

## The role of molecular predictors of the diagnosis and treatment in women with gynecological cancer

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### Abstract

*Tumor markers are substances that can be found in the body when cancer is present. The classic tumor marker is a protein that can be found in the blood in higher than normal amounts when a certain type of cancer is present, but not all tumor markers are like that. Some are found in urine or other body fluid, and others are found in tumors and other tissue. They may be made by the cancer cells themselves, or by the body in response to cancer or other conditions. The paper presents the current state of research for identification of clinically useful molecular markers of cancer of the reproductive system of women. Overview of all molecular indicators would have been no possible, so focus on those that seem to be the most promising and have a chance to clinical application.*

### 1. Introduction

Prevention is better than cure, so it is important to detect cancer in advance or at an early stage. The tests which are used to consist in the determination of the level of tumor antigens or tumor markers in the blood [1].

Gynecologic cancer is a group of cancers that affect the tissue and organs of the female reproductive system. Each type of cancer is named after the organ it originates. Types of gynecologic cancer include: cervical cancer (begins in the cervix, which is the lower, narrow end of the uterus), ovarian cancer (begins in the ovaries, which are located on each side of the uterus), uterine cancer (begins in the uterus, the pear-shaped organ in a woman's pelvis where the baby grows when a woman is pregnant), vaginal cancer (begins in the vagina, which is the hollow, tube-like channel between the bottom of the uterus and the outside of the body), vulvar cancer (begins in the vulva, the outer part of the female genital organs) [2].

Each gynecologic cancer is unique, with different signs and symptoms, different risk factors (things that may increase your chance of getting a disease), and different prevention strategies. All women are at risk for gynecologic cancers, and risk increases with age. When gynecologic cancers are found early, treatment is most effective [3].

In this paper we will present the current state of research on the identification of clinically useful molecular markers of cancer of the female reproductive. Overview of all molecular indicators would have been no possible or appropriate, therefore, focuses on those that seem to be the most promising and have a chance to clinical application.

## **2. Molecular, predictive and prognostic indicators in cancer**

Molecular prognostic indicators in cancer is defined as substances present in body fluids or associated with the surface of the tumor cells. Their identification and measurement are useful in the diagnosis of patients and treatment planning. Ideal marker characteristics are: high sensitivity and specificity, the predictive value and the presence in tissues or body fluids in concentrations proportional to the stage of the disease.

Molecular indicators are quantitatively related to the size of the tumor, they may be held in tumor tissue and in the serum of patients with cancer [4].

The importance of treatment of cancer molecular ratios differ depending on the substance that is being considered. We can distinguish some of their uses:

- a) determining the risk, by estimating the probability of an individual developing cancer,
- b) screening tests, which are used in large-scale programs of prevention and early detection of cancer,
- c) differential diagnosis, tissue specificity substance that helps to identify the disease,
- d) predicting the effects of therapy, by detecting potential therapeutic targets,
- e) forecast to determine the chances of a cure and the estimated life expectancy,
- f) monitoring of the patient's condition, such as sudden concentration of the evidence such as the progress of disease or risk of recurrence.

Effective diagnostic tool used in medicine is the modern technique of molecular biology. In recent years, its importance in clinic female reproductive system cancers (cervical and endometrial, ovarian, vulva) [5].

## **3. Ovarian cancer**

Is the most common cancer in women. This represents 1/3 of malignant tumors of female sexual organs and is the leading cause of female mortality due to these cancers. Despite the development of medicine, improvements in surgical techniques and the introduction of new chemotherapy, the protocols of five-year survival rate in the world has not fundamentally changed, and concerns in group of approximately 30-50% of patients in all stages of clinical disease. The main reason for this is the late diagnosis of the disease, because 75% of ovarian cancers are diagnosed in advanced stages [6].

At present, the primary biochemical marker of ovarian cancer is antigen CA 125, which is a membrane glycoprotein present on the surface of many types of epithelial cells. It includes, for markers of differentiation and it is useful in monitoring an effective therapy for patients with epithelial ovarian cancer, and for the detection of ovarian cancer in patients who are treated in a first phase.

The usefulness of this antigen has also been found in the diagnosis of cervical cancer [6].

#### 4. Cervical cancer

Is the second most common cancer that affects women and the second leading cause of death. In most cases, this type of cancer develops between 35 and 59 years of age. In cancer of the cervix is applicable determination of circulating markers: mainly SCC-Ag (*squamous cell carcinoma antigen-squamous cell carcinoma*) and less CYFRA 21-1, CEA (*carcino-embryonic antigen*), CA 125 and TPS (*tissue polypeptide antigen-specific tissue polypeptide specific antigen*) - although their usefulness in various stages of cancer has a different value. Rarely used markers for cervical carcinoma are cytokines [7].

SCC-Ag antigen belongs to the family of serine proteases, is one of the fractions tumor-associated antigen, is released from the cancer cells into the circulation - the standard serum in healthy women is 1.5-2.0 ng / ml. SCC-Ag is considered the marker of choice for cervical cancer, as in 20-70% of patients determined to have been elevated concentration, which is dependent on the degree of differentiation of cancer and the severity of the disease.

CYFRA 21-1 is a fragment of cytokeratin 19. Norm for healthy people does not exceed 2.5 ng / ml and higher concentrations of the marker observed in patients with-small cell lung cancer, head and neck cancers, and in 35-70% of patients with cervical cancer. It is assumed that the marker has a prognostic value in cancer of the cervix, but is no more useful for monitoring disease than SCC [8].

The higher concentration of carcinoembryonic antigen (CEA) are found in cancers of the colon and liver cancer. In cervical cancer higher concentration are found in 27-80% of patients in cancer adenocarcinoma. The effectiveness of the marker indications associated with detection of cervical cancer recurrence and metastases to the liver and lungs.

Although higher concentrations of the marker CA 125 is associated with a number of malignancies, but is primarily useful in the diagnosis and monitoring of ovarian cancer, and in 22% of patients with cervical cancer concentration of CA 125 is also increased.

Tissue polypeptide specific antigen - TPS, a part of TPA (*tissue polypeptide antigen*). In healthy people, the antigen concentration does not exceed 100 U/l. Its concentration is elevated in malignancies of different locations, but its sensitivity is lower than the SCC-Ag [9]. It is recommended that the simultaneous determination of TPS and SCC in evaluating the effectiveness of cancer treatment, especially with the use of radiation - the results of this marker is characterized by significant differences [9].

#### 5. Endometrial cancer

Is quite frequent cancer in women in Poland. Morbidity relates mainly to women over the age of menopause.

Endometrial cancer is the most common type of uterine cancer. Although the exact cause of endometrial cancer is unknown, increased levels of estrogen appear to play a role. Estrogen helps stimulate the buildup of the lining of the uterus. Studies have shown that high levels of estrogen in animals result in excessive endometrial growth and cancer. Most cases of endometrial cancer occur between the ages of 60 and 70 years, but a few cases may occur before age 40 [10].

Factors that may contribute to the growth of developing this type of cancer is:

- a) Diabetes
- b) Estrogen replacement therapy without the use of progesterone

- c) History of endometrial polyps
- d) Infertility (inability to become pregnant)
- e) Infrequent periods
- f) Tamoxifen, a drug for breast cancer treatment
- g) Never being pregnant
- h) Obesity
- i) Polycystic ovarian syndrome (PCOS)
- j) Starting menstruation at an early age (before age 12)
- k) Starting menopause after age 50

There is no effective screening test for endometrial (uterine) cancer. Women with any risk factors for endometrial cancer should be followed closely by their doctors. Frequent pelvic examinations and screening tests such as a Pap smear and endometrial biopsy may be considered in some cases. Use of birth control pills for over a year reduces the risk of endometrial cancer. Women who are taking estrogen replacement therapy without progesterone therapy or who have taken tamoxifen for more than 2 years have an increased risk of endometrial cancer and should have regular pelvic examinations and Pap smears.

In the best known markers of developing the cancer appears to be a glycoprotein antigen CA125, which is considered crucial for the diagnosis and monitoring of ovarian cancer, but it is also interesting that the elevated levels of the antigen CA125 in the serum is accompanied by a primary and recurrent endometrial cancers [10].

Another marker of cancer is antigen CA 19.9, which is currently used in the diagnosis of tumors of the gastrointestinal tract cells. Its function as an independent marker in monitoring the clinical course of endometrial cancer is limited. Correlating the value of the level of other markers, such as CA125 has a high sensitivity (83.3%) in the detection of cancer recurrence after surgery. From the point of view of clinical practice in patients with endometrial cancer seems to be interesting also serial determination of the levels of CA 15.3, originally used as a marker of ongoing cancer in the breast. The concentration of the marker has a high correlation with tumor stage. Increasing the concentration of CA 15.3 is associated with the spread of cancer outside the uterus. The higher levels of this marker was found in 28.1% of cases of cancer of the uterus only, while the spread of the disease outside the uterus gave the increase of CA 15.3 in 56.2% of cases.

CA 72.4 antigen for the diagnosis of endometrial cancer seems to be quite interesting, this study indicates that said elevated levels of serum antigen in 31.9% of patients with endometrial cancer and has been shown to correlate with the presence of a tumor in the appendages. A very important observation was also the statement in 10% of patients, elevated levels of CA 72.4, without an increase in the concentration of markers such as CA 125 and CA 19.9 [11].

Metalloproteinase 2 (gelatinase 2) - is a proteolytic enzyme, which is the most active to IV collagen type, which is the main component of the extracellular matrix. . Increased activity of this enzyme promotes a more rapid degradation of extracellular matrix components, allowing infiltration surrounding the tumor cells and stimulates their ability to form metastases.

It can be stated that the determination of the concentration of MMP-2 in serum may increase the sensitivity and specificity of the detection of recurrent disease due to the suitability of MMP-2 in monitoring the clinical course of several tumors of epithelial and glandular, including endometrial cancer [12].

## 6. Vulvar cancer

Vulvar cancer is a rare type of cancer. It forms in a woman's external genitals, called the vulva. The cancer usually develops slowly over several years. First, precancerous cells grow on vulvar skin. This is called vulvar intraepithelial neoplasia (VIN), or dysplasia. Not all VIN cases turn into cancer, but it is best to treat it early [13].

Often, vulvar cancer doesn't cause early symptoms. However, see your doctor for testing if you notice:

- A lump in the vulva
- Vulvar itching or tenderness
- Bleeding that is not your period

Being older and having a human papillomavirus infection are risk factors for vulvar cancer. Treatment varies, depending on your overall health and how advanced the cancer is. It might include laser therapy, surgery, radiation or chemotherapy [14].

As a non-specific marker of vulvar cancer can be  $\beta$ -hCG. This marker does not specify in this case of cancer. The research focused on vulvar cancer, found elevated levels of SCC-Ag. Prognostic markers for vulvar cancer also can be the expression of caspase-3 (no expression correlates with poor prognosis) and the expression of cyclooxygenase-2 (overexpression correlates with poor prognosis) [15].

## 7. Types of detections methods

Molecular diagnostics should be one of the most rapidly developing fields of biology and medicine. The impulse for the development of diagnostic methods in oncology is to seek to identify cancers at an early stage of the disease, therefore, besides the development of cancer imaging techniques are also developing molecular methods which include for example: PCR (*Polymerase chain reaction*) and ELISA (*enzyme-linked immunosorbent assay*) [16].

The polymerase chain reaction (PCR) is a biochemical technology in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. The advantages of PCR are :high sensitivity, only very small amount of DNA, which may come with any type of biological material and the short duration of the study. In this way it is possible to find a mutant cells that separated from tumor tissue in an early stage of its development, and get into the fluid secretions of fluids. This method is a simple method to demonstrate the presence of the mutation-specific tumor type. The disadvantage of this method is the possibility to obtain false positive results (for micro-pollutants) or negative (by the use of incorrect primer [17].

Amplification of the genetic material by PCR is also an output stage for a number of other test methods used in the diagnosis of cancer such as: RT-PCR, RFLP-PCR, Multiplex PCR, Asymmetric PCR, ASA PCR etc.

These techniques used in DNA sequencing, mutation detection, hybridization in situ identification of pathogens.

The enzyme-linked immunosorbent assay (ELISA) is a test that uses antibodies and color change to identify a substance. ELISA is a popular format of a "wet-lab" type analytic biochemistry assay that uses a solid-phase enzyme immunoassay (EIA) to detect the presence of a substance, usually an antigen, in a liquid sample or wet sample.

ELISA assays are performed on a standard polystyrene or Plexiglas, 96-well plates, consisting of 8 rows and 12 columns. All of the analyzed sample are located in separate wells. The plate coated with an antigen or an antibody. Connecting to antigen specific antibody (antigen-antibody reaction) reveals the color reaction, occurring through appropriate enzymes. The most commonly used enzymes are: Alkaline phosphatase (turns colorless p-nitrophenol phosphate in yellow p-nitrophenol), Horseradish peroxidase (have a blue color in the presence of tetramethylbenzidine), Glucose oxidase (from 5-aminosalicylic acid, giving a brown color) [18].

Changing the color of the solution is measured spectrophotometrically, and the result is compared to the controls forming a so-called. calibration curve.

The most common types of ELISA tests are: direct ELISA, indirect ELISA and sandwich ELISA.

ELISA is certainly one of the most important enzyme immunoassay techniques. Its advantages are high sensitivity, high throughput, the ability pólautomatyzacji, and above all a quantitative result. ELISA in routine diagnosis has been used primarily in medical microbiology, veterinary and diagnostics (detection and monitoring of treatment of pathogens), immunology and serology (detection of antibodies and autoantibodies), toxicology, oncology, and in the industry (strain types, detect fungal contamination of food products), and agriculture [19].

The development of a variety of molecular genetic techniques, enabling a more accurate analysis of nucleic acids is very important in modern medicine. Becoming more widely used in routine diagnostics are Cytogenetics and DNA analysis [20].

The diagnostic methods allow, in many cases, early diagnosis of the presence of cancer cells, determining the flow of disease, and many - choosing the most effective type of therapy.

## **8. Conclusions**

Laboratory diagnostic which are based on molecular biology, can be a valuable addition to basic research. Their constant modification and improvement would lead to a more precise determination extent of surgery, early detection of local recurrence and distant, and the implementation of appropriate treatment, chosen for the type of cancer .

The markers are important to find cancer early and be able to follow it during or after treatment, researchers are looking for new and better tumor markers. But as doctors have learned more about cancer, they've found that the level of a single protein or other substance in the blood may not be the best marker for the disease. They are looking for better ways to find and follow the course of different cancers.

We believe that in the near future, the factors are found that meet the criteria of markers that are perfect predictors of cancer.

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