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The clinical value of serum alpha-fetoprotein

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Abstract

Alpha fetoprotein (AFP) is a major fetal serum globulin structurally and functionally related to albumin. During fetal development, AFP is produced sequentially by the fetal yolk sac, gastrointestinal tract, and liver. Thus normal production of AFP is unique to fetal development, making it an ideal marker for early fetal evaluation. Since its introduction into obstetric practice, maternal serum alpha-fetoprotein (MSAFP) screening has become the earliest non-invasive biochemical test to provide information regarding the fetus, thereby promoting access to earlier diagnosis, enabling families to make informed reproductive choices, and designing appropriate strategies for prenatal care and delivery. Normally, fetal AFP concentration levels continue to decrease through infantile stage (0 to 2 years), down to adult levels (0 – 8ng/ml). However, AFP is frequently re-expressed in patients affected by hepatocellular carcinoma (HCC) and yolk-sac tumors (YST) therefore used in clinical practice as a tumor marker. To improve the specificity of AFP, glycoforms of AFP are determined and used in the differential/early diagnosis, follow up of treatment and prognostication of patients with AFP secreting tumors. Alpha-fetoprotein is also used as a biochemical diagnostic and prognostic marker for prolonged jaundice in newborns.

Keywords: Alpha-fetoprotein; Hepatocellular carcinoma; Yolk-sac tumors; Maternal serum alpha-fetoprotein; AFP glycoforms

1. Introduction

Alpha fetoprotein (AFP) is a major fetal serum globulin belonging to the family of oncofetal proteins, which is similar to a group of proteins referred as carcino-embryonic proteins. Normal production of AFP is unique to fetal development, making it an ideal marker for early fetal evaluation. The clinical significance of serum AFP measurement is well worked out for prenatal screening of open fetal defects during pregnancy [1]. Its utility has expanded from identifying pregnancies at a high risk of open fetal defects to detecting chromosomal abnormalities of the fetus [1].

AFP is frequently re-expressed in patients affected by hepatocellular carcinoma (HCC) and yolk-sac tumors (YST) therefore used in clinical practice as a tumor marker [2]. Alpha-fetoprotein has been a helpful aid in the screening, diagnosis, prognosis and monitoring of therapy in hepatocellular carcinoma (HCC) as well as germ cell tumors (GCTs). Alpha-fetoprotein is also used as a biochemical diagnostic and prognostic marker for prolonged jaundice in newborns [3].

Serum total AFP lacks specificity, since elevated values may be associated with pregnancy as well as a variety of benign and malignant diseases. To improve the specificity of AFP, efforts are made to determine the glycoforms of AFP used in differential/early diagnosis, follow up of treatment and prognostication of patients with AFP secreting tumors.

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2. Biochemistry and biology

Alpha-fetoprotein was first identified as a new fraction of alpha globulins in human fetal serum by Bergstrand and Czar in 1956 [4]. The name AFP reflects its location on protein electrophoresis (in the α_1 region between albumin and α_1-globulin) and its fetal origin. It is structurally and functionally related to albumin. Genes encoding AFP and albumin originate on chromosome 4, and both proteins have a molecular mass of 69,000 daltons [1]. AFP is a glycoprotein consisting of 591 amino acids and 4% carbohydrate moiety (N-glycan chain) that confers heterogeneity on the molecule [5].

Based on AFP heterogeneity, glycoforms are isolated in affinity electrophoresis and chromatography using the most appropriate lectins; concanavalin A (Con A, a mannose binding lectin) and *Lens culinaris* [lentil] agglutinin (LCA, recognizing α-1, 6-fucose) that interact with AFP's glycans [6]. The investigation of AFP binding to various lectins in cases of unexplained AFP elevation may be helpful in the differentiation of AFP source and disease. Increased production of LCA-reactive AFP has been proposed as a specific and early marker for HCC, while Con A non-reactive AFP could be a marker for YST and gastrointestinal malignancies [5]. LCA-reactive AFP could be further fractionated into three isoforms: AFP-L1, AFP-L2, and AFP-L3 based on their differences in affinity for LCA. AFP-L1 which is the main component of AFP in non-malignant liver disease does not contain the LCA-binding sites and does not bind to LCA. AFP-L1 isoform is typically associated with inflammatory liver diseases, such as chronic hepatitis and cirrhosis. The AFP-L2 fraction, which is known to be derived mostly from yolk sac and therefore abundant in maternal serum, shows intermediate affinity to LCA. In contrast, AFP-L3 also known as fucosylated AFP has LCA-reactive activity that reflects malignancy-associated changes in the AFP carbohydrate chains. Because the percentage of the ratio of AFP-L3 to total AFP (AFP-L3%) is frequently higher in HCC patients, AFP-L3% is used widely for early diagnosis for HCC. However, the applicability of AFP-L3% has been extended to non-seminomatous germ cell tumor patients.Therefore, irrespective of tumor site, serum AFP-L3 may be used to distinguish between benign and malignant AFP producing tumors [7]. Assay kits are now available commercially that specifically measure the AFP-L3 and AFP-P4 glycoforms [8]. Based on AFP affinity to Con A, AFP could be resolved into Con A reactive and non-reactive fractions. AFP isolated in the serum of GCT patients contain an additional sugar; N-acetyl glucosamine linked to the α-mannose blocking the Con A binding site on the AFP, making GCT AFP non reactive to Con A. Hence, it is possible to calculate the Con A binding ratio (Con A-BR) as the percentage of AFP not bound to Con A [9]. By the application of Con A-BR >15%, patients with liver disease (benign and malignant) are distinguished from patients with GCT with a sensitivity of 98% and specificity of 98%, using a cut-off value of 15% [9].

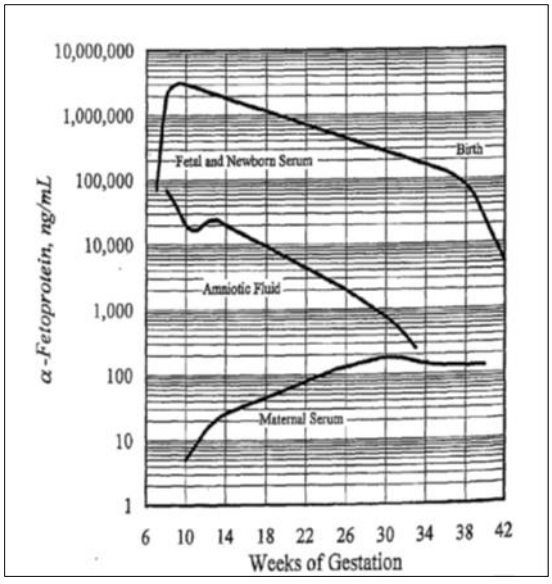

Figure 1 Alpha-fetoprotein values in different compartments: fetal & newborn serum, amniotic fluid, maternal serum

Several functions have been postulated for AFP. Like albumin, AFP may be an intravascular transport protein and may play a role in maintaining oncotic pressure. The primary role of AFP is to transport heavy metal ions and various insoluble molecules such as copper and nickel, fatty acids, bilirubin, and medications in fetal blood circulation [7]. An immunosuppressive effect of AFP has also been suggested as a mechanism for protecting paternally derived antigens in the fetus against maternal antibodies [10]. However, it is reported that when the serum AFP concentration is higher

than 30 ng/mL, the body's immune function will be inhibited [7]. By binding estrogens, AFP impairs the biological activity of estradiol thereby regulating the concentration of the unbound free hormone [7].

During pregnancy AFP is produced sequentially by the fetal yolk sac, gastrointestinal tract, and liver. During fetal development, the embryonic hepatocytes are the main site for the synthesis of AFP followed by the yolk sac. The gastrointestinal mucosa from the endoderm can also produce AFP in small amount. From 6 weeks of gestation, AFP begins to be produced. Concentrations in fetal plasma reach peak levels of 3 x 10^6 ng/ml in the 12 to 14 weeks by the end of the first trimester (figure 1). The fetal liver produces a constant amount of AFP through the 30th week of gestation with levels in the fetal blood decreasing to 10,000 - 200, 000 ng/ml as the pregnancy advances to term. This could be explained by a dilutional effect in the enlarging fetal intravascular compartment. After 30 weeks' gestation, fetal AFP production declines precipitously. Another explanation to fetal decline in AFP is that the AFP gene is almost completely repressed in fully matured fetus leading to disappearance of the protein soon after birth [11].

Alpha-fetoprotein is also found in high concentrations in amniotic fluid. Amniotic fluid AFP levels decline from a maximum of 80,000ng/ml in the 10th gestational week during the first trimester to 200-3000 ng/ml at term. The decrease in amniotic fluid AFP throughout the second and third trimester closely parallels the decrease in AFP in the fetal blood. A small proportion of AFP enters the amniotic fluid during fetal excretion after filtration of the fetal blood through the kidney. As the fetus swallows amniotic fluid, AFP is destroyed by gastrointestinal proteolytic enzymes. AFP concentration in amniotic fluid is approximately 150 times less than in fetal serum. First trimester amniotic fluid has a substantial amount of yolk sac derived AFP (Con A non-reactive). With advancing pregnancy, an increasing proportion of AFP is of liver origin which is Con A reactive. Even at the end of second trimester as much as 25% of amniotic fluid AFP may be of yolk sac origin [12].

Table 1 Normal serum AFP values of term babies from 0 to 24 months

Age (Days)	AFP mean (ng/ml)	AFP 95.5% interval (ng/ml)	Half-life (days)
0	41, 687	9, 120 – 190, 546	
1	36, 391	7, 943 – 165, 959	
2	31,769	6, 950 – 144, 544	
3	27, 733	6, 029 – 125, 893	
4	24, 210	5, 297 – 109, 648	
5	21, 135	4, 624 – 96, 605	5.1
6	18, 450	4, 037 – 84, 334	
7	16, 107	3, 524 – 73,621	
8-14	9, 333	1, 480 – 58, 887	
15-21	3, 631	575 – 22, 910	
22-28	1, 396	316 – 6, 310	
29-45	417	30 – 5, 754	14
46-60	178	19 – 1, 995	
61-90	80	6 – 1, 045	28
91-120	36	3 – 417	
121-150	20	2 – 216	42
151-180	13	1.25 – 129	
181-720	8	0.8 – 87	No correlation

Source; Blohm et al. 1998 [13]

In pregnant women, fetal AFP levels can be detected in maternal serum and urine. Since AFP could be quickly cleared from the mother's serum via her kidneys, maternal urine AFP levels correlate with fetal serum AFP levels. The AFP content in the blood and urine of pregnant women continues to increase. In maternal circulation, AFP levels rise above

non pregnant level at about 10th to 12th week of pregnancy and reach a peak between 30 to 32 weeks (figure 1). Thereafter, levels decline until term and drop precipitously after delivery. During the second trimester, maternal serum AFP (MSAFP) levels increases while fetal serum levels decline. This paradox is not completely understood, but it may result from the enlarging placenta allowing a greater capacity for diffusion of AFP or changes in the permeability of the placenta to AFP. The mechanism for transfer of AFP to the maternal circulation is trans-placental (two thirds) and trans-amniotic (one third).A comparison of AFP levels in the maternal and fetal compartments is shown in figure 1.

At birth mean serum AFP levels stands at 41,687 ng/ml in term babies and 158,125 ng/ml in 90 premature babies born before the 37th gestational week (table 1 & 2). In the first 4 weeks of life, circulating AFP levels decrease by 50% in 5.1 days (half-life) in term babies [13]. From 5 weeks as the infant ages, AFP concentration continues to decrease with increasing half-life rising through 14 and 42 days. Between day 180 and 720 of life, AFP levels up to 87 ng/ml is reached with a mean of 8 ng/ml without a further decline, falling to adult levels (0 – 8ng/ml). Serum AFP values of premature babies are higher than those of term babies (table 2). The physiologic values of AFP in both term and preterm babies are necessary when interpreting AFP results and must be taken into cognizance when using AFP as a marker in infants [11].

Table 2 Normal serum AFP values in premature babies from 0 to 24 months

Age (days)	AFP mean (ng/ml)	AFP 95.5% interval (ng/ml)	Half-life (days)
0	158, 125	31, 261 – 799, 834	
1	140, 605	27, 797 – 711, 724	
2	125, 026	24, 717 – 632, 412	
3	111, 173	21, 979 – 562, 341	
4	98, 855	19,543 – 500, 035	
5	87, 902	17, 378 – 444, 631	5.1
6	77, 625	15, 346 – 392, 645	
7	69, 183	12, 589 – 349, 945	
8-14	43, 401	6, 039 – 311, 889	
15-21	19, 230	2, 667 – 151, 356	
22-28	12, 246	1, 164 – 118, 850	
29-45	5, 129	389 – 79, 433	14
46-60	2, 443	91 – 39, 084	
61-90	1, 047	19 – 21, 878	28
91-120	398	9 – 18, 620	
121-150	193	4 – 8, 318	
151-180	108	3 – 4, 365	
181- 270	47	8 – 2, 630	
271 – 360	18	4 – 832	100
361 - 720	4	0 – 372	

Source; Blohm et al. 1998 [13]

3. Alpha fetoprotein as a tumor marker

Serum AFP measurement is a valuable clinical aid in diagnosis, prognosis, and monitoring primary hepatocellular carcinoma, hepatoblastoma, non seminomatous testicular germ cell tumors, embryonal carcinoma, teratomas, choriocarcinoma and yolk sac carcinoma, germ cell tumors of the ovary and extragonadal germ cell tumors [12].

3.1. AFP as a marker of hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is a leading cause of mortality among patients with chronic liver disease. Hepatocellular carcinoma is a common complication in patients with chronic viral hepatitis. In addition, non viral hepatitis like obesity associated non-alcoholic fatty liver disease also contributes to HCC. Since HCC takes many years to develop, emphasis is placed on surveillance of patients who are at high risk for HCC. If detected when HCC lesions are small and patients asymptomatic, the HCC is potentially curable [14].

AFP is reactivated by approximately 80% of HCC. Increased serum AFP levels have been approved and used as a clinical biomarker for liver cancer detection since 1980s. Approximately 60-90% of patients with primary HCC have serum AFP concentrations more than 500ng/ml [12]. Extremely high serum levels of AFP are mainly seen in patients with primary liver cancer, whereas the serum AFP levels in patients with metastatic liver cancer are generally lower than 424 ng/mL. The most well differentiated and highly anaplastic hepatomas do not produce AFP, since the AFP synthesis is associated with degree of liver cell differentiation. Before the onset of symptoms, AFP has gradually increased for many months in liver cancer patients. At that time, most of liver cancer patients have no obvious symptoms and the tumor sizes are relatively small [7].

Regenerated hepatic tissue (fibrosis) following liver injury/damage due to viral hepatitis, alcohol, chemically induced necrosis and liver surgery are also associated with mild to moderately raised AFP levels. An increase in serum AFP concentration below 400 ng/ml is also reported in 10-15% of cases of acute and chronic hepatitis, and liver cirrhosis. Serial AFP estimations help in distinguishing benign and malignant conditions3 as the steady and progressive rise in APF level is observed in malignancies whereas benign conditions show fluctuations and transient moderately elevated concentrations [12]. To enhance the sensitivity and specificity of AFP as a biomarker, the different glycoforms of AFP are determined.

3.2. AFP in screening and early detection of HCC

Detection of HCC at an early stage is critical for a good clinical outcome as treatment options become limited for advanced HCC patients. Therefore, a strategic surveillance of patients at risk for developing HCC should enable physicians to make an early diagnosis and, with proper treatment modalities, decrease the cancer-related mortality rate [14].

Although early detection of liver cancer leads to improved survival, the high false-negative rate makes the serum AFP level-based early detection strategies for liver cancer ineffective. Therefore, expert panels recommend the determination of serum AFP along with abdominal ultrasonography in the early detection of HCC in high risk patients [11]. Patients at risk for developing HCC are categorized into; super high risk group (hepatitis B and C related cirrhosis), and high risk group (chronic hepatitis B and C or liver cirrhosis with a cause other than hepatitis B/C) [15]. Surveillance using AFP is also recommended for individuals without cirrhosis who are hepatitis B carriers or have other risk factors; active viral replication, high hepatitis B virus DNA concentrations, family history of HCC, Asian males > 40 years old, females > 50 years old, Africans < 20 years old. Alpha-fetoprotein is determined together with an abdominal ultrasound at 6-month intervals in patients at high risk of HCC, especially in those with liver cirrhosis related to hepatitis B and hepatitis C virus [11]. Cirrhotic patients with AFP concentrations that are persistently elevated are at increased risk of developing HCC compared to those with AFP concentrations that fluctuate or remain within reference intervals [16].

Patients with AFP concentrations > 20 ng/ml and persistent elevations is indicative of increased risk of HCC even in the face of negative ultrasound. In this scenario a further investigation involving both fine-needle aspiration and core biopsy is recommended [11]. A serum AFP value within reference intervals does not rule out malignancy, since cases of low serum AFP concentrations are frequently encountered when HCC is detected during screening [17]. Also small HCC tumors are AFP negative in up to 40% of cases [18].

3.3. AFP in HCC Diagnosis

In general, increased serum AFP concentrations are considered to be reliable for the diagnosis of liver cancer. The use of AFP as an adjunct in the diagnosis of HCC is recommended by NACB panel, which also stresses the importance of serial AFP measurements together with consideration of sustained increases in AFP even at low concentrations [11]. In patients at risk for HCC, sustained increases in serum AFP even at low concentrations in conjunction with ultrasound aid early detection of HCC and guide further management. If the appearance of the nodules at ultrasound (lesions>2cm in size) is consistent with HCC, and AFP > 200 ng/ml, results may be considered diagnostic of HCC and biopsy not necessary [11]. However, the gold standard for the diagnosis of HCC remains the histopathological examination of one or more liver samples obtained by open surgery, laparoscopy, or ultrasound/CT-guided biopsy with the option of fine-

needle aspiration for cytology if liver biopsy is impossible. Elevated serum AFP concentrations are not specific for HCC because increased concentrations also occur in certain benign liver diseases and in some malignancies like non seminomatous germ cell tumors, stomach cancer, biliary tract cancer, and pancreatic cancers [19]. Elevated AFP concentrations exceeding 1000 μg/L are however, rare in non-HCC malignancies, occurring in <1% of cases. Alpha-fetoproteins has been reported to be higher in patients with HCC arising from chronic viral conditions compared to those with alcoholic liver disease and in younger and male patients [11]. Patients presenting with HCC may have AFP values ranging from within the reference interval to as high as 10×10^6 ng/ml (ie, 10 g/L), with pretreatment AFP concentrations >1,000 ng/ml in approximately 40% of patients [20]. Approximately 20%-40% of adult patients with hepatitis or liver cirrhosis have raised AFP concentrations (> 10 ng/ml) [21]. In these patients, an AFP concentration between 400 and 500 ng/ml is generally accepted as the optimal cut off to differentiate HCC from chronic liver disease [22].

The percentage of the ratio of AFP-L3 to total AFP (AFP-L3%) is frequently high in HCC patients even in the absence of elevated total AFP levels and thus, widely used for early diagnosis of HCC. AFP-L1 glycoform is specifically associated with chronic hepatitis and cirrhosis. Serum AFP-L3 levels are highest in liver cancer patients and can be used in the absence of elevated AFP levels to detect liver cancer at early stage [7]. AFP from HCC patient sera binds more strongly to Con A than does AFP from NSGCTs, and AFP from both tumors bind more strongly to LCA than does AFP from patients with benign liver disease. The affinity for LCA is slightly higher for AFP from HCC (AFP-L3) than that from NSGCTs (AFP-L2) [11].

3.4. AFP in HCC Prognosis

Many staging systems have been proposed by expert bodies using prognostic factors in the assessment of the progress of HCC [23, 24]. Prognostic factors adopted by these staging systems include tumor characteristics like tumor size/number, vascular/lymph node invasion, presence or absence of metastatic disease, liver function variables and tumor markers. Based on the stage of HCC, patients are classified as being at low, moderate, or high risk for death. Treatment choices are also made based on the stage of HCC.

In combination with other prognostic factors, AFP concentrations may provide prognostic information in untreated HCC patients and in those undergoing liver resection, with high concentrations indicating poor prognosis [11]. Classification systems that incorporate AFP, uses AFP concentration as indicators of tumor spread and burden, cellular differentiation, aggressive potential and patient survival rate [11]. Alpha-fetoprotein correlates with tumor size and have shown to be an independent predictor of survival [25]. Survival of patients with serum AFP > 10,000 ng/ml at diagnosis is significantly shorter than in those with AFP < 200 ng/ml [26]. Alpha-fetoprotein concentrations > 1,000 ng/ml predict a relatively worse prognosis, even after attempted curative resection [27]. Serum AFP concentrations < 12,000 ng/ml are required to meet the criteria for liver transplantation [28]. AFP doubling time has also been reported to be an important prognostic factor [29]. Persistence of a positive AFP-L3 fraction after curative intervention also has been reported to indicate residual or recurrent disease [30].

3.5. AFP in monitoring HCC patients after treatment

For patients with increased AFP concentrations before therapy (liver resection or liver transplantation, ablative therapies and palliative treatment), follow up with serial determinations of serum AFP to monitor efficacy of treatment, course of disease, and recurrence is recommended [11]. Current practice suggests follow up of patients for every 3 months for 2 years and subsequently every 6 months [11].

After complete removal of the tumor, AFP concentrations typically decrease, with a half-life of 3.5-4 days. Incomplete resection yields a longer half-life, which is associated with poorer survival, whereas failure of the AFP to normalize implies residual malignancy or severe liver damage [31]. However, normalization of AFP does not necessarily indicate complete clearance of the disease. Recurrence after transplantation may occur, even when AFP is stable and within normal limits, presumably reflecting the presence of micro-metastases too small to produce measurable serum concentrations [32].

Changes in AFP concentrations also reflect tumor response after chemotherapy, with longer survival in patients showing a significantly prolonged decrease in AFP than in those with slowly increasing concentrations [33]. In patients receiving new and effective combined systemic therapies, 75% have shown dramatic decreases in serum AFP, with concentrations normalizing completely in some patients. Progressive disease was found in patients with continued AFP increase [11]. It has been shown that in HCC patients undergoing systemic chemotherapy, serial AFP determinations may be useful both for prognosis and for monitoring treatment response, as well as providing a surrogate marker for

the evaluation of new therapeutic agents [34]. In another study, serum AFP changes may serve as a useful surrogate marker for clinical outcome in patients with advanced HCC receiving systemic therapy [35].

4. AFP value in germ cell tumors

Germ cell tumors (GCTs) are tumors involving the primordial germ cells of the gonads. The testis and ovaries are the most common primary sites where GCTs occur; however, the prevalence of GCTs is different at each of these sites. While over 90% of testicular tumors are GCTs and the most common cancers of young adult men, only 30% of ovarian tumors are GCTs. About 95% of all malignant testicular tumors are of germ-cell origin; most of the rest are lymphomas, Leydig or Sertoli cell tumors, and mesotheliomas. Primary extragonadal GCTs also occur in the mediastinum (chest region), cranium (pineal/suprasellar region) and retroperitoneum (abdominal region). Embryologic and histopathologic considerations suggest two different origins of extragonadal GCTs: metastases of germ cells from gonadal GCTs and primary GCTs originating from adhered primordial germ cells during migration in embryonic life to the primitive gonads [36]. The mediastinum is the second most common primary site affected by GCTs, with GCTs accounting for 15% of anterior mediastinal tumors in adults and 24% in children. GCTs also occur in the CNS, such as the pineal gland, neurohypophysis, and sacrococcygeal region [37]. GCTs metastasize by both lymphatic and vascular channels. Extracranial GCT commonly metastasizes to the regional lymph nodes, and lungs (pulmonary visceral metastasis). Non-pulmonary visceral metastatic (NPVM) GCT sites include; liver, brain, bone, kidney, skin, gastrointestinal tract. Mortality is high in untreated GCT patients.

Varying pathologic subtypes of GCTs has been classified regardless of location but based on tumor marker and histological findings (table 3). GCTs are classified into two categories: Seminomas and non-seminomatous GCTs. Seminomas are sub-typed into germinomas and dysgeminomas, while non-seminomatous GCTs (NSGCTs) comprise of embryonal carcinomas, endodermal sinus yolk sac tumors, choriocarcinoma and teratomas.

Table 3 Classification of GCTs with serum AFP, hCG, and LDH

Germ Cell Tumor	Incidence	Tumor marker
Seminoma		
Seminoma/Dysgerminoma/Germinoma	30%–60% of GCTs	hCG may be elevated, LDH elevated, AFP normal
Non-seminomatous/non-germinomatous GCTs		
Embryonal carcinoma	3%–4% of GCTs	hCG and AFP elevated, LDH may be elevated
Yolk sac tumor (endodermal sinus tumor)		100% secrete AFP, LDH may be elevated, hCG normal
Choriocarcinoma	1% of GCTs	100% secrete hCG, AFP and LDH normal
Teratoma	5%–10% of GCTs	Normal hCG and AFP in pure teratomas, immature teratomas may secrete AFP and LDH
Mixed tumors		
Subtypes existing in a tumor	60% of GCTs	Depends on tumor cells present

Germ cell tumors may occur as mixed or pure tumors. There is a high prevalence of mixed tumors, such that a GCT at a given site in a patient may consist of different histologic subtypes of tumors. Pure embryonal carcinomas, yolk sac tumors, or choriocarcinomas are extremely rare, although seminomas often appear in a pure form. Approximately 60% of testicular tumors are mixed GCTs. The prevalence of mixed GCTs is lower in the ovary than in the testis [37].

4.1. AFP in diagnosis of germ cell tumors

The diagnostic algorithm of GCTs includes a history and physical examination, cross-sectional imaging of the retroperitoneum, chest x-ray, and serum levels of AFP, hCG, and LDH [38]. Table 4 reviews some of the important characteristics of these common tumor markers.

Table 4 Characteristics of common GCT markers

Tumor marker	Half-life	Normal range	Tumor type
AFP	5–7 days	<40 ug/I	Embryonal, teratoma, yolk sac
hCG	24–36 hours	<5 IU/I	Seminoma, choriocarcinoma, embryonal,
LDH	Varies	1.5-3.2 ukat/I	Any tumor type

Determination of baseline serum hCG, AFP, and LDH before therapy is mandatory in all patients. The marker concentration in serum is dependent on histological type and tumor load (ie, stage). Serum AFP is elevated in NSGCTs and is used in differentiating seminomas from NSGCTs [39]. The classification of a GCT is based on histological examination, but if serum AFP is increased, a tumor classified as a seminoma is reclassified as NSGCT and treated accordingly [40]. More than 75% of patients with non-seminomatous testicular germ cell tumors have elevated serum concentration of AFP [12]. Among germ cell tumors of the ovary, dysgerminomas are known to be AFP non-secretors. AFP expression is almost entirely confined to ovarian yolk sac tumors (ovarian hepatoid and endometrioid yolk sac tumor), although focal expression can be seen in embryonal carcinoma and in hepatic or enteric tissues in teratomas [41]. The rapidly growing and highly malignant endodermal sinus tumors of the ovary are invariably associated with elevated AFP. The concentration of AFP correlates very well with the quantity of endodermal sinus elements in yolk sac tumor of ovary.

For screening purposes, serum AFP and hCG levels revealed a prevalence rate of 60% in untreated stage-I, 70% in untreated stage-II and 90% in untreated stage-III non-seminomatous testicular carcinoma [12]. Depending on histological type, 30-70% of patients with extragonadal germ cell tumors have an elevated serum AFP levels. Presence of AFP has been reported in 25.8% serum, 26.3% CSF of intracranial germ cell tumors. Elevated serum AFP concentration is reported in a case of endodermal sinus tumor of nasopharynx in a 4-year child [12].

The percentage of AFP binding to Lectin can differentiate elevated serum AFP levels due to testicular cancer and liver disease [42], Considering a cut-off value of Con A binding ratio (Con A-BR) of 15%, AFP produced by GCT (> 15%) could be distinguished from AFP produced by tumoral and non-tumoral liver diseases (≤15%) with a sensitivity of 98% and specificity of 100% [43]. However, the applicability of Con A-BR is limited due to its lack of specificity since gastrinomas also produce Con A non reactive AFP. Therefore, the use of Con A-BR can only be effective after ruling out the presence of GIT tumors. AFP-L3% is a sensitive and specific marker of testicular cancer, especially in cases where the AFP level is only slightly increased or at the cut-off value of 20 ng/mL [9]. AFP-L3% with a value of >50% has been shown to correctly identify 96% of patients with various types of NSGCTs, i.e., embryonal carcinoma (n = 9), yolk sac tumour (n = 4), mixed type without seminomas (n = 5) and a mixed type with seminoma (n = 7), irrespective of the AFP level in serum [9].

4.2. AFP in GCT prognosis and treatment decisions

Besides AFP use in differential diagnosis, serum AFP together with hCG are valuable markers of NSGCTs and have contributed significantly in terms of prognosis, staging, and making treatment decisions in enhancing the cure rate of these cancers [39].

Prognosis of GCTs relates not only to anatomic extent of spread, as is true for most cancers, but also relate to the primary site (extragonadal or gonadal) as well as the extent of production of the tumor markers; AFP, hCG, and LDH, which may reflect the underlying biologic aggressiveness. The degree of AFP, hCG, LDH elevation is directly proportional to tumor burden, these tumor markers thus serve as poor prognostic indicators associated with adverse outcome. The extent of biomarker elevation in patients with NSGCTs has been incorporated into the cancer staging criteria of expert bodies (table 5). In this staging criterion, patients are categorized into good (S1), intermediate (S2), and poor (S3) risk groups [44]. In prognostication, testicular GCTs could be classified using AFP levels of 500 KU/l into low, medium and high risk groups giving a five year survival rate as 95%, 85% and 54% respectively [39]. The International Germ Cell Cancer

Collaborative Group classified NSGCT into prognostic groups using tumor markers, site of primary tumor (mediastinal or not)/NPVM, and classified seminoma using NPVM alone (table 6) [45]. The tumor type, cancer staging, and prognostic groups are used for risk stratification which aid in selection of appropriate therapy for patients in whom the diagnosis of cancer has been established.

Table 5 American Joint Committee on Cancer Stage parameters

Stage	AFP (ug/l)	hCG (IU/l)	LDH
S0	wnl	Wnl	Wnl
S1	<1000	<5000	<1.5 x ULN
S2	1000 – 10,000	5000– 50,000	1.5 – 10 x ULN
S3	>10,000	>50,000	>10 x ULN

Table 6 Classification of advanced (metastatic) GCTs into various risk groups using tumor markers

Good prognosis	
Non-seminoma (NS)	**Seminoma**
Primary testicular/retroperitoneal NS and Absence of non-pulmonary visceral metastases and Good markers - all of: AFP <1000 ng/ml and hCG<5000 iu/l (1000 ng/ml) and LDH <1.5 x ULN (upper limit of normal)	Any primary site and Absence of non-pulmonary visceral metastases and Normal AFP, any hCG level, any LDH level.
INTERMEDIATE PROGNOSIS	
Non-seminoma	**Seminoma**
Primary testicular/retroperitoneal NS and Absence of non-pulmonary visceral metastases and Intermediate markers-any of: AFP 1000 ng/ml to10,000 ng/ml or hCG 5000 iu/l to 50,000 iu/l or LDH 1.5 x ULN to 10 x ULN	Any primary site and Presence of non-pulmonary visceral metastases and Normal AFP, any hCG level, any LDH level.
POOR PROGNOSIS	
Non-seminoma	**Seminoma**
Primary mediastinal NS or Presence of non-pulmonary visceral metastases or Poor markers- any of: AFP >10,000 ng/ml or hCG>50,000 iu/l or LDH >10 x ULN	No patients classified as poor prognosis

Stage I seminomas and non-seminomas may be treated by surgery (orchiectomy or oophorectomy). Surgery in combination with radiotherapy leads to a higher cure rate than surgery alone. Without radiotherapy some patients may relapse, but mostly cured by second-line therapy.

4.3. AFP in monitoring GCT therapy and follow-up surveillance

In all therapies, surveillance using tumor markers at an increased frequency to detect relapse is recommended. If AFP in serum is increased before therapy, the rate of marker decline reflects the response to therapy. Persistent AFP elevation after chemotherapy indicates residual disease and the need for further therapy [46]. Chemotherapy may induce a transient increase or surge in AFP concentrations during the first week of treatment [47]. In the absence of residual disease after orchidectomy for testicular GCT, the half-life of hCG is approximately 1.5 days and that of AFP 5 days [48]. During chemotherapy, half-lives >3.5 days for hCG or >7 days for AFP predict recurrence and adverse prognosis [49]. Marker half-life is calculated from the slope of the logarithm of the marker concentration versus time. It is preferable to use marker concentrations from several time points and to calculate the half-life from the slope of the regression line. Marker levels exceeding the upper reference limit after therapy suggest residual disease.

The determination of AFP-L3%, appears to be a better biomarker for identifying a recurrence of the yolk sac tumor than an analysis of total AFP in serum for a neo-natal patient during therapeutic monitoring [9]. The other very important outcome of using AFP-L3% is that neonates without any disease, but having high AFP levels, are correctly identified as healthy by measuring AFP-L3%. An AFP-L3% assay might provide better information about future disease recurrence/relapse for NSGCT, teratocarcinoma and embryonal cell carcinoma adult patients than the total AFP level in serum [9].

After successful primary therapy, all patients are monitored with physical examination, tumor marker determinations and imaging test. With such surveillance, relapse is in most cases detected before clinical symptoms appear. The surveillance is tailored to take into account tumor type, stage, treatment, and likelihood of relapse. Continuous and serial monitoring with AFP, hCG, and LDH is recommended even when they are not raised before therapy, because marker expression can change during therapy. Frequency of measurement depends on the stage and pathology of disease but should be determined according to standard protocols. Because baseline levels are dependent on the individual, and increases are more important than absolute concentrations. A single increasing value must be confirmed with a second sample and the possibility of transient elevation due to non-specific interference should be actively considered.

5. Alpha fetoprotein in prenatal screening of fetal defects

Maternal serum alpha-fetoprotein (MSAFP) screening is a non-invasive biochemical test that provides information about the health status of the fetus. Maternal AFP could be measured alone in the prenatal screening of fetal abnormalities presenting as open neural tube defects (spina bifida, anencephaly) or ventral wall defects (gastroschisis, omphalocele). Elevated MSAFP is observed in open fetal defects due to leakage of a large amount of fetal AFP through the defective skin coverings of the brain, spine and abdomen into the amniotic fluid which subsequently diffuse via the placenta into maternal circulation. Generally, MSAFP screening programs detect approximately 85% of open fetal NTDs: 80% of open spina bifida and 90% of anencephaly. Almost all of these open lesions can then be diagnosed by amniotic fluid testing [1]. More than 90% of anencephaly cases can be detected by MSAFP screening, and 99% can be detected by ultrasound examination. Approximately 99% of anencephaly cases can also be detected by amniotic fluid AFP and acetylcholinesterase (AChE) testing. In contrast, most encephaloceles are skin covered and therefore are less likely to be identified by MSAFP screening or amniocentesis and are most often detected by ultrasound [1].

For higher sensitivity, AFP is measured in combination with other biochemical markers; unconjugated estriol (uE_3), hCG, inhibin A, pregnancy-associated plasma protein-A (PAPPA) in the assessment of pregnancies at risk of aneuplodies; Down's syndrome (trisomy 21), Edwards syndrome (trisomy 18), and Patau syndrome (trisomy 13). Low MSAFP levels are observed in aneuploid pregnancies, the reduced levels is explained by the impaired liver AFP synthesis by the aneuploid fetus. It should be noted that a significant amount of patients with elevated maternal AFP do not develop birth defects. Therefore patients should understand that a normal MSAFP result does not gurantee a child without an abnormality (including an NTD), and that an elevated MSAFP level does not specifically diagnose an abnormality. Instead, an elevated value places the patient in a high-risk group that necessitates further evaluation.

Besides screening for open fetal defects and aneupoidies, MSAFP levels have a high predictive value in predicting other adverse pregnancy outcomes like; low birth weight, intrauterine growth retardation, premature delivery, placental abruption, intrauterine fetal death, preeclampsia and increased risk of perinatal death [50].

A combination of biomarker testing as well as high resolution imaging techniques ensures that more patients obtain accurate information about their personal risk status [51]. MSAFP screening decreases morbidity and mortality by promoting access to early diagnosis, enabling families to make informed reproductive choices, and designing appropriate strategies for prenatal care and delivery

Alpha-fetoprotein along with other biochemical testing could be performed using amniotic fluid and fetal serum when necessary, in this instance samples are obtained through invasive techniques in the diagnosis of prenatal fetal defects [51]. Invasive sampling techniques such as chorionic villus biopsy or amniocentesis can have serious consequences including bleeding, preterm labor, and fetal loss [52]. The development of non invasive biomarker prenatal screening tests has reduced the need for these invasive diagnostic procedures.

5.1. Multiples of the median (MoM) AFP value for gestation age

MSAFP test measurement is typically reported as a multiple of the median (MoM). Multiples of the median literally refers to the number of times a patient's AFP value is higher or lesser than the reference median value. This statistical convention was introduced in the first U.K. collaborative study on AFP, as a method in which participating laboratories compared individual test results [53]. Measurements of AFP can be affected by laboratory technique resulting in difficulty comparing absolute results between centers. Also standard deviations are influenced by data spread. Because MoMs are a reflection of an individual patient's AFP value compared with the median, it is not influenced by outlying values. Each laboratory is expected to develop reference data with a median MSAFP value (table 7) from unaffected pregnancies calculated for each week of gestation [51, 54].

Table 7 Median AFP values obtained from a population of normal pregnancies

Gestation age (weeks)	Median Maternal AFP (ng/ml)
14	27.2
15	31.8
16	36.5
17	41.7
18	48.0
19	56.0
20	63.1

Source; Vranken et al, 2006 [54]

The AFP calculation for an individual patient is adjusted by other variables (table 8) that affect the interpretation of the result; these factors include maternal weight, gestational age, race, multiple gestation, and insulin dependent diabetes mellitus [1].

Table 8 Common causes of false maternal serum Alpha-Fetoprotein Levels

False-Positive	False-Negative
Inaccurate gestational dating (patient has a more advanced gestation than estimated)	Inaccurate gestational dating (patient has less advanced gestation than estimated)
Multiple gestation	Maternal insulin-dependent diabetes mellitus
Underweight patients (less than 40.9 kg)	Obesity
Spontaneous fetal to maternal bleeding	
Two weeks after radio diagnosis involving the use of radioactive tracers	

Maternal serum AFP screening is most accurate when performed between 16 and 18 weeks gestation: however, testing can be performed between 15 and 22 weeks. Under estimation of gestational age as a result of screening patients earlier or later than the optimal gestational age leads to false MSAFP results. The adjusted MSAFP result is expressed as a MoM by dividing the AFP concentration by the median AFP value for the appropriate week of gestation. The log gaussian distribution of MSAFP levels in unaffected pregnancies and in those with open spina bifida and Down syndrome is shown in Figure 2.

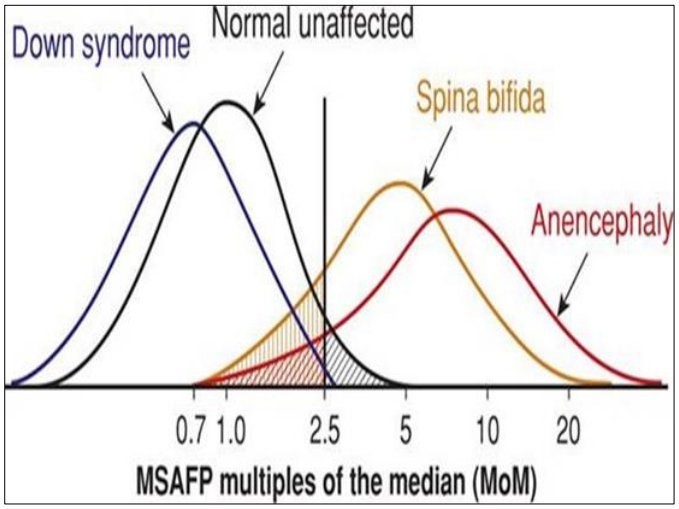

Figure 2 The log Gaussian distribution curve of MSAFP MoM in unaffected and affected pregnancies

The median MSAFP value for each week of gestation is designated as 1.0 MoM. An MSAFP level is considered elevated if the value is greater than 2.0 or 2.5 times the median value (2.0 or 2.5 MoMs) for normal controls at the same week of gestation.

Case study 1. MoM AFP of patients at 16 weeks gestation

Patients ID No	Patient's AFP (ng/mL)	Median AFP (ng/mL)	Ratio	Patients MoM	Reference MoM
1	20.0	36.5	20/36.5	0.60	1.00
2	36.0		36.0/36.5	0.99	
3	131.0		131.0/36.5	3.60	

A case study (case study 1) involving a patient at 16 weeks gestation for prenatal screening with an AFP value of 131.0 ng/ml will have a MoM AFP of 3.6, when calculated using a reference median AFP value of 36.5 ng/ml (case study 1). The MoM AFP value of 3.6 implies that the patients AFP value of 131.0 ng/ml is 3.6 times greater than the median reference value of 36.5 ng/ml at 16 weeks gestation, and is predictive of carrying a fetus with open neural defects. Conversely a patient with an AFP value of 20 ng/ml (case study 1) will have a calculated MoM AFP of 0.6 which implies that the patients AFP level of 20 ng/ml is o.6 times less the median reference value of 36.5 ng/ml and is considered to be at risk of carrying an aneuploid fetus (case study 1). If the patients AFP were 36.0 ng/ml (case study 1) then her MoM AFP would be 0.99 which is approximately equal to the reference MoM 1.00, implyingthat the woman is carrying a normal fetus in regards fetal assessment.

During prenatal screening women with MSAFP level >2.0 MoM and ≤ 0.8 MoM are considered abnormal and further subjected to invasive diagnostic procedures since their pregnancies are at high risk of fetal defect (figure 2) [50]. Most commonly, MSAFP in open spina bifida has a MoM 3.3 to 3.8; anencephaly 7.7 MoM; gastroschisis 7.8 MoM; and omphalocele 4.5 MoM. MSAFP is not used as a diagnostic test, but rather as a screening test [51]. Screening differs from diagnostic testing in that a positive MSAFP result does not mean that the patient has an affected fetus but that the patient is in a category of sufficient risk to warrant further studies such as ultrasound or amniocentesis.

6. AFP as a biochemical marker of prolonged neonatal jaundice

Prolonged neonatal jaundice is defined as jaundice lasting more than 14 days of life in the full-term infants and more than 21 days in premature newborns [55]. Prolonged neonatal jaundice includes; prolonged unconjugated hyperbilirubinemia due to breastfeeding or secondary to other pathologic conditions and conjugated hyperbilirubinemia primarily due to cholestasis (biliary atresia). Breast milk jaundice usually does not require medical intervention and need to be differentiated from prolonged pathologic jaundice. The differential diagnosis of neonatal jaundice is therefore necessary and early recognition of pathologic jaundice is essential to ensure timely treatment and optimal prognosis [3]. AFP as a biochemical marker predicts the development of prolonged jaundice in newborns and as well valuable in the differential diagnosis of neonatal jaundice [3]. AFP is also used in conjunction with fractionated bilirubin as a prognostic marker in determining the prognostic outcome of pathologic jaundice.

7. Conclusion

Serum alpha-fetoprotein is a biomarker of HCC, GCTs, fetal defects and prolonged neonatal jaundice. It's testing is clinically utilized in the screening, diagnosis, prognosis and therapeutic monitoring of AFP producing tumors. The determination of MSAFP is utilized in the screening of fetal defects. Serum AFP is also used in the prediction of the development of prolonged pathologic jaundice in neonates. It is important to note the age variation in serum AFP values, when interpreting results in babies aged 0 to 2 years. The physiologic elevation of MSAFP should be recognized when interpreting results in pregnant women. The clinical utility of serum AFP is limited due its lack of disease specificity. However, a combination of other biomarkers and determination of isoforms of AFP has improved the specificity of AFP.

Compliance with ethical standards

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