

A Dictionary to Tumor Markers and The Methods of Estimation

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Abstract

Tumor markers are substances produced by tumor cells or by other cells of the body in response to cancer or certain benign (noncancerous) conditions. These substances can be found in the blood, in the urine, in the tumor tissue, or in other tissues. Different tumor markers are found in different types of cancer, and levels of the same tumor marker can be altered in more than one type of cancer. In addition, tumor marker levels are not altered in all people with cancer, especially if the cancer is early stage. Some tumor marker levels can also be altered in patients with noncancerous conditions. To date, researchers have identified more than a dozen substances that seem to be expressed abnormally when some types of cancer are present. Some of these substances are also found in other conditions and diseases. Scientists have not found markers for every type of cancer. ELISA and RIA are the extensively used techniques to estimate the concentration of tumor markers. As such they are not suitable for tumor screening and localization, but valuable as adjuncts for medical follow-up care of tumor patients, where their serum level alterations may anticipate the clinical detection of tumor behavior by a lead time of 1 to 6 months before other methods.

Key words: Tumor marker, malignancy, clinical relevance, Reference range.

1 Introduction

Tumor markers are biochemical substances, measurable and associated with a malignancy. They are either produced by tumor cells (biochemical's that tumor-derived) or by the body in response to tumor cells (tumor-associated). They are typically substances that are released into the circulation and thus measured in the blood. There are a few exceptions to this, such as tissue-bound receptors that must be measured in a biopsy from the solid tumor or proteins that are secreted into the urine. Though tumor markers are rarely specific enough to be used alone to diagnose cancer, they do have a number of clinical uses. They can be used to stage cancer, to indicate a prognosis, to monitor treatment, or in follow-up to watch for cancer recurrence. Changes in some tumor markers have been sensitive enough to be used as targets in clinical trials. When used for diagnosis, tumor markers are used in conjunction with other clinical parameters such as biopsy and radiological findings. Although there are a multitude of tumor

markers, very few of them have found their way into clinical practice because of their lack of specificity. However, some of these non-specific markers have found a place in monitoring cancer treatment rather than in diagnosis.

1.1 Classification:

A. General Scheme

1. Based on biochemical structure
2. Based on function
3. Based on combination of biochemical structure and function.
4. Based on discovery of oncofetal markers.

B. Most tumor markers are present normally in peripheral blood in low concentration. Some oncofetal markers may cross the placenta and circulate in maternal serum in a concentration higher than in the non - pregnant female (i.e. -AFP).

C. Common Types of Tumor Markers

- Oncofetal antigens (e.g. Alpha-feto

protein(AFP), Carcinoembryonic antigen(CEA), Pancreatic oncofetal antigen)

- Tumor associated antigens or cancer antigens (CA125, CA19-9, CA15-3, CA72-4, CA50 etc).
- Enzymes (e.g. Prostate Specific Antigen (PSA), glycosyl transferases, terminal deoxy nucleotide transferases (TDT), lysozyme, neuron specific enolase, alpha amylase).
- Hormone receptors (e.g. estrogen and progesterone receptors).
- Hormones (Beta human chorionic gonadotropin, calcitonin, placental lactogen) etc.
- Other biomolecules (e.g. poly amines).

Quantitative as well as qualitative examination of these markers can be done through modern techniques of sensitive immunoassays (RIA, ELFA, ELISA) using monoclonal or polyclonal antibodies immunoassays in majority of cases and biochemical and molecular biological techniques in other cases.

1.2 Ideal Characteristics of Tumor Markers

1. Organ specific and Tumor specific.
2. Positive only when malignancy is present.
3. Positive early in the development of malignancy.
4. Easy to measure in blood

1.3 Clinical Use of Tumor Markers:

1. Most Tumor Markers are non-specific for a single cancer; they are found with different tumors of the same tissue type (tumor-associated markers).

- Most tumor markers should not be used as a “cancer screening tool”.
- Most common use: clinical staging, monitoring therapy, detecting recurrence or presence of residual disease.

1.4 Sensitivity and Specificity:

- Some categories of tumor antigens (i.e. cell surface markers: CA-125, etc) have better clinical sensitivity and specificity than others such as oncofetal antigens [Alpha-Fetoprotein (AFP); Carcinoembryonic Antigen (CEA); etc].
- Use of monoclonal antibody techniques with immunoassays has improved laboratory sensitivity and specificity.
- It is most important to learn about tumor markers having high sensitivity and specificity versus those that are relatively in-sensitive and non-specific. [4-6,9,13]

2 Methods of Estimation:

- ELISA (Enzyme Linked Immunosorbent Assay)
- RIA (Radio Immuno Assay)

2.1 ELISA test:

The enzyme-linked immunosorbent assay (ELISA) is typically used to detect and quantify antigen within biological fluids, in which the Dual- Antibody Sandwich ELISA is being used for measuring the concentration of 80% of tumor markers in blood or serum.

The Dual-Antibody Sandwich ELISA:

The dual-antibody sandwich ELISA (DAS ELISA) is one of the most sensitive and specific techniques for quantifying molecules in solution. The DAS ELISA requires two antibodies that recognize separate epitopes on the antigen to be measured such that they are able to bind to the molecule simultaneously. The “capture” antibody, which is specific for the substance to be measured, is first coated onto a high-capacity protein-binding microtiter plate (an ELISA plate). Following the coating stage, any vacant binding sites on the plate are then blocked with the use of an irrelevant protein such as bovine serum albumin (BSA). This creates a solid-phase antigen-binding surface that should not nonspecifically bind other molecules.

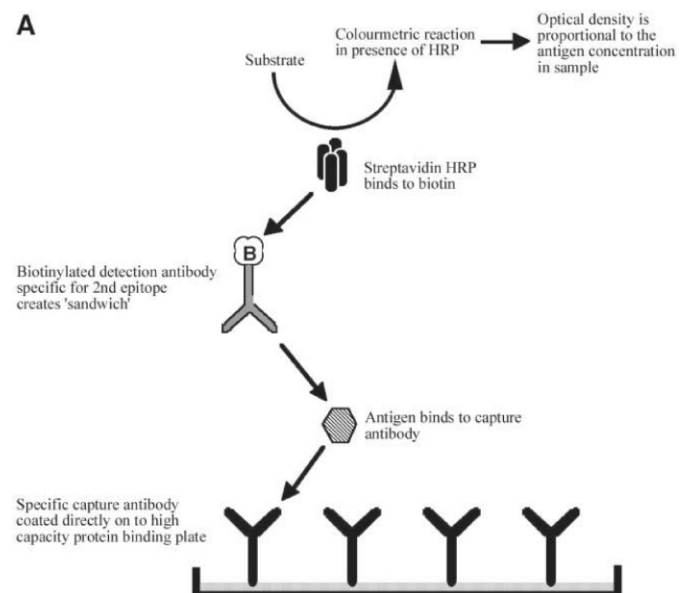
Samples, standards, and controls are then incubated on the plate, and any antigen present subsequently binds to the capture antibody. The bound antigen is detected using a secondary antibody (recognizing a different epitope on the antigen), thus creating the “sandwich.” The detection antibody is most often directly conjugated to biotin. Biotin conjugation allows an amplification process to be carried out with the use of streptavidin conjugated to an enzyme such as horse radish peroxidase (HRP). As streptavidin is a tetrameric protein, binding four biotin molecules, the threshold of detection is greatly enhanced. Developing the assay into a readable format involves the addition of a substrate such as 3, 3', 5, 5'-tetramethylbenzidine (TMB) for the HRP enzyme. In the presence of the HRP enzyme, TMB begins a colorimetric reaction that can then be measured using a spectrophotometer. The resulting color (optical density [OD]) relates directly to the amount of antigen present within the sample. Comparison of the OD within a sample to those obtained using a standard curve of known concentrations allows an estimate of antigen concentration within that sample to be gained.

2.1.1 Basic DAS ELISA Protocol

- Coating with capture antibody:** Capture antibody diluted in coating buffer is added to a high capacity-protein binding 96-well microtiter plate. The plate is then incubated, allowing the antibody to bind to the plate; subsequently, the plate is washed to remove any excess or unbound antibody.
- Blocking vacant binding sites:** Vacant binding sites on the plate are then blocked with an irrelevant protein such as BSA. Following incubation, the plate is washed once more to remove excess unbound protein.
- Addition of samples/standards:** A titration series of known standards

must be prepared. Ideally, these should be diluted in a matrix representing that of the samples to help identify false positives (e.g., if there are any substances in the matrix that bind nonspecifically to the plate that have enzyme activity). A negative control must also be included (e.g., culture medium only or serum known to be negative for the antigen to be measured). Samples and antigen standards are then incubated on the ELISA plate, allowing any antigen present to bind to the coating antibody.

- Addition of detection antibody:** Following the addition of samples, the biotinylated detection antibody is added, which binds to any antigen bound to the plate. Following incubation, the plate is once again washed thoroughly to remove unbound reagents.
- Addition of enzyme conjugate:** Streptavidin conjugated to an enzyme such as HRP is then incubated onto the plate. This binds to biotin molecules on the detection antibody. The plate is once again washed thoroughly to remove unbound reagents.
- Development and analysis:** The ELISA is then developed using a suitable substrate (e.g., TMB to detect the HRP enzyme). The OD can be measured using a spectrophotometer. The OD values from the standard titration of antigen are then used to determine an estimate of antigen within samples. [36].



2.2 Radio Immuno Assay:

One of the most sensitive technique for detecting antigen or antibody is Radio immunoassay. The principle involves competitive binding of radiolabelled antigen and unlabelled antigen to a high-affinity antibody. The antibody and labeled antigen are mixed at a concentration to enable the saturation of antigen binding sites of the antibody. The test samples of unlabelled antigen of unknown concentration are added progressively. As a result of the competition between two antigens for available binding sites on the antibody, as the concentration of unlabelled antigen increases, more labeled antigen will be displaced from the binding sites. Gamma emitting isotope such as I and beta emitting isotope such as tritium are also routinely used as labels. The important step in the RIA is the determination of the amount of antibody needed to bind 60% - 70% of a fixed quantity of radioactive antigen. Determination of amount of bound labeled antigen can be done by precipitating the Ag-Ab complex to separate it from free antigen and the radio activity in the precipitate can be measured. Amount of antigen in the test mixture can be found out by generating a graph, using unlabelled samples of known concentrations. To separate bound antigen from the free antigen in RIA, Ag-Ab complex is precipitated with secondary anti-isotype antiserum. After removal of the complex, using radiation counter, the amount of free labeled antigen remaining in the supernatant can be measured, subtracting this value from the total amount of labeled antigen added gives the amount of labeled antigen bound. [1, 11, 60]

3.0 Clinically Relevant Tumor Markers

3.1 AFP (Alpha Feto Protein):

AFP is a molecule produced in the developing embryo and fetus. In humans, AFP levels decrease gradually after birth, reaching adult levels by 8 to 12 months. Normal adult AFP levels are low, but detectable; however, AFP has no known function in normal adults. In normal fetuses, AFP binds the hormone estradiol. AFP is measured in pregnant women, using maternal blood or amniotic fluid, as a screening test for subset

developmental abnormalities, principally open neural tube defects. It is also measured in pregnant women, other adults, and children, serving as a biomarker to detect a subset of tumors, principally hepatocellular carcinoma and endodermal sinus tumors. Many functions have been proposed for AFP; an anti-cancer active site peptide has been identified and is referred to as AFPep. AFP is normally produced by the fetal yolk sac, the fetal gastrointestinal tract, and eventually by the fetal liver. Levels of AFP in fetal serum rise until the end of the first trimester of gestation and then fall. Because the fetus excretes AFP into its urine, amniotic fluid levels of AFP tend to mirror fetal serum levels. In contrast, maternal serum levels of fetal AFP are much lower but continue to rise until about week 32. LabCorp, a large US clinical laboratory testing company, began offering AFP screening tests in the early 1980s. The normal range of AFP for adults and children is variously reported as under 50, under 10, and under 5 ng/mL. At birth, normal infants have AFP levels 4 or more orders of magnitude above this normal range, decreasing to within it over the first 12 years of life. During this time, the normal range of AFP levels spans approximately 2 orders of magnitude. Correct evaluation of abnormal AFP levels in infants must take into account these normal patterns. Very high AFP levels may be subject to hooking, resulting in a reported high level that is nonetheless significantly lower than the actual level. This is important for analysis of a series of AFP tumor marker tests, e.g. in the context of post-treatment early surveillance of cancer survivors, where the rate of decrease of AFP has diagnostic value.

3.1.1 AFP tests:

There are two categories of AFP tests: tests performed on serum (blood plasma), and tests performed on amniotic fluid. Tests performed on serum are further categorized by the reason for performing the test: maternal serum, adult tumor marker, and pediatric tumor marker.

Tests performed on serum: The standard is a quantitative test, reporting a measured concentration of AFP in the sample, but there is also a less expensive qualitative test, reporting only that the concentration is normal or high. The qualitative test is appropriate only in some circumstances. The

resulting test report should specify the assay method and equipment used, and the report of a quantitative test should also provide a reference range for the test result. Many laboratories report reference ranges that are based on all other samples tested in that laboratory, necessarily including samples with abnormal AFP concentrations due to disease. Superior reference ranges are produced by research on healthy subjects.

Maternal serum: Maternal serum AFP tests need to be interpreted according to the gestational age, as levels rise until about 32 weeks gestation. Typically, such measurements are done in the middle of the second trimester (14-16 weeks). Elevated levels are seen in multiple gestation as well as in a number of fetal abnormalities, such as neural tube defects including spina bifida and anencephaly, and abdominal wall defects. Other possibilities are errors in the date of the gestation or fetal demise. In contrast, low levels of maternal serum AFP are associated with Down syndrome and Trisomy 18. Diabetic patients also have lower levels. Patients with abnormal levels need to undergo detailed obstetric ultrasonography. The information is then used to decide whether to proceed with amniocentesis. Maternal serum AFP may be measured as part of a routine prenatal screening test:

- Triple test: AFP, hCG and estriol
- Quad test: AFP, hCG, estriol, and Inhibin
- Genetic counseling usually is offered when the screening test result is positive.

Like any elevated tumor marker, elevated AFP by itself is not diagnostic, only suggestive. Tumor markers are used primarily to monitor the result of a treatment (e.g. chemotherapy). If levels of AFP go down after treatment, the tumor is not growing. In the case of babies, after treatment AFP should go down faster than it would normally. A temporary increase in AFP immediately following chemotherapy may indicate not that the tumor is growing but rather that it is shrinking (and releasing AFP as the tumor cells die). AFP-L3, an isoform of AFP which binds Lens culinaris agglutinin, can be particularly useful in early identification of aggressive tumors associated with hepatocellular carcinoma (HCC). AFP is the main tumor marker (sometimes with HCG)

used to monitor testicular cancer, ovarian cancer, and malignant teratoma in any location: values of AFP over time can have significant effect on the treatment plan. AFP is normally elevated in infants, and because teratoma is the single most common kind of tumor in infants, several studies have provided reference ranges for AFP in normal infants. Perhaps the most useful is this equation:

$$\log Y = 7.397 - 2.622 \cdot \log(X + 10)$$

where X = age in days and Y = AFP level in nanograms per milliliter.

Tests performed on cerebrospinal fluid (CSF). In normal infants, AFP in CSF is:

- median 61 kIU/L (5th-95th centile: 2-889 kIU/L) in infants -69 to 31 days old
- median 1.2 kIU/L (5th-95th centile: 0.1-12.5 kIU/L) in infants 32 to 110 days old

Levels of AFP in CSF decline with gestational age in proportion to levels of AFP in serum.

Interpretation of AFP test results: AFP test results often are reported as either ng/ml or MoM (multiple of the median, where the median is calculated for an appropriate reference population).

Maternal serum: Abnormally elevated AFP in the serum of a pregnant woman can have one or more of these sources:

- a problem with the fetus
- a problem with the placenta
- a tumor or liver disease in the woman
- a normally elevated AFP in the fetus or woman (some people naturally have very high AFP)

Usual follow-up steps include: - (1) a prenatal ultrasound exam to look for fetal abnormalities and/or (2) measurement of AFP in amniotic fluid obtained via amniocentesis.

Amniotic fluid: AFP in amniotic fluid has one or two sources. The fetus normally excretes AFP into its urine, hence into the amniotic fluid. A fetus with one of three broad categories of defects also releases AFP by other means. These categories are open neural tube defect, open abdominal wall defect, and skin disease or other failure of the interior or exterior body surface.

Abnormally elevated AFP in amniotic fluid can have one or more of many different causes:

- Normal elevation. 75% of AFP test results in the range 2.0 to 4.9 MoM are false positives: the baby is normal.
- open neural tube defect
- open abdominal wall defect
- congenital nephrosis
- others

3.1.2 Sources of AFP:

Serum alpha-fetoprotein is a fetal serum protein produced by the yolk sac and liver. Tumors-Principal tumors that secrete AFP are endodermal sinus tumor (yolk sac carcinoma), neuroblastoma, hepatoblastoma, and hepatocellular carcinoma. With regard to hepatocellular carcinoma, AFP is not useful for screening but is somewhat useful for surveillance after treatment. Rare AFP-secreting tumor types include carcinoma in a malignant mixed Müllerian tumor. In Wilms tumor AFP is rarely elevated, but when it is elevated it may serve as a marker of disease progression or recurrence. There are case reports of elevated AFP associated with teratoma. However, some of these case reports involve infants but do not correct for the normal elevation of AFP in infants, while others ignore the likelihood that teratoma (and other germ cell tumors) may in fact be mixed tumors containing elements of endodermal sinus tumor. In patients with AFP-secreting tumors, serum levels of AFP often correlate with tumor size. Resection is usually associated with a fall in serum levels. Serum levels are useful in assessing response to treatment. Increased serum levels in adults are also seen in acute hepatitis, colitis and ataxia telangiectasia.

3.1.3 Molecular variants of AFP:

Because of its affinity to the lectin, the AFP could be resolved into concanavalin A reactive (R Con A) and non-reactive (NR Con A) fractions. The AFP molecules synthesized by the yolk sac contain an additional sugar; N acetyl glucosamine linked to the _-mannose blocking the Con A binding site on the AFP. Quantitative as well as qualitative evaluation of AFP molecular variants revealed two types

of patterns, one specific to "liver" and the other to "yolk sac". Remarkable consistency and reproducibility of each pattern was observed in many cases of HCC and in germ cell tumors occurring either in gonads or at extra-gonadal sites. [24, 28, 38]

3.2 CEA (Carcinogenic Embryonic Antigen):

Carcinoembryonic antigen (CEA), first described in 1965 by Gold and Freedman, was characterized as a glycoprotein of 200 KD. Extensive studies of patients bearing primary and metastatic colorectal neoplasms have determined that its primary use is in the detection of local and metastatic cancer recurrence after initial resection of the primary tumor, through periodic postoperative analysis of CEA in serum or plasma. It is normally produced during fetal development, but the production of CEA stops before birth. Therefore, it is not usually present in the blood of healthy adults, although levels are raised in heavy smokers. CEA was first identified in 1965 by Phil Gold and Samuel O. Freedman in human colon cancer tissue extracts. It was found that serum from individuals with colorectal, gastric, pancreatic, lung and cervical carcinomas had higher levels of CEA than healthy individuals. CEA testing is of significant value in the monitoring of patients with diagnosed malignancies in whom changing concentrations of CEA are observed. CEA is encoded by the cea gene which is a member of the immunoglobulin super family. The human CEA gene family is clustered on chromosome 19q. In humans, the carcinoembryonic antigen family consists of 29 genes; of these, 18 are expressed, with 7 belonging to the CEA subgroup and 11 to the pregnancy-specific glycoprotein subgroup. CEA measurement is mainly used to identify recurrences after surgical resection. Elevated CEA levels should return to normal after surgical resection, as elevation of CEA during follow up is an indicator of recurrence of tumor. CEA is a substance normally found in a fetus which, when found at elevated levels in the blood of adults, may indicate the presence of colorectal cancer or other types of cancer. CEA is therefore called a tumor marker. It has been used to monitor patients for the recurrence of a number of different cancers, including breast, thyroid, lung, cervical,

pancreatic, stomach, and colon/rectal. It is also referred to as an "oncofetal antigen" because of its similarity to fetal tissue. CEA levels can also be an indication of the effectiveness of treatment. Tests for its presence in the serum of the cancer patients aid in screening, in evaluating recurrent or disseminated disease, and in gauging the success of surgical removal of malignant tumors. CEA is most frequently tested in blood. It can also be tested in body fluids and in biopsy tissue. The best use of CEA is as a tumor marker, especially for cancers of the gastrointestinal tract and cervical cancers. When the CEA level is abnormally high before surgery or other treatment, it is expected to fall to normal following successful surgery to remove all of the cancer. A rising CEA level indicates progression or recurrence of the cancer. In addition, levels >5 ng/ml before therapy are associated with cancer which has already spread (metastatic disease). Both benign and malignant (harmless and cancerous) conditions can increase the CEA level. The most frequent cancer which causes an increased CEA is cancer of the colon and rectum. Others include cancers of the pancreas, stomach, breast, lung, and certain types of thyroid and cervical cancer. Benign conditions which can elevate CEA include smoking, infections, inflammatory bowel disease, pancreatitis, cirrhosis of the liver, and some benign tumors in the same organs in which an elevated CEA indicates cancer. Chemotherapy and radiation therapy can cause a temporary rise in CEA due to the death of tumor cells and release of CEA into the blood stream. The presence of carcinoembryonic antigen (CEA) in the serum of the cancer patients can be detected with the help of ELFA (Enzyme linked fluorescent assay). The test measures the amount of a protein that may appear in the blood of some people who have certain kinds of cancers, especially large intestine (colon and rectal) and breast cancer. It may also be present in people with cancer of the pancreas, ovary, or lung. Results are usually available in 1 to 3 days. Normal values may vary from lab to lab. [4, 6, 18, 35, 41, 48, 55, 56].

Carcinoembryonic antigen	
Nonsmokers:	Less than 3 units per milliliter
Smokers:	Less than 5 ng/mL (or 5ng/mL).

3.2.1 High values:

1. Cancer of the colon, lung, pancreas, breast, or cervix may be present.
2. Cancer may not be responding to treatment.
3. May have returned after treatment. A steadily rising CEA may be the first sign that cancer has come back after treatment. Also, people with advanced cancer or cancer that have spread to other parts of the body (metastatic cancer) may have high CEA levels if their original cancer produced this protein before treatment.
4. Another condition or disease is present, such as cirrhosis, pancreatitis, kidney failure, inflammatory bowel disease, peptic ulcer disease, chronic obstructive pulmonary disease (COPD), or an obstructed bile duct.

3.2.2 Low values:

People with small cancers or early-stage disease usually have low, or even normal, CEA levels.

3.3 Human Chorionic Gonadotropin:

HCG, composed of two dissimilar subunits the alpha chain (14 KD) and beta chain (24KD, glycoprotein hormone produced in pregnancy that is made by the embryo soon after conception and later by the syncytiotrophoblast (part of the placenta). The beta subunit of human chorionic gonadotropin (b-hCG) normally is produced by the placenta. Elevated b-hCG levels most commonly are associated with pregnancy, germ cell tumors, and gestational trophoblastic disease. The reference values in serum of healthy men and non pregnant women are less than 5 IU/ml and post menopausal women are less than 10IU/ml. False-positive levels occur in hypogonadal states and with marijuana use. Both AFP and b-hCG play crucial roles in the management of patients with nonseminomatous germ cell tumors. The AFP or b-hCG level is elevated in 85 percent of patients with these tumors, but in only 20 percent of patients with stage I disease. Hence, these markers have no role in screening. Marked elevations of AFP or b-hCG are associated with very few disease states. In patients with extragonadal disease or metastasis at the time of diagnosis, highly

elevated AFP or b-hCG values can be used in place of biopsy to establish a diagnosis of nonseminomatous germ cell tumor. AFP values in excess of 10,000 ng per mL or b-hCG levels above 50,000 mIU per mL at initial diagnosis portend a poor prognosis, with a five-year survival rate of 50 percent. Similarly staged patients with lower AFP and b-hCG levels have a cure rate higher than 90 percent. Following AFP and b-hCG levels is imperative in monitoring response to treatment in patients who have nonseminomatous germ cell tumors. Patients with AFP and b-hCG levels that do not decline as expected after treatment have a significantly worse prognosis, and changes in therapy should be considered. Because curative salvage therapy is possible, the tumor markers are followed every one to two months for one year after treatment, then quarterly for one year, and less frequently thereafter. AFP or b-hCG elevation is frequently the first evidence of germ cell tumor recurrence; a confirmed elevation should prompt reinstitution of therapy. The b-hCG level is used to diagnose gestational trophoblastic disease, a rare neoplastic complication of pregnancy. The b-hCG value is followed to assess response to treatment and to detect relapse in a manner similar to that for germ cell tumors. [19]

3.4 Prostate Specific Antigen:

Prostate-specific antigen (PSA) is a glycoprotein produced by prostatic epithelium. The PSA level can be elevated in prostate cancer, prostatitis, benign prostatic hypertrophy, and prostatic trauma, as well as after ejaculation. In men with prostatitis, PSA levels return to normal within eight weeks of symptom resolution. Waiting 48 hours after ejaculation to measure the PSA level has been recommended. Digital rectal examination does not elevate PSA levels above normal values. In men who have been taking finasteride (Proscar) for more than six months, reported PSA levels should be doubled to accurately reflect true values, because the drug is an enzyme inhibitor that suppresses normal production of PSA by the prostate gland. In prostate cancer, the positive predictive value of PSA levels greater than 4 ng per mL is 20 to 30 percent and rises to 50 percent when PSA levels exceed 10 ng per mL. Nevertheless, 20 to 30 percent of men

with prostate cancer have PSA levels within normal ranges. Modifications to improve the positive predictive value of PSA testing include revised limits of normal based on age, race, velocity, density, and percentage of unbound (free) antigen. To date, these modifications have not resulted in improved outcomes. However, in patients with PSA values between 4 and 10 ng per mL, the PSA velocity and percentage of free PSA have been helpful in making clinical decisions. A velocity of 0.75 ng per mL per year is predictive of cancer. When less than 10 percent of PSA is unbound, the positive predictive value for prostate cancer is 55 percent, compared with 8 percent when more than 25 percent of PSA is unbound. Prostate cancer screening remains controversial. Surrogate evidence of screening benefits includes lower PSA levels and earlier stage of disease at the time of initial diagnosis. Limitations of screening include uncertainty about outcome benefit after treatment of localized prostate cancer, potential identification of clinically insignificant tumors, and attendant morbidity of treatment. Experts from the American Urological Association suggest that patients should be given sufficient information to allow them to make an informed decision about prostate cancer screening using PSA levels. If PSA testing is undertaken, an age of 40 years has been suggested for initiation of screening in black men and in all men with a family history of prostate cancer. In patients without established risk factors and a minimum life expectancy of 10 years, screening could begin at age 50. If elevated PSA values are confirmed, patients should be referred for biopsy. Randomized clinical trials are being conducted to assess the validity of these recommendations. PSA levels predict the presence of metastatic disease. Patients with newly diagnosed prostate cancer and PSA levels below 20 ng per mL rarely have osseous metastasis and do not need bone scanning, because the incidence of metastatic disease in these men is lower than 2 percent. In addition, computed tomographic scanning is unnecessary in men with PSA levels below 25 ng per mL. At our institution, if a prostate nodule is detected, the bone scan is widely positive, and the PSA level exceeds 100 ng per mL, treatment is often instituted without performance of biopsy. After treatment of

prostate cancer, PSA levels should be obtained every six months for five years, and then annually. In men who have undergone radical prostatectomy, any detectable PSA is significant. Salvage radiotherapy may be appropriate in these patients if recurrence is limited to the prostate bed as determined by ProstaScint scanning, a nuclear medicine test using a radio labeled antibody that targets only prostate tissue. After radiotherapy, a PSA nadir is not reached for one to two years. Three consecutive elevations of the PSA level indicate biochemical relapse in previously irradiated patients. Metastases do not become clinically evident for an average of eight years, and death does not occur for an average of 13 years. Thus, management decisions must include consideration of a patient's age and comorbid conditions. [10, 43, 54]

3.5 Prostate Acid Phosphatase:

Prostatic acid phosphatase (PAP), also prostatic specific acid phosphatase (PSAP), is an enzyme produced by the prostate. AFP is a glycoprotein of 590 amino acids and a carbohydrate moiety. It may be found in increased amounts in men who have prostate cancer or other diseases. The highest levels of acid phosphatase are found in metastasized prostate cancer. Diseases of the bone, such as Paget's disease or hyperparathyroidism, diseases of blood cells, such as sickle-cell disease or multiple myeloma or lysosomal storage diseases, such as Gaucher's disease, will show moderately increased levels. Certain medications can cause temporary increases or decreases in acid phosphatase levels. Manipulation of the prostate gland through massage, biopsy or rectal exam before a test may increase the level.

3.6 Cancer Antigen 125:

Cancer Antigen 125 (CA 125) is an antigenic determinant on a glycoprotein recognized by a monoclonal antibody. It is expressed in the amnion and its derivatives of fetal coelomic epithelia. The antigen is also found in several adult tissues such as the epithelium of the fallopian tubes, the endometrium, the endocervix, the pleura, and the peritoneum. Thus, the normal tissue of the body, namely the endometrium, produces a basal level of CA 125 which can contribute significantly to the level of circulatory or serum CA 125. While a basal level of circulating CA 125 the

antigen may be elevated. These conditions may be better understood by classifying them into non-gynecological and gynecological processes. Various studies have documented an elevation in CA 125 in a few non-gynecological conditions, including cirrhosis of the liver and tuberculosis. Cancers of the pancreas, breast, colon, and lung have also been found to express higher levels of CA 125. Studies are currently underway to determine the efficacy of using CA 125 in the diagnosis and management of various types of cancers. Meanwhile, gynecological processes such as pelvic inflammatory disease, endometriosis, and menstruation have been implicated in raising the serum level of CA 125. Other conditions such as benign ovarian cysts, tubo-ovarian abscess, hyper stimulation syndrome, ectopic pregnancy, and fibroids also have been correlated with elevated levels of CA 125. The sensitivity of serum CA 125 for pretreatment ovarian carcinoma varied from 43%-97% depending on the stage of ovarian malignancy. Finally, when compared with the normal, non-pregnant state, the antigen levels in pregnant women have been observed to be significantly higher during the first trimester, but not during the second and third trimesters. While these non-gynecological and gynecological conditions have been associated with increased levels of CA 125, the highest serum levels of the antigen are found in ovarian cancer patients. CA 125 estimation is of clinical value in the pre-operative diagnosis and monitoring of ovarian malignancies. Available data suggests that CA 125 is elevated in the majority of epithelial ovarian malignancies prior to clinical presentation. Large trials of screening for ovarian cancer indicate that using a CA 125 cutoff value of 30 U/ml has good sensitivity, but inadequate specificity for detecting pre-clinical disease. The sensitivity of CA 125 is related to stage (40-95 percent) and histologic type (lower levels in mucinous adenocarcinoma). Use of transvaginal ultrasonography as a second-line test in women with elevated CA 125 levels improves specificity to acceptable levels, as does use of a mathematical algorithm which analyses rates of change of CA 125. The best-established application of the CA 125 assay is in monitoring ovarian cancer. Doubling or halving of CA 125 serum values correlated with tumor progression or regression,

respectively. The rate of decline in CA 125 during primary chemotherapy has been an important independent prognostic factor in several multivariate analyses. A deviation from the ideal CA 125-regression curve predicts poor outcome within three months of treatment. Persistent elevation of CA 125 at the time of a second look surgical surveillance procedure predicts residual disease with greater than 95 percent specificity. Rising CA 125 values have preceded clinical detection of recurrent disease by at least three months in most, but not all studies. Given the modest activity of salvage chemotherapy, this information is not yet impacted on survival. Rising CA 125 during subsequent chemotherapy has been associated with progressive disease in more than 90 percent of cases. Combined assay of either CEA or CA 19-9 or both along with CA 125 did not increase diagnostic sensitivity compared to sensitivity achieved by CA 125 alone for epithelial tumors of the ovary. CA 125 is a tumor associated antigen whose presence in the serum of the cancer patients can be detected by directing specific monoclonal antibodies against them through the ELISA test. [5, 6, 35, 12, 16, 45, 47, 48, 49, 56]

3.7. Cancer Antigen 19-9:

The CA 19.9 cancer antigen is a glycoprotein associated with malignant neoplasms. The CA 19.9 assay is based on the use of a monoclonal mouse 116-NS-19-9 antibody. It is measured to aid in the management of patient with malignancies. CA 19.9 antigen is most often followed in the patient with pancreatic and colon cancer. The CA 19.9 may also be elevated in stomach and hepatobiliary malignancies. Sometimes in benign inflammatory disease of pancreas, gallbladder and liver CA 19.9 may also be elevated. The CA 19.9 assay level decreases after therapy and increases in cases of relapse, residual disease and metastasis. The CA 19.9 assay is used as an additional test for the prognosis and monitoring of therapy of patients with diagnosed malignant tumors. A decrease in the CA 19.9 assay level can indicate a positive response to therapy and therefore good prognosis. A constant increase of the CA 19.9 assay value often reflects evolution of the tumor and a poor response to therapy. In 99.6% of healthy adults, serum CA 19-9 levels are lower than 37 u/ml. [13, 48, 52]

3.8 Cancer Antigen 549:

CA-549 is a circulating breast cancer-associated antigen that reacts with monoclonal antibody BC4E 549. Biochemical characterization of CA-549 revealed that it is an acidic (isoelectric point 5.2) glycoprotein that exhibits two bands by sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing conditions of apparent molecular weights of 400,000 and 512,000. Immunohistochemical staining of unfixed frozen tissue sections of human breast tumors and a variety of benign tissues with BC4E 549 revealed no preferential staining of tumor over benign breast tissue and cross-reactivity with a wide range of other benign tissues including kidney, liver, lung, colon, pancreas, ovary, and spleen. [14]

3.9 Cancer Antigen 15-3:

Cancer antigen 15-3 is a glycoprotein antigen (300KD) reacts with two monoclonal antibodies 115D8 and DF3 raised against breast carcinoma cells. Elevated levels are also reported in liver cirrhosis, acute and chronic hepatitis. In rare cases, increased levels are found in cancers of ovary, lung, stomach and uterus. [13, 39, 55]

3.10 Cyfra 21-1:

CYFRA 21-1 is a cytokeratin 19 fragment found in serum of cancer patients. Precise recognition of this fragment is made with two monoclonal antibodies (BM 19-21 and KS 19-1) which were obtained after immunization of mice with MCF-7 cells. Cytokeratin 19 (CK19) is a member of the intermediate filament group of proteins, whose physiological role remains unclear. It is an acid-type cytoplasmic protein, with a molecular weight of 40 000 D, expressed in simple epithelium. On the death of the cell, it is released into the serum in the form of soluble fragments. In immunohistochemistry, CK19 is found in the cytoplasm of the epithelial tumor cell, including that of bronchial cancers. Preliminary clinical studies of bronchial cancer patients sera have shown that CYFRA 21-1 assay is useful in the diagnosis and follow-up of non-small cell lung carcinoma and particularly of squamous cell carcinoma of the lung.

3.11 Cancer Antigen 72-4

CA 72-4 is defined by two monoclonal

antibodies: B 72-3 and CC 49. B 72-3 was obtained by mouse immunization with a membrane fraction enriched with human metastatic breast cancer. In immunohistochemistry, it presents good affinity for human gastro-intestinal and mammary carcinomas compared to the corresponding benign or normal tissues. The 72-3 antigen is also known as Tumor Associated Glycoprotein 72 or TAG 72. CC 49 was generated after mouse immunization with TAG 72 purified by affinity chromatography. It recognizes a different epitope from that of B72.3. The assay of 72-4 has been evaluated in many clinical studies which have demonstrated its high sensitivity and excellent specificity in cases of gastric cancer. [35]

3.12 Cancer Antigen 50

Tumor cells express antigenic substances in the cell membrane which are not usually produced by healthy cell membranes. The detection of these tumor-associated substances is a valuable tool in the diagnosis of malignant disorders. Using the hybridoma technique of Köhler and Milstein, specific immunological reagents (monoclonal antibodies, or MAbs) can be obtained which recognize tumor-associated antigens. A monoclonal antibody of this type, C-50 MAb, was obtained after immunization using a colorectal adenocarcinoma cell line Colo 205. The C-50 MAb recognizes two different carbohydrate chains, the sialylated Lewis-a and the hitherto unknown sialylated lactotetraose. Structures containing CA-50 are mainly found in gastro-intestinal carcinomas (e.g. pancreatic, gastric, colorectal and hepatic carcinomas), but also in other malignant growths (endometrial carcinomas). The CA-50 antigens occur in the cell membrane in a lipid-bound form (as ganglioside) and in a form bound to a high molecular weight protein (as glycoprotein). Tumor passes the CA-50 antigens into the blood stream, where they can be specifically determined by means of immunological techniques based on the C-50 MAb. [26]

3.13 Cancer Antigen 27.29

Cancer antigen (CA) 27.29 is a monoclonal antibody to a glycoprotein (MUC1) that is present on the apical surface of normal epithelial cells. CA 27.29 is highly associated with breast cancer, although levels are

elevated in several other malignancies. CA 27.29 also can be found in patients with benign disorders of the breast, liver, and kidney, and in patients with ovarian cysts. However, CA 27.29 levels higher than 100 units per mL are rare in benign conditions. Because of superior sensitivity and specificity, CA 27.29 has supplanted CA 15-3 as the preferred tumor marker in breast cancer. The CA 27.29 level is elevated in approximately one third of women with early-stage breast cancer (stage I or II) and in two thirds of women with late-stage disease (stage III or IV). CA 27.29 lacks predictive value in the earliest stages of breast cancer and thus has no role in screening for or diagnosing the malignancy. Disagreement exists about the ability of CA 27.29 to detect asymptomatic recurrence after curative treatment. One trial in patients at high risk for recurrence of breast cancer (stage II or III) found that CA 27.29 was highly specific and sensitive in detecting preclinical metastasis. The average time from initial elevation of CA 27.29 to onset of symptoms was five months. Because CA 27.29 testing may lead to prompt imaging of probable sites of metastasis, it may be possible to decrease morbidity through earlier institution of therapy. [2]

3.14 Neuron-specific Enolase

Neuron Specific Enolase is a glycolytic enzyme normally present in neurons, peripheral nerve tissues and neuroendocrinal tissues, especially in the cells of the APUD (Amine Precursor Uptake Decarboxylation) system. It is in the form of dimers with a molecular weight of approximately 95000D. NSE is found at high seric levels in tumors of neuroectodermic or neuroendocrine origin: small-cell carcinoma of the lung cancer and neuroblastoma are two examples. Bronchial cancers have many histological varieties. Small-cell anaplastic cancer is the most feared due to its rapid growth and the - presence of frequently early metastases. NSE is a clear indicator of this microcellular variety of cancer. Determining the NSE level at the time of diagnosis allows orientation of the anatomopathological assessment of such tumors. Several studies have shown the relationship between the NSE level and the spread of the illness: high levels are associated with advanced cancer. Repeated measurements of NSE during cytotoxic

treatment allow evaluation of its effectiveness and the prediction of a possible relapse. Neuroblastoma is another type of neuroendocrinal tumor. Measurement of NSE is useful in differential diagnosis between a WILMS tumor and neuroblastoma when dealing with a child exhibiting an abdominal mass syndrome. Should a high level of NSE indicate the presence of neuroblastoma, repeated measurements enable its development to be closely followed. [29, 46]

3.15 Chromogranin A

Chromogranin A (CGA) is an acid protein of 439 aa (49kD). One of the granin family, it is located in the secretion granules of the neuroendocrine cells. CGA is a pro-hormone which undergoes maturation by proteolytic cleavage. This gives bio-active peptides (vasostatins, chromostatin, pancreastatin, parastatin etc.) which have paracrine and autocrine functions. Circulating CGA is present in healthy subjects, and the values obtained are independent of age and of sex. CGA is a specific marker for neuroendocrine tumors. The interest of seric CGA assay was first shown in pheochromocytoma, and then rapidly extended to other endocrine cancers with particularly significant high rates in intestinal carcinoids and in neuroendocrine tumors of the pancreas. Unlike other biological markers, for example plasmatic catecholamines, the rates of CGA are affected by neither stress nor the drugs used in the treatment of pheochromocytomas. The rate of circulating CGA is associated with a neuroendocrine differentiation and linked to the tumor mass. Some authors have also shown that the presence of CGA in prostate cancers indicates an unfavorable evolution. These pathological rates can be associated with a decreased survival, independently of the disease's stage. [40]

3.16 Keratins:

Keratins are proteins ranging from 40 to 68 KD are known to form intermediate filaments of 8-10 nm in diameter. Keratin 18 is a type I cytokeratin. It is, together with its filament partner keratin 8, perhaps the most commonly found products of the intermediate filament gene family. They are expressed in single layer epithelial tissues of the body. Mutations in this gene have been linked to cryptogenic cirrhosis. Two transcript variants encoding

the same protein have been found for this gene. Keratin K8 is considered to be a marker of malignancy in skin tumors, including human cancer. Increased levels or ectopic expression of simple epithelium keratins in invasive tumors of different origin have been understood to be a consequence of malignancy. No causative role for K8/K18 in tumorigenesis had been identified.. The ectopic expression of keratin 8 in skin causes major alterations in its morphology, including epidermis and hair follicles hyperplasia, dysplasia, and ultimately preneoplastic changes of differentiated epidermal cells in aging. The dysplastic changes observed in hair follicles expressing K8 are self-renewing and contain reservoirs of multipotent stem cells capable of regenerating the epidermis. These are the origin of many neoplasms, including carcinomas and pilomatricomas, arising through the inappropriate activation of signaling pathways that regulate hair follicle morphogenesis and the hair cycle. They serve as tumor markers for undifferentiated and anaplastic carcinomas, disparately growing infiltrating carcinoma cells, thyroid tumors, prostate tumor and breast tumor. [22, 23]

3.17 Hydroxy Indole Acetic Acid:

It is tumor marker of first choice for diagnosing indole secreting tumors. This is a metabolite of serotonin that is excreted in the urine. Serotonin is a neurotransmitter that is synthesized from the amino acid tryptophan by enterochromaffin cells in the gut and bronchi. It is metabolized to 5-hydroxyindole acetic acid in the liver and then excreted in the urine. Elevations in 5-hydroxyindole acetic acid can indicate carcinoid tumor. The normal range in a 24 hour urine collection is 3 to 15 mg per 24 hours.

3.18 Interleukin-2 Receptor:

The interleukin-2 receptor (IL-2R) is heterotrimeric protein expressed on the surface of certain immune cells, such as lymphocytes, that binds and responds to a cytokine called interleukin 2. IL-2 receptor is a glycosylated protein and it is a good tumor marker for some types of lymphoid malignancies

3.19 Ferritin:

Ferritin, a 450 kDa protein consisting of 24 subunits is present in every cell type. In

vertebrates, these subunits are both the light (L) and the heavy (H) type with an apparent molecular weight of 19 kDA or 21 kDA respectively. In plants and bacteria the complex only consists of the H-chain type. Inside the ferritin shell, iron ions form crystallites together with phosphate and hydroxide ions. The resulting particle is similar to the mineral ferrihydrite. Each ferritin complex can store about 4500 iron (Fe^{3+}) ions. Elevated ferritin levels are reported in advanced cancers of breast, ovaries, lungs and esophagus.

3.20 Tumor Suppressor Gene P53:

The p53 gene like the Rb gene is a tumor suppressor gene, i.e., its activity stops the formation of tumors. If a person inherits only one functional copy of the p53 gene from their parents, they are predisposed to cancer and usually develop several independent tumors in a variety of tissues in early adulthood. This condition is rare, and is known as Li-Fraumeni syndrome. However, mutations in p53 are found in most tumor types, and so contribute to the complex network of molecular events leading to tumor formation. [2, 25]

3.21 Oncogene P21 Ras:

The ras oncogene p21 antigen (p21) has been identified in several epithelial malignancies, including breast, colon, bladder, and prostate. The pattern and intensity of immunoreactivity between normal and neoplastic tissues has been distinctly different. Studies on thyroid lesions from different cases by immunohistochemistry for the expression of p21 with a monoclonal antibody (RAP-5) had shown that normal thyroid tissues has the least immunoreactivity, while papillary carcinomas, Hurthle cell carcinomas, and follicular carcinomas as showed slightly more intense staining than Hurthle cell adenomas or follicular adenomas. Anaplastic carcinomas showed much less intense staining than most other carcinomas, while medullary thyroid carcinomas showed only slight immunoreactivity. Inflammatory thyroid lesions associated with goiters, including Hashimoto's thyroiditis and Graves's disease, showed moderate to intense expression of p21 as did multinodular goiters. Semi quantitative analysis of staining intensity by serial dilution of the primary

antibody showed significant differences in staining between normal thyroid and some carcinomas (P less than 0.05), but not between carcinomas and adenomas. These results show that while antibody RAP-5 detects an antigen that is only weakly expressed in normal thyroids, this antigen is more strongly expressed in benign and malignant thyroid tumors, as well as in inflammatory and non-neoplastic proliferative thyroid lesions. It is thus not helpful in identifying differences between neoplastic and non-neoplastic thyroid lesions. [50]

3.22 Squamous Cell Carcinoma (SCC) Antigen:

Elevated levels of SCC antigen can be found in advanced cervical cancers, extensive liver diseases and lung tumors. It is a 48 KD protein, derived from uterine cervix. SCC antigen is useful for monitoring cancer recurrence following the removal of squamous cell carcinoma, and its half-life has been reported to be 2.2 hours. Concentrations that remain persistently elevated or begin to increase following tumor removal suggest persistent or recurrent disease. In cases where SCC antigen levels will be used to monitor progress of patients, testing is indicated four weeks prior to surgery. [37]

3.23 BRCA 1 and BRCA 2:

It is gene which, when damaged (mutated), places a woman at greater risk of developing breast and/or ovarian cancer, compared with women who do not have the mutation. The types of mutations are frame shift, nonsense and splice site including deletions and duplications. [15]

3.24 PS2:

PS2 is a cysteine rich, 6.5kDa protein found in both estrogen-dependent (breast tumors) and estrogen-independent tissues (normal stomach mucosa). About 60% of breast carcinomas are positive for PS2. Staining is cytoplasmic; often with localization to the Golgi apparatus. PS2 is primarily expressed in estrogen receptor-positive breast tumors. Antibody to PS2 is reportedly useful in identifying a subset of estrogen-dependent breast tumors which may respond to endocrine therapy. [44]

3.25 Calcitonin:

Calcitonin is a 32-amino acid linear polypeptide hormone that is produced in humans primarily by the parafollicular (also known as C-cells) of the thyroid, and in many other animals in the ultimobranchial body. It may be used diagnostically as a tumor marker for a form of thyroid cancer (medullary thyroid adenocarcinoma), in which high calcitonin levels may be present and elevated levels after surgery may indicate recurrence. It may even be used on biopsy samples from suspicious lesions (e.g. swollen lymph nodes) to establish whether they are metastasis of the original cancer. [3]

3.26 Tissue Polypeptide Antigen:

Serological tumor marker composed of a molecular complex of cytokeratins 8, 18, and 19. It is used in the diagnosis and staging of bronchogenic carcinoma. Elevated serum TPA levels are reported in breast cancer, lung cancer and urological cancer. [31]

3.27 Beta-2-Microglobulin:

BETA-2-MICROGLOBULIN (11kd) is the best tumor marker for lymphomas and multiple Myeloma. Elevated levels are also reported in CNS mets, CSF and other lymph proliferative disorders. [8, 17]

4.0 Conclusion

Tumor markers are often circulating tumor-associated indicators of tumor development. There are only a handful of well-established serums and tissue based tumor markers that are being routinely used by doctors in the diagnosis and management of cancer patients. Many other potential markers are still being researched. Each tumor marker has a variable profile of usefulness for screening, determining diagnosis and prognosis, assessing response to therapy, and monitoring for cancer recurrence. The goal is to be able to screen for and diagnose cancer early, when it is the most treatable and before it has had a chance to grow and spread. So far, no tumor marker has gained acceptance as a general screen. The markers are either not specific enough (too many false positives, leading to expensive and unnecessary follow-up testing) or they are not elevated early enough in the disease process. As such they are not suitable for tumor screening and localization, but

valuable as adjuncts for medical follow-up care of tumor patients, where their serum level alterations may anticipate the clinical detection of tumor behavior by a lead time of 1 to 6 months before other methods.

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