Tumor markers

ویژگی تومور مارکرها

اختصاصی باشند

توانایی تشخیص زود رس تومور را داشته باشد

تاریخچه

- بنس جونز 1847
- AFP 1963
- CEA 1965

آنزیم ها

- اولین تومور مارکرها
- اغلب فاقد ویژگی و حساسیت

ALP

- کبد
- متاستاز استخوان
- ایزوآنزیم جفتی در سرطان تخمدان، نای، دستگاه گوارش

LDH

- غیر اختصاصی
- سنجش ایزوآنزیم ها بیشتر بیانگر درگیری بافتها است

NSE

The glycolytic enzyme enolase (2-phospho-D-glycerate hydrolase, EC 4.2.1.11) exists as several dimeric isoenzymes ($\alpha\alpha$, $\alpha\beta$, $\alpha\gamma$, $\beta\beta$ and $\gamma\gamma$) composed of three distinct subunits α, β and γ. The γ unit is found either in a homologous $\gamma\gamma$- or in a heterologous $\alpha\gamma$-isoenzyme and is known as neuron-specific enolase (NSE). The monoclonal antibodies used in the CanAg NSE EIA bind to the γ-subunit of the enzyme and thereby detects both the $\gamma\gamma$ and the $\alpha\gamma$ forms (1, 2). The NSE levels are low in healthy subjects and subjects with benign diseases. Elevated levels are commonly found in patients with malignant tumours with neuroendocrine differentiation, especially small cell lung cancer (SCLC) (3) and neuroblastoma (4). Quantitative determination of NSE in serum may be valuable in the management of patients with suspected or diagnosed SCLC or neuroblastoma, to aid in the differential diagnosis and to monitor the effect of treatment (5, 6).

PAP

PSA

PSA is a 32 kDa single chain glycoprotein serine protease with a chymotrypsin like specificity produced by the secretory epithelium of the prostate gland (1). PSA is normally secreted into the seminal fluid and plays a functional role in the cleavage of the seminal vesicle proteins and the liquefaction of the seminal coagulum (2). Only low levels of PSA are normally present in the blood stream, and increasing serum concentrations indicate prostatic pathology, including benign prostatic hyperplasia and cancer of the prostate. Determination of PSA is now widely used for detection and management of patients with prostatic cancer and considered as the superior serological marker for cancer of the prostate (3, 8).

PSA has been shown to form stable complexes with different antiproteases and the dominating portion of PSA in patient serum occurs in complex with α_1-antichymotrypsin (PSA-ACT) (4). However there are large variations in the relation between free PSA and PSA-ACT complex between different individuals. Studies have also indicated that the proportion of free PSA is higher in benign prostatic disease as compared to prostatic cancer (5).

PSA

- total PSA = Complex PSA + free PSA
- Complex PSA= PSA-A_1CT & PSA-A_2MG
- Total PSA assay often fPSA+ PSA-ACT

PSA non prostatic

- NAFPSA
- High risk for Breast cancer= lower NAFPSA

Level of PSA and fPSA in BPH & PCa

Diagnosis (n)	FPSA/TPSA			FPSA/TPSA	
	Median	Min.	Max.	Mean	(95% confidence interval)
BPH (52)	0.18	0.04	0.42	0.19	(0.17–0.21)
PCa (77)	0.09	0.02	0.53	0.12	(0.10–0.14)

Sensitivity & specificity of PSA and fPSA

FPSA/TPSA cut-off	Clinical specificity (BPH > cut-off) n	%	(95% confidence interval)	Clinical sensitivity (PCa ≤ cut-off) n	%	(95% confidence interval)
0.23	14 (52)	27	(16–41)	69 (77)	90	(81–95)
0.16	36 (52)	69	(55–81)	64 (77)	83	(73–91)
0.08	48 (52)	92	(81–98)	30 (77)	39	(28–51)

آنتی ژن های جنینی

AFP

α-Fetoprotein (AFP), the foetal equivalent to albumin, is a 67 kDa glycoprotein produced during embryonic development and found in high concentrations in foetal serum and amniotic fluid. In normal non-pregnant adults AFP is present in low concentrations in serum. However AFP may be markedly increased in the serum from patients with cancer of the liver, testis or ovary. Quantitative determination of AFP in serum may be valuable in the management of patients with suspected or diagnosed liver cancer or germ cell tumours of the testis or ovary (1, 2).

CEA

Carcinoembryonic antigen (CEA) is a glycoprotein, which was first identified in patients with colonic carcinoma and in epithelial tumours of endodermal origin (gastrointestinal tract) by Gold and Freedman (1). The CEA molecule is quite heterogeneous due to the carbohydrate contents (50-60%) and depending on the purification procedure employed. It is soluble in perchloric acid and has a molecular weight of about 175.000–200.000 Daltons (2). Immunological and genetic characterization of CEA has identified a family of CEA-like molecules sharing common antigenic determinants. The most relevant CEA-like molecule is NCA (non-specific cross-reacting antigen) synthesized both by normal and pathological tissues. The problem of cross-reacting CEA-like molecules when assaying CEA is possible to overcome by the use of monoclonal antibodies.

CEA is secreted from tumour cells and is a widely used serological marker of gastrointestinal carcinomas, lung cancer and breast cancer. In colorectal cancer, the clinical use of CEA testing for monitoring response to therapy and for documenting progressive disease is well established (5, 6). CEA may also be present in benign gastrointestinal inflammatory diseases or in hepatobiliary diseases. These observations make it necessary to emphasize that the CEA assay should not be used as a cancer-screening test.

مارکرهای کربوهیدراتی

CA 15-3

The MUC-1 antigen is a membrane-anchored mucin-type glycoprotein present in malignant and normal epithelial cells of certain organs, e.g. breast, lung, ovary, pancreas and colon (1). The apoprotein of the MUC-1 mucin contains a transmembrane domain, a cytoplasmic domain, and an extracellular carbohydrate rich domain. The extracellular domain is characterized by polymorphism with respect to the number of a 20 amino acid tandem repeat (VNTR polymorphism).

The MUC-1 breast cancer mucin (CA15-3 antigen) is secreted from tumor cells and is a well-established serological marker for monitoring the clinical course of breast cancer patients (6).

CA 19-9

C192,
highly specific for the sialyl Lewis[a] epitope, also known as CA19-9 antigen (1). In adults, the epitope is typically expressed in trace amounts on mucosal cells of gastrointestinal epithelia. In patients with malignant disease, the epitope may appear associated with high molecular weight mucin in blood. Assays for CA19-9 are frequently used to monitor gastrointestinal malignancies such as pancreatic, gall bladder, gastric, and colorectal cancers (2, 3).

CA 125

CA125 is a high molecular weight mucin type glycoprotein, originally defined by the Oc125 monoclonal antibody (MAb) established by Bast et al. (1). Different epitopes, co-expressed with the Oc125 epitope on the CA125 antigen, have been used for the development of heterologous assays for determination of the CA125 antigen (2).

Assays for CA125 are frequently used to monitor patients with gynecological malignancies such as epithelial ovarian cancer (5).

CA 19-9

The tumor marker CA242 is defined by the monoclonal antibody C242. The chemical structure of the antigenic determinant is not exactly known, but the determinant have been shown to be a sialylated carbohydrate structure. In serum, CA242 is found on the same mucin-complex as CA50 and sialylated Lewis^a (CA19-9). Thus, CA242 is related, but not identical to the epitope of CA19-9 (1, 2). Serum levels of CA242 are low in healthy subjects and subjects with benign diseases, while elevated levels are commonly found in serum from patients with gastro-intestinal cancer (3).

The CA242 marker may be used as an aid in the diagnosis and management of patients with known or suspected gastro-intestinal carcinomas (4-9).

CA 242

The tumor marker CA242 is defined by the monoclonal antibody C242. The chemical structure of the antigenic determinant is not exactly known, but the determinant have been shown to be a sialylated carbohydrate structure. In serum, CA242 is found on the same mucin-complex as CA50 and sialylated Lewis^a (CA19-9). Thus, CA242 is related, but not identical to the epitope of CA19-9 (1, 2). Serum levels of CA242 are low in healthy subjects and subjects with benign diseases, while elevated levels are commonly found in serum from patients with gastro-intestinal cancer (3).

The CA242 marker may be used as an aid in the diagnosis and management of patients with known or suspected gastro-intestinal carcinomas (4-9).

CA 27-29

- Metastatic breast cancer

CA 549

- Active brest cancer

CA 72-4

- کارسینومای مجاری معدی-روده ای و تخمدان

CA 72-4

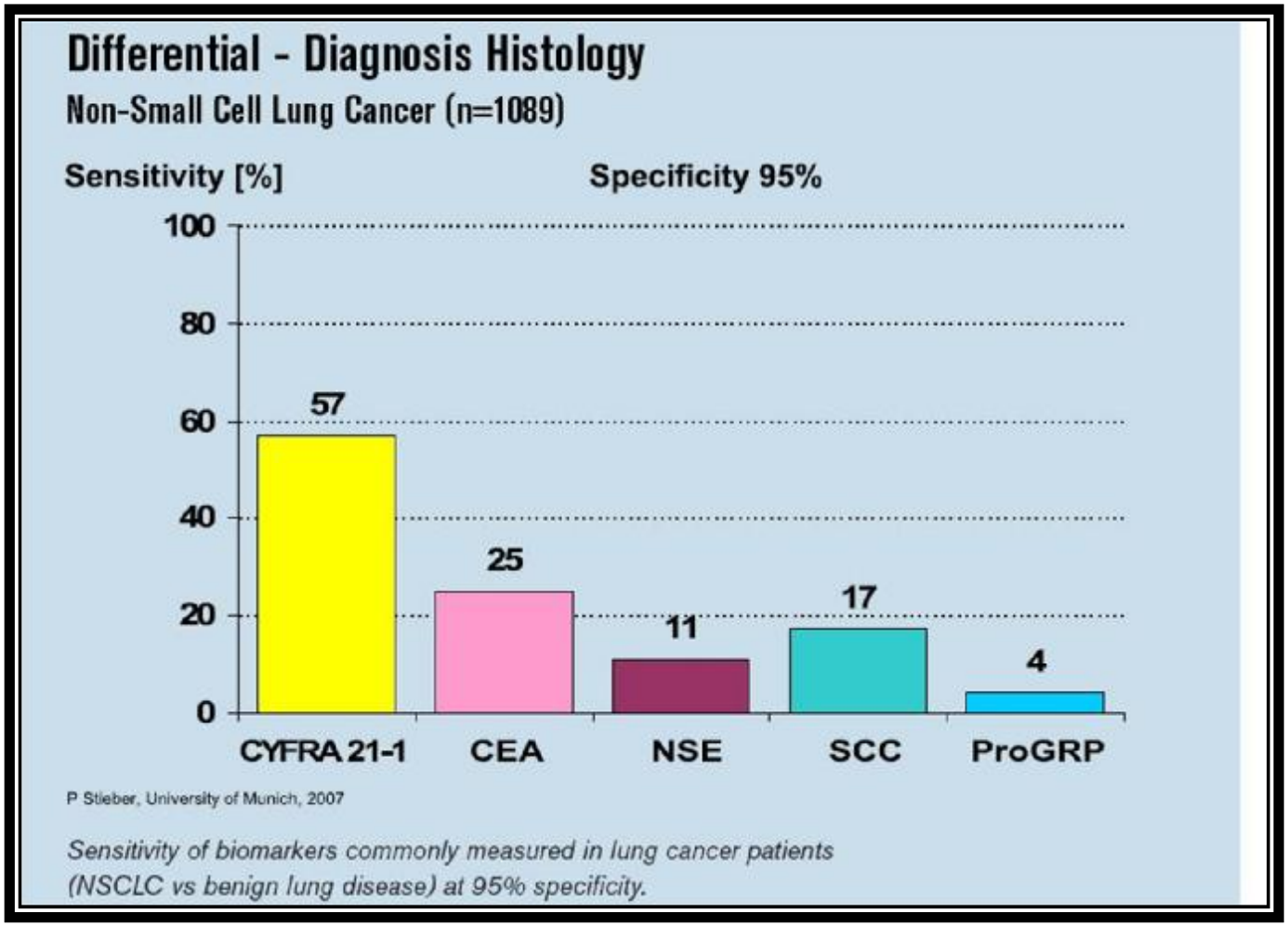
Hormones

سیتوکراتین ها

CYFRA 21-1

The CYFRA 21-1 EIA is a one-step, solid phase, quantitative assay for the measurement of soluble cytokeratin 19 fragments in serum. The measurement of CYFRA 21-1 levels may be useful in the monitoring and prognosis of non-small cell lung cancer. CYFRA 21-1 continues to be investigated in patients with bladder, head-& neck and breast cancer.

Lung cancer


Differential - Diagnosis Histology
Non-Small Cell Lung Cancer (n=1089)
Sensitivity [%]
Specificity 95%
100
80
60
40
20
0
57
25
11
17
4
CYFRA 21-1
CEA
NSE
SCC
ProGRP
P Stieber, University of Munich, 2007
Sensitivity of biomarkers commonly measured in lung cancer patients
(NSCLC vs benign lung disease) at 95% specificity.

SCC

Squamous cell carcinoma antigen (SCC ag) is a group of glycoproteins with molecular weight ~45 kDa, belonging to the family of serine/cysteine -protease inhibitors (1). The protein was originally isolated by Kato and co-workers from human squamous cell carcinoma tissue and shown to consist of at least 10 subfractions differing in isoelectric point (2). More recent studies have shown that SCC antigen is composed of two distinct but highly homologous gene products, SCCA1 and SCCA2 with different inhibitor specificities (3).

SCC antigen is a serological marker of squamous cell carcinomas of the uterine cervix, vulva, lung, head & neck, and oesophagus (4-6). In squamous cell carcinoma of the uterine cervix, pre-treatment serum SCC ag may be used as an early stage prognostic factor (7) and the use of pre-treatment SCC ag have been suggested in order to select high-risk patients for adjuvant therapy (4). Further, for patients with elevated levels of SCC ag before start of treatment, the profile of SCC ag correlates with the response to radio- and chemo-therapy and measurement of SCC ag may thus be used to monitor the effect of therapy and for early detection of recurrent disease (4).

Other tumor markers

HE4

Human epididymis protein 4 (HE4) belongs to the family of whey acidic four-disulfide core (WFDC) proteins with suspected trypsin inhibitor properties. Other proteins in this family include SLPI, Elafin, and PS20 (WFDC1) (1, 2). The HE4 gene codes for a 13kD protein, although in its mature glycosylated form the protein is approximately 20-25 kD, and consists of a single peptide containing two WFDC domains (3). HE4 was first identified in the epithelium of the distal epididymis and originally predicted to be a protease inhibitor involved in sperm maturation (4, 5). HE4 has since been reported to be expressed in several normal tissues including epithelia of respiratory and reproductive tissues and also in ovarian cancer tissue (6-10). In addition to expression on a cellular level, secreted HE4 has been detected in high levels in the serum of ovarian cancer patients. In a case/control study comparing patients with ovarian cancer to healthy and benign conditions, Hellström et al. found that HE4 detected ovarian cancer with 67% sensitivity at a specificity level of 96% (11). In a subsequent study evaluating numerous known biomarkers for ovarian cancer, HE4 showed the highest sensitivity for the detection of ovarian cancer, particularly in early stage disease. In this study, the combination of HE4 and CA 125 was a more accurate predictor of malignancy than either marker alone, with a sensitivity of 76% and a specificity of 95% (12).

Malignant mesothelioma

Malignant mesothelioma (MM) is a highly aggressive neoplasm with poor prognosis. It arises primarily from the surface serosal cells (mesothelial cells) of the pleura and, less commonly, of the pericardium or peritoneum. The estimated annual incidence is approximately 7 to 13 per million white males in the United States (U.S.). Epidemiologic studies have established that exposure to asbestos fibers is the primary cause of MM with as many as 80% of the mesothelioma patients having been exposed to asbestos.[1,2] Up to 8 million people in the U.S. have been occupationally exposed to asbestos over the last five decades during mining and milling of asbestos and in diverse manufacturing processes that use the material. Today, many public and private buildings contain asbestos, including 10% to 15% of schools in the U.S. Because of occupational asbestos exposure and the long latency period of 30 to 40 years, the current annual incidence of approximately 3000 new cases (U.S. only) is expected to increase by more than 50% in the coming decade, most of which will be associated with prior asbestos exposure.[3]

Although malignant mesothelioma remains a relatively uncommon malignancy, it continues to represent an important cause of mortality in numerous areas worldwide;

MESOMARK

MESOMARK measures soluble molecules that are related to the mesothelin/Megakaryocyte Potentiating Factor (MPF) family of proteins and recognized by the monoclonal antibody OV569.[11] The reactivity of OV569 is low for normal human tissues except for the mesothelium. Soluble members of the mesothelin/MPF family of proteins have been reported in the sera of patients with tumors of mesothelial origin.[11,12]

ProGRP

ProGRP is a stable precursor of the gut hormone GRP (Gastrin Releasing Peptide). GRP, originally isolated from porcine stomach, is secreted from Small Cell Lung Cancer (SCLC) cells. Although detection of serum GRP has been expected to be useful for diagnosis of SCLC, determination of serum GRP has not been feasible owing to its instability in blood. The ProGRP peptide however, is stable in serum and can be used as a serological marker for GRP. Serum levels of proGRP have been shown to be elevated in patients diagnosed with SCLC.

S100

S100 is a 20 kDa protein belonging to the S100/calmodulin/troponin C superfamily of EF-hand calcium-binding proteins. S100 was originally isolated from human brain and considered a glial-cell specific protein (1). Today, 20 monomers of the S100 family have been identified based on structural and functional similarities (2, 3). Most of the S100 proteins exist as dimers and are expressed in a cell-specific manner. Two of the S100 monomers, designated S100A1 and S100B (4) are highly conserved between species and are found as homo- (BB) and heterodimers (A1B) in central nervous system glial cells and in certain peripheral cells eg. Schwann cells, melanocytes, adipocytes, and chondrocytes (5). S100A1B and S100BB are also present in malignant tissues, most notably in melanoma and to a lesser extent in glioma, thyroid cell carcinoma and renal cell carcinoma (2).

Determination of S100B in serum has been shown to be clinically useful for prognosis and treatment monitoring of patients diagnosed with malignant melanoma (6-9). Studies also suggest that S100B may be useful in the management of patients with brain damage from eg. traumatic head injury, perinatal asphyxia, cardiac arrest, cardiac surgery and stroke (10-13).

Sample collection

Collect blood by venipuncture and separate the serum according to common procedures. Samples can be stored at 2–8° C for 2 days. For longer periods it is recommended to store the samples at –20°C or below. Avoid repeated freezing and thawing of the samples. Allow frozen samples to thaw slowly, preferably at 2–8° C over night and then bring the samples to room temperature before analysis.

International standard

The 1st International Standard IS 72/225 may be used as a reference standard. Values for AFP Calibrators and Controls were assigned against a set of in-house reference standards whose values are traceable to IS 72/225 using the conversion factor 0.83, i.e. 1µg/L corresponds to 0.83 kIU/L.

Detection limit

Detection limit

The detection limit of the CanAg AFP EIA is $\leq$ 0.5 µg/L defined as the concentration corresponding to the mean of the absorbance values of the AFP calibrator 0 plus 2 standard deviations according to formula:

$$\frac{2 \times SD\ CAL\ 0}{OD\ CAL\ 5 - OD\ CAL\ 0} \times 5\ \mu g/L$$

Hook effect

Hook effect

No hook effect has been noticed for samples up to 40 000 µg/L. However, since patients with advanced hepatocellular carcinoma may show extremely high levels, false low results due to a high dose hook effect may be seen in specimens from these patients. In order to avoid reporting misleadingly low results due to a hook effect at higher concentrations, particularly in patients for whom markers are being measured for the first time, or when very high AFP values may be expected, it is recommended to assay specimens at two dilutions (i.e. neat and diluted 1:100 with normal human serum).

Interference

	Concentration with no significant (± 10%) interference
Lipemia (Intralipid®)	10 mg/mL
Bilirubin, unconjugated	0.6 mg/mL
Hemoglobin	2 mg/mL