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TUMOUR MARKERS: RATIONALE USE

Mathias M. Müller, Marietta Vogl

Corresponding author's address:
Mathias M. Müller
Institute of Laboratory Diagnostics
Kaiser Franz Joseph Hospital
Kundratstrasse 3
A-1100 Vienna - Austria

7.1 Introduction

Tumour markers are substances associated with malignancy that can be measured in body fluids or tissues. They may reflect both the tumour burden and tumour biology. Tumour markers are molecules produced by cancer cells or are metabolic and immunologic products of healthy tissues, produced in response to the presence of cancer. Their concentration results from marker expression, synthesis, release, catabolism and blood-supply of the tumour. They can be identified in intact cells by immunohistopathology, flow cytometry, cancer genetics and molecular biology or they can be released into the circulation and measured with immunochemical techniques.

Tumour markers have a long history. In 1846, the first tumour marker was identified. It is the Bence-Jones protein, which is an immunoglobuline light chain produced in excess by about half of the patients with multiple myeloma. Since its identification, the hunt for the ideal tumour marker is going on. A high amount of different molecules and analysis linked with events associated with cancer emerged in the last decades: enzymes, isoenzymes, glycoproteins, glycolipids, amino acids, metabolites, hormones, differentiation antigens, oncofetal antigens, cytokeratins, transmembrane and nuclear receptors, adhesion molecules, cytokines, oncogenes, tumour suppressor genes, angiogenesis and metastasis factors as well as the analysis of markers of cell kinetics and DNA ploidy. The increasing understanding of the normal biological growth processes and their control mechanisms such as the cell cycle, angiogenesis and apoptosis, as well as the investigations of the mechanisms of tumour progression, invasiveness and metastasis will enlarge considerably the variety of markers and techniques used in this field.

According to European and national statistics app. 25 % of the mortality rates is due to cancer and malignancies. In females breast cancer is the most frequent, followed by colon carcinoma. In men cancer of the prostate is the most frequent, followed again by colon carcinoma. The incidence for the different kinds of cancer is very similar in women and men. Just the incidence for the cancer of the lung differs: 6.7 % in women and 15.4 % in men.

Table 1. History of tumour markers

Year	Author	Marker	Carcinoma
1846	H. Bence-Jones	BJ Protein	Multiple myeloma
1930	B. Zondek	hCG	Chorion carcinoma
1932	H. Cushing	ACTH	Lung, small cells
1959	C. Markert	AP,CK- isoenzymes	Ovaria, lung, colon
1963	G. I. Abelev	AFP	Hepatoma, testis
1965	P. Gold, S. Freeman	CEA	Colorectal
1969	R. Heubner	Oncogenes	Blood malignomes
1975	H. Kohler	Monoclonal Antibodies	Blood malignomes
1985	H. Harris	Suppressor Genes	Colon, breast

According to the structure and origin different types of tumour markers exist. Enzymes, hormones, oncofoetal antigens, glycoproteins, sialylated blood group antigens, proteins, oncogenes and suppressor genes.

7.2 Criteria for clinical usefulness

7.2.1 Selection criteria for tumour markers

There are two main points to be considered when measurements of tumour markers are performed:
- selection of the most appropriate marker
- time point of blood sampling

Based on clinical studies and on the collaboration between laboratorians and clinicians a kind of accepted list (Table 2) of markers to be used for management of cancer patients has been established. Most of these markers are useful for monitor the effect of therapy and for the detection of recurrence.

Table 2. Markers for different kind of cancers

Carcinoma/Malignoma	Marker of 1st Choice
Gastrointestinal	CEA, CA 19-9
Pancreas	CA 19-9
Liver	CA 19-9, AFP
Lung small cells Breast	Cyfra, SCC NSE CA 15-3, Estr-, Pr-Receptors
Ovar	CA 125
Uterus	SCC
Prostate	PSA, fPSA
Testis Seminoma	AFP, ß-HCG hPLAP, ß-HCG

It is general practice that the 1st measurement should be performed immediately after the diagnosis of the cancer. The 2nd measurement is usually performed one week after initiation of any kind of therapy as based on the biological half time (Table 3) of tumour markers. During the next year and without any clinical signs of relapse patients should be investigated for the specific tumour markers which had been occurred in the pre-treatment phase. Later on staying in remission every 3 months are recommended for follow-up. In case of signs of recurrence of the tumour shorter sampling times have to be considered.

Table 3. Biological half life of tumour markers

Marker	t/2 in Days
AFP	2 – 8
CA 125	5
CA 19-9	4 – 8
CA 15-3	5 – 7
CEA	2 – 8
CYFRA 21-1	1
HCG	1
NSE	1
PSA	2 - 4
SCC	1
TAG 72	3 – 7

7.2.2 Diagnostic Validities

An "ideal tumour marker" has to exhibit the following criteria derived from their clinical application:
- Specific: the capacity to recognize healthy people as healthy. This means no false positive results. This item will allow to screen a population.
Sensitive: the capacity to recognize a disease as a disease. This means no false negative result. This will allow diagnosis of cancer and monitoring patients under therapy.
Relative: a good correlation between tumour burden and/or malignant cell proliferation and tumour marker concentration should exist. This is essential for monitoring symptomatic patients.
- Effective: the measurement of tumour maker concentrations has to be reproducible over space and time at relative low costs.

Therefore, before using in the clinical arena a tumour marker a thorough review of evidence based literature has to be performed. For each of the markers the reference intervals for healthy individuals, for cancer patients in remission must be established in order to know the diagnostic validities such as sensitivity, specificity, positive and negative predictive values.

Since there is a lack of standardization for tumour marker assays, the diagnostic validities are dependent from the measurement procedure applied and cannot easily accepted from published data. It is strongly recommended that in collaboration with the clinicians diagnostic studies should be performed before introducing a tumour marker assay in a given environment. On basis of these studies the cut-off levels, decision levels and reference ranges can then be computed and decided upon. An essential part in the statistical treatment of data is the computing of "ROC-curves" taking clinical criteria into consideration for deciding on the best diagnostic validities. Figure 1 shows an example of a "ROC Curves" comparing the tumour markers CEA and CA 549 for the detection and the monitoring of breast cancer. The best sensitivities and specificities were achieved at a cut-off of 11,000 U/L and 5 µg/L for CA 549 and CEA respectively. Moreover, these curves show that CA 549 seems to be more useful than CEA.

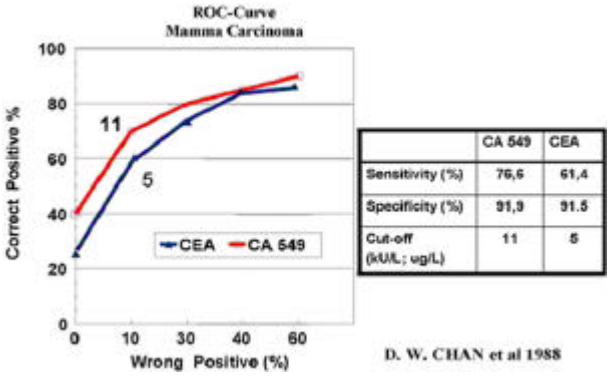


Figure 1. Diagnostic validities of CEA and CA 549 for mamma carcinoma

7.2.3 Screening for Prostate Carcinoma

In spite of great progress in research only AFP and PSA are useful for screening. PSA has been accepted for screening males between 50 and 70 years of age for prostate carcinoma. A few years ago, we screened in our hospital a collective comprised of 2840 patients without a known prostate disease by using PSA. The outcome of this screening is shown in figure 2. In summary in 1.37 % of the males aged 50 to 98 years an unknown carcinoma of the prostate gland was found. In patients with a PSA concentration > 4 ng/ml 10.1 % exhibited a cancer.

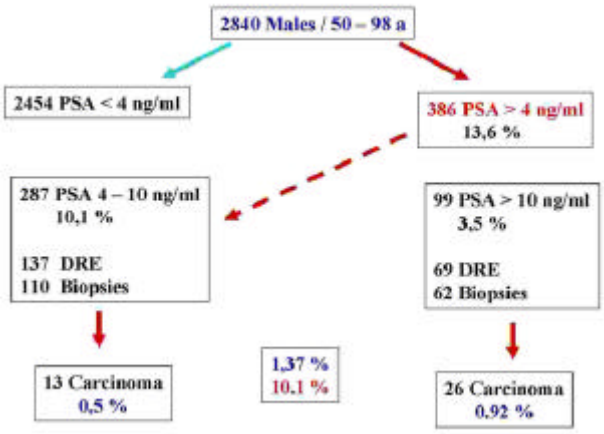
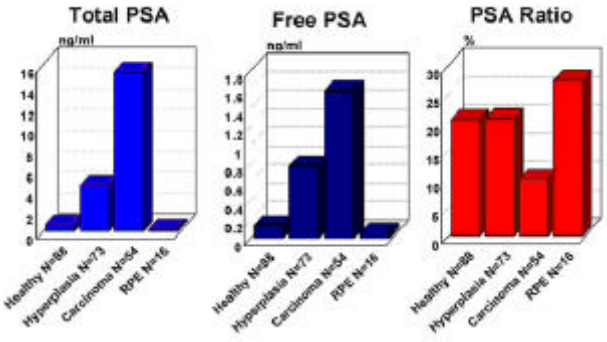


Figure 2. Screening for prostate carcinoma

The measurement of free PSA and the determination of the percentage FPSA (PSA ratio) is an additional helpful diagnostic tool to distinguish between cancer and benign disease of the prostate. The combination of total and free PSA measurements added to the DRE gives more safety to establish the correct diagnosis. Nevertheless, in some cases only the biopsy is able to conclude. In another study, we measured PSA and free PSA in 231 patients to prove the ability of free PSA and the PSA ratio to differentiate between malign and benign disease of the prostate. Healthy controls, males with prostate hyperplasia, with histological proofed prostate carcinoma and after radical prostatectomy were investigated. Results (medians) are shown in figure 3. In males with carcinoma the highest concentrations of total PSA and free PSA were measured. The lowest PSA ratio was computed. For differential diagnosis between hyperplasia and carcinoma the PSA ratio using a cut-off level of 0.21 (or 21 % free PSA) showed the best diagnostic validity. In contrast neither PSA nor free PSA contributed to a better discrimination.



For diagnosis and follow-up of males the combination of PSA and free PSA (ratio) are nowadays well-established diagnostic tools. In a thorough clinical investigation Finne P. et al. examined the usefulness of PSA, free PSA, DRE and the prostate volume for prostate carcinoma risk-estimation. The combination of laboratory and clinical investigations showed better results than using the PSA results alone.

7.2.4 Reference intervals

IOne of the biggest problems concerning tumour markers is the definition of reference intervals and defining cut-off levels for making decisions. For screening and diagnostic purpose the usual procedure to investigate a large group of healthy individuals seems to be sufficient. However, for follow-up of patients with malignancies individual reference intervals are much more appropriate. Even if the marker is below the “normal” or usual cut-off value, any increase must be interpreted as a possible relapse. In many cases, a lot of time can be gained this way. Of course this long-term observation implies that the measurement procedure applied is not changed.

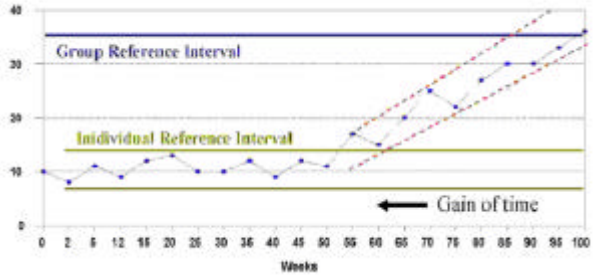


Figure 4. Reference intervals

In 1995, we conducted a study with 55 women who had been treated for a mammary carcinoma and who were followed-up and monitored for 4 years. During this period individual reference intervals during the relapse free period were established for CEA and CA 15-3. The diagnostic validities using individual reference intervals for metastases and for local relapse were computed; using this approach the specificities indicating a relapse free period were 97 and 99 %.

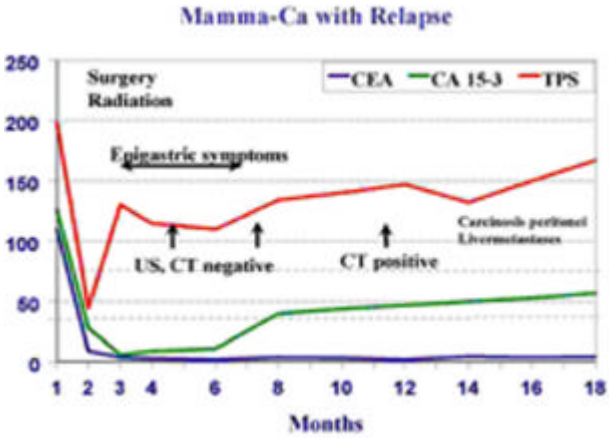
Table 4. Individual reference intervals for CEA and CA 15-3 in patients with mammary carcinoma

	CEA (ug/L)	CA 15-3 (kU/L)
Mean / Xi	1,07	20,9
SD / Xi	0,88	9,6
Range	0,1 – 3,93	7,1 – 46,8

Table 5. Diagnostic validities of CEA and CA 15-3 in patients with mammary carcinoma

Marker	Sensitivity (Metastases)	Sensitivity (Local Relapse)	Specificity
CEA	71 (10/14)	75 (3/8)	97
CA 15-3	86 (12/14)	38 (6/8)	99

In the following figures the follow-up of various patients suffering from mammary, rectum and lung carcinoma is shown. In all these cases the tumour markers investigated correlate well with the clinical course and the therapeutic concepts used. As can be seen an increase in serum marker concentrations occurred usually earlier than signs of relapse diagnosed by image techniques.



US - Ultrasound, CT - Computer tomography

Figure 5. Monitoring of mammary carcinoma (CEA, CA 16-3, TPS)

4 months after therapy, the markers CEA and CA 15-3 have nearly disappeared. Only TPS, a very unspecific marker for cell proliferation, began to rise. At the same time, the patient complained about epigastric distress. Sonography and CT were negative. CA 15-3 began to rise. 5 months later by means of CT liver metastases were detected. In this patient TPS was the right marker to indicate earlier than CA 15-3 the relapse of the malignancy.

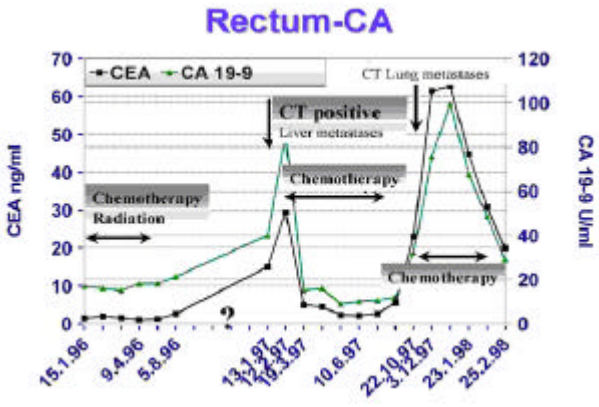


Figure 6. Monitoring of rectum carcinoma (CEA, CA 19-9)

This patient suffered on a carcinoma of the rectum staged as Duke C. This is an example for the good monitoring using CEA and CA 19-9. Chemotherapy is followed by a decrease of the two markers. The two marker peaks reflect the metastases detected by CT.

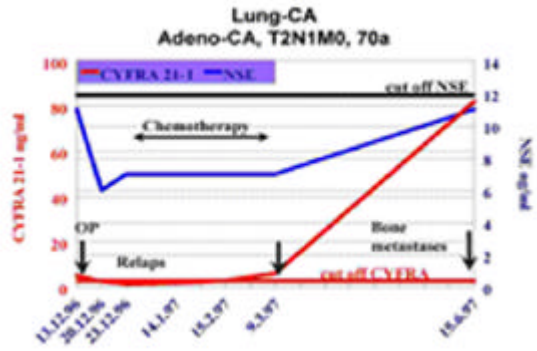


Figure 7. Monitoring of lung carcinoma (CYFRA, NSE)

This carcinoma was classified as an adeno-carcinoma of the lung. NSE and Cyfra 21-1 were measured. NSE has practically no sensitivity for this kind of carcinoma and therefore it makes no sense to measure it; it is an example of not rationale use. The increase of CYFRA 21-1 correlates with the appearance of bone metastases.

7.3 Cellular markers

As malignancy is essentially a disorder of growth control, any substance or process involved in the regulation of the cell cycle, apoptosis, angiogenesis and spread of metastases may become a useful tumour marker. The emerging tumour markers discovered during the elucidation of the human genome may revolutionize the management of cancer disease. In 1936 Casperson concluded that tumour cells contain increased amounts of nucleic acids as compared with normal cells by using quantitative image cytometry. Since then, cytometric analysis has a significant impact on our understanding of genetic changes in tumours. The technique of flow cytometry allows rapid measurements of physical and biochemical properties of cells. Cells were labeled with fluorescing compounds binding quantitatively to DNA. This technique allows quantifying DNA in tumour cells, which frequently show gains, or losses in genomic size. Usually fast proliferating malignant cells exhibit hyperploid DNA characteristics.

The functional status and the origin of cells in body fluids (liquor, ascites) can now easily be detected by means of flow cytometry in combing fluorescence conjugated monoclonal antibodies recognising specific surface cell markers with cell-cycle analysis after intracellular staining of DNA with probidium-iodide. With this approach an inflammatory leukocytosis in the spinal fluid can be accurately distinguished from a meningial carcinomatosis. The cells investigated in the liquor cerebrospinalis showed two features characteristic for malignant cells found in glioblastomas: surface expression pattern of the epithelial cells' CAM 5.2 epitope and 76 % of hyperploid DNA concomitant with an increased synthesis rate of 13 % (figure 8).

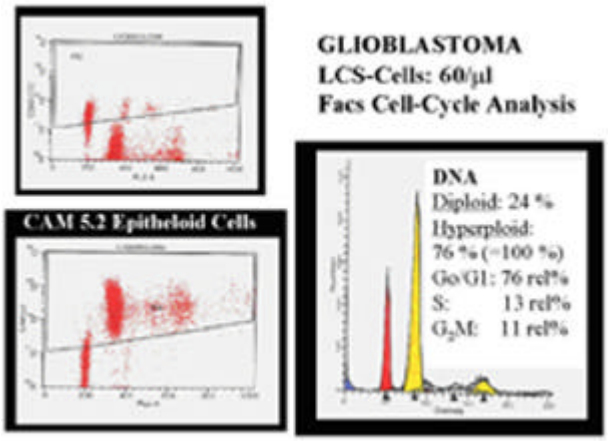


Figure 8. Flowcytometric investigation of meningial carcinomatosis

Tumour specific genetic material in blood, far from the tumour site, might be a useful diagnostic tool for diagnosis and prognosis. The wild type p53 protein seems to modulate cellular responses to cytotoxic stresses by contributing to both cell-cycle arrest and programmed cell death. Mutant p53 protein is associated with loss of this surveillance mechanism and therefore plays a role in the genesis of diverse types of tumours. Zhi-Ming Shao et al. performed a study to determine the presence of p53 mutations in the peripheral blood of breast cancer patients and its prognostic value in these patients. In breast cancer patients a mean concentrations of 211 ng/ml of plasma DNA were measured whereas in healthy controls only 21 ng/ml were detected. In patients exhibiting p53 mutations in their primary tumour 65 % also showed p53 mutations in their plasma DNA. Patients with tumour and plasma DNA p53 mutations had the worst prognosis with respect to recurrence and distant metastasis. Furthermore, p53 mutations in plasma DNA were strongly correlated with clinical stage, tumour size, lymph node metastasis and oestrogen receptor status.

7.4 Conclusion

In conclusion, we can say that at present, tumour markers are primarily used to monitor the success of therapy in cancer patients. The relatively poor sensitivities, the insufficient correlations with low tumour-burden as well as the missing organ specificity, in most cases do not allow screening or detection of high-risk patients. Exceptions are the PSA for the cancer of the prostate and the AFP and HCG for the cancer of testes. In the future, the emerging tumour markers might offer new strategies concerning screening, prognostic statements, early detection of relapse and new therapeutic options. Our success in treating cancer will depend on our ability to understand and control the regulatory events of the cellular growth mechanisms and interactions.

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