review

Tumor markers in clinical oncology

Srdjan Novaković

Department of Tumor Biology, Institute of Oncology, Ljubljana, Slovenia

The subtle differences between normal and tumor cells are exploited in the detection and treatment of cancer. These differences are designated as tumor markers and can be either qualitative or quantitative in their nature. That means that both the structures that are produced by tumor cells as well as the structures that are produced in excessive amounts by host tissues under the influence of tumor cells can function as tumor markers. Speaking in general, the tumor markers are the specific molecules appearing in the blood or tissues and the occurrence of which is associated with cancer.

According to their application, tumor markers can be roughly divided as markers in clinical oncology and markers in pathology. In this review, only tumor markers in clinical oncology are going to be discussed. Current tumor markers in clinical oncology include (i) oncofetal antigens, (ii) placental proteins, (iii) hormones, (iv) enzymes, (v) tumor-associated antigens, (vi) special serum proteins, (vii) catecholamine metabolites, and (viii) miscellaneous markers.

As to the literature, an ideal tumor marker should fulfil certain criteria - when using it as a test for detection of cancer disease: (1) positive results should occur in the early stages of the disease, (2) positive results should occur only in the patients with a specific type of malignancy, (3) positive results should occur in all patients with the same malignancy, (4) the measured values should correlate with the stage of the disease, (5) the measured values should correlate to the response to treatment, (6) the marker should be easy to measure. Most tumor markers available today meet several, but not all criteria. As a consequence of that, some criteria were chosen for the validation and proper selection of the most appropriate marker in a particular malignancy, and these are: (1) markers' sensitivity, (2) specificity, and (3) predictive values. Sensitivity expresses the mean probability of determining an elevated tumor marker level (over the "cut-off value") in a tumor-bearing patient. Specificity expresses the mean probability that a normal tumor marker value derives from a tumor-free individual. The predictive value shows the applicability of a tumor marker in a mixed group of patients.

Many theoretical applications exist for tumor markers in clinical oncology. Clinically important utilization of markers includes (i) early detection of the tumor, (ii) differentiating benign from malignant conditions, (iii) evaluating the extent of the disease, (iv) monitoring the response of the tumor to therapy, and (v) predicting or detecting the recurrence of the tumor. Since no ideal tumor markers with adequate sensitivity and specificity currently exist, they are only exceptionally used in screening (prostate specific antigen - PSA). Nevertheless, tumor markers can play a crucial role in the detection of an early disease relapse and assessment of response to therapy in selected groups of patients. In monitoring the patients for disease recurrence, tumor marker levels should be determined only when meaningful treatment is possible.

Key words: neoplasms-diagnosis; tumor markers, biological

What exactly are tumor markers?

The result of malignant transformation is a malignant cell that, in each cycle of cell division, generates a new malignant cell. In this process, the malignantly transformed cells acquire some new properties through which they differ from nonmalignant cells of the same origin. The acquired properties can be either the changes in cellular morphology, physiology, or the changes in cell growth (behavior).[1] These subtle differences between normal and malignant cells are therefore being exploited in the detection of malignant cells (or malignancy in general), and the substances that are being determined in this process are termed tumor markers.

According to the fact that the differences between tumor cells and normal ones appear on various levels (see above), also tumor markers differ one from another and represent a rather broad conception that comprises both various substances and various cellular processes. Consequently, membrane antigens, hormones, enzymes, polyamines, nucleosides, products of oncogenes, products of tumor suppressor genes, or DNA ploidy and proportion of cells in S phase of the cell cycle (proliferative activity) can be considered as tumor markers.[2,3] Different fields in oncology utilize different tumor markers according to their needs and their techniques of follow-up: tumor markers in clinical oncology differ from the ones in molecular biology, and these differ from the ones in immunohistochemistry, in physiology, etc.

Table 1. Utilization of tumor markers in oncology

I) FOR FOLLOW-UP OF THE DISEASE

   1) determination in body fluids
      a) to monitor the treatment response
      b) to detect early the disease recurrence
      c) to evaluate the extent of disease
      d) to differentiate benign from malignant conditions
      e) as screening method for some types of cancer

   2) immunoscintigraphy and lymphoscintigraphy

   3) immunohistochemistry
      a) to set the diagnosis
      b) to determine the prognosis
      c) to predict the treatment response

II) FOR TREATMENT

   1) direct cytotoxicity of specific monoclonal antibodies (MoAb)
      a) binding of complement to specific MoAb
      b) binding of cytotoxic cells to specific markers-receptors

   2) binding of drugs to specific MoAb

   3) binding of toxins to specific MoAb

   4) binding of radioactive isotopes to specific MoAb

   5) inhibition of growth factor receptors

The application of tumor markers

Theoretically, the possibilities for the application of tumor markers in oncology are numerous, but the utilization depends upon the sensitivity and specificity of the marker and upon reliability of other methods that are being used for the same purpose (Table 1).

Tumor markers in clinical oncology

The standard definition of tumor markers in clinical oncology comprises predominately the substances that are produced by malignant cells or the substances that are produced by other cells under the influence of malignant cells and that can be determined in body fluids. Tumor markers can be either newly synthesized substances (that are not commonly produced by normal healthy cells) or the substances that can be found in normal organisms in much lower concentrations.[2] The determination of tumor markers in clinical oncology is helpful in many processes: in the process of diagnose setting and prognose prediction, in determining the disease extent and planning the treatment, as well as in the early detection of disease recurrence or metastasis. However, the markers in clinical oncology are nowadays, due to their incompetences, only exceptionally applied as screening methods for the detection of malignant diseases.

Different markers are used for different purposes – namely, some of them are more appropriate for the follow-up of the disease and the others for the early detection of the disease recurrence.[3-5] In addition to the above applications, tumor markers can also serve as predictors of a disease outcome. The follow-up of malignant disease before, during and after treatment, and careful processing of the data, namely, gives us a valuable information about the nature of malignancy, and thus also about the patient's prognosis. In general, extremely high concentrations of tumor markers are predictors of poor outcome.[6]

Determination of tumor marker in patient's sera

In body fluids, tumor markers are found in low concentrations and for their determination highly sensitive technology is needed. The techniques that are being used are more or less based on the same principle – *i.e.* the determination of antigen-antibody complexes. Most widely used techniques are the radio-immune assay, the enzyme-immune assay, and the luminometric-immune assay, which differ in the compound bound to the detection antibodies, and in the method of detection of the formed complexes.[7]

Sensitivity and specificity of tumor markers

The ideal tumor marker should be (i) present only in tumor cells, (ii) specific for the organ and type of tumor, (iii) assessable in the sera of all patients with the same type of tumor, (iv) assessable in the serum at the very beginning of tumor development and its concentrations in the serum should correlate with the tumor burden. Besides, the serum concentrations of the marker should be regarded as a valid predictor of disease in patients with the specific type of tumor.[2]

Up till now, no antigenic structure is known that would be present only in tumor cells – and that means that the antibodies against certain tumor markers crossreact also with other antigenic structures. We can therefore conclude that no tumor marker and no methods of following up the presence of malignant cells are 100% specific. When assessing these results, we have to bear in mind that not only a malignant disease causes elevated levels of tumor markers, but that there are also other factors that affect their concentration. The most com-

mon incompetences of tumor markers are (i) inadequate specificity for the type of malignancy, (ii) production of markers in high concentrations in nonmalignant diseases (different inflammatory processes, benign tumors, nonmalignant diseases of the liver and pancreas), (iii) production of markers in different physiological conditions (pregnancy, menstruation, lactation), and (iv) production in perfectly healthy tissues.[2,3,7]

To determine as accurately as possible the role and applicability of a specific tumor marker and the method in a specific type of malignancy, some new terms were introduced – including the sensitivity and specificity. The sensitivity of a marker reports the proportion of patients bearing a specific type of tumor in which the serum (urine, plasma, cerebrospinal liquor) level of marker is elevated. The more patients with the same type of tumor have an elevated level, the more sensitive is the marker, and the lower is the expected number of false negative determinations. The specificity of a marker represents the proportion of patients who do not have a certain type of malignancy and in whom the marker level is normal. That means that the lower is the number of patients not bearing a certain type of tumor and having an elevated marker level, the higher is the specificity, and the lower is the expected number of false negative determinations.

In case there are more tumor markers elevated in one type of malignancy, it is possible to increase the sensitivity of detection by combining these markers' determinations, but we have to bear in mind that, in this way, the specificity of detection is decreased. By combining properly the marker determinations, the specificity of detection can be only slightly lowered, while the sensitivity is significantly increased. The prerequisite for a proper combination of different markers is their high specificity and complementarity for the type of tumor. An example of such proper combination is the determination of

ßHCG (human chorionic-gonadotropin) and AFP (alpha fetoprotein) in non-seminoma germ tumors. Each of the above-mentioned markers has a specificity of more than 90% and the sensitivity of approximately 60% for this type of tumors. Due to their complementarity (meaning that they are elevated in different patients), the sensitivity of the combination of the two markers reaches approximately 95%.[8-10]

Classification of tumor markers

Tumor markers can be classified in several ways: according to their chemical structure, their tissue of origin, types of malignancies in which they are elevated, etc. The most common classification tries to combine their biochemical properties, tissue of origin, and functionality. According to this classification, we distinguish: oncofetal proteins, hormones and/or carcinoplacental antigens, enzymes, tumor-associated antigens, special serum proteins, and miscellaneous markers.[3,11]

Oncofetal proteins

Oncofetal proteins are antigens that are normally produced during the embrional development. In adults, their production is limited or completely absent (stopped). Elevated concentrations in adults result from reactivation of certain genes that control cellular growth and are directly connected to malignant process.

Carcinoembryonic antigen (CEA) is a typical representative of this group and one of the first known tumor markers. During embrional development, it is produced in epithelial cells of the gastrointestinal tract, liver, and pancreas. CEA is important for the follow-up of patients with colorectal cancer because 65% of all patients (including those with localized disease and stage I), and as much as 100% of patients with metastatic disease have elevated serum levels of CEA.[2,4]

Besides, this marker is convenient also for the follow-up of patients with other malignancies – especially breast, ovarian, pancreatic, lung, liver, and endometrial cancer.[12,13] Serum concentrations between 4 - 10 ng/ml can be found either in the patients with malignant or in the patients with benign diseases, and even in some heavy smokers, while concentrations above 10 ng/ml speak more in favor of a malignancy.[14] Elevated serum concentrations can be found also in the patients with bronchitis, gastritis, duodenal ulcer, liver diseases, pancreatitis, colorectal polyposis, etc.[14]

Alpha-fetoprotein (AFP) is known for approximately as long as CEA. It was discovered in 1963 in the sera of mice with hepatocellular carcinoma.[15] AFP is a glycoprotein produced in yolk sac, in the epithelial cells of the gastrointestinal tract and liver during embrional development.[16] In pregnancy, AFP enters amniotic fluid through fetal blood, and passes the placenta, thus going into maternal blood. In healthy adults, AFP can be found in the blood in very minute concentrations. Normal serum concentrations appear approximately 9 months after birth. Elevated serum AFP levels (above 10 ng/ml) in adults can be found in the patients with acute viral hepatitis, liver cirrhosis, obstructive icterus, and in some malignant diseases, as pancreatic cancer, lung cancer or gastric cancer. The main role of AFP is to follow up the patients with hepatocellular carcinoma (95 to 100% specificity and sensitivity) in whom the concentrations above 1200 ng/ml practically confirm the diagnosis of primary liver (hepatocellular) cancer, and the patients with non-seminoma germ tumors (specificity 60%).[16,17]

Hormones

Malignant formations can alter the synthesis and secretion of various hormones. Quantitative and qualitative alterations of the synthesis and hormone secretion can there-fore be the indicators of a malignant process and can be monitored as tumor markers. Quantitative alterations occur when tumors develop in the tissue of endocrine glands, thus influencing the normal production of hormones by either increasing or decreasing it. This group comprises hormones of malignant endocrine tumors as *parathyroid hormone, insulin, prolactin, catecholamines* and others. Qualitative changes take place when malignantly transformed cells of some organs (lungs, breast, stomach, central nervous system, ovaries) start producing hormones – i.e. the so called ectopic hormone production (e.g. *calcitonin* and *parathyroid hormone* in breast cancer, *lipotropin* in carcinoid tumors, *calcitonin, insulin, parathyroid hormone* in thymic malignomas).[2,3]

Among all hormones, *human chorionic gonadotropin (ßHCG)* is one of the most applicable tumor markers. This is a protein with the molecular mass of 45 KD. It belongs to the group of carcinoplacental antigens – proteins that are synthesized in placenta during pregnancy (most early pregnancy tests are based on the detection of ßHCG) and can be found in adults only exceptionally.[18] Elevated serum concentrations of ßHCG can be found in almost all female patients with germ tumors with trophoblastic component (choriocarcinomas), hydatidiform moles, and in the majority of male patients with germ tumors. ßHCG has a very short serum half-life (36-48 hours) and is therefore functional to follow up treatment response, as well as to predict prognosis. In combination with AFP, ßHCG is an excellent marker for monitoring the patients with germ tumors. On the other hand, approximately 10% of germ tumors do not synthesize tumor markers; hence, they cannot be used for diagnostic purposes and in the follow-up of these patients. Slightly elevated levels of ßHCG can be found also in the patients with breast, gastric, lung, liver, or colorectal cancer, but are irrelevant in clinical monitoring of these patients.[19]

Enzymes

Certain enzymes that are produced more intensely if a malignant process is occurring in the organism can also be used as tumor markers.

Prostatic acid phosphatase is an enzyme that is produced in normal prostatic tissue. Elevated serum concentrations (above 3 ng/ml) can be observed in the patients with prostatic cancer and usually correlate with an advanced phase of the disease when the tumor penetrates the prostatic capsule. The determination of prostatic acid phosphatase is therefore convenient for the discrimination of benign (hypertrophy) from malignant processes.[20]

Alkaline phosphatase exists in the form of iso-enzymes that are synthesized in the liver, bones or placenta. Elevated serum concentrations in patients with malignant disease usually indicate a metastatic spread of the disease into the liver and/or bones, and/or the presence of primary bone tumors (osteosarcoma).[20]

Neuron specific enolase (NSE) is a cytoplasmic glycolytic enzyme that was primarily detected in the cells of neuroectodermal origin and in neuronal cells. The latter were proved to be in the tumor tissue of the tumors with neuroectodermal or neuroendocrine differentiation.[21-23]

Among other (nonspecific) markers in this group, we should not leave out *lactic dehydrogenase (LDH)* that is quite often elevated in the sera of patients with malignant lymphomas and germ tumors (seminomas), *γ glutamyl transpeptidase (GGT)* that indicates cholestasis (often elevated because of liver metastasis), and *thymidine kinase (TK)* that helps to evaluate the disease spread in the patients with leukemia, lymphoma, brain tumors, small cell lung cancer, and breast cancer.

Tumor-associated antigens

This is a heterogeneous group of markers that comprises various membrane structures of tumor cells. Updated technology exploits the possibility of producing specific monoclonal antibodies against certain antigenic structure that is most characteristic for the type of tumor cells. Therefore, the markers in this group are more specific for the type of malignancy than the others and quite often their serum concentrations reflect more accurately the growth or regression of the tumor mass.

Carcinomic antigen 15-3 (CA 15-3) is produced in the secretory epithelium (of the breast, lungs, gastrointestinal tract, uterus, etc.) and can be found frequently in the excretions of healthy adults. Elevated serum concentrations of this marker (above 30 U/ml) are detected predominately in the patients with breast cancer; however, raised levels of CA 15-3 can be observed also in other malignancies as lung, prostatic, ovarian, cervical, and gastrointestinal cancer.[13, 24] Hence, CA 15-3 is no specific marker either for the organ or for the type of tumor. Despite that, CA 15-3 is a good indicator of treatment response and disease course in breast cancer patients (whose tumors produce that antigen). The serum level of CA 15-3 can be influenced also by different non-tumoral diseases of the breast, by benign breast tumors, but the values rarely exceed 40 U/ml. Raised concentrations can be observed in approximately 8% of pregnant women between 38th and 40th week of gestation.[3]

Concomitant determination of CA 15-3 and CEA in breast cancer patients increases the sensitivity (reduces the number of false negative determinations), while retaining a substantial specificity of the procedure (rather low number of false positive determinations).

Carcinomic antigen 125 (CA 125) is a characteristic marker for ovarian cancer (it is elevated in more than 80% of patients with non-mucinous ovarian cancer). During embrional development, CA 125 is produced in celomic epithelium, Muellerian ducts, epithelial cells of the pleura, pericardium, and peritoneum.[25,26] In adults, CA 125 can be found

in mucosa of the cervix uteri and in the lung parenchyma, however, it is not produced by healthy ovarian tissue. Elevated concentrations of CA 125 (above 35 U/ml) can be found not only in the patients with ovarian cancer but also in the patients with benign or malignant gynecological diseases (endometriosis, ovarian cysts, endometrial cancer, cervical cancer) as well as in the patients with non-gynecological malignancies (lung cancer, prostatic cancer, peritoneal malignant mesothelioma). In the group of patients with ovarian cancer, CA 125 is most reliable and applicable in the follow-up of patients with epithelial and undifferentiated ovarian cancer.[27]

Carcinomic antigen 19-9 (CA19-9) is a glycolipid and actually represents a modified Lewis's hapten from the blood group system. CA 19-9 is frequently elevated in the serum of patients with gastrointestinal tumors. The marker is slightly more specific for pancreatic and liver cancer, yet quite often raised concentrations can be found in patients with colorectal, gastric, and ovarian cancer. In relatively high concentrations, it can be detected in healthy adults in the prostatic fluid, gastric fluid, amniotic fluid, and in the excretions of the pancreas and duodenum. Therefore, only determinations in the serum or plasma are rational because there the concentrations will be elevated only in case of disease.[28]

Prostate specific antigen (PSA) is a serine protease first isolated from the tissue extract of the prostate and sperm. It is produced in the prostate tissue and excreted in the prostate fluid where it can have very high concentrations. The role of this serine protease is to prevent coagulation of sperm. In healthy persons, very minute amounts of PSA enter the bloodstream so that its concentration in serum is rather low. In the patients with prostatic disease, the amounts of PSA that enter bloodstream increase significantly (especially in case of prostatic malignancy), thus generating high serum concentrations. This marker is substantially specific for prostatic cancer and

its serum concentrations reflect very well the tumor burden. Due to its high sensitivity and extraordinary correlation with tumor burden we prefer applying PSA to prostatic acid phosphatase in the follow-up of prostatic cancer patients.[20,29] Besides, the method (together with other procedures) is being utilized in the screening of prostatic cancer in the group of males over 50 years old who have more risk factors (e.g. prostatic cancer in patient's father, breast cancer in patient's mother or sister, obesity, prostatic cancer in more than one generation). With the determination of different forms of PSA (*i.e.* total, bound or free), and with a proper evaluation of free to total PSA ratio, it is possible to predict quite confidently if the patient suffers from a benign or malignant prostatic disease.[30]

Special serum proteins

This group comprises various proteins. One of the best known is *feritin* that binds iron intracellularly and is responsible for detoxication (e.g. binding of free radicals). Under normal circumstances, high concentrations of feritin can be found in the liver, spleen, and bone marrow. Normal serum level of feritin ranges from 8 to 440 ng/ml. Raised concentrations can be observed in the patients with acute leukemia, lymphomas (especially Hodgkin's lymphomas), lung, liver, and prostatic cancer.[2]

Thyroglobulin is an intracellular glycoprotein responsible for the production and storage of thyroxine. In low concentrations, it can be found in the sera of most healthy persons (0-75 ng/ml), while extremely high concentrations can be detected in the patients with well differentiated follicular (rarely anaplastic) thyroid carcinoma. Conversely, in the patients with medullary thyroid carcinoma, the serum levels of thyroglobulin do not follow the development and course of malignancy.[2,3]

Beta-2-microglobulin is a protein that is identical with the HLA light chain and thus appears

on the cell membrane of almost all differentiated cells. Raised serum concentrations can be observed in the patients with lung, breast, pancreatic, colorectal cancer, as well as lymphomas and chronic lymphoid leukemia (CLL).[2,3]

S-100 protein was first isolated from bovine brain. Normal serum concentration of this marker is below 0.3 ng/ml. In addition to being a good indicator of traumas to CNS, S-100 can be applied as tumor marker in the patients with neurinoma, glioblastoma, astrocytoma, and meningeoma. It has a special role as prognostic factor and in the follow-up of the patients with malignant melanoma (<0.3 ng/ml - 85% 3 years' survival; 0.3-0.6 ng/ml - 50% 3 years' survival; >0.6 ng/ml - 10% 3 years' survival).[31,32]

Miscellaneous markers

This group involves markers the production of which correlates perfectly with the changes in velocity of cellular proliferation. It is a heterogeneous group of substances which are not specific for the type of tumor but generally indicate the presence of a malignant process. The group comprises polyamines, nucleosides and tissue polypeptide antigen (TPA).

Polyamines like spermine, spermidine and putrescine were detected in elevated concentrations in urine in cases of a rapid regeneration of cells of certain tissue.

Nucleosides as dimethylguanosine and pseudouridine are components of RNA that (like polyamines) enter the circulation in larger amounts in cases of enhanced cellular proliferation.

Tissue polypeptide antigen (TPA) is likewise a nonspecific marker of enhanced cellular proliferation. The molecular mass of this polypeptide is approximately 180 KD and the molecule is composed of different cytokeratin units – *i.e.* of cytokeratin 19 (44%), cytokeratin 18 (36%), and cytokeratin 8 (30%). During embrional development, it is produced in various embrional tissues as well as in the placenta. In adults, TPA is a part of cellular membranes (cytoskeleton) of normal and tumor cells. It is synthesized during S phase of the cell cycle and its concentrations reflect the velocity of cellular proliferation. A more rapid cellular proliferation demands a more rapid synthesis of TPA, and consequently, larger amounts of this polypeptide enter the circulation. Therefore, TPA is a common (universal) marker that goes together with pathological cellular proliferation (that is usually present in malignant transformation) regardless of the type or localization of the tissue. Unlike in other markers, the serum concentrations of TPA poorly reflect the tumor burden. Normal serum concentrations of TPA are below 90 U/ml and concentrations higher than 120 U/ml can be either a consequence of a malignant or of a benign process.[2]

Biological factors that affect serum concentrations of tumor markers

Regarding the facts, that no tumor marker is ideal and that the substances that are being applied as tumor markers are not synthesized exclusively as a consequence of malignancy, I present some of the most common factors that affect serum concentrations of tumor markers (Table 2).

When to determine tumor markers?

Tumor markers are determined in such a way that we gain as much as possible of clinically useful data. We have to prepare an approximate concept that includes the determination of markers (i) prior to surgery or any kind of treatment (chemotherapy, radiotherapy, biological therapy, or hormonal therapy); (ii) after the surgery, during the treatment, and after the treatment once in 3 to 6 months' period during the first and second year, then once yearly or at regular controls; (iii) in case of sus-

Table 2. Factors (in addition to malignant disease) that affect serum concentrations of tumor markers

False positive results

- ➢ presence of inflammatory processes;
- ➢ benign liver diseases and consequential disturbancies in metabolism and excretion (AFP, TPA, CEA, CA 19-9, CA 15-3);
- ➢ disturbancies of renal function (beta-2-microglobulin, calcitonin, PSA, CEA, CA 19-9, CA 15-3);
- ➢ extensive tumor necrosis;
- ➢ as a consequence of diagnostic and therapeutic procedures (digitorectal examination, mamography, surgery, radio and chemotherapy);
- ➢ as a consequence of different physiological conditions (pregnancy - ßHCG, CA 125, CA 15-3, MCA, AFP, menstrual cycle - CA 125).

False negative results

- ➢ complete absence of production (e.g. CA 19-9 in Le[(a-b-)] persons);
- ➢ insufficient expression of a certain antigenic determinant (or production in only some of tumor cells);
- ➢ insufficient blood circulation in the tumor;
- ➢ production of immune complexes with autoantibodies;
- ➢ rapid degradation and clearance of antigens;

pected relapse or disease progression; (iv) prior to introduction of a novel treatment; (v) at least 3 weeks after the introduction of a novel treatment; (vi) 2 to 3 weeks after the determination of raised concentrations of the marker.

The proposed concept of tumor marker determinations is applicable in the majority of tumor markers. Certainly, the demands in clinical oncology are quite often different and we have to adjust the dynamics of determinations in accordance with the type and properties of the tumor, and with the planned methods of treatment. In other words, the above mentioned concept represents only an approximate model that has to be further modified in each patient specifically regarding the fact that each patient is a control to himself (monitoring of the dynamics of serum concentrations).

Conclusion

Determination of tumor markers for monitoring the course of malignant disease is an established and often an irreplaceable oncological laboratory method. Tumor markers

are reliable predominately in monitoring the treatment response, as well as in early detection of disease recurrence (prior to development of clinically notable signs). Due to their incompetences, tumor markers' determinations can be only exceptionally applied as screening methods or as the sole diagnostic tool; however, in combination with other diagnostic methods, they play an important role in the diagnostic process and in treatment planning. Besides, by combining various tumor markers we can achieve a greater specificity and sensitivity in the follow up of one type of malignancy. The simplicity and noninvasiveness of the method for the determination of tumor markers enable monitoring the disease also in the patients not eligible for other types of diagnostic procedures.

On account of individual differences in the serum concentrations of each individual tumor marker, we recommend multiple determinations of tumor markers and monitoring the dynamics of serum concentrations (even in cases when serum concentrations are below the cutoff values). It would be ideal to determine the level of tumor markers in each patient before

treatment, several times between the treatment (depending upon the type of treatment, type of malignancy, and the sort of tumor marker), and after the treatment. Tumor markers should be monitored also for a certain period after the treatment has been finished, best at regular control examinations (once in six months or once yearly). This kind of follow up will enable a timely detection of disease recurrence even in asymptomatic patients. From a single determination of tumor markers we can find out whether the malignancy has developed or not and, if it has, what is its extent, but only if the concentrations are very high. In patients who are undergoing just symptomatic (palliative) therapy, the determination of tumor markers no longer makes sense. If the patient had normal concentrations of tumor markers prior to any kind of treatment, and if these concentrations did not change (increase or decrease) at disease progression or regression, it is very unlikely that concentrations will be elevated in case of disease recurrence. In these patients, tumor marker levels need not to be followed either during treatment or after treatment. Hence, it is important to make the first determination of tumor markers prior to any treatment in order to disclose the group of patients in whom tumor marker determinations are not sensible.

References

1. Hill RP, Tannock IF. Introduction: cancer as a cellular disease. In: Tannock IF, Hill RP, eds. *The basic science of oncology.* New York: Mc Graw-Hill, 1992: 1- 4.

2. Rubin P, ed. *Clinical oncology: A multidisciplinary approach for physicians and students*, 7th ed. Philadelphia: W.B. Saunders, 1993: 791.

3. Novaković S. *Pregled pomembnejših tumorskih označevalcev v klinični onkologiji.* Ljubljana: Onkološki inštitut, 2000: 64.

4. Clinical practice guidelines for the use of tumor markers in breast and colorectal cancer. Adopted on May 17, 1996, by the American Society of Clinical Oncology. *J Clin Oncol* 1996; **14:** 2843-77.

5. Bast RC, Ravdin P, Hayes DF, Bates S, Fritsche H, Jessup JM, *et al.* 2000 update of recommendations for the use of tumor markers in breast and colorectal cancer: Clinical practice guidelines of the American Society of Clinical Oncology. *J Clin Oncol* 2001; **19:** 1865-78.

6. Perkins LG, Slater DE, Sanders KG, Prichard GJ. Serum tumor markers. *Am Fam Physician* 2003; **68:** 1075-82.

7. Malkin A. Tumor markers. In: Tannock IF, Hill RP, eds. *The basic science of oncology.* New York: Mc Graw-Hill, 1992: 196-206.

8. International germ cell consensus classification: A prognostic factor-based staging system for metastatic germ cell cancers. International Germ Cell Cancer Collaborative Group. *J Clin Oncol* 1997; **15:** 594-603.

9. Mazumdar M, Bajorin DF, Bacik J, Higgins G, Motzer RJ, Bosl GJ. Predicting outcome to chemotherapy in patients with germ cell tumors: The value of the rate of decline of human chorionic gonadotropin and alpha-fetoprotein during therapy. *J Clin Oncol* 2001; **19:** 2534-41.

10. Einhorn LH. Treatment of testicular cancer: A new and improved model. *J Clin Oncol* 1990; **8:** 1777-81.

11. Novaković S, Serša G. Tumor markers in clinical oncology. In: Benulič T, Serša G, Kovač V, eds. *Advances in Radiology and Oncology.* Ljubljana: Radiologia Iugoslavica, 1992: 214-20.

12. Ballesta AM, Molina R, Filella X, Jo J, Gimenez N. Carcinoembryonic antigen in stanging and follow-up of patients with solid tumors. *Tumour Biol* 1995; **16:** 32-41.

13. Jezeršek B, Červek J, Rudolf Z, Novakovič S. Clinical evaluation of potential usefulness of CEA, CA 15-3, and MCA in follow-up of breast cancer patients. *Cancer Lett* 1996; **110:** 137-44.

14. Fletcher RH. Carcionembryonic antigen. *Ann Inter Med* 1996; **104:** 66-74.

15. Abelev GI. Study of the antigenic structure of tumors. *Acta Int Cancer* 1963; **19:** 80-5.

16. Johnson PJ. The role of serum alpha-fetoprotein estimation in the diagnosis and management of hepatocellular carcinoma. *Clin Liver Dis* 2001; **5:** 145-59.

17. Yuen MF, Cheng CC, Lauder IJ, Lam SK, Ooi CG, Lai CL. Early detection of hepatocellular carcinoma increases the chance of treatment: Hongkong experience. *Hepatology* 2000; **31:** 330-5.

18. Fowler JE, Platoff GE, Kubrock CA, Stutzman RE. Commercial radioimmuno assay for beta subunit of human chorionic gonadotropin: Falsely positive determinations due to elevated serum luteinizing hormone. *Cancer* 1982; **49:** 136-9.

19. Bosl GJ, Bajorin DF, Sheinfeld J, Motzer RJ, Chaganti RS. Cancer of the testis. In: DeVita VT, Hellman S, Rosenberg SA, eds. *Cancer. Principles and Practice of Oncology*, 6th ed. Philadelphia: Lippincott, Williams & Wilkins, 2001: 1491-518.

20. Cooper HC, Robinson MRG, Whelan P, Ferro AM. Tumor markers in prostate cancer. *Cancer* 1992; **70:** 225-9.

21. Schmechel D, Marangos PJ, Zis AP, Brightman M, Goodwin FK. Brain enolase as specific markers of neuronal and glial cells. *Science* 1978; **199:** 313-5.

22. Carney DN, Ihde DC, Cohen MH, Marangos PJ. Serum neuron-specific enolase: a marker for disease extent and response to therapy of small-cell lung cancer. *Lancet* 1982; **I:** 583-5.

23. Wick MR, Brend MD, Scheithauer W, Kovacs K. Neuron specific enolase in neuroendocrine tumors of the thymus, bronchia and skin. *Am J Pathol* 1983; **79:** 703-7.

24. Geraghty JG, Coveney CE, Sherry F, O'Higgins JN, Duffy JM. CA 15-3 in patients with locoregional and metastatic breast carcinoma. *Cancer* 1992; **70:** 2831-4.

25. Tuxen MK, Soletormos G, Dombernowsky P. Tumor markers in the management of patients with ovarian cancer. *Cancer Treat Rev* 1995; **21:** 215-45.

26. Novaković S, Serša G, Krošl G, Plesničar S. CEA, MCA and CA 125 tumor markers in pregnant women. *Radiol Iugosl* 1990; **24:** 79-84.

27. Jacobs IJ, Skates SJ, MacDonald N, Menon U, Rosenthal AN, Davies AP, *et al.* Screening for ovarian cancer: A pilot randomized controlled trial. *Lancet* 1999; **353:** 1207-10.

28. Steinberg W. The clinical utility of the CA 19-9 tumor-associated antigen. *Am J Gastroenterol* 1990; **85:** 350-5.

29. Woodrum LD, Brawer KM, Partin WA, Catalona JW, Southwick CP. Interpretation of free prostate specific antigen clinical research studies for the detection of prostate cancer. *J Urol* 1998; **159:** 5-12.

30. Catalona WJ, Partin AW, Slawin KM, Brawer MK, Flanigan RC, Patel A, *et al.* Use of the percentage of free prostate-specific antigen to enhance differentiation of prostate cancer from benign prostatic disease: A prospective multicenter clinical trial. *JAMA* 1998; **279:** 1542-7.

31. Ghanem G, Loir B, Morandini R, Sales F, Lienard D, Eggermont A, Lejeune F. On the release and half-life of S100B protein in the peripheral blood of melanoma patients. *Int J Cancer* 2001; **94:** 586-90.

32. Jury SC, McAllister JE, MacKie MR. Rising levels of serum S100 protein procedure other evidence of disease progression in patients with malignant melanoma. *Br J Derm* 2000; **143:** 269-74.