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SHORT COMMUNICATION

Re-evaluation of Alpha-fetoprotein as a Tumor Marker for Breast Cancer: A Meta-Analysis

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Abstract

Alpha-fetoprotein (AFP) has long been recognized and accepted in the clinic as a valuable tumor biomarker for liver and germ cell cancers. Correspondingly, AFP has also been utilized in combinations with other tumor markers for the diagnosis of breast cancer (BC). Over the last four decades, AFP has demonstrated only a lackluster contribution and limited value in utility as a member of various tumor marker assay combination panels for BC. This limited value in assay combination panels can be attributed to a lack of sensitivity and specificity in screening. It is approaching a time when AFP could possibly be deleted, without consequences, as a biomarker for breast cancer. Thus, the usefulness of AFP as a tumor biomarker for breast cancer should be re-evaluated and quantitively assessed in view of its 40 years of assay utilization in the cancer clinic.

Introduction:

Alpha-fetoprotein (AFP) is the "gold standard" biomarker for hepatocellular carcinoma and other liver distress disorders such as alcoholic cirrhosis, viral hepatitis, and hepatic necrosis [1, 2]. In addition, AFP has also been utilized as a biomarker for head and neck germ cell tumors and testicular cancers [3]. Because AFP is a biomarker of aggressive malignant disease, the fetal protein has been employed as one of the constituent members of multiple biomarker assay combination panels for breast cancer (BC) as well as other cancers.

During the last four decades AFP has remained as a component biomarker panel member in classical assays for BC. Hence, the tumor marker constituents for breast cancer assays are frequently composed of four members: AFP, carcinoembryonic antigen (CEA), CA 27.29, and CA 15-3 [4]. However, AFP has performed poorly and contributed the least benefit as a breast cancer tumor marker in these quad-combination clinical assays. Therefore, it is presently proposed that AFP should be re-evaluated for its usefulness as a tumor marker for breast cancer. In other words, is it time for AFP to be considered for deletion in the clinical assay combination panels for breast cancer?

Aims and Objectives:

The objectives of the present treatise are three-fold in nature. First, the various conventional diagnostic biomarkers employed in human breast cancer assays in the clinic are presented in a meta-analysis discussion backdrop. Historically, serum levels of AFP are included as part of a four- member combination assay panel for BC. Secondly, the contributions, weaknesses, limitations, and outcomes of the performance of AFP as a member of the assay combination panels are presented throughout the discussion. Thirdly, a re-evaluation of the reliability and usefulness of AFP as a suitable BC biomarker, alone or in combination with other biomarkers, is forwarded and critiqued. Having served as a component biomarker assay member for BC since the early 1980s, logic would dictate that if AFP contributes little if any value to the four-member assay panel for BC, its deletion should be under consideration.

For those seeking additional background information and insight into topics related to AFP and breast cancer, readers should consult References 5-8. Such topics would include the following: A) breast cancer risk subsequent to pregnancy; B) the role of the AFP receptor in BC signal transduction; C) the BC cell uptake of AFP and receptor-mediated endocytosis, and D) breast cancer during pregnancy.

A) Historical Background: Alpha-fetoprotein:

Human alpha-fetoprotein (AFP), discovered in 1956, has achieved a notable history in clinical utility as a tumor-associated fetal protein or "oncofetal protein" [9]. Hence, AFP has been utilized both as a fetal defect biomarker during

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pregnancy and as a malignant tumor marker in certain juvenile and adult cancers [10]. The oncofetal protein represents a single chain poly-peptide with a molecular mass of 69 kDa depending on its intrinsic bound carbohydrate moieties [1]. The tertiary-folded structure of this fetal protein reflects a helical V- or U- shaped molecule configured from 15 disulfide bridges. AFP has been classified as a member of the albuminoid gene family consisting of albumin, alpha-albumin, AFP, and Vitamin-D binding protein [11]. Human AFP binds to multiple hydrophobic ligands including various drugs, dyes, steroids, hormones, heavy-metals, flavonoids, fatty acids, and retinoids. Finally, AFP has been recognized as a critical pregnancy regulatory growth factor capable of either enhancement or inhibition depending on its immediate surrounding microenvironment [12].

B) Breast Cancer Traits and Characteristics.

Breast cancer is the leading cause of cancer in women and the third most common cancer worldwide [13]. The majority of cancer mortalities can be attributed to cancer cell metastasis to distal organ/tissue targets rather than the primary mass itself. As in most cancers, breast cancer is a chromosomal/genetic instability disorder resulting from an accumulative series of DNA mutations, variations, and alterations in the cells and tissues of the breast. Such chromosomal and DNA alterations could include: 1) gene amplifications; 2) point mutations; and 3) chromosomal translocations [14]. These alterations and mutations lead to the initiation and expression of certain malignant behaviors. Such behaviors often manifest as: a) protein expression of oncogenes and transcription factors; b) inhibition of cell death (apoptotic) factors; c) promotion of cancer cell growth and progression; d) enhancement of drug resistance; and e) increased cell invasion, migration, and metastasis [15]. Thus, multiple factors can contribute to the processes of cell transformation, carcinogenesis, and developmental progression of malignant tumors such as breast cancer.

C) Circulating and Intracellular Proteins as Tumor Biomarkers

Circulating tumor-associated antigens (TAAs) have been successfully employed as minimally invasive tools for monitoring cancer growth and progression [16, 17]. Thus, tumor biomarker TAAs have been shown to play increasingly important roles in cancer detection and treatment management [18]. Monoclonal antibodies have been developed for use in detecting TAAs as tumor biomarkers in certain solid tissue malignancies such as breast cancer for monitoring responses to therapy and for detecting early relapse. Furthermore, assay panels of multiplexed tumor biomarkers have been utilized in assays to assess tumor growth, invasion, metastasis, cell death, immune activation, and angiogenesis. Such clinical-based assays are potentially useful in screening for early malignancy, aiding in cancer diagnosis, surveillance following surgery, predicting drug response and/or resistance, and monitoring therapy in advanced disease. Some biomarkers have also been used in evaluating the pre-diagnostic risk assessment for breast cancer [20].

Multiple tumor biomarkers have been developed for a variety of different cancers, some being used as single markers while others being employed in biomarker combination panels [21, 23] [Table 1a, 1b, Table-2]. For such examples, a single stand-alone biomarker for prostate cancer is prostate-specific antigen (PSA); carcinoembryonic antigen (CEA) for colorectal cancer; cancer antigen CA19-9 for pancreatic cancer; cancer antigen CA125 for ovarian cancer, and AFP for liver cancer. Examples employing biomarkers in combination panels have included: A) beta-human chorionic gonadotrophin (β-HCG) in combination with AFP for gestational trophoblastic cancers and nonseminomatous germ cell tumors, and B) cancer antigen

Table-1A: Conventional Tumor Biomarkers for Various Cancers: Data were derived and extracted from References #16-18, 20, 24-25.

Tumor Biomarker	Organ or Tissue Cancer Type
1. CA 15-3	Breast Cancer (glandular, ductal)
2. CA 27.29	Breast Cancer Metastases
3. CEA	Colorectal Cancer
4. AFP	Hepatocellular Carcinoma, germ cell cancers
5. CA 19-9	Pancreatic Cancer Masses
6. CA 125	Ovarian Cancer
7. Beta-HCG	Gestational Trophoblastic Cancer
8. PSA	Prostate Cancer
9. Beta-HCG plus AFP	Non-seminomatous Germ Cell Cancers

Table 1B: Less Conventional Tumor Biomarker for Various Cancers: Data were derived and extracted from References #18, 20-22, 25, 28-29.

1. EGFR-2	Breast Cancer (HER2 positive)
2. MIF	Liver Cancer, Innate Immunity
3. HGF	Head and neck squamous cell carcinoma, gastric cancer
4. sFAS	Bladder cancer, colorectal cancer
5. TNF-alpha	Intestinal inflammation, pre-cancer adhesion
6. OSPT	General (tumor) cancer progression marker
7. ER-alpha	Breast Cancer (receptor positive)
8. HPG	Non-specific renal cell carcinoma
9. PLT	Pituitary gland cancer
10. LPT	Melanoma cancer
11. IL-6	Expression in most cancers, inflammatory factor
12. PD-L1	Hides tumor cells from immune attack

Total Biomarker Abbreviations:
AFP = Alpha-fetoprotein; β-HCG = beta human chorionic gonadotrophic; BMG = Beta – 2 – microglobulin; CA-19-9 = Pancreatic cancer biomarker; CA 15-3 = breast cancer biomarker; CA 27.29 = breast cancer metastases biomarker; CA – 125 = Ovarian cancer biomarker; CEA = carcinoembryonic antigen; EGFR-2 = epidermal growth factor receptor; ER-alpha = estrogen receptor alpha; FER = Ferritt; HGF = hepatocyte growth factor; HPG = haptoglobin; IL-6 = Interleukin – 6; LPT = leptin; MIF = migration inhibitory factor; OSPT = osteopontin; PLT = prolactin; PSA = prostate specific antigen; PD – L1 = programmed cell death protein ligand-1; sFAS = soluble FAS ligand; TNF-alpha = Tumor Necrosis Factor (transmembrane protein).

Table-2: Selected assay parameters for four commonly utilized tumor biomarkers for breast cancer. Values were derived and extracted from Refs. #4 and #21.

Tumor Biomarker	Percent Sensitivity (%)	Percent Specificity (%)	Percent Predictive Positive Value (%)	Diagnostic Index of Sensitivity
1. CA 15-3	63.38	80.64	34.35	0.27
2. CA 27.29	39.29	83.56	23.40	N.R.
3. CEA	22.82	79.66	16.67	0.27
4. AFP	21.82	79.01	20.34	0.16

Legend: CA 15-3 = Breast Cancer Glandular/ductal Biomarker; CA 27.29 = Breast Cancer Metastatic Biomarker; CEA = carcinoembryonic antigen, Colorectal Biomarker; AFP = Alpha-fetoprotein Liver Cancer Biomarker, NR = not reported.

CA27.29 and CA-15 for metastatic and solid breast cancers, respectively. Various combinations of tumor biomarkers such as AFP, CA 15-3, CEA, Neu oncoprotein (p 185) tissue-polypeptide antigen (TPA) have further been exploited for use in breast cancer.

Some of these single and combined biomarkers for cancer have shown only limited value due-to their lack of sensitivity and specificity in screening. Such has been observed for serum AFP, both in singleton and in combination, as a biomarker for breast cancer diagnosis and monitoring [22]. In various biomarker combination assays, AFP has frequently contributed the least value toward the diagnosis of breast cancer, while CA 15-3 proved that most effective. Perry et al found the most effective biomarker for BC metastases was CA 27.29 [16]. Interestingly, various combinations of biomarkers in stepwise discriminant analyses in an early clinical study that showed 90% of breast cancer patients could be diagnosed with a combination of CA 15-3, beta-2 microglobulin (BMG), and ferritin although some subsequent studies varied in agreement [13]. In a later study the BC diagnostic efficacy (sensitivity) for AFP was nearly 22%, compared to over 63% for CA 15-3 [4] [Table-2]. Moreover, in a combination panel, CA 15-3 proved to be the single most significant biomarker for BC with an 88% diagnostic specificity with AFP being less effective [Table-2]. Of the many biomarkers surveyed, only CA 15-3 was found to best correlate with all four diagnostic stages of breast cancer [4].

Selected Studies of Various Biomarkers for Breast Cancer:

As discussed above, some studies have employed four tumor antigens (CA 15-3,

CA 27.29, AFP, and CEA for their diagnostic efficacy in breast cancer patients [Table-2]. In one study, it was proposed and confirmed that CA 15-3 would prove superior to CA 27.29, CEA, and AFP in assay performance for breast cancer. In that study, a total of 554 patient samples were obtained from hospitals, while 200 healthily adult samples were used for the determination of normal reference values. The patient population included patients with no disease (N = 184), with non-malignant disease (N = 11), with breast cancer (N = 87), and other types of non-breast cancer (N = 272). Diagnostic percent sensitivities for each marker are listed in Table-2. It was concluded that CA 15-3 emerged as the best tumor antigen for use as a diagnostic aid and monitoring agent for BC. It was from studies such as the above that AFP should be considered for re-examination as a useful tumor marker for breast cancer [See Ref. 2,3,5,12].

Another study assessed the clinical value of five serum tumor markers (AFP, CA 15-3, CEA, TPA, and Neu oncoprotein P 185) in the diagnosis of breast cancer [21]. The serum values were measured in a prospective series of patients with breast cancer (n = 233), benign breast disease (n = 176) and healthy control subjects (n = 215) with cut-off levels determined for each test group. Using those cut-off levels, the diagnostic index of sensitivity of the CEA test was 0.27, while AFP was 0.16, CA 15-3 was 0.27, TPA was 0.18, and the Neu oncoprotein was 0.19 in detecting breast cancer. [Table-2] Correlation coefficients were statistically significant between CEA and CA 15-3, and between CA 15-3 and Neu oncoprotein however, no AFP correlation combinations regarding breast cancer patients were reported. In patients with benign breast disease the serum levels of AFP, CA 15-3, CEA and TPA correlated with age, being somewhat higher in older patients [21]. Similarly, in population control samples, higher age correlated with higher levels of AFP, CA 15-3, Neu and TPA. For breast cancer patients, no correlation was found between the age at diagnosis and any of the measured biomarkers. The only marker found to be statistically associated with the diagnosed stage of breast cancer was CA 15-3. In conclusion, the results indicated that combination of serum CEA, AFP, TPA, and Neu oncoprotein were only of limited value in the diagnosis of breast cancer. Again, this study demonstrated no advantage of AFP as a tumor marker for breast cancer [See Refs. 21-24].

For the diagnosis of bone metastasis in breast cancer patients during systemic treatment, the serum tumor markers including CA 15-3, CA 19-9, CA 125, AFP, CEA, beta-2 microglobulin (BMG), ferritin, and TPA were measured in 22 patients with known bone metastases (stage-4) together with 30 patients without disseminated metastases [25]. The most useful single marker for breast cancer metastasis proved to be CA 15-3 as presented above. The study also found that some patients could be diagnosed by using the combined markers of CA 15-3 and BMG [13]. Although CA 27.29 was detected in BC metastases, it was true for many other different tumors as well [25]. Although AFP showed lesser or little value, it has been continually used in monitoring with other combinations

of tumor markers at regular intervals in hopes of improving the overall diagnostic efficiency of BC diagnosis in the screen [See Refs 26-29]. Although CA 27.29 has been employed for metastatic BC testing, this biomarker lacks specificity being utilized for other metastatic tumors such as colon, stomach, ovarian and pancreatic cancers [25].

Even though AFP has been demonstrated to be somewhat specific in liver cancer detection dating back to 1959-1964 [26-28], varying degrees of elevated serum AFP samples have recently been reported in multiple types of malignant and benign tumor diseases. Though not reported until 2019, AFP concentrations were found to be significantly different (P-Values) from normal patients' blood levels in 47 different types of cancer and non-cancer diseases [28]. Elevated AFP levels were found in cancer-bearing patients that exhibited liver, breast, esophagus, cervical, pancreatic, endometrial, gastric, lung, and rectal cancers. In non-cancer disease patients, AFP blood levels were statistically above normal in disorders such as cirrhosis, hepatitis, nephrotic syndrome, and gastritis. In comparison, patients with multiple myeloma, Wilm's tumor, and 22 other types of non-cancer diseases have AFP values significantly lower than in normal patients [28]. Thus, increased serum AFP levels were highly evident for liver cancer patients but were not specific to only hepatomas. In light of such data, the specificity of AFP in cancers such as breast malignancy add further credence to the ineffectiveness and non-specificity of AFP as a tumor-associated biomarker for non-hepatic cancers such as BC.

In separate studies, AFP and Beta-HCG assay results were compared in infiltrating ductal breast carcinoma at different stages of malignancy [24]. Discovery of an HCG-like material in normal breast tissue (not serum) extracts demonstrated a limited value of the Beta-HCG assay in this study. In contrast, the measurement of histologically observed AFP warranted further consideration of AFP as a breast tissue biomarker for an in vitro evaluation of the migratory spread in all 4 stages of BC malignancy [24]

Summary Findings:

It becomes evident from the above data, findings, and assay descriptions, that human AFP as a tumor biomarker for breast cancer contributes little, if any, value as an assay component in combination assay panels. Such is not true for AFP as biomarker for liver cancer and hepatic disorders which have been known for many decades [26, 27]. It is the opinion of the present author (GJM) that AFP could be safely considered for possible deletion as a biomarker for breast cancer from various combination assay panels presently utilized in the cancer clinic.

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