CHAPTER: TUMOR MARKERS

**Cancer:** A relatively autonomous growth of tissue.

**Cancer Staging:** The process by which cancer is divided into groups of early and late disease; useful for prognosis and guiding therapy.

**Carbohydrate Tumor Marker:** Antigens containing a major carbohydrate component usually found on the surface of cells or secreted by cells (e.g., mucins or blood group antigens).

**Ectopic Syndrome:** Production of a hormone by non-endocrine cancerous tissue that normally does not produce the hormone (e.g., ADH production by small-cell lung carcinoma).

**Microarray:** A small piece of silicon, plastic, or glass onto whose surface has been fabricated a structured, two dimensional array of compartments that are accessed by their position in the array (the compartments can contain DNA, RNA, protein, antibodies, or small pieces of tissue).

**Oncofetal Antigen:** Proteins produced during fetal life that decrease to low or undetectable concentrations after birth. They reappear in some forms of cancer because of the reactivation of a gene in the transformed malignant cells.

**Oncogene:** A mutated normal cellular gene (protooncogene) that causes the malignant transformation of normal cells when activated.

**Prognosis:** A prediction of the future course and outcome of a patient’s disease based on currently known indicators (e.g., age, sex, tumor age, tumor marker concentration, etc.).

**Tumor Markers:** A substance produced by a tumor found in blood, body fluids, or tissue that may be used to predict the tumor’s presence, size, and response to therapy.

**Tumor- Suppressor gene:** A gene involved in the regulation of cellular growth; loss of a tumor-suppressor gene has the potential to allow autonomous growth.

A tumor marker is a substance produced by a tumor, or by the host in response to a tumor, that is used to differentiate a tumor from normal tissue or to determine the presence of a tumor based on measurements in the blood or secretions.

**TUMOR MARKER** substances are found in cells, tissue, or body fluids and are measured qualitatively or quantitatively by chemical, immunological, or molecular diagnostic methods.

Morphologically, cancer tissue has been recognized by pathologists as resembling fetal tissue more than normal adult differentiated tissue. Tumors are graded according to their degree of differentiation as being (1) well differentiated, (2) poorly differentiated, or (3) anaplastic (without form). Tumor markers are the biochemical or immunological counterparts of the differentiation state of the tumor. In general, some tumor markers represent re-expression of substances produced normally by embryogenically closely related tissue (Table 20-1).

Some tumor markers are specific for one type of cancer, while others are seen in several cancer types. Many of the well-known markers are seen in both noncancerous conditions and cancer. Consequently, these tumor markers are not diagnostic for cancer. However, in many cases blood concentrations of tumor markers reflect tumor activity an volume.

Clinically an ideal tumor marker should be both specific for a given type of cancer and sensitive enough to detect small tumors to allow early diagnosis or use in screening.
DEFINITION-TUMOR MARKER

Any macro-molecule regardless of function can be called tumor marker.
1. Polypeptide
2. Protein
3. Nucleic acid
4. Enzyme
5. Hormone

CHARACTERISTICS:

• Produced exclusively by a cancer cell as a response to tumor development
  – Sensitivity
• Not exclusively by a cancer cell, but has a sufficient quantities to be distinguished from production by a normal tissue cell
  – Specificity

An Ideal Tumor Maker

• The quality should be included
  – High sensitivity
  – High specificity
  – Can be qualified
  – Safe
  – Convenience
  – Low price

• How to identify tumor marker?

• On cell
  – Cytochemistry, Flow cytometry
• On tissue
  – Histochemistry, Cytosol assays
• In body fluids
  – Blood, urine, CSF, Amniotic fluid
Assay technology

- **Monoclonal antibody (MoAb)**
  - Specificity
  - Mass production
- **Sandwich technique**
  - MoAbs1 + Tumor marker
  - MoAbs2 + Tracer

**Monoclonal Antibody**

- 1975, Kohler and Milstein reported the development of the hybridoma technology
- How to create the MoAb?
  - Antibody-forming B cell from an immunized animal (mouse, usually)
  - Tissue-culture adapted malignant plasma cell (myeloma)
  - Fused them in order to make hybrids that retain the properties of both the immunized specific antibody-forming and immortal myeloma fusion partner

**Sandwich Techniques**

- Tumor markers as an antigen between two MoAbs
- The first is bound onto a solid support (tubes or wells...)
- The second, unbound upon introduction into the assay carries the signal (tracer: radionuclide, enzyme, fluorochrome...)
Tumor marker in Oncology

• Screening
• Diagnosis
• Staging
• Prognosis

Screening

• Tumor markers play a limited role for tumor screening, just because….
  – relatively low sensitivity
  – lack of specificity and relation to tumor size
• Inappropriate for the detection of small in situ cancer
• In some cases, tumor markers can be equal to other examinations envisioned for screening
  – PSA & prostate cancer
  – calcitonin & medullary thyroid cancer

Diagnosis

• Tumor is not the key diagnostic examination, but can be a complementary sign to clinical finding or medical imaging
  – AFP alpha-fetoprotein & hepatoma
• Sometimes implicate the existence within the tumor of an exclusive secretary histological contingent
  – NSE & SCLC

Staging

• The tumor markers and medical imaging are complementary in the pre-therapeutic and post-therapeutic staging.
Prognosis

- The pre-therapeutic level of certain tumor marker can contributes a prognostic factor because of links with...
  - Metabolic activity
  - Tumor size
  - Invasion
- More valuable in that it is independent or other usual prognostic factors for the pathology
- Allow doctors to refine therapeutic strategy by selecting groups with risk of failure response to treatment
- This property is one of the major aspects of current use of the tumor marker
  - **CEA & colon cancer**
    The carcinoembryonic antigen (CEA) test measures the amount of this protein that may appear in the blood of some people who have certain kinds of cancers, especially cancer of the large intestine (colon and rectal cancer). It may also be present in people with cancer of the pancreas, breast, ovary, or lung.
  - **CA19-9 & pancreatic cancer**
  - **CYFRA 21-1 & lung squamous cell cancer**

**During treatment**

- High markers level before treatment generally
  - Not only correlate very well with the therapeutic result but are sometimes superior to this result in the assessment of complete remission
- The assay must be taking into account the marker half-life when during treatment and all post-therapeutic re-evaluation

**During monitoring**

- Contribute to a valuable mean and lead to suspicion for...
  - local or metastasis
  - curable recurrence
  - much earlier before clinical or radiological detection
- The protocol of the follow-up must be very strict
  - **CA15-3 measured every 3 months for 1 year and then every 6 months in breast cancer patient**

**CLASSIFICATION**

- **Carcino-embryonic protein**
  - AFP
  - CEA
- **Carcino- associated protein**
- **B2M** beta-2-microglobulin

- **PSA**
  - Prostate-specific antigen, or PSA, is a protein produced by cells of the prostate gland. The PSA test measures the level of PSA in a man's blood. For this test, a blood sample is sent to a laboratory for analysis. The results are usually reported as nanograms of PSA per milliliter (ng/mL) of blood.

- **TG**

**How is it used?**

The thyroglobulin test is primarily used as a tumor marker to evaluate the effectiveness of treatment for thyroid cancer and to monitor for recurrence. Not every thyroid cancer will produce thyroglobulin, but the most common types, the well-differentiated papillary and follicular thyroid cancers, frequently do, resulting in increased levels of thyroglobulin in the blood.

Thyroglobulin testing may be used, along with a TSH test, prior to thyroid cancer treatment to determine whether the cancer is producing thyroglobulin. If it is, then the test can be ordered at intervals after treatment to monitor for cancer recurrence. Several thyroglobulin levels may be ordered over a period of time (serial samples) to look at the change in concentration. The change often provides more information than a single value.

Thyroglobulin testing is also occasionally ordered to help determine the cause of hyperthyroidism and to monitor the effectiveness of treatment for conditions such as Graves disease. Rarely, the test may be ordered to help differentiate between subacute thyroiditis and thyrotoxicosis factitia and to determine the cause of congenital hypothyroidism in infants.

- **TPA** Tissue plasminogen activator (abbreviated tPA or PLAT) is a protein involved in the breakdown of blood clots. It is a serine protease (EC 3.4.21.68) found on endothelial cells, the cells that line the blood vessels.

- **Carbohydrate antigen**
  - CA125 – OVARIAN CANCER
  - CA19-9 – COLON CANCER
  - CA15-3 – BREAST CANCER
  - SCC

- **Hormone**
  - **CT**

  **Calcitonin is a hormone that is produced and released by the C-cells of the thyroid gland. Its biological function in humans is unclear.**
MARKERS PRODUCED BY VARIOUS TISSUES

**AFP Alpha-fetoprotein**

- **Origin**
  - From fetal GI tract, liver, yolk sac, kidney
- **Reference value**
  - 99% of general population
  - AFP < 10-20ng/ml
- **Indication**
  - HCC
    - Sensitivity between 30% to 80% according to stage and pathological status
    - Screening for cirrhotic patient: every 6 months
    - Highly suspicion is AFP > 200ng/ml
  - Non-seminomatous testicular germ cell tumors
    - Sensitivity between 60% to 80% in combination with free β-HCG
- **Follow-up**
  - Post-op assay: one month after operation
  - AFP decreased below normal value: complete remission
  - AFP increased: incomplete resection or recurrence
- **Neonatal neural tube defects**
  - AFP in amniotic fluid > 2.5 times of the normal value
- **Non-specific increases**
  - Rare
  - Acute and chronic hepatitis, cirrhosis
    - AFP seldom higher than 4 times of the normal value
  - Other metastatic carcinomas and multiple pregnancy

**CEA carcino-embryonic antigen**

- **Reference value:** CEA < 5 ng/ml
- **Indication:** Adenocarcinoma
  - Digestive tract carcinomas
    - In combination with CA19-9 assay
  - Colorectal cancer
    - Sensitivity varies according to the stage and differentiation of the tumor, between 25% to 80%
  - Pancreatic, small intestine and stomach
    - Level correlated with stage and differentiation of the tumor
  - Pre-op assay
    - Poor prognosis if CEA > 10 ng/ml
  - Post-op assay
    - 6 weeks after surgery, CEA within normal range means complete remission
Follow-up
  - Every three months
  - CEA increased > 50% means recurrence/metastasis
    Before clinical symptoms happened

Lung cancer
  - Adenocarcinoma
  - Non-specific increases: usually twice above normal range
    - Smoking: CEA positive in 4.5% cases
    - Benign digestive tract lesion: liver cirrhosis
    - Lung benign lesion: emphysema
    - Advance chronic renal failure

**B2M β2-microglobulin**

- Reference value: 1000-2400 ng/ml
- Increased B2M indicates increasing cell reproduction rate
  commonly in inflammation, autoimmune disease, lymphoma and
  viral infection

- The marker for write
  - Non-Hodgkin's lymphoma
  - AIDS
  - Lymphocytic leukemia
  - Viral hepatitis
  - Renal transplantation

**PSA prostate specific antigen**

- Reference value
  - < 50 yrs: PSA < 2.5 ng/ml
  - > 50 yrs: PSA < 5 ng/ml
  - fPSA/PSA > 0.19
- Indication: prostate cancer
  - Screening & diagnosis
    - DRE + PSA = 96% sensitivity
  - Follow-up
    - After operation: PSA should be undetectable within 6 wks
  - Clinical follow-up
    - PSA assay every 3 months
    - The re-increase of PSA reveals early diagnosis of
      recurrence/metastasis
- Non-specific increases
  - Acute pancreatitis
  - Prostate adenoma
1g of adenoma = 1/3 ng/ml of PSA
1g of cancer = 3 ng/ml of PSA

TG thyroid globulin

- **Reference value**
  - 90% general population < 25 ng/ml
- **Indication:** Differentiated thyroid cancer
  - Follow-up therapy
    - After operation, TG should be undetectable
    - High TG level indicates incomplete resection or metastasis
  - Clinical follow-up
    - Monthly assay for six months then every 3 months
    - TG > 5 ng/ml requires a complete survey looking for recurrence or metastasis
- **Non-specific increases**
  - Grave’s disease
  - Toxic nodular goiter or simple goiter
  - Acute or subacute thyroiditis

TPA tissue polypeptide antigen

- **Origin**
  - Exist in Endothelial cell covering in respiratory, digestive, reproductive system
  - One of the cyto-skeletal component
- **Reference range**
  - < 100 U/I
- **Indication:** Malignancy of fetus and placenta
  - Assay by IRMA
  - Nonspecific due to normally exist in blood stream and increases rapidly when large amount of cell reproduction happen
  - Diagnostic
    - Bladder cancer
  - Monitoring marker
    - Breast, digestive, lung and ovarian cancer
CA125 cancer-antigen 125

- Reference value
  - 95% general population < 35 U/ml
- Indication: ovarian cancer
  - High sensitivity to serous adenocarcinoma, lower to mucinous adenocarcinoma (associated with CEA and CA72-4)
  - Screening
    - not suggested for ovarian cancer but for ovarian tumor
  - Follow-up:
    - Post-op: tumor residues is good response to CA125
    - Second look surgery: CA125 increase means bulky peritoneal residues or metastasis, but normal CA125 does not exclude the second look surgery
    - Early detection of recurrence: increased more than 50% of CA125 level precedes the clinical diagnosis of recurrence
- Non-specific increases
  - Liver cirrhosis with ascites
  - Pleural effusion
  - Peritonitis and Pericarditis
  - During menstruation
  - Third trimester
  - Endometriosis
  - Ovarian cysts

CA15-3 cancer antigen 15-3

- Reference value
  - 98.7% general population < 30 U/ml
- Indication: breast cancer
  - Most specific tumor marker
  - At the time of suspected breast cancer
    - Unable to detect localized or metastatic breast cancer
  - Prognostic value
• **CA15-3 > 50 U/ml** = high suspicion of metastasis with poor prognosis
  - Follow-up: 6 weeks after surgery
  - Clinical follow-up
    • 3 yrs a year then every 6 months
    • > 50% of reference value predict recurrence or metastasis
    • The association of CA15-3 and CEA assays = increase sensitivity by 10%
    • Monthly assay during chemotherapy in metastasis stages
    • High correlation with the clinical response to treatment
  • Non-specific increases
    • Liver cirrhosis, acute hepatitis, severe chronic hepatitis (< 50 U/ml)
    • Other metastasis: pancreas, ovary, colorectal, lung, stomach and uterus = rarely > 50 U/ml except pancreas adenocarcinoma

**CA19-9 carbohydrate antigen 19-9**

• **Reference value**
  - 99.6% general population < 37 U/ml

• **Indication**
  - Digestive tract carcinoma
    • Pancreatic and biliary tract cancer: sensitivity 85%, specificity 95%
    • Colorectal cancer: associated with CEA
    • Gastric cancer: associated with CEA and CA72-4
  - Follow-up
    • Monthly assay during the first year, then every two months during two years, then every six months
    • CA19-9 > 1000 ng/ml indicates the metastasis
  - Remarks
    • Combination of CEA and CA19-9 increase the early diagnostic rate to 90% in patient with high risk with a mean lead time of 4-6 months before clinical response
    • No relation associated with tumor size
  • Non-specific increases: benign pathology
    • Lung: acute cystic fibrosis
    • Digestive tract:
      • 10% of cholecystitis and 8% of pancreatitis (< 3 times of normal value)
      • Liver cirrhosis
Other metastatic adenocarcinoma
- usually < 3 times of normal value

SCC squamous cell carcinoma associated antigen

- Known as TA-4 (SCC antigen)
- Origin
  - Separate and purify from cervical epithelial cell
- Reference value
  - < 1.5 ng/ml
- Indication: SCC, especial in cervical cancer
  - > 2.5 ng/ml in 53.6% of cervical cancer
  - Increase according to the disease progression and stage
- Follow-up
  - Should downhill to normal range within 72 hours after operation
  - Increasing persist indicating incomplete resection
- Remark
  - TA-4 in Lung SCC is 3-4 times to normal range, but is normal in other types of lung cancer
  - Helping tracing tumor and early diagnose in recurrence

CT Calcitonin

- Reference value
  - 99% general population < 10 ng/ml
- Indication: Medullary thyroid cancer
  - Screening and diagnosis
    - very sensitive in screening and early diagnosis in high risk group (familial and multiple endocrine neoplasia)
  - Follow-up
    - Therapy follow-up: repeat assay after operation, high level indicates incomplete resection or metastasis
    - Clinical follow-up: monthly assay, then every three months
- Non-specific increases
  - Neuroendocrine tumors: pheochromocytomas, carcinoid tumors
– Digestive tract and pancreatic endocrine tumors
– SCLC
– Differentiated thyroid cancer (< 5% of cases)
– Benign condition
  • CRF, hyperparathyroidism, paget’s bone disease

**Conclusion**

- The tumor markers contribute to cancer detection, diagnosis and prognosis is unquestionable, but they need to be estimated considerably
- The tumor markers in oncology should be used depending on knowledge and clinical experience

**CLINICAL APPLICATION**

- Tumor markers are assuming a growing role in all aspects of cancer care, starting from screening to follow-up after treatment, and their judicious application in clinical practice needs a thorough understanding of the basics of pathophysiology, techniques of identification or testing, reasons for out-of-range levels of tumor markers, as well as the knowledge of evidence of their role in any given malignancy.

- These are, at the most, just an adjunct to diagnosis, and establishing a diagnosis on the basis of tumor markers alone (especially a single result) is fraught with associated pitfalls because of the problem of nonspecificity.

- **In reality an ideal tumor marker does not exist.**

- **Detection can be done either in tissue or in body fluids like ascitic or pleural fluid or serum.**
• Clinical uses can be broadly classified into 4 groups: 

- screening and early detection,
- diagnostic confirmation,
- prognosis and prediction of therapeutic response and
- monitoring disease and recurrence.

• In addition to variable sensitivity and specificity, the prevalence of a particular malignancy may be a major determinant in the application of a particular test as a screening tool.

• Serum levels, in certain situations, can be used in staging, prognostication or prediction of response to therapy.

• Monitoring disease is, perhaps, the most common clinical use of serum tumor markers.