Contents

Tumor markers in general .................................................................................. 3
  What are tumor markers? ............................................................................. 4
  Factors affecting tumor marker evaluation ................................................. 4
  Cut off, clinical sensitivity, clinical specificity ............................................. 6
  Use of tumor markers .................................................................................. 12

Selected tumor markers and their clinical applicability ...................... 17
  AFP ............................................................................................................... 17
  CEA .............................................................................................................. 18
  PSA, Free PSA ............................................................................................ 19
  hCG .............................................................................................................. 20
  CA 15-3 ....................................................................................................... 21
  CA 125 ......................................................................................................... 22
  CA 19-9 ....................................................................................................... 23
  NSE .............................................................................................................. 24
  TK ................................................................................................................ 25
  Cytokeratin 19 Fragment ............................................................................ 26
  B2M .............................................................................................................. 26
  Calcitonin .................................................................................................. 28
  Thyroglobulin ............................................................................................ 29
  α Subunit .................................................................................................... 30
TUMOR MARKERS

Tumor markers in general

Neoplastic diseases have accompanied humankind since the beginning of times. Although cancer survival has been improving within last decades, the number of cancer deaths has increased due to the expanding and aging of population, as well as a result of decrease in mortality due to other cause like e.g. infection diseases. In consequence, health related and social significance of neoplastic diseases is on the rise. Early and high quality diagnostics enables to start suitable treatment in time, and to decrease the risk of unfavorable impacts of these diseases.

Although the prevalence and incidence of cancer diseases is predominant in developed countries, it is rising quickly in developing countries. The table below shows mortality rate in some selected countries:

**Prevalence:** The total number of cases of the disease in the population at a given time (or, in percentage, the total number of cases in the population, divided by the number of individuals in the population)

**Incidence:** A measure of the rate of developing some new condition within a specified period of time. In other words, it is the number of new cases during some time period per certain amount of people (e.g. 25 new cases per 100 000 people per year).

Table 1: Percentage of death due to cancer disease from the total number of deaths (from “WHO global health estimates 2014; WHO web site, page http://www.who.int/healthinfo/global_burden_disease/estimates/en/index1.html”)

<table>
<thead>
<tr>
<th>Country</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Netherlands</td>
<td>33.3</td>
</tr>
<tr>
<td>France</td>
<td>30.8</td>
</tr>
<tr>
<td>Japan</td>
<td>30.3</td>
</tr>
<tr>
<td>Japan</td>
<td>30.3</td>
</tr>
<tr>
<td>South Korea</td>
<td>30.3</td>
</tr>
<tr>
<td>Australia</td>
<td>29.5</td>
</tr>
<tr>
<td>Italy</td>
<td>29.2</td>
</tr>
<tr>
<td>Portugal</td>
<td>28.2</td>
</tr>
<tr>
<td>Israel</td>
<td>27.3</td>
</tr>
<tr>
<td>Austria</td>
<td>26.9</td>
</tr>
<tr>
<td>Germany</td>
<td>26.5</td>
</tr>
<tr>
<td>Czech Republic</td>
<td>25.8</td>
</tr>
<tr>
<td>Greece</td>
<td>25.7</td>
</tr>
<tr>
<td>United States</td>
<td>23.6</td>
</tr>
<tr>
<td>China</td>
<td>22.4</td>
</tr>
<tr>
<td>Turkey</td>
<td>21.6</td>
</tr>
<tr>
<td>Argentina</td>
<td>21.3</td>
</tr>
<tr>
<td>North Korea</td>
<td>17.1</td>
</tr>
<tr>
<td>Brazil</td>
<td>17.0</td>
</tr>
<tr>
<td>Paraguay</td>
<td>15.8</td>
</tr>
<tr>
<td>Russia</td>
<td>15.5</td>
</tr>
<tr>
<td>Egypt</td>
<td>13.8</td>
</tr>
<tr>
<td>Ukraine</td>
<td>12.8</td>
</tr>
<tr>
<td>Mexico</td>
<td>11.9</td>
</tr>
<tr>
<td>Pakistan</td>
<td>7.6</td>
</tr>
<tr>
<td>India</td>
<td>7.0</td>
</tr>
<tr>
<td>South Africa</td>
<td>6.8</td>
</tr>
<tr>
<td>Congo</td>
<td>3.3</td>
</tr>
</tbody>
</table>
Nowadays, the determination of tumor markers is an integral part of diagnostic procedures, together with e.g. x-ray studies, endoscopic examinations, bone marrow aspirations, ultrasound imaging, magnetic resonance imaging (MRI), computed tomography (CT) scan, and positron-emission tomography (PET) scan.

Tumor markers can contribute to distinguishing between malignant and benign tumors, to assessing the stage of the disease, to evaluating the efficacy of treatment, and they are best used in the early detection of recurrence. Indicated use of the appropriate marker can significantly affect treatment outcome and therefore improve the patient's survival. In ideal cases, tumor markers may detect tumor mass of 1 mg (approx. $10^6$ cells), whilst clinical diagnosis by means of imaging techniques is possible in tumor consisting of not less than approximately $10^9$ tumor cells.

**What are tumor markers?**

They are substances produced:
- by cancer cells
- by organism, in reaction to the tumor growth

They are localized on the cell membrane of tumor cell, or in its cytoplasm (enzymes of metabolic pathways, fragments of cytoplasmic structures), and may be determined either directly in the cells, or in blood and other body fluids.

**Factors affecting tumor marker evaluation**

There are several factors that should be taken into account when evaluating tumor markers. Some of them are listed in the following paragraphs, to suggest the necessity of complex view on the matter. It is necessary to count with:

A) Biological half-life
Respect to biological half-life is necessary, especially if tumor markers are used for the control of therapy. Time period after surgery or chemotherapy must be at least double or triple of the biological half-time.

B) Biological variability
Important for evaluating of significance of tumor marker increase/decrease in the course of follow-up.

C) Clinical specificity (ability of the assay to recognize correctly healthy individuals)
Increased levels of tumor markers themselves do not prove cancer disease. They may be increased in some benign conditions, but also in certain percentage of healthy people.
D) Clinical sensitivity (ability of the assay to detect individuals with the disease)
Low levels of tumor markers themselves do not prove the absence of cancer
disease, due to the reasons summarized above.

E) Dependence of tumor marker values on:

- **Histological type of the tumor** – However tumor markers are not
strictly organ or tissue dependent, there is certain correlation between
histological type and tumor marker appearance.

- **Differentiation of cells** - Well differentiated cells are able to produce
complicated peptide or mucine structures. With de-differentiation, only
less complicated substances necessary for proliferation or cellular skeleton
transformation (growth, necrosis, apoptosis) are created.

- **Size of the tumor** – Level of many markers increases with enlargement
of tumor mass. On the other hand, there are markers like thymidine
kinase or cytokeratin fragments, correlating rather with extent of
proliferation than with tumor mass itself.

- **Blood supply of tumor mass** – vascularization facilitates the release of
tumor marker into circulation.

- **Therapy** – Therapy may influence tumor marker levels, too. For instance,
therapy may alter metabolic pathway of enzyme tumor markers, or cause
temporary release of tumor markers in response to chemotherapy (lysis
phenomenon).

- **Preanalytical factors** – Preanalytical step may influence tumor marker
levels, too. Typical example is false increase of NSE levels due to
hemolysis of sample (release of NSE from erythrocytes).

- **Analytical factors** – Like in other immunoassays, the determination of
tumor markers is susceptible to the influence of factors like heterophilic
antibodies, e.g. HAMA (human anti-mouse antibodies). The probability of
presence of these antibodies is higher in patients with oncology disease, as
they may develop in response to diagnostic procedures (scintigraphy) or
treatment (antibody treatment).

Commercial assays are usually designed to suppress the effect of such
antibodies. Nevertheless, this interference cannot be eliminated absolutely
so there may still be found a few samples which are affected by this
phenomenon.

- **Other specific factors** – the level of tumor markers may be affected by
several other factors. For example, it is reported that cycling or digital
rectal examination may cause increase in PSA levels, smoking increases
levels of CEA etc.
**Cut off, clinical sensitivity, clinical specificity**

Utility of tumor marker determination is very dependent on its correct indication and interpretation. Because of inter-individual differences, it is always problematic to adapt some single threshold value that could distinguish between population of healthy and diseased people. This is even truer in case of tumor markers, where all the factors listed in previous chapter may play certain role.

**Ideal (hypothetical) Tumor marker**

In ideal case, there exists single threshold value (so called cut off) that enables to precisely distinguish between population of healthy and diseased people. Unfortunately, such ideal behavior is theoretical only.

**Cut off:** Discrimination level between positive and negative values at given clinical specificity
Real case

Cut off enables to distinguish between population of healthy and diseased people only with certain probability. On the one hand, there are healthy individuals with tumor marker level above cut off, on the other hand there are diseased individuals with level below cut off. Such cut off should be therefore selected so as to minimize this number of falsely negative and falsely positive cases. And it is clear that there should be different cut off adapted in dependence on the intended use of the marker.

Real tumor marker

The cut off value is frequently established as 95\textsuperscript{th} percentile of the levels in healthy population. If such cut off value is adapted, that means:

- 5 % of the individuals who are healthy would be found falsely positive;
- the percentage of false positive results may be even much higher within group of individuals with some benign conditions, as this population tends to have increased levels of tumor markers;
- the percentage of false positive results may be higher (or lower) also due to some other factors. Typical example is the influence of ethnical origin, which may be seen in case of some markers, resulting in increased number of false positive or false negative result rate comparing to the primarily selected population.

\textbf{Percentile}: The value of a variable (e.g. tumor marker concentration) below which a certain percentage of observations fall. So the 95\textsuperscript{th} percentile is the value below which 95 % of the observations may be found.
There is an example of Cytokeratin 19 fragment results. In this case, 95th percentile of the healthy population corresponds to 2.36 ng/mL. Nevertheless, when we consider the results of benign group, as well as the results of non-small cell lung cancer patients in remission and progression, cut off value 3.3 seems to correspond better than the 95th percentile value.

Of course, the adaptation of individual cut off, taking into account different types of tumors and intended clinical use (screening, primary diagnostics, secondary diagnostics etc.) would be the best way. If it is not possible for some reason, it is at least necessary to be aware of the limited applicability of the single cut off in all diagnostic applications.
In establishing the cut off value, it is desirable to achieve the best possible clinical sensitivity and specificity.

**Clinical sensitivity (SN):** The ability of the assay to find true positive patients in diseased population.

\[ SN = \frac{TP}{TP + FN} \]

Where TP is true positivity and FN is false negativity

**Clinical specificity (SP):** The ability of the assay to find true negative patients in healthy population.

\[ SP = \frac{TN}{TN + FP} \]

Where TN is true negativity and FP is false positivity
For instance for the purpose of screening healthy population, i.e. situation when absolute majority of examined individuals do not suffer from the disease, it seems reasonable to increase the cut off value to avoid high number of falsely positive cases, even with the risk of some missed positive cases. In other words, it is desirable to have the cut off ensuring very good specificity of the assay, even if the sensitivity would be decreased at certain extent. We compare here “healthy” individuals with those having oncological disease. Application of usual cut off (95th percentile of healthy people) would lead in 5% false positivity (even without any influence of the other factors mentioned above). As we expect that the vast majority of population do not suffer from the screened cancer, such percentage seems to be quite high. It is possible to diminish the number of false positive cases e.g. by shifting the cut off value to the level of 99th percentile, leaving thus only 1% of falsely positive results.

Completely different situation is in primary diagnosis. The examined individuals are people who suffer from some kind of healthy problems. So, the "healthy" population may be hardly used as reference one. It would be desirable to have a reference group of individuals with benign diseases, but such group tends to be very heterogeneous and not so easy to define. In such a case, the intention is to miss as few cancer positive cases as possible. We therefore focus on sensitivity in this case, though specificity should still remain reasonably high as well.

Other terms that may be met in connection of tumor marker evaluation

**PPV (positive predictive value):** The probability of disease presence in case of a positive test result given in per cents.

\[ PPV = \frac{TP}{TP + FP} \]

Where TP is true positivity and FP is false positivity

**NPV (negative predictive value):** The probability of disease absence in case of a negative test results given in per cents.

\[ NPV = \frac{TN}{TN + FN} \]

Where TN is true negativity and FN is false negativity
Usefulness of tumor marker for specific diagnostic purpose may be evaluated on bases of so called receiver operating characteristics curve (ROC curve). It is created by plotting the fraction of true positives (clinical sensitivity) vs. the fraction of false positives (1 – clinical specificity). The graphs below show comparison of ideal marker, real marker, and marker without any diagnostic value. Quality of the marker may be characterized by calculated Area under curve (AUC), with maximal value 1 for ideal marker, minimal value 0.5 for marker without any significance, and value between these extremes for the real marker.
Use of tumor markers

It is clear that it is not possible to base the diagnoses on tumor markers only. Optimal interpretation of tumor marker assessments requires the knowledge of methodology on the one hand and the knowledge of clinical course of the disease in the diagnosed individual on the other hand. Possible use of tumor markers is shortly summarized in the following paragraphs.

Screening
General requirement for screening test is minimal sensitivity 97 % at the level of 95% specificity. Moreover, this should be accomplished in early stages of the disease, when prospect for the patient is more promising. There was an intention to use CEA for the screening of colorectal cancer, CA 125 for the screening of ovarian cancer, and PSA for the screening of prostate cancer. Unfortunately, none of these markers fulfilled the requirement of minimal specificity and sensitivity. In fact, the so called “PSA screening”, which has been implemented in many countries, also is not the screening in the real sense of the term. It is performed only in certain population (men over certain age), and thus it should be rather considered as an early diagnosis at the selected (high risk) group.

Primary diagnostics
Suitability of tumor markers for primary diagnostics is limited, too. The main reasons are again their low sensitivity and specificity.

Differential diagnostics
Differential diagnostics helps in specification of histological type of tumors. It may be useful when there is limited possibility to get this information by other means, and especially in cases when the exact identification of tumor is crucial for the decision about treatment. The use of tumor markers may be profitable in certain cancers, mainly in testicular tumors, chorioepitelioma, neuroendocrine tumors and lung tumors.
Follow-up
Long term follow-up appears to be the most important indication for tumor markers. Increasing levels of tumor markers suggest progression and generalization of the disease. Moreover, tumor marker levels may increase several months before clinical manifestation of the disease (so called lead-time). Random indication and investigation of tumor markers is almost useless. The dynamics of tumor marker changes gives more useful information than absolute level itself. And, although the follow-up itself starts only after the therapy, it is reasonable to determine tumor marker level even before. The reasons are:

1. Pre-treatment level may help with the selection of the right marker. The markers that were increased before treatment tend to increase in progression as well.
2. Pre-treatment level may be useful for evaluating of therapy effect
3. Pre-treatment level give evidence about prognosis. In general, the higher pre-treatment levels of tumor markers are, the worse prognosis is.

Scheme for tumor marker follow-up intervals may be modified in dependence on cancer and on the markers used. Example of such scheme is below:

First determination before treatment (surgery) ↓
Post-operative determination 14 days after surgery ↓
Three determinations with one month interval ↓
Determination each 3-4 months up to three years after surgery ↓
After that, twice a year.

Note: Frequency of testing has to be increased when signs of progression appear.
Monitoring of therapy
The use of tumor markers for therapy control is on the rise. Appropriate markers are now available in many types of malignancy.
Right timing of blood sample collection is necessary to reflect the real effect of therapy, not only so called “lysis phenomenon” (temporary increase of tumor marker level in response to therapy). As it is necessary to respect biological half-life of tumor marker, the sample is usually collected three or four weeks after therapy application.

Example of the tumor marker determination in the course of disease. Patient with ovarian cancer, values determined by Beckman Coulter assay.
Tab. 2: Positivity of tumor markers in benign conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>Change in tumor marker value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequently (in more than 30 %)</td>
</tr>
<tr>
<td>Smoking</td>
<td>CEA</td>
</tr>
<tr>
<td>Examination per rectum</td>
<td>CEA, PSA</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>hCG, AFP, CA 125, CA 19-9</td>
</tr>
<tr>
<td>Pernicious anemia</td>
<td>TK, Ferritin</td>
</tr>
<tr>
<td>Regeneration processes connected with rapid cell growth</td>
<td>TK, TPS</td>
</tr>
<tr>
<td>Bacterial infection Local</td>
<td>TK, TPS</td>
</tr>
<tr>
<td>Bacterial infection Systemic</td>
<td>TK, TPS</td>
</tr>
<tr>
<td>Viral infection</td>
<td>TK</td>
</tr>
<tr>
<td>Heart failure Without exudate and ascites</td>
<td>CA 125, APF, B2M</td>
</tr>
<tr>
<td>Heart failure With exudate and ascites</td>
<td>CA 125, TPS, TPA, B2M, MT, Cytok. 19</td>
</tr>
<tr>
<td>Exudate of benign etiology Pleural</td>
<td>CA 125, TPS, TPA, MT, Cytok. 19</td>
</tr>
<tr>
<td>Exudate of benign etiology Abdominal</td>
<td>CA 125, TPS, TPA</td>
</tr>
<tr>
<td>Atherosclerosis</td>
<td>TPS</td>
</tr>
<tr>
<td>Renal failure</td>
<td>CEA, Chg A, B2M, Ferritin, AFP</td>
</tr>
<tr>
<td>Liver and bile tract disorders Acute</td>
<td>CA 19-9, CA 125, AFP</td>
</tr>
<tr>
<td>Liver and bile tract disorders Chronic</td>
<td>CA 19-9, CA 125, AFP</td>
</tr>
<tr>
<td>Liver failure</td>
<td>Chg A, APF, CA 19-9</td>
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<tr>
<td>Lung diseases Inflammatory</td>
<td>CEA, CA 19-9</td>
</tr>
<tr>
<td>Lung diseases Benign tumor</td>
<td>CEA, CA 19-9, Cytok. 19</td>
</tr>
<tr>
<td>Breast diseases Inflammatory</td>
<td>CEA, CA 15-3</td>
</tr>
<tr>
<td>Breast diseases Benign tumor</td>
<td>CA 19-9, CA 72-4, AFP, CEA</td>
</tr>
<tr>
<td>Bowel disease Inflammatory</td>
<td>TK, TPS</td>
</tr>
<tr>
<td>Bowel disease Benign tumor</td>
<td>CEA, CA 19-9, CA 72-4</td>
</tr>
<tr>
<td>Urinary tract Inflammatory</td>
<td>TPA, TPS, Cytok. 19</td>
</tr>
<tr>
<td>Urinary tract Benign tumor</td>
<td>TPA, TPS, Cytok. 19</td>
</tr>
</tbody>
</table>
Tab. 3: Changes of tumor marker values in cancer

<table>
<thead>
<tr>
<th>Localization and type of cancer</th>
<th>Tumor marker</th>
<th>With biggest diagnostic value</th>
<th>Supplementary marker</th>
<th>Limited diag. value (though level changes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head and neck</td>
<td></td>
<td>TPS, Cytok. 19</td>
<td>TPA,MT</td>
<td>TK, CEA</td>
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<tr>
<td>Esophageal</td>
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<td>SCC, CA 72-4</td>
<td>CEA, TPA, TK</td>
<td>TPS, CA 19-9, Cytok. 19</td>
</tr>
<tr>
<td>Lung - NSCLC</td>
<td>Squamous</td>
<td>Cytok. 19, TPA, MT</td>
<td>SCC</td>
<td>PSA, TPS, NSE, CA 125</td>
</tr>
<tr>
<td></td>
<td>Adenocarcinoma</td>
<td>TPA, MT, Cytok. 19</td>
<td>CA 125</td>
<td>PSA, TPS, NSE, CA 125</td>
</tr>
<tr>
<td></td>
<td>Large cell</td>
<td>TPA, MT, Cytok. 19</td>
<td>TK</td>
<td>PSA, CA 125, TPS</td>
</tr>
<tr>
<td>Lung - SCLC</td>
<td>NSE, proGRP</td>
<td>MT, Cytok. 19</td>
<td></td>
<td>TPA, SCC</td>
</tr>
<tr>
<td>Gastric</td>
<td>CA 72-4</td>
<td>CEA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorectal</td>
<td>CEA</td>
<td>CA 19-9, TPS, TK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bile duct and liver</td>
<td>CA 19-9, CEA, Chg A, AFP</td>
<td>CA 15-3, SCC, Cytok. 19</td>
<td>TK, TPS, TPA, MT</td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>CA 19-9</td>
<td>TK</td>
<td></td>
<td>CA 72-4, TPS, CA 15-3, CA 125</td>
</tr>
<tr>
<td>Neuroendocrine</td>
<td>Chg A, NSE</td>
<td>TK</td>
<td></td>
<td>TPS, TPA, CEA</td>
</tr>
<tr>
<td>Renal</td>
<td></td>
<td>NSE, TPS, Chg A, Cytok. 19</td>
<td></td>
<td>Several markers</td>
</tr>
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<td>Urinary tract</td>
<td>TPA, Cytok. 19</td>
<td>TPS, TK</td>
<td></td>
<td>CEA, MT, SCC</td>
</tr>
<tr>
<td>Prostate</td>
<td>PSA, fPSA</td>
<td>Chg A</td>
<td></td>
<td>CA 19-9</td>
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<tr>
<td>Breast</td>
<td>CA 15-3, CEA</td>
<td>TK, TPA, Chg A, Cytok. 19</td>
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<td>TPS, MT, CA 19-9</td>
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<tr>
<td>Testicular</td>
<td>AFP, hCG, TK, NSE</td>
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<td>Cytokeratins, CA 72-4</td>
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<td>Ovarian</td>
<td>CA 125, CA 72-4, HE-4</td>
<td>TPA, CA 19-9, Cytok. 19, AFP, MT, CEA</td>
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<td>CA 15-3, Chg A, PSA</td>
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<td>Uterus</td>
<td>SCC, Cytok. 19</td>
<td>TK, TPA</td>
<td></td>
<td>CEA, CA 19-9, CA 125, TPS, MT</td>
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<tr>
<td>Haemoblastosis</td>
<td>TK, B2M, Ferritin</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Chg A – chromogranin A
SCC – squamous cell cancer antigen
MT – Monototal
TPA – Tissue Polypeptide Antigen
TPS - Tissue Polypeptide Specific Antigen

Other abbreviations are explained further in the text.
Selected tumor markers and their clinical applicability

**AFP (alpha fetoprotein)**

**Characteristics:**
AFP is a glycoprotein of molecular weight approximately 70 kDa. It consists mostly of proteins and contains only 4.5% of saccharides. It belongs to the group of oncofetal antigens. Its structure is very similar to that of albumin.

**Occurrence:**
AFP is released by the yolk sac and later by fetal liver. In the initial stage of the fetal development, AFP acts as a substitute for albumin and its transport functions. AFP concentrations in fetal plasma reach their peak between weeks 10 and 13 of the gestation at the concentration level approximately 3 g/L and then gradually decrease until the term of delivery when the levels represent about 80 mg/L. The decrease continues after the child is born and up to 2 years of age the levels fall below 10 µg/L. The same values are observed in healthy adults.

**Biological half-life:** 3 - 6 days

**Clinical interest**
- Primary hepatocellular carcinoma	diagnosis, monitoring
- Germ cell tumors (ovarian cancer, testicular cancer)	diagnosis, monitoring, prognosis

**Increased levels in benign conditions**
- Liver cirrhosis
- Acute viral hepatitis
- Chronic hepatitis
- Chronic renal failure
- Pregnancy
CEA (carcinoembryonic antigen)

Characteristics:
CEA is a glycoprotein with molecular weight of approximately 180 kDa, which consists of saccharides (55%) and proteins (45%). The high degree of heterogeneity of its molecule is due to the heterogeneity of its saccharide component, the protein component is constant (homogeneous).

Occurrence:
CEA is an oncofetal protein which can be found in the epithelial cells primarily in the gastrointestinal tract and bronchi. In the first trimester of pregnancy, it is contained in the cell cytoplasm. In the later stages of fetal development, it is found on the surface of cellular membranes. In adult individuals, it is released in limited amounts by the epithelium cells in the bronchi, breasts and the gastrointestinal tract. CEA is found in minimal concentration in blood, pleural fluid, ascites and CNS fluid. The highest levels are found in salivary glands and their ducts.
CEA is metabolized in the liver.

Biological half-life: 14 days

Clinical interest
- Colorectal cancer
- Lung cancer
- Breast cancer
- Prostate cancer
- Cancer of stomach
- Ovarian cancer
- Medullar cancer of thyroid gland

Increased levels in benign conditions
- Smoking
- Chronic renal failure
- Chronic liver disease
- Chronic hepatitis
- Chronic pancreatitis
- Chronic bronchitis
- Crohn's disease
- Ulcerous colitis
- Bronchopneumonia
- Mucoviscidosis
- Autoimmune diseases
PSA (prostate specific antigen)
free PSA (free prostate specific antigen)

Characteristics:
PSA is a glycoprotein with molecular weight of 34 kDa which consists of a simple polypeptide chain of 238 amino-acids (90 %) and saccharides (10%). It belongs to the group of kallikreins. It is released by both healthy and malignant prostatic tissue as a specific product.

Occurrence:
PSA is a serine protease which causes the spermatic plasma liquidisation. PSA occurs in prostatic fluid, seminal plasma, healthy prostate tissue, in hyperplastic tissue, as well as in malignant prostatic tissue and in metastases of prostatic origin.
PSA is also released by paraurethral glands and in very low concentrations it is observed in women.
PSA occurs in three main forms in serum - free (fPSA), PSA bound on \( \alpha_2 \)-macroglobulin and PSA bound on \( \alpha_1 \)-antichymotripsin. The complex of PSA and \( \alpha_2 \)-microglobulin is immunologically inactive, and thus non-detectable by immunoassays, while two other forms are immunologically detected.

Biological half-life:  
PSA 2 - 3 days  
fPSA 7 hours

Clinical interest
- Prostate cancer (early diagnostics in risk group, differential diagnosis, follow-up, effect of treatment)
  - Total PSA
    - If total PSA is between 4-10 ng/mL, than ratio Free/Total PSA
      - Saving of unnecessary prostate biopsies

Increased levels in benign conditions
- BPH (benign prostate hyperplasia)
- Prostatitis
- Infarct of the prostate
- Mechanical irritation (rectal examination, catheterization, cystoscopy, cycling, horse-riding etc.)
HCG (Human chorionic gonadotropin)

Characteristics:
hCG is a glycoprotein with relative molecular weight of about 40 kDa. It belongs to the group of glycoprotein hormones, together with lutropin (LH), follitropin (FSH) and thyrotropin (TSH). Similar structure can be observed in all above mentioned hormones. They are made up of two non-covalently bound subunits α and β. α-subunits are identical in all these glycoprotein hormones. Their biological specificity is determined by β-subunits, which more or less differ from one another although they show a significant degree of homology (e.g. in β-hCG and β-LH the sequences of amino-acids differ only in 18%).

Occurrence:
Unlike the other glycoprotein hormones, which are released by adenohypophysis, hCG is produced by trophoblastic cells in placenta during gestation and stimulates the growth of the corpus luteum. hCG facilitates the immunological tolerance of the fetus by mothers’ immunity system. In blood, as well as in urine, both intact hCG molecules, free α and β-subunits and β-subunit cleavage products (free-β and β-core) can be found. Only complete intact hCG molecule is biologically active. For tumor diagnostics, free β-subunit (β-hCG) assessment seems to be more specific.

Biological half-life: 1.5 – 2.5 days

Clinical interest
- Non-seminomatous germ cell tumors
  Diagnosis, monitoring, prognosis
- Choriocarcinomas, hydatiform moles, seminomas
  Diagnosis, monitoring, prognosis
- Discrimination between malignant and benign trophoblastic tumors
  Lower ratio free β-hCG to hCG for benign tumors
- Levels may be increased also in other cancers
  Bladder, pancreas, uterus, lung breast, stomach or colon tumors

Increased levels in benign conditions
- Pregnancy
- After menopause
- In women with myoma or ovarian cysts
- Endometriosis
- Liver cirrhosis
CA 15-3

Characteristics:
CA 15-3 is glycoprotein of mucine type, defined by means of a monoclonal antibody, with molecular weight of 300 - 400 kDa. It belongs to oncofetal proteins.

Occurrence:
CA 15-3 occurs in fetal epithelial cells of bronchi and in hepatocytes. In adults, it is present as a surface antigen of ductal epithelium orientated into lumen of breast.

Biological half-life: 7 days

Clinical interest
- Breast cancer
  Prognosis, follow-up, effect of treatment

Increased levels in benign conditions
- In pregnancy
- Cirrhosis
- Hepatitis
- Benign breast involvement
- Benign disease of gastrointestinal tract
- Chronic bronchitis
- Pneumonia and bronchopneumonia
- Tuberculosis
- Rheumatic diseases
CA 125

Characteristics:
CA 125 is glycoprotein of mucine type, defined by means of a monoclonal antibody, with molecular weight of 200 kDa. It belongs to oncofetal proteins.

Occurrence:
CA 125 is supposed to act as a differentiating antigen of coelomic tissue during fetal development. CA 125 occurs in fetal epithelial cells of respiratory and gastrointestinal tract. In adults, similarly as in fetus, it occurs in epithelial cells of respiratory tract. There are extremely high levels in serum during pregnancy and in maternal milk.

Biological half-life: 4 days

Clinical interest
- Ovarian cancer (especially in combination with HE-4 and calculation of ROMA index)
  Staging, prognosis, follow-up, effect of treatment
- Increased also in endometrial, breast, lung and colorectal cancer

Increased levels in benign conditions
- Pregnancy
- Cirrhosis
- Hepatitis
- Pancreatitis
- Serous inflammation
- Endometriosis
- Renal failure
- Benign ovarian and endometrial diseases
- Ovarian cysts
- 1st half of menstrual cycle
CA 19-9

Characteristics:
CA 19-9 is by means of a monoclonal antibody defined mucin-sialo-glycolipid with molecular weight of more than 200 - 1000 kDa. It belongs to oncofetal proteins.

Occurrence:
CA 19-9 occurrence is closely connected with Lewis (a) determinant of blood group. This tumor marker is not released in rarely observed Lewis (a-/b-) group. In fetus, CA 19-9 occurs mainly in epithelial cells of gastrointestinal tract, pancreas and liver. In adults, it is released by epithelial cells of bronchi and gastrointestinal tract in limited amounts.

Biological half-life: 5 days

Clinical interest
- Pancreatic cancers
- Colorectal cancer (in combination with CEA)
- Liver and bile duct cancer

Increased levels in benign conditions
- Acute pancreatitis
- Chronic pancreatitis
- Cholecystitis
- Obstructive jaundice
- Cholelithiasis
- Hepatitis
- Liver cirrhosis
- Primary biliary cirrhosis
- Benign gastric and intestine disease (mainly inflammatory)
**NSE (neuron specific enolase)**

**Characteristics:**
This enzyme catalyses the transformation of 2-phosphoglycerate to phosphoenolpyruvate in course of glycolysis. It is a protein of 78 kDa molecular weight.

**Occurrence:**
NSE can be detected in nervous and lung tissue of fetus, in adults mainly in neuroendocrine structures.

**Clinical interest**
- Tumors of neuroendocrine origin
  - Small cell lung cancer - Histological typing and monitoring
- Neuroblastomas – monitoring, prognosis
- Seminoma, renal cancer, medullary carcinoma of thyroid gland, tumors of neuroendocrine origin

**Increased levels in benign conditions**
- Benign lung diseases
- Renal failure
- Benign liver diseases

Note: When full blood is left for a long time at laboratory temperature, but also at 4–8 °C, the NSE levels are significantly and non-specifically elevated, due to the release of NSE from erythrocytes.
TK (thymidine kinase)

Characteristics:
Thymidine kinase catalyses the transformation of thymidine to thymidine monophosphate (TMP) in the presence of adenosine triphosphate (ATP). TMP is transformed to triphosphate in several steps and then incorporated into DNA. TK activity in the cell is increased in S-phase of cellular division when DNA is synthetized. Thus, TK activity reflects cellular proliferation rate and it is a non-specific tumor marker capable of detecting malignant growth in various organs.

Occurrence:
There are two thymidine kinase isoenzymes which have different biochemical and electrophoretic features in eukaryotic cells – TK1 and TK2. TK2 activity is relatively stable during the whole process of cell division, TK1 activity appears mainly in S-phase. Higher values of TK1 can occur in neoplastic disease, as well as in some viral infections.

Biological half-life: 2 days

Clinical interest
- Haemoblastomas (diagnosis, follow-up and effect of treatment)
- Malignant lymphomas (diagnosis, follow-up and effect of treatment)
- Supplementary marker in assessment of tumor process aggressivity, it means grade of proliferation
  - bronchogenic CA, breast CA, colorectal CA, prostate CA, testicular CA, urinary bladder CA etc.
Note: TK Reflects tumor growth (dividing of cells), not only tumor mass. Compared to other markers, it gives additional information about activity of the disease.

Increased levels in benign conditions
- Viral infections
- Psoriasis
- Tuberculous pleuritis
- Inflammatory processes of lung and gastrointestinal tract
- Sarcoidosis
- Rheumatic diseases
- Pernicious and megaloblastic anaemias
- Collagenoses
Cytokeratin 19 Fragment

Characteristics:
Cytokeratin 19 fragments (known also as Cyfra 21-1 – trade mark of company Roche) are specific fragments of cytokeratin subunits of intermediate filaments, similarly as e.g. TPA and TPS. Cytokeratin 19 is an acidic protein with molecular weight of 40 kDa.

Occurrence:
Cytokeratin 19 fragments occur in cells of the lung, uterus and gastrointestinal tract. It is used as a marker of malignant tissue degradation and cell necrosis.

Biological half-life: 2 days

Clinical interest
- Non-small cell lung cancer (NSCLC)
  - Differential diagnostics (especially if biopsy is not possible)
  - Prognostic factor for NSCLC (SCLC)
  - Monitoring of treatment
  - Follow-up
- Monitoring of uterine cancer (mainly cervical cancer)
- Monitoring of urinary bladder cancer
- Monitoring of head and neck cancer

Increased levels in benign conditions
- Liver cirrhosis
- Chronic renal failure
- Asthma
- Tuberculosis
- Respiratory tract infection
B2M (β₂-microglobulin)

Characteristics:
β₂-microglobulin is a single chain globular protein composed of 100 amino-acids with one disulfidic bridge and with molecular weight of 11 800 Da.
β₂-microglobulin forms a light chain of Human Leukocyte Antigen (HLA) of the class I. Its sequence and tridimensional structure is homologous with the constant part of heavy and light immunoglobulin chain.
β₂-microglobulin assessment is useful mainly in oncology and nephrology.

Occurrence:
Physiological β₂-microglobulin synthesis is most intensive in B-lymphocytes and plasma cells. Its high concentrations can be found in macrophages and endothelial cells. In general, β₂-microglobulin can be detected in all the cells except erythrocytes and trophoblastic cells.

Biological half-life: 40 minutes

Clinical interest in oncology
- Diagnostics of multiple myeloma (mainly in progression of the disease, possibility of the therapy monitoring)
- Decision about the aimed therapy in chronic lymphatic leukaemia

Increased levels in benign conditions
- Inflammatory processes
- Massive cell necrosis (during chemotherapy and actinotherapy of tumors)
- Chronic liver and renal diseases
- Renal failure, mainly in hemodialysis
- Rejection of renal graft
Calcitonin

Characteristics:
Calcitonin is a peptide composed of 32 amino-acids with molecular weight of 3.5 kDa. It occurs in different forms in circulation, including monomeric, oxidized monomeric, dimeric and polymeric forms, and also in the form of 17.5 kDa calcitonin precursor.

Occurrence:
Calcitonin is secreted by the parafollicular cells (also referred to as calcitonin cells or C cells) of the thyroid gland. The main action of calcitonin is the inhibition of bone resorption by regulating the number and activity of osteoclasts. Calcitonin is secreted in direct response to serum hypercalcemia and may prevent large oscillations in serum calcium levels and excessive loss of body calcium. However, the role of calcitonin in the regulation of serum calcium in humans is minor in comparison with parathyroid hormone and 1,25-dihydroxyvitamin D, and its determination is not used for the diagnosis of calcium homeostasis disorders.

Biological half-life: 12 minutes

Clinical interest in oncology
- Monitoring of medullary thyroid carcinoma
- Monitoring of tumors with ectopic production of calcitonin

Increased levels in benign conditions
- Hypercalcemia
- C-cell hyperplasia
- Hyperparathyreosis
- Paget’s disease
- Lung diseases
- Pernicious anemia
- Pancreatitis
Thyroglobulin

Characteristics:
Thyroglobulin (Tg) is a big dimeric glycoprotein consisting of two identical subunits. It has 2,748 amino acids in total, and a molecular weight of approximately 670 kDa. It contains iodine, bound via 140 tyrosines and its content varies between 0.2 and 1%.

Occurrence:
Thyroglobulin is the unique product of thyroid follicular cells or malignantly transformed cells originating in the thyroid. The physiological function of Tg is to provide the substrate for the synthesis of the thyroid hormones – thyroxine (T4) and triiodothyronine (T3).

Biological half-life: 65 hours

Clinical interest in oncology
- Monitoring of differentiated thyroid carcinomas after surgical resection of thyroid gland and radioisotope ablation

Increased levels in benign conditions
- Goiter
- Hyperthyroidism
- Thyroid adenoma
- Subacute thyroiditis
- Hashimoto's thyroiditis
- Graves’ disease

Note: Anti-Tg antibodies may be present in approx. 25% of thyroid cancer patients. These antibodies may interfere in immunoassay and affect the determined results. For correct interpretation, it is always necessary to determine anti-Tg antibodies, or to perform a recovery test, in order to exclude the possibility of antibody interference.
α Subunit

Characteristics:
Glycoprotein hormones lutropin (LH), follitropin (FSH) and thyrotropin (TSH) and human chorionic gonadotropin (hCG) are made up of two non-covalently bound subunits α and β. Their biological specificity is determined by β-subunits, their α-subunits are almost identical, containing the same 92 amino acids and differing only partly in their glycosylation (molecular weight 13 – 18 kDa).

Occurrence:
The α-subunit is produced by pituitary gland (together with TSH, LH and FSH) and by placenta (together with hCG). Although only complete intact hormones are biologically active, certain amount of α-subunit may be found in circulation in free form, too. The amount of free α-subunit may increase in certain pathologies like pituitary tumors or trophoblastic diseases.

Clinical interest in oncology
- Diagnosis and follow-up of pituitary tumors
- Differential diagnosis of thyrotropin-secreting pituitary tumor versus thyroid hormone resistance
- Differential diagnosis of constitutional delay of puberty versus hypogonadotrophic hypogonadism

Increased levels in benign conditions
- Pregnancy
- Within 24 hours after ovulation
- Hyperparathyreosis
- Renal failure
- Assisted reproduction with ovarian hyperstimulation or in vitro fertilization