# Activity of telomerase in ovarian cancer cells. Clinical implications

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**Summary**

Estimation of telomerase activity in cell nuclei of ovarian malignant tumours may provide an independent prognostic index. The test for telomerase activity in tumour cell nuclei may be accepted as a useful diagnostic test with application for differential diagnoses of benign ovarian tumours vs tumours of a borderline or malignant character.

*Key words:* Ovarian cancer; Telomerase; Telomeres.

## Introduction

Ovarian cancer accounts for 4% of all female tumours and represents one of main causes of death due to female genital tumours [1]. In 2004 in Poland the malignancy was the sixth most important tumour if morbidity was considered and the fourth if tumour-induced mortality of women was considered. In the year ovarian cancer developed in 364 women and resulted in death of 2,273 women. The standardised coefficient of its incidence was 10.93 per 100,000 women [2]. Comparable values of the standardised coefficient of its incidence are also detected in highly developed countries such as the United States of North America (13.5 of new cases per 100,000 females annually), Australia (12 new cases per 100,000 females annually), Canada (12 new cases per 100,000 females annually) [3, 4]. Despite the increasing trend of incidence in the disease a growing percentage of 5-year survival is observed, linked both to growing health awareness of patients and to improved detectability of the tumours. The search continues for new tumour markers which would allow for a rapid and reliable diagnosis of ovarian cancer.

In recent years an increased interest in telomeres and telomerase has been seen. Muller and McClintock were the first to isolate redundant sequences of purine and pyrimidine bases at the ends of chromosomes in eukaryotic cells and they termed them telomeres (Greek: telos = end; *meros* = part) [5, 6]. It was not until the 1980s that human telomeres were found to be formed by double-stranded 5' TTAGGG 3' sequences, rich in guanine single-stranded sequences, in TRF 1, TRF 2, Rap 1p, sir proteins, in Cdc 13p and Est 1p [7]. The number of telomere repeats varies among species and in humans it amounts to around 2000. The number decreases with the unavoidable process of cellular senescence. Shortening of the telomeres provides a specific type of clock of cellular senescence, counting down a specific score of cell divisions before the cell stops dividing in the resting phase [8]. The repetitive telomeric sequences are most numerous in young dividing cells, i.e., in germinal, foetal cells, in the stratum basale of epidermis, and in haemopoetic as well as neoplastic cells.

The role of telomeres involves protection of chromosomes from activity of exonucleases and, thus, maintenance of genomes in an intact form. In the absence of telomeres random recombinations might lead to various pathological processes. Telomeres function as stabilizers of chromosomal structure and in this way they prevent formation of abnormal ring and centromeric forms in terminal segments of chromatin [9].

In cases of neoplastic cells telomere length depends on the activity of telomerase, the enzyme capable of telomere synthesis. The enzyme was isolated for the first time by Greider in 1985 while four years later Morin described it in human neoplastic cells, linking the fact with cell immortality [10, 11]. In 1997 Meyerson *et al.* [12] cloned the gene of telomerase reverse transcriptase while Bodnar with his research team introduced it to human cells and demonstrated that this was followed by augmented synthesis of telomerase and the cells acquired neoplastic traits [13].

Telomerase consists of template RNA *(TR)* formed by approximately 395-450 base pairs, the catalytic unit of reverse transcriptase *(hTERT),* and by TP1, p23, HSP 90 proteins. The extent of TP1 expression manifests a significant correlation with telomerase activity in neoplastic cells although factors which induce cell differentiation (retinoic acid, phorbol esters) decrease expression of hTERT but exert no effect on TP1 expression.

In 1994 Counter *et al.* [14] in their studies on neoplastic cells isolated from ascites sampled from patients with advanced ovarian cancer discovered the potential for detection of telomerase activity in cells *in vivo*. The authors demonstrated that short stable telomere sequences

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were present in cells of ovarian cancer but not in cells of ovarian surface epithelium. Such observations pertained also to the activity of telomerase, which was markedly higher in cells fractionated from ascites of patients with ovarian cancer than in cells of healthy women.

High interest in telomerase is linked to its involvement in processes of cell senescence and neoplasia [15-17]. In tumour cells telomerase prevents shortening of telomeres. It is assumed to be capable of influencing the neoplastic process: its activity is supposed to appear when the cells fully lose control over cell proliferation [18].

## Materials and Methods

Our studies were conducted on a group of 34 patients treated for ovarian cancer. The mean age of the patients was 57.4 years (the youngest patient was 33 years old and the oldest was 78 years old).

The patients were qualified for the studies when ovarian cancer was histologically confirmed, the patient carried no other coexisting malignancies in the past, was subjected to primary surgical treatment according to standard medical procedures defined for ovarian cancer and to first lapse chemotherapy in line with therapeutic standards based on *paclitaxel* and platinum derivatives. On the day when the observation ended all patients completed their first lapse therapy.

The studies aimed at evaluating telomerase activity in tumour cell nuclei in patients treated for ovarian cancer and examining the relationships between the obtained results and clinical variables (extent of tumour clinical advancement, grade of cell differentiation, histological type of the tumour, size of tumour remains following primary surgery, presence of ascites, results of treatment following primary surgery and first lapse chemotherapy, results of *second-look* operation, relapse of the tumour and death due to ovarian cancer). The relation was also examined between activity of telomerase in ovarian cancer cell nuclei and concentration of CA 125 tumour marker, determined at four time points and presence of mutations in the *BRCA1* gene in patients. Activity of telomerase in tumour cell nuclei was also examined in five patients treated for marginal forms of ovarian cancer (mean age of the patients: 48.4 years; the youngest patient was 34 years old and the oldest 76 years old) and in a group of the same size including benign ovarian tumours, such as endometrial cysts (mean age of the patients: 36 years; the youngest patient was 23 years old and the oldest 50 years old).

Telomerase activity was estimated using in situ hybridization with DNA/RNA probe (Telomere PNA FISH Kit/FITC Code No K5325, DAKO Polska Sp. z o.o.).

## Results

Activity of telomerase was demonstrated in cancer cell nuclei in 24 patients. Taking into account telomerase activity in tumour cell nuclei the patients were recruited to three groups. Patients in group 1 manifested no telomerase activity in cell nuclei (n = 10 patients), patients in group 2 showed telomerase activity in less than ten cell nuclei per visual field of a microscope (n = 17 patients), patients in group 3 manifested telomerase activity in 10-50 cell nuclei per microscope field (n = 7 patients). Telomerase activity was demonstrated in two patients with a marginal form of ovarian cancer (in one patient the activity was detected in 10 tumour cell nuclei and in the other in 10-50 tumour cell nuclei per microscope field), while in three patients with this form of ovarian cancer no telomerase activity could be shown in tumour cell nuclei. In none of the five patients with endometrial cysts could activity of the enzyme be demonstrated in cell nuclei of cells in the cyst wall.

In 27 patients with ovarian cancer an elevated level of CA 125 marker was documented. The mean value of the marker level, tested at four time points amounted to:

1) 1301.0 U/ml before the primary surgery (minimum value of 35.8 U/ml/maximum value of 4875.6 U/ml);

2) 778.9 U/ml before the first course of chemotherapy, administered up to ten days following the surgery (minimum value of 3.9 U/ml/maximum value of 4875.6 U/ml);

3) 28.9 U/ml before the fourth course of chemotherapy (minimum value of 3.2 U/ml/maximum value of 119.4 U/ml);

4) 18.1 U/ml following the last course of chemotherapy (in a single case the patient received just four courses of chemotherapy and in three cases the patients received five courses of chemotherapy, thus the estimation was conducted following the fourth and following the fifth chemotherapy course, respectively) (minimum value of 2.2 U/ml/maximum value of 132.8 U/ml).

Mutations in *BRCA1* gene were detected in 20 patients, including two patients with 5382*insC*.

A detailed analysis of data resulted in the following conclusions:

1) Activity of telomerase in tumour cell nuclei could be detected more frequently in patients treated for ovarian cancer in high stages of clinical advancement (FIGO Stages II and IV), of low tumour cell differentiation (G2, G3) and of serous adenocarcinoma type;

2) Augmented activity of telomerase was detected in tumour cell nuclei in patients treated for ovarian cancer with remnants of tumour masses below 1 cm following the primary operation and with presence of ascites detected during the surgery;

3) No relationship could be detected between activity of telomerase in tumour cell nuclei in patients treated for ovarian cancer, and results of treatment following primary surgery and a standard first lapse chemotherapy.

Estimation of telomerase activity in cell nuclei of ovarian malignant tumours may provide an independent prognostic index. Elevated activity of telomerase develops in patients with a higher number of tumour relapses and in patients who die due to ovarian cancer. A markedly higher probability of survival is manifested by patients demonstrating no telomerase activity in cancer cell nuclei as compared to patients with detectable activity of the enzyme. This has been confirmed by statistical analysis using the survival probability test of Kaplan-Meier ($p$ = 0.015) (Figure 1).

The test for telomerase activity in tumour cell nuclei may be accepted as a useful diagnostic test with application for differential diagnoses of benign ovarian tumours vs tumours of a borderline or malignant character. Coex-

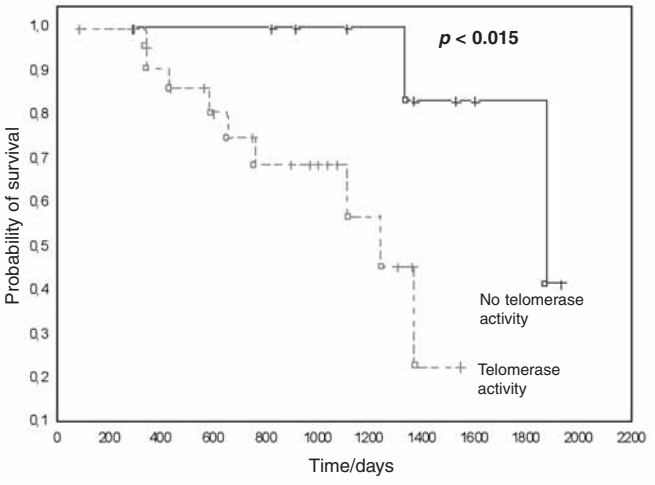


Figure 1. — Probability of survival in patients demonstrating no telomerase activity in cancer cell nuclei in comparison to patients with detectable activity of the enzyme.

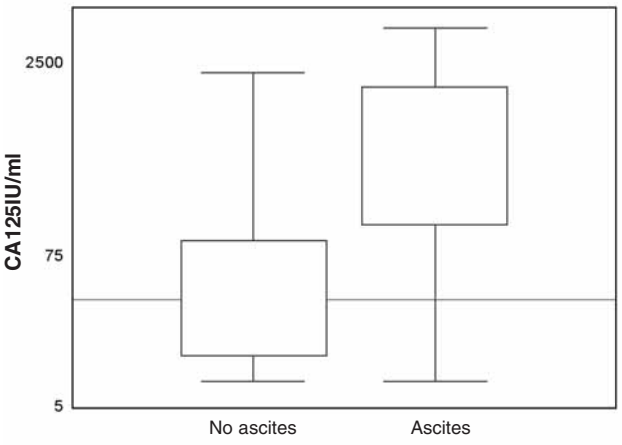


Figure 2. — Level of CA 125 in patients with ascites vs without ascites.

isting mutations in the *BRCA1* gene and telomerase activity in tumour cell nuclei provide a positive prognostic factor since no relapses of the neoplastic process or deaths due to ovarian cancer have been detected in patients with such characteristics of tumour genome.

In the Mann-Whitney U test, in which the level of CA 125 tumour marker served as a variable, significant differences were detected between patients with vs those without ascites ($p < 0.05$) (Figure 2).

## Disscusion

In their report of 1995, Kim *et al.* [19] reported that telomerase activity was detected in the sensitive TRAP test in 85% of examined cases of malignant tumours (in 100 biopsies in 12 variable locations). A similar conclusion was drawn also by Unate *et al.* [20] following their analysis of telomerase activity in tumour cell nuclei. In the analysis the authors reported that 90% of tumours manifested activity of the enzyme but a proportion of tumour cells lacked such activity. Studies conducted on patients with breast cancer demonstrated telomerase activity in 95% of breast cancer cases, 65% of cases with non-malignant adenofibromas but not in a normal tissue [21, 22].

Similar observations were made in prostate cancers: telomerase activity was detected in 90% prostate cancer cases, in approximately 40% cases of benign prostatic hypertrophy but not in the normal tissue of prostate [23]. The investigators suggest that telomerase may provide a tumour marker used for early detection of cancer cells, when they cannot yet be detected by routine histopathology. Despite such an optimistic view on telomerase, doubts may appear whether the marker can differentiate malignant lesions from benign hypertrophies.

Similar data originate from other studies on tumours of the liver [24, 25], urinary bladder [26, 27] and thyroid gland [17].

In ovarian cancer telomerase activity is elevated but in most cases the elevation is not particularly pronounced. This can be illustrated by the group of patients studied in the present investigation. The group is not very numerous but analysis of the clinical data and results on the activity of the studied enzyme provides a few significant pieces of information.

In the studied group of 34 patients telomerase activity was demonstrated in 24 cases (70.6%). In parallel, activity of the enzyme was demonstrated in 40% patients with the borderline form of ovarian cancer while lack of this activity was detected in patients with benign endometrial tumours. Therefore, telomerase activity may be accepted as a diagnostic marker applicable in the differential diagnosis of benign ovarian tumours, preneoplastic lesions and tumours of the organ.

Development of sensitive tests for detection of telomerase activity, e.g., the TRAP test has provided hope for new potential in diagnosis and prognosis in oncological diseases. Intense studies have also started on mechanisms which control the activity of the enzyme, including the potential for blocking its activity. The search has started for telomerase inhibitors, which might find application in the therapy of tumours. The anti-sense technology has also been applied [28]. Currently, also other possibilities of switching off telomerase are known. One of them involves elimination of tumour cells with telomerase activity linked to induction of the specific immune response of cytotoxic T lymphocytes, activated following immunisation with telomerase reverse transcriptase [29, 30].

New reports which appear in the world literature make it mandatory to treat with a distance the new therapeutic solutions. Also this study confirms the need for moderate optimism in this question. First, demonstration of telomerase activity in cell nuclei of ovarian cancer only in 70.6% patients does not allow us to use the enzyme as a tumour marker specific for the tumour (specificity of a

marker is defined by a proportion of individuals with fully negative result among persons in whom no tumour is detected) since it is manifested in many tumours [31]. For the second, this is not a very sensitive marker (sensitivity of a marker is defined by the number of patients with a positive result to the number of patients suffering from a given type of tumour) and it provides no chances to detect it in patients with a low mass of neoplastic tumour. For the third, the marker carries no predictive value (the value is defined by the ratio of patients suffering from a given type of tumour with a truly positive result to the number of all individuals with a truly or falsely positive result) and it cannot be used in screening studies. Thus, telomerase does not exhaust the principal requirements posed to neoplastic markers and its activity should not be routinely estimated in cell nuclei of malignant tumours of the ovary.

A similar conclusion was drawn by Nagai *et al.* [32] in their report of 1998. The authors analyzed patients with malignant tumours of the uterine cervix, uterine body or ovary. Telomerase activity in tumour cells was detected using the TRAP test. The activity was detected in 91.7% of patients with cancer of the uterine cervix, in 85.2% of patients with cancer of the uterine corpus and in 90.9% of patients with ovarian cancer. The authors confirmed trace activity of the enzyme in 52.9% of cases of preneoplastic lesions in the vagina of the *high grade SIL* type and in 15.4% *low-grade SIL* type lesions. No relationship could be detected with the histological type of the studied tumours and clinical advancement stage, and no significant differences could be disclosed between patients with cancer of the uterine corpus and those with ovarian cancer. Significant differences were confirmed between malignant tumours and preneoplastic lesions of the uterine cervix, and between cancers and benign tumours of the ovary. In view of the above, the authors concluded that determination of telomerase activity might prove useful in diagnoses of cancers and in identification of groups with a high risk of developing a tumour.

In 2002 Sapi *et al*. [33] analysed the relationship between CA125 marker level and activity of telomerase in cell nuclei of ovarian cancer cells isolated from blood using an immunomagnetic procedure. The authors found a significantly higher concentration of CA125 in patients manifesting telomerase activity in cell nuclei (100% of patients with Stage IV tumour advancement according to FIGO and 35% of patients with Stage III tumour advancement according to FIGO) as compared to patients who manifested no activity of the enzyme in cell nuclei (65% patients with Stage III tumour advancement according to FIGO and 100% of healthy women). The authors concluded that telomerase provides a potential marker for detection of circulating cells of ovarian cancer.

In our hands, analysis of the group of 34 patients with ovarian cancer has demonstrated that significant differences, detected by the ANOVA rank test of Kruskal-Wallis at the level of $p < 0.05$, could be detected only for estimations of CA125 concentrations tested before surgery and for activity of telomerase in tumour cell nuclei. No significant differences have been detected between CA125 concentrations tested before the first, fourth and after the last course of standard first lapse chemotherapy or in telomerase activity in cell nuclei. Thus, the reduced tumour mass due to the primary surgery coexistent with the decrease in CA125 concentration in serum is significantly correlated with activity of telomerase activity in cell nuclei. However, the above does not justify the statement that telomerase represents a sensitive, specific tumour marker with prognostic potential since telomerase fails to exhaust criteria for a good marker and may be useful only in certain time points (it may be estimated before primary surgery), as mentioned in the earlier discussion.

As many as 75% of the studied group of patients have demonstrated telomerase activity in tumour cell nuclei in advanced stages of the disease (Stage III and IV of clinical advancement according to FIGO). When grade of tumour cell differentiation is concerned, higher activity of the enzyme has been observed in poorly differentiated ovarian cancers: such activity has been detected in 66.7% patients with G3 cancers and 29.2% patients with G2 cancers. It is also worth noting that 76% of patients with telomerase activity in tumour cell nuclei have ascites.

In 1998 Duggan *et al.* [34] claimed that evaluation of telomerase activity in cell nuclei of tumour cells contained in ascites in ovarian cancer patients represents a more sensitive test than cytological evaluation of the ascites fluid. Therefore, it is worthwhile considering application of the test in analyses of the advancement of neoplastic disease.

Considering the fact that following the adjuvant treatment of ovarian cancer with cytostatic drugs the *second-look* procedure for evaluation of results of treatment is performed with decreasing frequency [35], evaluation of telomerase activity could be applied for detection of the remains of subclinical neoplastic disease, as mentioned by Nouriani *et al.* in 2004 [36]. In parallel, the authors suggested that evaluation of the enzyme activity in cell nuclei of cells obtained from washings during *second-look* surgery in patients with ovarian cancer may increase sensitivity of the surgical procedure in detection of residues on the neoplastic disease.

In this study no evaluation of telomerase activity was performed in cell nuclei of tumour cells obtained from washings resulting from the *second-look* operation but the relationship was evaluated between telomerase activity estimated in the material from primary surgery obtained before the adjuvant treatment with cytostatic drugs and results of *second-look* surgery. Activity of telomerase in cell nuclei of ovarian cancer cells has been detected much more frequently in the group of patients with negative results of second-look operation. The conclusion follows that activity of the enzyme should be estimated in cell nuclei of tumour cells obtained from washings resulting from *second-look* surgery, as was done by Nouriani *et al.* [36].

As far as histological type of ovarian cancers is concerned, most involve tumours of a serous structure

(serous adenocarcinoma) and Rosai *et al.* [37], in a 2004 publication, claimed that such tumours comprise 60% to 80% of diagnosed ovarian tumours. Mucous and endometrial cancers are much less frequent. Also this study provides evidence that most of detected ovarian cancers involve serous cancers, which comprised 53% of studied cases (18 patients of the examined group). In analysis of the material, tumours of serous adenocarcinoma type have been shown to carry higher telomerase activity in cell nuclei of tumour cells. Thus, a relationship could have been noted between histological type of ovarian cancer and telomerase activity in cell nuclei of neoplastic cells.

The polish and world literature contains few reports on the clinical fate of ovarian cancer patients in whom a coexistence was documented of mutations in the *BRCA1* gene with telomerase activity in cell nuclei in tumour cells. In one recent report an inhibitory effect was detected in the *BRCA1* gene on the catalytic subunit of human telomerase, hTERT [38].

In this study the relationship between mutations detected in *BRCA1* gene age activity of telomerase in cell nuclei of ovarian cancer cells was examined. Mutations were detected in two cases of studied patients and both of the patients also manifested activity of the enzyme in cell nuclei of tumour cells. Analysis of survival in the cases has justified the conclusion that coexistence of mutations in the *BRCA1* gene and telomerase activity in tumour cell nuclei represents a positive prognostic index since both patients with a mutation in the *BRCA1* gene have reached complete clinical remission following surgical treatment and standard first lapse chemotherapy, and the neoplastic process has shown no relapse nor has it resulted in patient death.

It is also worth drawing attention to the simplicity of the applied technique. Most estimations used in contemporary diagnosis are based on the TRAP (Telomere Repeat Amplification Protocol) technique involving amplification of telomerase products. The method is sensitive permitting detection of telomerase activity in cell nuclei from one to ten cells, which may comprise not more than 0.01% of the studied cell population [39], but preparation of the procedure involves the use of an expensive polymerase chain reaction procedure. In this study the estim microscope was used to detect the reaction in tumour cells, equipment accessible in any pathology lab. The economy of the test clearly facilitates access to tumour diagnosis.

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