close to the albumin gene.

While AFP is synthesized only in fetal tissue, it is found not only in fetal serum but in amniotic fluid and maternal serum with increasing gestational age. The increase in amniotic fluid AFP concentration results from passage by AFP through the fetal glomeruli then excreted into the amniotic fluid; the rise in the maternal s.AFP level is due to increase in placental permeability to protein. At
birth, the newborn s.AFP level is approximately 5,000 ng per mL\textsuperscript{8,12} and then it slowly decreases to adult levels (ca. 5 ng per mL) by the end of the first year.\textsuperscript{15} Amniotic fluid and maternal s.AFP levels above established gestational period levels may be indicative of fetal maldevelopment such as neural tube defects, omphalocele, and congenital nephrosis which may allow for leakage of CSF and/or fetal serum into the amniotic fluid, crossing the permeable placenta into the maternal serum.\textsuperscript{13,16,23}

Abelev et al\textsuperscript{1,2} reported findings of increased s.AFP in cases of hepatocellular carcinoma; subsequently, it has been demonstrated that serum AFP elevations are noted in 50 percent of those with germ cell tumors and in all children with hepatoblastomas. Also, deviations have been found in testicular and ovarian tumors.\textsuperscript{26} In addition, s.AFP may be elevated in chronic or acute hepatitis and in cirrhosis.\textsuperscript{5,6,14,25} With such wide-ranging occurrence of findings, it becomes difficult to utilize s.AFP as a specific tumor marker until a diagnosis is established. However, s.AFP has been found useful for clinical monitoring of patients having nonseminomatous germ cell tumors, and serial sampling may indicate response to therapy or may signal recurrence of active tumor.\textsuperscript{27}

The upper reference limit for s.AFP has not been definitively established. Hunter and Knight,\textsuperscript{12} using an RIA method, found most healthy individuals had an s.AFP value of two ng per mL or less. Employing ‘sandwich’ type enzymeimmunoassay techniques, two kit manufacturers\textsuperscript{18,19} have shown that 99 percent of healthy individuals had s.AFP levels of 15 ng per mL or less and 100 percent of the subjects had levels of 20 ng per mL or less. For nonspecific tumor marker screening purposes, it appears reasonable to use a s.AFP value of the 20 ng per mL for the upper limit of the reference range.

**Methods of Analysis**

All analyses for AFP are based upon the immunoassay principle. The final separation technique, detection, and quantitation will vary by the method. Radioimmunoassay (RIA) is based upon the principle of competitive binding. The separation of the free and bound ligand (usually \(^{125}\text{I}\) labelled antibody) may be by precipitation with polyethylene glycol (PEG) and/or with addition of a second antibody. Quantitation of patient sample AFP is inversely related to the amount of radiolabelled AFP bound. The immuno-radiometric method is based upon the ‘sandwich’ principle wherein the sample AFP binds to a solid phase immobilized anti-AFP antibody. A second anti-AFP \(^{125}\text{I}\)-antibody is added completing the ‘sandwich’. Quantitation is based directly upon the amount of \(^{125}\text{I}\)-antibody bound. Enzymeimmunoassay (EIA) is based on the ‘sandwich’ principle and is essentially identical to that for the immuno-radiometric technique except that the second antibody is anti-AFP enzyme labelled-antibody. Enzymes most frequently used are alkaline phosphatase or peroxidase. Quantitation of the patient sample AFP is directly related to the activity of the bound second antibody enzyme.

**STANDARDS**

Reimer et al\textsuperscript{21} provide information concerning a U.S. National AFP preparation for an in mid-pregnancy maternal serum. Other quality defined materials are: CDC Biological Lot standard (lot #101780); the British Standard (72–227); and the World Health Organization Reference\textsuperscript{24} for human AFP(72–225). It has been recommended\textsuperscript{7} that interlaboratory comparison should be based only on international unit(s) per mL (IU per mL). It is noted that commercially available
methodologies for the most part state calibrations in mass units, ng per mL. Vendors should provide the source of calibration and relationship of IU per mL to ng per mL. Reported range for varying source materials is 0.83 to 1.29 ng per IU.

This report uses the mass units, ng/mL, and all methods are calibrated to the WHO reference 72/225 (1.2 ng per IU).

Methods

Clinical Assays Gammadab [125I]Alpha-fetoprotein Radioimmunoassay Kit.* This assay will be referred to hereafter in the text as CA-AFP; it served as the method of reference for this study. This is a competitive binding radioimmunoassay which, subsequently, employs a precipitating antiserum (sheep anti-rabbit serum and PEG) to separate polyclonal antibody bound tracer from unbound tracer. The patient sample (standards & controls) AFP competes with [125I]-human AFP for binding sites on the antibody. The amount of [125I]-human AFP bound is inversely proportional to the amount of AFP present in the patient sample (standard and control). This method had been utilized in our laboratory for approximately nine years. Isotope measurement, data reduction and calculation of results were carried out using the Packard Cobra 5010 autogamma counting system.†

Abbott IMx AFP.‡ The analysis requires the availability of the Abbott IMx automated immunochemistry analyzer system which is described in depth by Fiore et al.§ Basically, the IMx incorporates both the Microparticle Capture Enzyme Immunoassay (MEIA) technology for high-molecular mass/low concentration analytes and the reflectance fluorescence technology for hapten assay. This assay is referred to hereafter in the text as IMx-AFP. The IMx-AFP is a solid phase ‘sandwich’ assay. The microparticles are covalently coated with monoclonal AFP-antibody which serves to ‘capture’ the sample AFP. An alkaline phosphatase (ALP) labeled anti-AFP antibody is added to form the ‘sandwich’ complex. The enzymatic lysis of added substrate, 4-methylumbelliferyl phosphate, results in the fluorescent product (4-methylumbelliferone) and is measured on the IMx by front surface fluorometry. Quantitation of the sample AFP is directly related to the fluorescent signal equivalent to the AFP calibration.

Boehringer Mannheim Diagnostics (BMD) Enzymum-Test AFP.§ This assay may be performed manually or may be automated. This study is based on assays using the ES300 Immunoassay Analyzer available from BMD. The assay will be referred to hereafter in the text as ES300.

The ES300 assay is based upon the ‘sandwich’ principle. The sample AFP is bound to the polyclonal anti-AFP antibody coated tube. The addition of a peroxidase (POD)-labelled anti-AFP antibody results in the formation of the ‘sandwich’. Subsequent addition of chromogen (diaminonium-2,2-azinobis(3-ethyl-benzothiazoline-6-sulfonate)) and substrate, H2O2 (from Naperborate), results in the formation of a chromophore. The concentration of the chromophore is proportional to the POD activity of the ‘sandwich’ AFP-complex bound to the walls of the reaction tube. Quantitation of sample AFP is based upon AFP calibrators subjected to the reaction sequence.

* Cat. No. CA585, Baxter Healthcare Corp., Dade Div., 620 Memorial Drive, Cambridge, MA 02139.
† Packard Instrument Co., One State Street, Meriden, CT 06450.
‡ Reagent pack, cat. no. 2271-20; AFP calibrators and sample diluent are available separately; Abbott Laboratories, Abbott Park, IL 60064.
§ Cat. no. 1135236/200 tests; Boehringer Mannheim Corp, 9115 Hague Road, Indianapolis, IN 46250.
STANDARDS: The vendors supply AFP standards with their kits. These are: Clinical Assays, 0 to 300 ng per mL; Abbott Laboratories, 0 to 350 ng per mL; and Boehringer Mannheim Corporation, 0 to 255 ng per mL. This will vary on a lot-to-lot basis.

QUALITY CONTROL: Common lyophilized serum quality control material was used for all assays. This was Ciba-Corning Ligand Control. Three levels were used with the following data acceptable ranges; Control A, 24 to 36 ng per mL AFP (#016901); Control B, 68 to 81 ng per mL AFP (017901); and Control C, 204 to 231 ng per mL AFP (#018901). This was incorporated in all analytical runs and also used as blind controls as further quality assurance.

SAMPLES: Sera were analyzed prospectively. They were harvested from clotted samples from females registered in the “MUSC Maternal Serum AFP Screening Program”. The samples were analyzed in duplicate by each method and the data analyzed statistically.

Results

In figures 1, 2, and 3 are illustrated the precision of the s.AFP assays studied. All methods prove to have high levels of precision. This may be noted by the agreement of the mean for the duplicate assays (n = 313) by each method.

In figures 4, 5, and 6 are illustrated the correlation of data from the assays studied. It is noted that the inter-assay correlations are highly acceptable. The correlation of the Abbott IMx method correlates less favorably with that of Clinical Assay than does that for the BMD ES300. Also, note the mean difference of the Abbott data of 4.01 ng per mL compared to that of Clinical Assays and 4.93 ng per mL compared to the BMD ES300. This is reflected also in the biases and slopes of the Abbott IMx method compared to the other methods. With all
methods, there does appear to be a tendency of data "scatter" at elevated concentrations of s.AFP, i.e., levels above 180 ng per mL. This does not diminish the degree of correlation for the methods.

The linear regression equation for the data is expressed in the lower right quadrant of figures 1 through 6. Statistical analyses were performed according to methods provided by Daniel.7
**Discussion**

The Clinical Assays’ Gammadab and BMD Enzymum-Test for s.AFP may be employed as manual tests with the latter adaptable to automation (ES300). The Abbott IMx-MEIA assay does require the IMx analyzer.
For this study, the Clinical Assays' assay was carried out in the manual mode with automation of the RIA data reduction. The BMD and Abbott assays were automated. The methods applied analytically were found to correlate well. The Clinical Assays and BMD are directly related and thus directly transferable. The Abbott method has a tendency to quantitate higher but may be transferable with the Clinical Assays method requiring adjustment when applied to maternity testing.

The total throughput (time for analysis) of the s.AFP assays are comparable. However, the manual Clinical Assays' method does require a significantly greater expenditure of technician time than the automated methods. The Abbott IMx is a batch analyzer, whereas the BMD ES300 is a random access analyzer. The latter may be devoted to multiple analyte assays within an analytical run.

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References


