TUMOR MARKERS

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USE OF TUMOR MARKERS

- **Screening** (Hb in faeces)
- **Dg and diff. dg** in symptomatic individuals
- **Clinical staging of cancer**, is aided by quantitation of the marker, i. e. the serum level of the marker reflects the number of cancer cells present in the body
- **Monitoring** of the disease, **monitoring of responses to the therapy**
- **Prognostic indicator** of disease progression and patient survival
- **Detection of cancer recurrence**, permits early treatment or a change in therapy

Tumor Markers in the Clinical Biochemistry Lab

CLASSIFICATION OF TUMOR MARKERS

- **According to proof:** humoral (serum), cellular (tissue)
- **According to chemical structure** (glykoproteins, glykolipids, polypeptides, imunoglobulins, polyamines)
- **According to visceral specificity**
- **According to** physiological **function** (oncofetal antigens, oncoplacental antigens, enzymes, hormones, serum proteins, receptors and others)

Visceral specificity

- **high:** ***calcitonin*** **-** medullary carcinoma of the thyroid
  PSA **-** prostate cancer
  NSE **-** small cell lung cancer
  hCG **-** germ-cell tumors
  AFP **-** hepatocellular and germ-cell carcinoma
- **moderate:** ***CA 19-9*** **-** pancreatic cancer
  CA 125 **-** ovarian cancer
  CA 15-3 **-** breast cancer
- **low:** ***CEA***
  TPA

Oncofetal antigens

- **substances produced during fetal life** (present in high concentrations in the sera of fetuses, decrease to low levels or disappear after the birth)
- **reappear in patients with cancer**
- Their production demonstrates that certain genes are reactivated as a result of the malignant transformation of the cell.

- **CEA**
- **CA (carbohydrate antigens)**
- **AFP**

- **SCC** (squamous cell carcinoma)
- **MCA** (mucinous carcinoma antigen)
- **MSA** (mammary serum antigen)
- **TATI** (tumor associated trypsin inhibitor)

CEA (carcinoembryonic antigen)

- **family of related oncofetal cell-surface glycoproteins,** the 1st used Tu marker (discovery 1965)
- nonspecific

- ↑: liver cirrhosis, pulmonary emphysema, benign breast cysts disease, ulcerative colitis, rectal polyps

colorectal, lung, ovarian, pancreatic, gastric and bile ducts Ca

- **marker for colorectal and breast carcinoma, pancreatic, gastric and bile ducts Ca**

- **cut off value < 5.0 ng/ml**

CEA – 1st choise marker of colored Ca (CRCA)

- **One of the most common malignancies in both sexes** in economically developed countries
- Incidence has increased more than 3 times during last 30 years.
- Prevalence is increasing annually by 2–3%.
- CR – newly diagnosed around 8 000 patients per year and about half of them die from CRCA.
- CR – 3rd most common malignancy in ♂, 4th in ♀
- 25% is diagnosed metastasized!

2017 data.

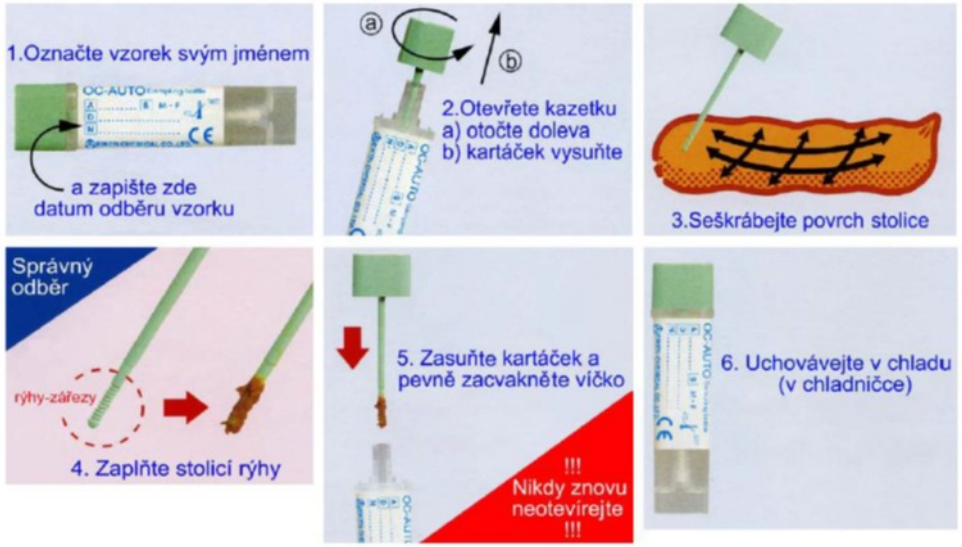
Colorectal carcinoma

- possibilities of prevention :

Target population participation
~ 20 - 30%

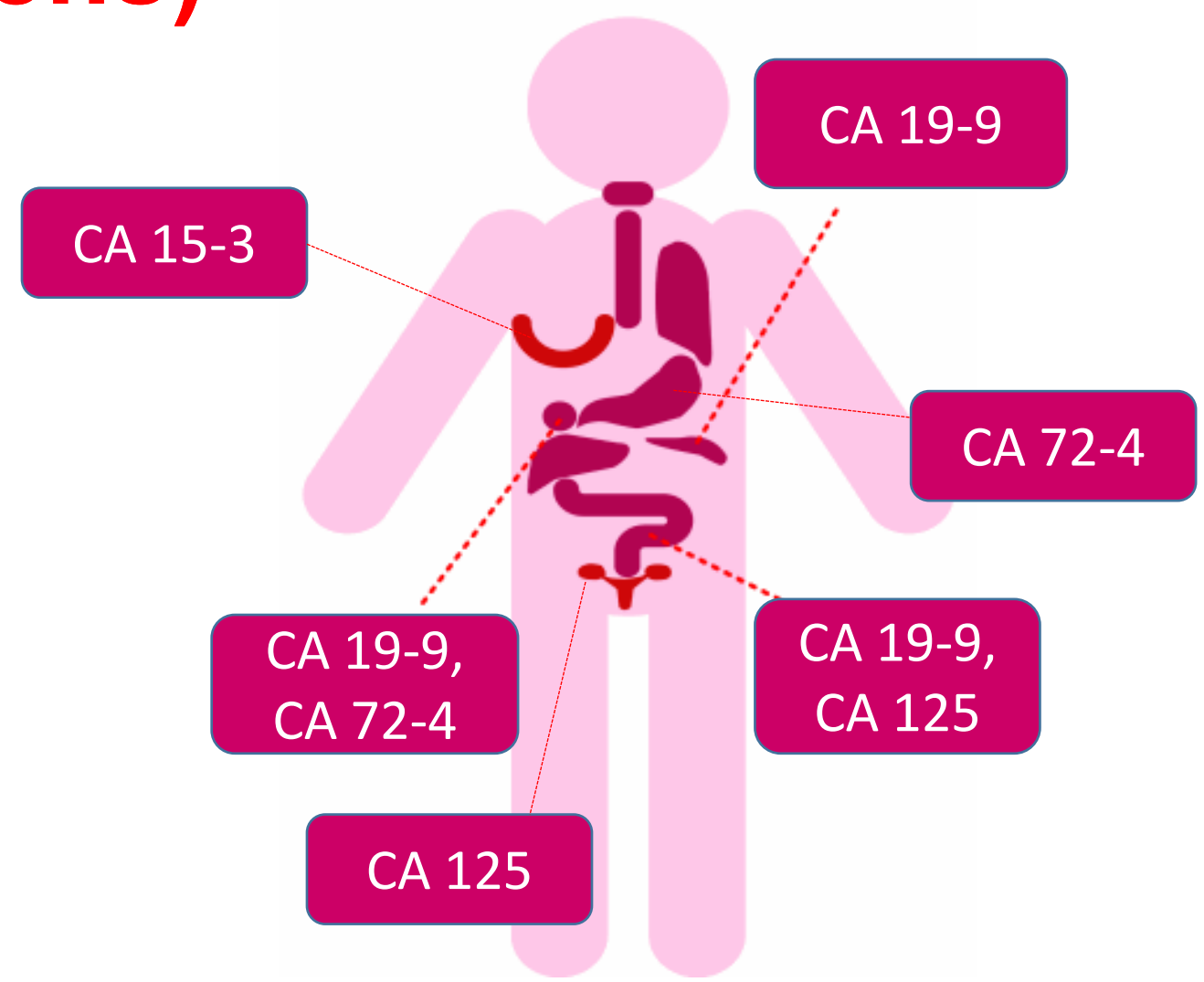
Primary
Lifestyle, nutrition

Secondary
Broadcast screening from 1.7.2000 (CR)-
cyclic fecal occult blood testing in asymptomatic individuals from age 50
or
screening colonoscopy from age 55.



CA (carbohydrate, carcinoma antigens)

- high-molecular-weight glycoproteins (mucins) or just glycoprotein epitopes
- produced physiologically prenatally or postnatally typically in tumor cells
- Some CAs are big glycoproteins (72-4, 125), other only different epitopes of the same glycoprotein (CA 15-3 and CA 27-29).



CA 72-4 (carbohydrate antigen 72-4)
TAG 72 (tumor associated glycoprotein 72)

- glycoprotein produced by oesophageal, gastric and pancreatic epithelium

- in adults ↑: liver diseases, acute pancreatitis, gastric ulcer, inflammations of GIT
  Ca of stomach, colon, uterus, lung (NSCLC)

- **marker for monitoring of gastric Ca (1st choice marker), pancreatic, oesophageal and ovarian Ca**

- **cut off ≤ 7 IU/ml**

Target of the anti-cancer drugs
anatumomab, mafenatox, minretumomab.

CA 19-9 (carbohydrate antigen 19-9)

- glycoprotein of fetal GIT, pancreas and liver epithelium; in adults it is produced by GIT and bronchial epithelium.
- **marker for pancreatic, colorectal and gastric carcinoma**
- **cut off value ≤ 40 IU/ml**

CA 19-9

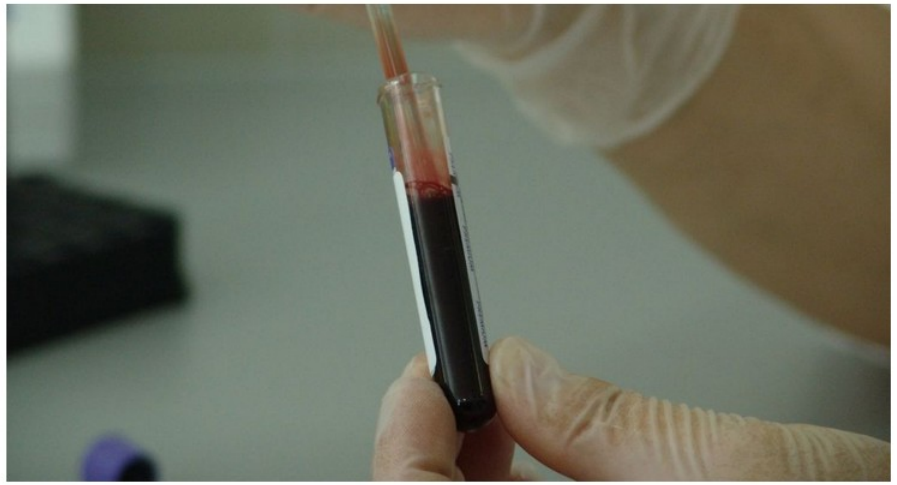
- **Sensitivity** in selected Tu (Klinická biochemie a metabolismus, 2009)

Ca	Sensitivity / %
pancreatic	70-90
colorectal	18-58
cholangiocellular	22-49
bile ducts	55-79
gastric	25-60

CA 19-9 negative patients

- patients who lack the Lewis antigen (blood type Le-a,b antigen on ercs; secreted by endothelium, GIT)
- about 10% of the Caucasian population
- CA 19-9 is not expressed even in large tumors
- deficiency of fucosyltransferase (transfers L-fucose from GDP-fucose to oligosaccharide substrate ; reaction needed for synthesis of both Le-a,b and CA 19-9)

Universitas
magazín vysokých škol



aktuality 26. října 2020 | Věra Přibylová | 2 minuty

Rakovinu slinivky odhalí analýza krve. Univerzita Pardubice získala patent

Metoda diagnostiky rakoviny slinivky analytického chemika Michala Holčapka z Fakulty chemicko-technologické Univerzity Pardubice získala evropský patent. Jeho skupina popsala možnost, jak zjistit nemoc již v počáteční fázi a pouze z analýzy krve. Tím se rýsuje šance pro miliony lidí, kteří se dnes o tomto onemocnění dozvědí příliš pozdě.

Špičkový chemik se i díky tomuto objevu letos **dostal mezi 60 nejlepších analytických chemiků světa** podle The Power List 2020 v časopise The Analytical Scientist.

„Patent slouží k ochraně naší metodiky na diagnózu karcinomu slinivky na základě lipidomické analýzy tělních tekutin. Věříme, že je také dalším krokem k využití v klinické praxi. Metodika je vhodná pro velkokapacitní screening až dvaceti tisíců vzorků za rok a dokáže detekovat nemoc se správností přes devadesát procent," říká profesor Holčapek.

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Patentovaná metodika profesora Holčapka využívá pro diagnostiku analýzu krve, ve které se kvantitativně a s využitím interních standardů stanovuje alespoň 60 lipidů pomocí hmotnostní spektrometrie.

Karcinom slinivky je maligní nádorové onemocnění, u kterého je (podle **American Cancer Society**) pouze desetiprocentní šance na pětileté dožití. To je vůbec nejméně ze všech známých typů rakoviny. Tento typ rakoviny je navíc v počáteční fázi bez jakýchkoliv příznaků, a ve chvíli, kdy se diagnóza stanoví, již pacientovi většinou nelze pomoci. Včasný screening karcinomu slinivky je proto nejdůležitější.

Úspěšné výsledky chemiků z Univerzity Pardubice se podařilo zopakovat i na spolupracujících pracovištích v Německu a v Singapuru. Nedávno si vědecký tým Michala Holčapka také ověřil, že je možné metodiku použít i v klinické laboratoři, která nemá předchozí zkušenosti s analýzou lipidů.

„Dalším důležitým krokem bude klinická validace, což je studie, kterou se musí ověřit reálný přínos včasné diagnostiky karcinomu slinivky před vlastním využití v praxi. Bez silného partnera by organizace takové studie byla obtížná. Proto v současné době ve spolupráci s univerzitním Centrem pro transfer technologií a znalostí jednáme s možným strategickým partnerem, který pomůže zavedení diagnostiky do klinické praxe," dodal Holčapek.

Metodika z Univerzity Pardubice by se podle klinické praxe využívala nejčastěji u tří skupin osob. Zvýšené riziko onemocnění rakovinou slinivky mají nově diagnostikovaní pacienti s cukrovkou 2. typu starší 50 let, lidé s dědičných výskytem onemocnění v rodině, a lidé, u kterých se projevily tzv. nespecifické symptomy, tedy příznaky neodpovídající určité nemoci.

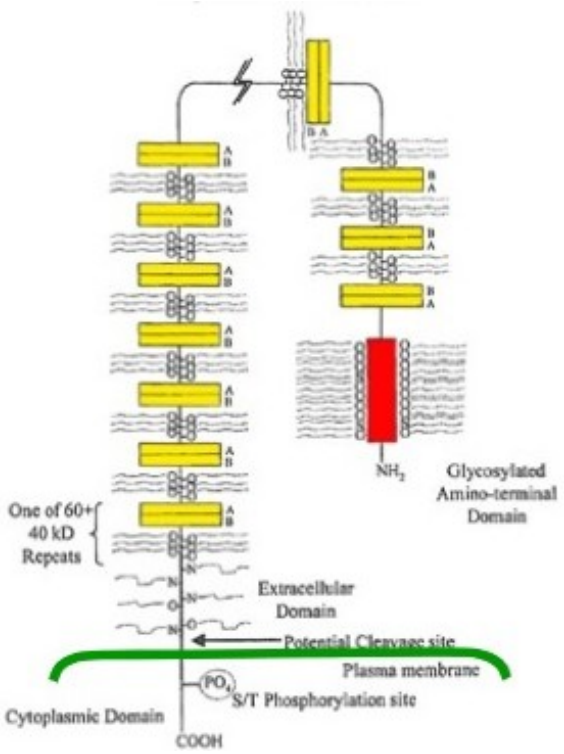
Jak zlepšit léčbu rakoviny? V Ostravě vymýšlejí, jak ji zlevnit a ušít lidem na míru
Rozhovor s Michalem Šimíčkem a Julim R. Bagóem z Blood Cancer Research Group při Lékařské fakultě Ostravské univerzity

Nechci se ohlédnout a vidět ztracený čas, říká biolog Marek Mráz
Seriál: Představujeme české držitele prestižních ERC grantů

Teorie her zvyšuje šanci na přežití metastatické rakoviny
Rozhovor s matematičkou Kateřinou Staňkovou o zefektivňování léčby onkologických pacientů.

Věda nezná hranice. A k tomu musíme vést i studenty
Rozhovor s novým rektorem Univerzity Pardubice Jiřím Málkem.

CA 125 (carbohydrate antigen 125)



- glycoprotein of the cornea and conjuctiva, the respiratory tract and the female reproductive tract epithelia of both fetuses and adults (lubricating barrier)

- ↑: ***ovarian, colorectal Ca**
  * endometrial, breast, pancreatic, liver and pulmonary Ca
  * pregnancy, breast milk
  * benign diseases of ovaries and endometrium, hepatitis, icterus, pancreatitis

- **marker for dg and monitoring of therapy of non-mucinous ovarian Ca; additional marker for pancreatic and colorectal Ca**

- **cut off ≤ 35 IU/ml**

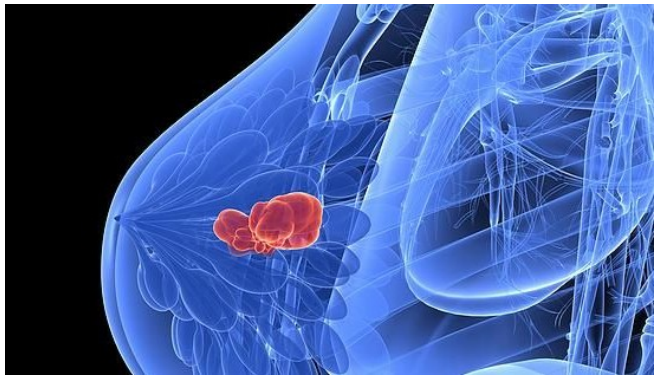
CA 15-3 (carbohydrate antigen 15-3)

- glycoprotein of fetal bronchial and hepatic cells, adult mammary cells

- **in adults ↑: pregnancy**
  rheumatic dis., chronic dis. of liver, stomach, pancreas, ovaries, uterus, prostatic gland, AIDS
  Ca of organs mentioned above
-
- **marker for breast Ca monitoring**

- **cut off ≤ 35 IU/ml**

CA 15-3 and CEA – 1st choice markers for breast carcinoma



- **The 2nd most common Ca in females**
- Incidence – 1 million of woman worldwide
- 90 - 95% sporadic
- 5 – 10% inherited - BCRA1 and 2 gene mutations – possibility of DNA testing from peripheral lymphocytes
- The lifetime risk of developing cancer for BRCA1/2 is 87%, in women without mutation 8-10%.

- Secondary prevention –mammography or ultrasound examination from 45 years of age

AFP (α1-fetoprotein)

- glycoprotein synthesized in large quantities by the fetal yolk sac and liver
- one of the major proteins in the fetal circulation

- **in adults AFP /S ↑:** **pregnancy**
  liver diseases

- **marker for hepatocellular and germ-cell carcinoma**
- **cut off value < 10 μg/l**

AFP (α1-fetoprotein)

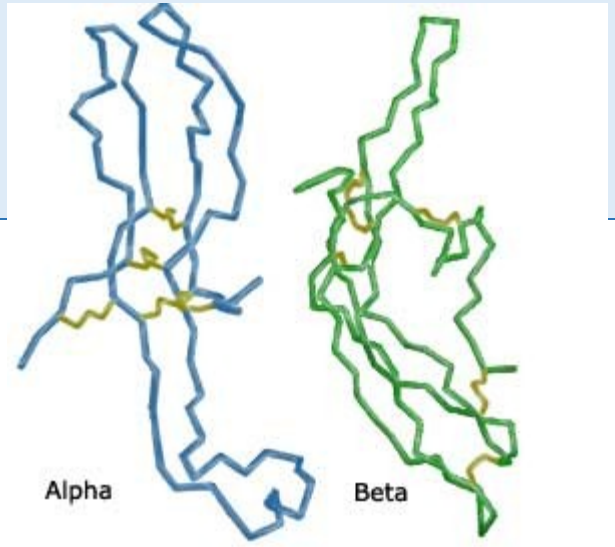
- **Sensitivity in selected Tu** (Klinická biochemie a metabolismus, 2009)

Tumor	Sensitivity / %
Hepatocellular Ca	80
Embryonal Tu	80
Teratoma	20
Yolk sac Tu	80

Oncoplacental antigens

- Substances produced by the trophoblastic cells of the placenta in both pregnancy and pathological conditions and also by germinative tumors as a mark of malignant dedifferentiation
- ↑ levels show evidence of ↑ malignancy and metastatic potency of the given tumor

- **hCG**



hCG subunits

- **SP-1**

hCG (human chorionic gonadotropin)

- glycoprotein secreted by the syncytiotrophoblastic cells of the placenta

α–subunit	**β –subunit**
common to several other hormones, e. g. FSH, LH or TSH	unique tohCG can be cleaved to urinary peptide β-core fragment

- ↑: **pregnant women**
  hydatidiform mole

- **marker for tumors of placenta (trophoblastic tumors, particularly choriocarcinoma), and germ-cell tumors of the testis and ovary**

- **cut off value < 2.00 IU/l males, < 10.00 IU/l females** (βhCG)

Enzymes

- present in much higher concentrations **inside cells**
- **released into circulation as the result of tumor necrosis or a change in the membrane permeability of the cancer cells**

• **PSA** • **ALP** • **NSE**	• **TK (thymidinkinase)** • **LD** • **kathepsins**

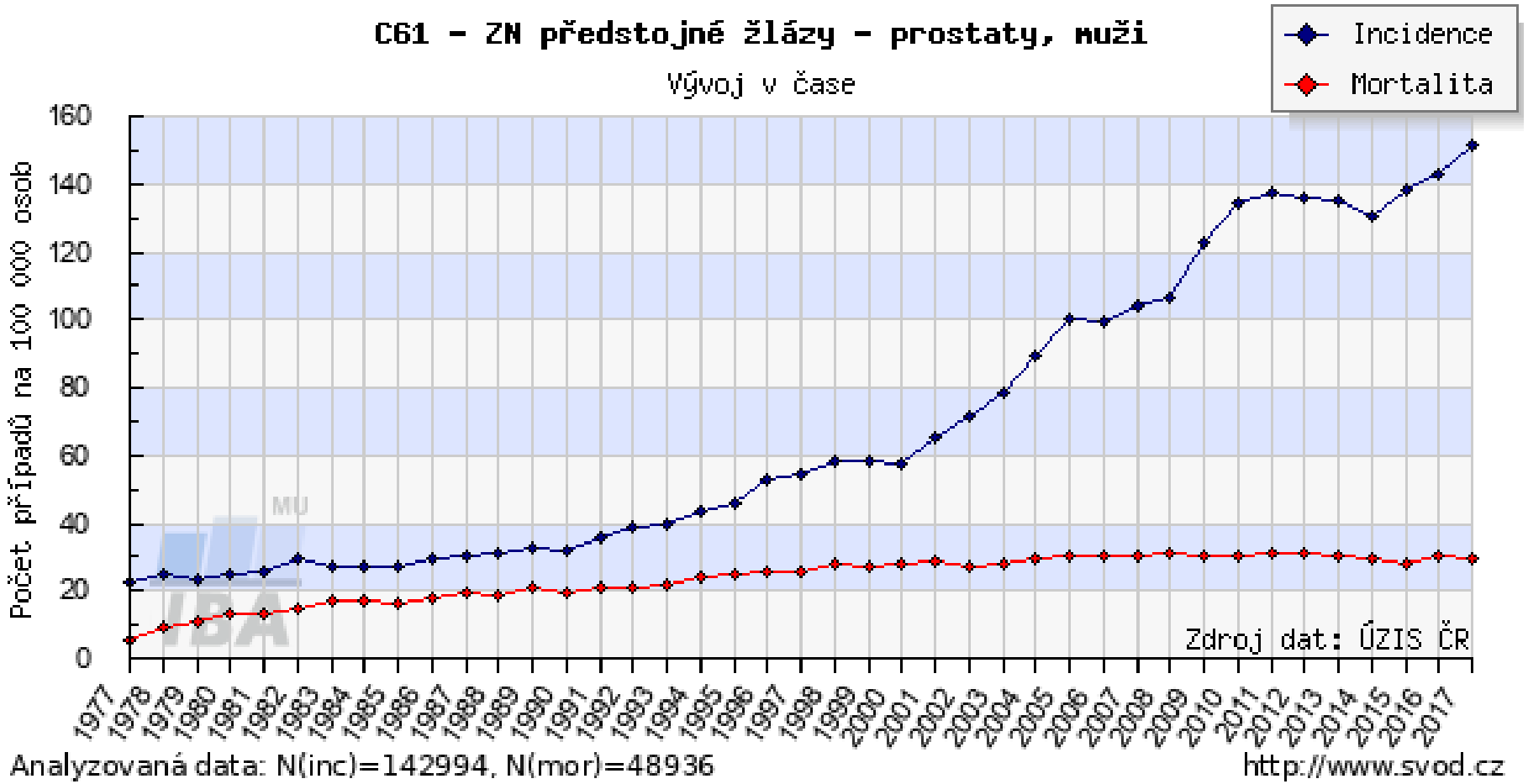
Prostate carcinoma

- 2nd most common malignancy in men, CR
- 7 305 new cases in 2016 (141 new cases/ 100 000 males)
- Risk factors:

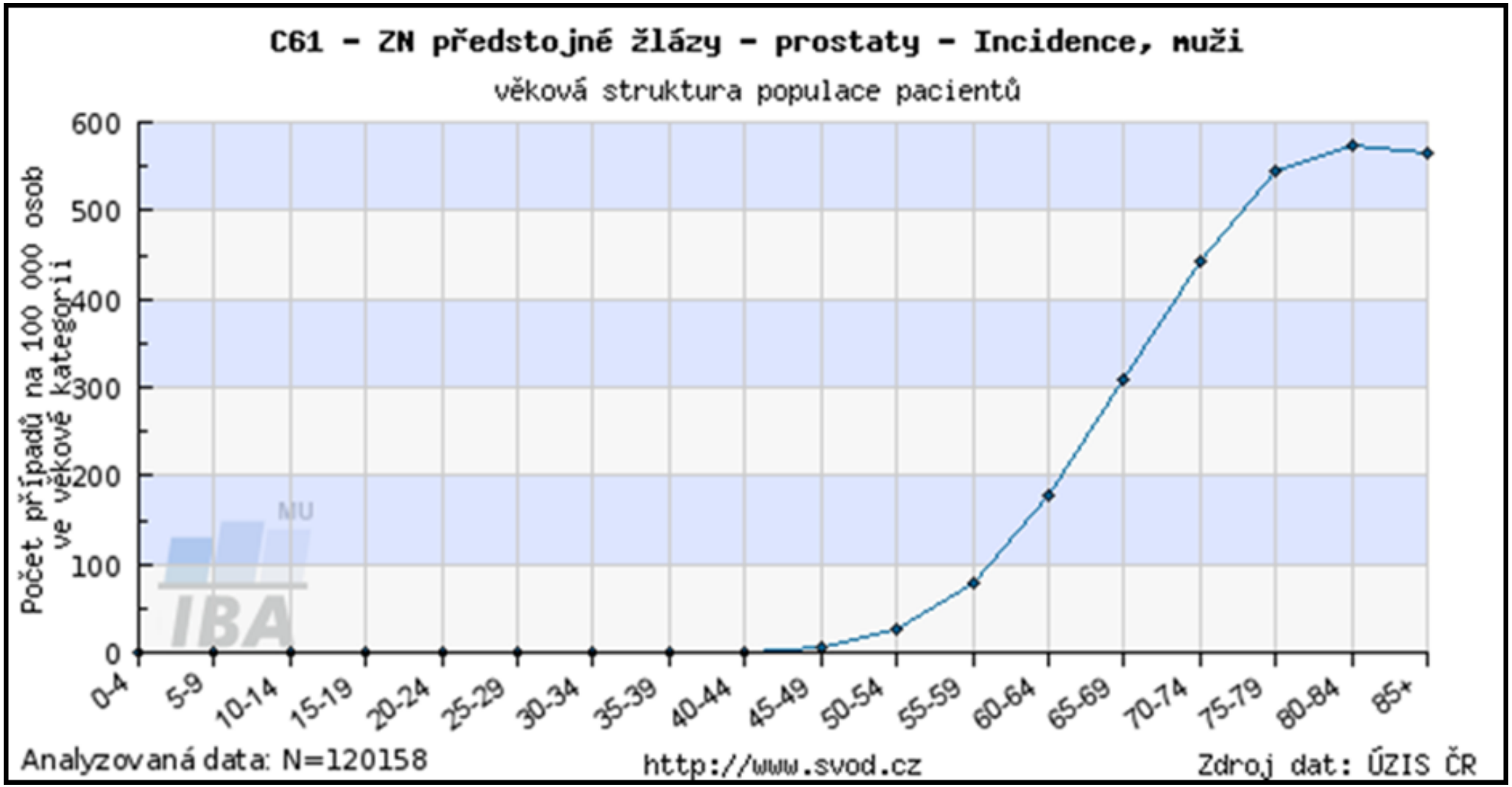
  age,

  life style,

  genetic factors



Epidemiology of prostate cancer



PSA (prostate-specific antigen)

- glykoprotein **protease** (237 AA, Mr = 33 000) **produced exlusively by the epitelial cells of the prostate gland, secreted into seminal fluid** (liquefaction).
- Produced as inactive proPSA → PSA.
- **In serum,** it occurs **as free fPSA and** α_1-antichymotrypsin or α_2-macroglobulin **bound** (55-95%).

- **↑: benign prostatic hyperplasia BPH, prostate infammation, urological manipulations**

- **marker for screening** (men > 50y, urinating difficulties), **dg and monitoring of course and treatment of prostate cancer**

- **cut off value < 4.0 µg/l (= ng/ml) (> 50 y), 2.5 µg/l (< 50 y,** see more in ***age specific levels***)

Increased levels of total PSA in plasma / serum

- **age specific levels:**

cut off	40-49 y. 2,5 ng/ml,	50-59 y. 3,5 ng/ml,
	60-69 y. 4,5 ng/ml,	70 and more y. 6,5 ng/ml

- tPSA > 10 ng/ml: very suspicious PCa, we perform another examinations
- tPSA 4 – 10 ng/ml: both PCa & BPH likely, we perform another examinations

Derived parameters

- ***index f/t PSA – free/total PSA:*** fPSA< 15%: probable PCa,
  fPSA > 20% probable benign condition
- ***tPSAD (tPSA density):***
- ratio [tPSA]/$_{UTS}$ prostate volume in cm^3
- adjustment of BPH and PCa: cut off 0.15 ng/ml
- ***PSAV (tPSA velocity):***
- increase of [tPSA] / year
- healthy 0.04 ng/ml/y, BPH 0.07-0.27 ng/ml/y, PCa ≥ 0.75 ng/ml/y
- ***tPSA doubling time:***
- time to double [tPSA]
- ***tPSA-TZ:***
- [tPSA] / transition zone volume

Other derived parameters

- **proPSA**
  - isoforms **(-2)proPSA** and (-4)proPSA typical for PCa, clinical significance(-2)proPSA

- **PHI (Prostate health index)**
  - $PHI = \frac{(-2)proPSA}{fPSA} . \sqrt{tPSA}$
  - higher specificity than fPSA/tPSA

Other causes of PSA increase in blood

- **Other prostate diseases:** benign prostate hyperplasia, prostatic inflammation
- **Mechanical stimulation** (fPSA is more susceptible): biopsy, cystoscopy, catetrization, per rectum examination
- Ejaculation
- PSA is a prostate-specific biochemical marker but is not specific for cancer.

ALP (alkaline phosphatase)

- **Zn^{2+} glycoprotein, in alkaline environment (pH= 8-10) it catalyses the hydrolysis of H_3PO_4 monoesters and transphosphorylation**

- **bone isoform (b-ALP)**
- **↑: osteoSa, bone metastases**
  other bone affections; growth
- **liver isoform (l-ALP)**
- **↑: liver metastases**
  other liver diseases

- **ref.values : adults 0.6-2.6 μkat/l, children and youth ≤ 8 μkat/l**

NSE (neuron-specific enolase)

- **enolase - enzyme of glycolysis** (2-phosphoglycerate → phosphoenolpyruvate)
- **NSE - form of enolase found in neuronal and neuroendocrine tissues**

- **↑: lung and liver dis., renal failure**

- **marker for small-cell lung cancer (SCLC), pheochromocytoma, medullary carcinoma of the thyroid, neuroblastoma, melanoma, and pancreatic endocrine tumors**

- **cut off value < 15 μg/l**

Hormones

The production of hormones in cancer involves two separate routes:

1. the endocrine tissue that normally produces the given hormone can produce its **excess amounts**
2. **ectopic syndrome -** hormone produced by a distant nonendocrine tissue that normally does not produce this hormone (for instance: ***ACTH*** normally produced by the **pituitary gland,** ectopicaly produced by the **lung small cells)**
   elevation of a hormone is not specific ← it may be produced by a variety of cancers

• **prolactin** • **calcitonin** • **PTH** 1.	• **ACTH** • **ADH** 2.

Serum proteins

produced either by tumor cells or by an organism in the presence of tumor	
• paraproteins 	• ferritin • β_2-microglobulin

Monoclonal immunoglobulins (paraproteins)

- **produced by neoplastic plasma cells in monoclonal gammapathies. In serum, we can identify whole Ig, heavy chains (IgG, M, A; D, E) and *κ, λ light chains (Bence Jones proteins)* -** these are small enough (22 kD) to pass through the kidney into the urine → **prerenal „over-flow“ proteinuria.**

- **↑: multiple myeloma and other monoclonal gammapathies,** lymphomas and leukemias, osteogenic sarcoma, bone metastases

- **marker for multiple myeloma and other monoclonal gammapathies**

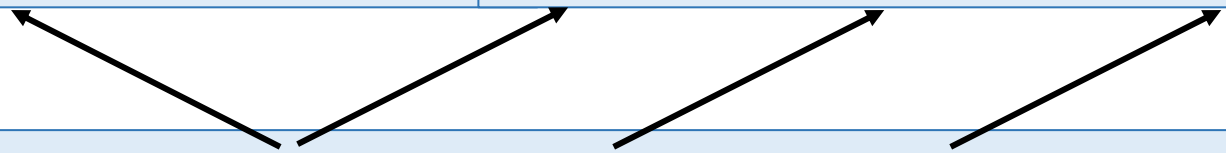
- **ref. values: FLC (free light chains)/S:** κ = 3.3-19.4 mg/l, λ = 5.7-26.3 mg/l, index κ/λ = 0.26-1.65;
  FLC/U = 1-10 mg/24h; κ/U = 1.25-5.5 mg/l, λ/U = 0.51-3.2 mg/l, index κ/λ = 0.82-3.0

Receptors

Cellular (tissue) markers

- Estrogen rec.
- Progesterone rec.

- Growth factors receptors (HER1, HER2/neu)
- DNA aneuploidy



The main usage: breast Ca, colorectal Ca; brain tumors

Estrogen and progesterone receptors

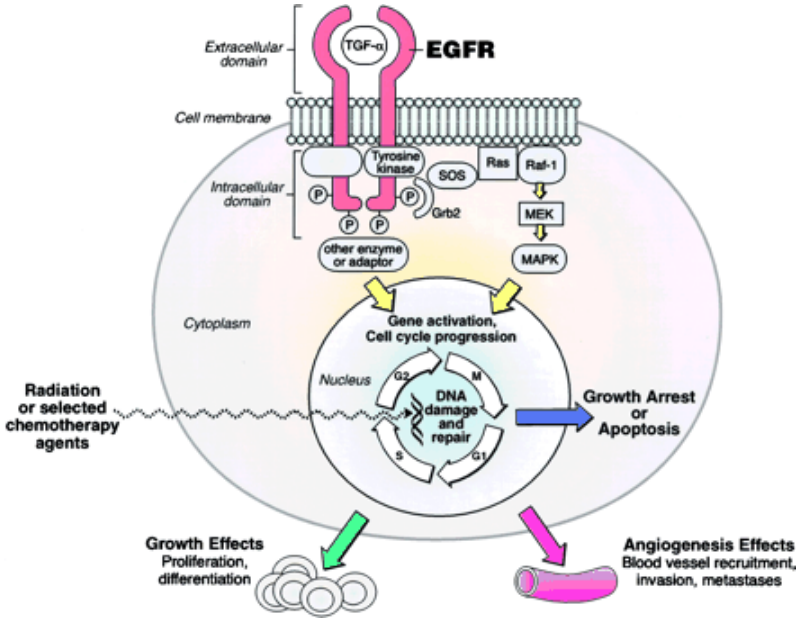
- **The most important prognostic markers for breast Ca;** detected in tumor tissue.

- **positivity =** ↑ cell diferentiation, ↓ invasivity, **better prognosis;** = antiestrogen therapy indication

- **immunohistochemical determination - ELISA**

Growth factors receptors

- Transmembrane receptors with tyrosinkinase activity – phosphorylation of Tyr residues of protein substrates
- The binding of substrate to the extracellular domain causes a conformational change of the receptor, its autophosphorylation and activation of downstream signaling pathways → influence of cel. proliferation, inhibition of apoptosis
- **HER1 (EGFR), HER2/neu,** HER3, HER4



Growth factors receptors

Type of receptor	HER1	HER2/neu*
Ligands	EGF, TGFα, amphiregulin, betacelulin, epigene, epiregulin	?
Blocked by	Monoclonal AB - tyrosin kinase inhibitors EGFR TKI (cetuximab, erlotinib, gefitinib)	Monoclonal AB (trastuzumab)

* Nomenclature: HER 2 in humans, neu in rodents

Other tumor markers

substances, which we cannot class with the previously mentioned groups

- Ki-67
- TPA, TPS
- CYFRA 21-1
- HE4

- Mesothelin
- Chromogranin A
- Neuropeptide Y
- S-100 β
- 5-hydroxyindolacetic acid

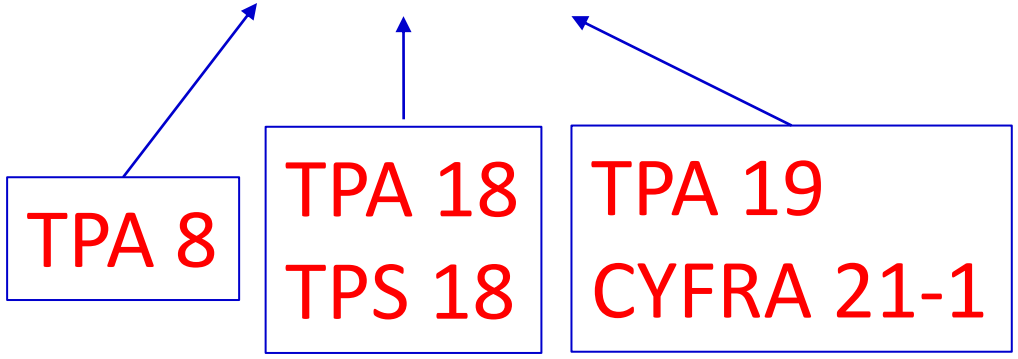
Proliferative antigen Ki-67

- The non-histone nuclear protein expressed during active cell cycle phases (max at the G2 interface and mitosis, is absent in the G0 phase).
- It affects the spatial layout of chromatin - gene expression control.
- Immunohistochemistry detection in biopsy tissue - Anti-Ki-67 antibody.
- Ki-67 expression = proliferative tumor activity.
- Proliferative activity in cancer correlates with grade and prognosis.

= prognostic marker determined in tumor tissue of solid tumors

Cytokeratins and their fragments

- **Cytokeratins** = keratins found in the intracytoplasmic cytoskeloeton of epithelial cells.
- Clinical significance: fragments of (cyto)keratins No. 8, 18, 19



TPA, TPS (tissue polypeptide (specific) antigen)

- non-specific cytokeratins fragments produced by both normal and tumor cells
- ↑ **levels seen in increased cell proliferation** → its estimation is useful for **monitoring of the disease**

- ↑: **liver dis., DM, rheumatoid dis. breast and GIT tumors**

- **marker for urinary bladder carcinoma**

- cut off value ≤ 140 IU/l

Breast carcinoma markers - summary

- Basic markers: CEA and CA 15-3
- Receptor markers:
  - Growth factors receptors
  - Estrogene receptor
  - Progesterone receptor
- Proliferative antigen Ki-67
- Other markers: TPA

CYFRA 21-1 (cytokeratin fragment)

- **Cytokeratin 19 fragment present in lung, uterine and GIT cells. Marker of degradation of malignant tissues and necrosis.**
- **↑: cirrhosis, asthma, respiratory infections, renal failure**
- **marker for cervical and pulmonary (NSCLC) carcinoma**
- **cut off value ≤ 3.3 μg/l**

CYFRA 21-1

- **Sensitivity in selected Tu** (Klinická biochemie a metabolismus, 2009)

Tumor type	Sensitivity / %
epidermoid pulmonary Ca	55
big-cell pulmonary Ca	35
pulmonary adenoCa	28
urinary bladder Ca	30

Basic tumor markers – lungs, bronchi, trachea, pleura

Non-parvicellular Ca (NSCLC)

CEA

CYFRA 21-1

HER1

Parvicellular Ca (SCLC)

NSE

CYFRA 21 -1

Mesothelioma

Mesothelin

Ovarian tumor markers

Non-mucinous Ca

CA 125

HE4

Mucinous Ca

CA 19-9

CA 72-4

Germinative Ca

TPA/TPS

CEA

hCG

HE4 – Human Epidydimal Protein 4

- in clinical practice since 2014 (CR)
- glycoprotein, protease inhibitor with antimicrobial and antiinflammatory effects, first found in epidydimal epithelium (probable role in sperm maturation)
- specificity for ovarian Ca 92%, sensitivity 70-80%
- **level increases as soon as in I. and II. stage of the disease, even in patients with non-elevated CA 125**
- cut off = 80 pmol/l in postmenopausal women
  50 pmol/l in premenopausal women

HE4 in clinical practice

- suitable for monitoring the effectiveness of anticancer treatment
- can not be used alone but always as part of other investigation method:
- **combination with CA 125**

ROMA score (Risk of Ovarian Malignancy Algorithm)

- since 2010
- **predictive index** - risk assessment of ovarian carcinoma in the presence of resistance of unclear nature in a small pelvis
  The high risk of epithelial ovarian Ca is indicated by the ROMA score ≥ 11.4% in premenopausal and ≥ 29.9% in postmenopausal women.

Markers for dg and monitoring of bone metastases

Bone metastases: tumors of lungs, prostate, breast

Monoclonal gamapathies

New bone formation markers

Usage: monitoring the effect of treatment on osteoblastic metastases

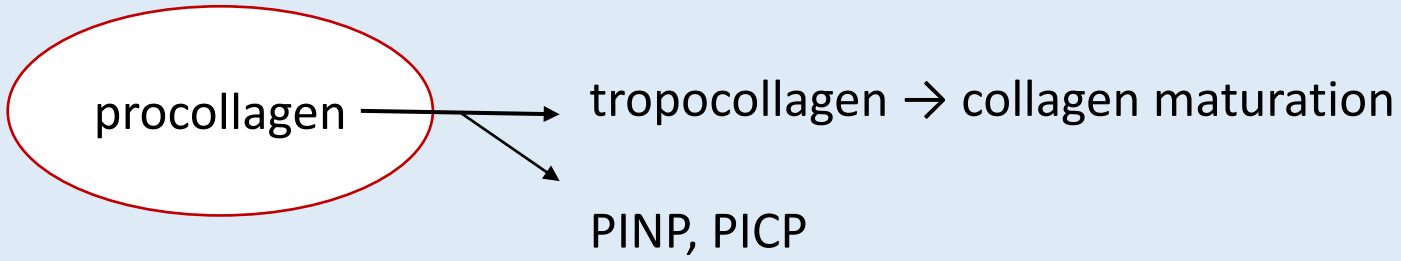
Bone resorption markers

Usage: dg bone mass distribution of solid tumors (PCa), monitoring the effect of antiresorptive treatment

Markers in bone metastases

Bone formation markers

- **PINP (N-terminal propeptide of type I procollagen)**



- **Osteocalcin -** serum levels are proportional to its formation in osteoblasts.

- **Bone ALP –** bone izoform of ALP, serum levels are proportional to osteoblasts activity

Markers in bone metastases

Bone resorption markers

- **ICTP (C-telopeptide of type I collagen):** marker of collagen degradation by action of MMP 9
- **CTX-I (β-CTX β-Cross Laps, C-terminal telopeptide of type I collagen):** marker of collagen degradation by action of enzymes from osteoclasts