Original paper

Immunohistochemical expression of PARP-1 in triple-negative endometrial cancer – a comparison of different score systems

Michał Bulsa[1], Sylwia Romana Jankowska-Szabłowska[2], Elżbieta Urasińska[2]

[1]Obstetrics and Gynecology Department with the Oncological Gynecology and Infertility Diagnostics and Treatment Sub-Departments, Multidisciplinary Provincial Hospital, Gorzów Wielkopolski, Poland
[2]Department of Pathology, Pomeranian Medical University, Szczecin, Poland

Endometrial cancer is the most common malignant neoplasm of the female reproductive system. The number of diagnosed cases is increasing every year. In recent years, the triple-negative phenomenon (TNP) has been identified as one of the determinants of shorter survival in endometrial cancer patients. The aim of the study was to compare the PARP-1 protein expression in triple-negative (TNEC) and non-triple-negative (NTNEC) endometrial cancer patients and determine the relationship between the PARP-1 protein expression in endometrial cancer cells and patient's overall survival depending on the adopted scale (H-SCORE < 75, H-SCORE < 50, Allred scale).
The study involved 265 patients with histopathologically confirmed endometrial cancer. The patients were divided into 2 groups: patients with TNEC and patients with NTNEC. The study was conducted using a tissue microarray technique. Expression of PARP-1 protein was determined by immunohistochemistry.
Protein expression evaluation was performed using virtual microscopy and the Image Scope computer image analysis system.
The following conclusions were reached: total and individual levels of nuclear or cytoplasmic PARP-1 expression varied with the presence or absence of TNP, and PARP-1 nuclear expression at the 2+ level had a significant effect on the increased risk of death (according to H-SCORE < 75).

Key words: endometrial cancer, PARP-1, triple-negative cancer.

Introduction

Endometrial cancer is the most common cancer of the female reproductive system in developed countries. According to a World Health Organization report, the number of new cases of endometrial cancer was 417,376 in 2020, which is an increase of 90,117 cases compared to 2018 [1]. Endometrial cancer is the sixth most common type of cancer found in women in the world. Enzyme PARP-1 (poli ADP-ribose) polymerase is considered to be involved in the processes of maintaining genome stability, chromatin remodeling, regulation of proliferation, differentiation, neoplastic transformation, and cell death [2]. Clinical trials are currently underway on the use of PARP-1 inhibitors in the treatment of endometrial cancer [3–6].

For the first time, a triple-negative phenomenon (TNP) was described for breast cancer. It is characterized by the lack of expression of PR, oestrogen receptor, and human epidermal growth factor receptor 2 (HER-2). The second cancer for which the TNP was described was endometrial cancer. It should be noted

that the literature on triple-negative endometrial cancer is sparse [7–12]. There are different criteria for diagnosing triple-negative endometrial cancer [13–18]. In our study, we have compared TNP criteria (H-SCORE < 75, H-SCORE < 50 and Allred scale) and its impact on survival [19]. Based on that study, we compare herein the PARP-1 protein expression in endometrial cancer for triple-negative (TNEC) and non-triple-negative (NTNEC) endometrial cancer patients and determine the relationship between the PARP-1 protein expression in endometrial cancer cells and patients' overall survival (OS) depending on the adopted scale (H-SCORE < 75, H-SCORE < 50, Allred scale).

Material and methods

Study group

The study included a group of 265 women with histopathologically confirmed diagnosis of endometrial cancer. The patients were divided into 2 groups: patients with TNEC and patients with NTNEC, depending on the score system used (H-SCORE < 75, H-SCORE < 50 or Allred scale). Histological examinations of patients who underwent surgery in the years 2004–2016 at the Department of Gynaecology, Endocrinology, and Gynaecological Oncology at Pomeranian Medical University in Szczecin, the Department of Perinatology, Obstetrics and Gynaecology at Pomeranian Medical University in Szczecin, and the Gynaecological Department of the Ministry of Internal Affairs and Administration Hospital in Szczecin were performed at the Department of Pathomorphology at Pomeranian Medical University in Szczecin. At the end of the observation (9 October 2018), 53 patients did not survive and for 26 it was not possible to obtain information on the date of death. For prognosis assessment, the OS was defined as the time from surgery until the end of observation or until the patient's death. The data were obtained from the death register by the Systems Management Department of Ministry of Digitization of the Republic of Poland. The study was approved by the Bioethics Committee of the Pomeranian Medical University (no. KB-0012/01/01/2015 of 07.01.2015). The clinical and morphological characteristics of the study group are presented in Table I.

Immunohistochemical staining

The technique of tissue microarray (TMA) made of paraffin blocks containing material collected for routine histopathological examination from a postoperative preparation fixed in 10% formalin and embedded in paraffin was used in the study. The expression of PARP-1 was determined by immunohistochemistry. The preparations were dewaxed in an incubator (temperature 60°C), then, to unmask the antigen, the preparations were heat treated at 96°C, pH = 9. The sections cooled down to 65°C and then were incubated with peroxidase solution and then with the tested antibody (monoclonal mouse anty-PARP-1, clone F-2, dilution 1:500; Santa Cruz Biotechnology) Visualization of the antigen-antibody reaction was done with detection system EnVision™ FLEX (Dako). Slides were stained with Mayer's haematoxylin, dehydrated, and covered with coverslips.

Evaluation of protein expression

The percentage of endometrial cancer cells expressing PARP-1 was calculated using virtual microscopy and the ImageScope computer image analysis system. Material from 3 tissue cores taken from 3 different places of the cancer was analysed for each patient. The total number of cells analysed from the material collected from one patient was equivalent to the number of cells from 3 different tissue cores. The level of nuclear and cytoplasmic PARP-1 expression in terms of weak (1+), moderate (2+), and strong (3+) reaction was analysed by the system. We looked for a relationship between the degree of PARP-1 nuclear expression at particular expression levels (1+, 2+, 3+) and the total nuclear expression of PARP-1, depending on the presence or absence of TNP. Similar analysis was performed in relation to the intensity of PARP-1 cytoplasmic expression. The stages of computer image analysis are presented in Figure 1.

Statistical analyses

The normality of the distributions of all variables was checked by the Kolmogorov-Smirnov test. These variables were described by means, standard deviations, and medians. The statistical differences between the 2 groups were checked with Student's t-test or the Mann-Whitney *U* test. The results were described by the probability (p). A Cox regression model was used to estimate which factors increase the chance of survival. The results were described by the hazard ratio along with the 95% confidence interval and the probability. The statistically significant differences in all the tests performed were those for which the probability was lower than 0.05.

Results

According to H-SCORE < 75, we found higher PARP-1 nuclear expression at the level of 1+ in TNEC. There were no other significant differences in nuclear or cytoplasmic expression of PARP-1 in TNEC and NTNEC. Cox regression analysis revealed that PARP-1 nuclear expression at the 2+ level had a significant effect on the increased risk of death.

Table I. Characteristics of the study group

Parameters	Characteristics	$N = 265$	Percentage
Age at diagnosis	≤ 57	65	24.53
	57–70	135	50.94
	> 70	65	24.53
FIGO and WHO histological type	Endometrioid carcinoma	254	95.85
	Mucinous carcinoma	0	0.00
	Serous carcinoma	4	1.51
	Clear-cell carcinoma	3	1.13
	Neuroendocrine neoplasm	1	0.38
	Mixed-cell adenocarcinoma	1	0.38
	Undifferentiated carcinoma	2	0.75
Grading	G1	144	54.34
	G2	92	34.72
	G3	29	10.94
FIGO clinical stage	IA	155	58.49
	IB	68	25.66
	II	24	9.06
	IIIA	15	5.66
	IIIB	0	0.00
	IIIC 1	1	0.38
	IIIC 2	0	0.00
	IVA	1	0.38
	IVB	1	0.38

TNP		H-SCORE < 75		H-SCORE < 50		Allred scale	
		n	%	n	%	n	%
	–	225	84.91	236	89.06	238	89.81
	+	40	15.09	29	10.94	27	10.19

FIGO – Federation of Gynaecology and Obstetrics, TNP – triple-negative phenomenon, WHO – World Health Organization

According to H-SCORE < 50, there was a lower percentage of cells expressing PARP-1 nuclear expression in TNEC. The higher percentage of cells with nuclear expression at the 1+ level and a reduced percentage at the level of 3+ was found in TNEC. In TNEC there was also a higher percentage of cells with PARP-1 cytoplasmic expression at the 1+ level and a reduced percentage at the level of 3+. There were no other significant differences in nuclear or cytoplasmic expression of PARP-1 in TNEC and NTNEC. Cox regression analysis did not show that PARP-1 expression increases the risk of death. According to the Allred scale, TNEC showed a greater percentage of cells with PARP-1 nuclear expression at the 1+ level and a decreased percentage at the level of 3+. There were no other significant differences in nuclear or cytoplasmic expression of PARP-1 in TNEC and NTNEC. Cox regression analysis showed that PARP-1 expression did not increase the risk of death. All results are shown in Tables II–V.

Discussion

Considering the fact that commonly used chemotherapeutic agents act in the mechanism of damaging the DNA of cancer cells as well as normal cells, it is important to look for other methods of eliminating cancer cells. One way of reducing the side effects of the therapy is to administer a chemotherapeutic drug to the blood vessel responsible for nourishing

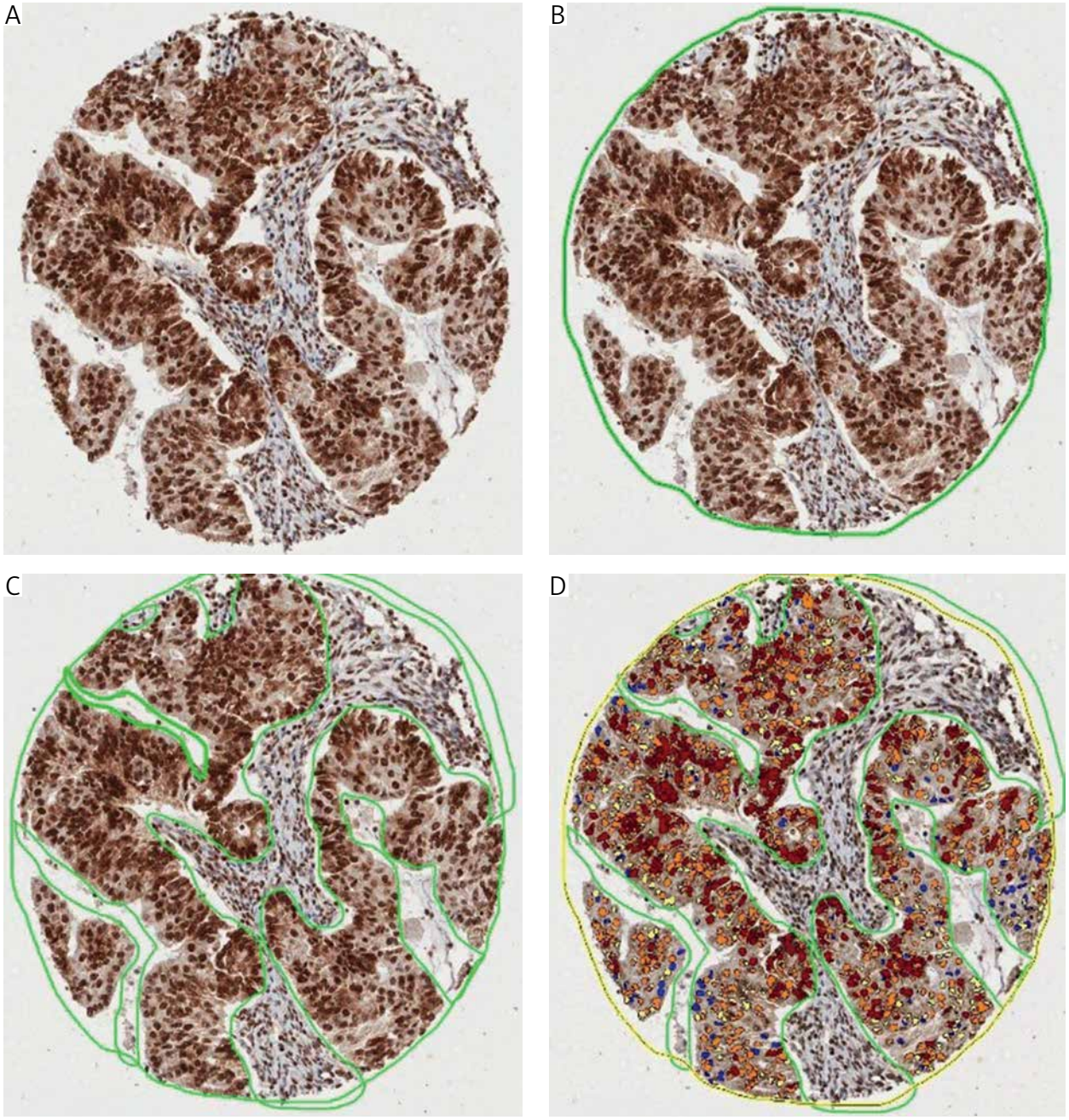

Fig. 1. Immunohistochemical staining for PARP-1 in endometrial cancer cells (stages of computer image analysis). A) Slide before computer analysis; B) Marked area for computer analysis; C) Marking the areas excluded from computer analysis; D) Computer analysis of the selected area

the tumour tissue. Another promising line of research is the use of drugs that selectively suppress genes that are responsible for DNA reparation. It has been observed that cells are killed when 2 DNA damage repair pathways are blocked. Therefore, the researchers' interest was attracted by the genes and proteins responsible for DNA reparation, including the BRCA 1/2 genes and the PTEN or PARP-1 proteins. In this study, the expression of PARP-1 protein was analysed with regard to the TNP. It is noteworthy that no reports of PARP-1 expression in TNP (+) endometrial cancer were published at the time of the studies described in this paper. Bryant *et al*. found the lethal effect of the use of PARP-1 inhibitors on cell lines defective of the protein involved in DNA repair in the mechanism of homologous recombination [20]. Another team of researchers found higher mortality of cells with a mutation in the PTEN gene after the use of olaparib [21]. Moreover, it has been proven that administration of PARP-1 inhibitors leads to sensitization of neoplastic cells to the action of chemotherapeutic agents. According to Magan *et al*. Administration of PARP-1 inhibitors accelerates cell death in response to doxorubicin therapy [22]. It was observed that

Table II. Nuclear expression of PARP-1 in endometrial cancer patients

Parameters		H-SCORE < 75					H-SCORE < 50					Allred scale				
		N = 265	Mean	SD	Median	*p*	*N* = 265	Mean	SD	Median	*p*	N = 265	Mean	SD	Median	*p*
All cells with positive nuclear PARP-1 expression	NTNEC	225	89.58	9.7	92.84	0.0806	236	89.64	9.61	92.87	0.017	238	89.46	9.72	92.85	0.1085
	TNEC	40	86.58	11.32	89.01		29	84.95	12.15	87.82		27	86.2	12.04	88.97	
Cells with weak positive nuclear PARP-1 expression (1+)	NTNEC	225	13.57	8.19	12	0.02	236	13.58	8.24	12.06	0.006	238	13.73	8.39	12.15	0.042
	TNEC	40	16.98	9.94	17.09		29	18.18	9.96	18.97		27	17.26	9.36	18.29	
Cells with moderate positive nuclear PARP-1 expression (2+)	NTNEC	225	32.91	9.49	32.92	0.6911	236	32.87	9.47	32.98	0.7565	238	32.77	9.48	32.76	0.872
	TNEC	40	32.23	11.81	33.44		29	32.27	12.75	34.35		27	33.09	12.92	36.63	
Cells with strong positive nuclear PARP-1 expression (3+)	NTNEC	225	43.1	20.39	42.67	0.1157	236	43.18	20.48	42.69	0.038	238	42.96	20.68	42.58	0.049
	TNEC	40	37.37	25.17	28.55		29	34.51	25.68	24.64		27	35.84	25.09	27.41	

NTNEC – non-triple-negative endometrial cancer, TNEC – triple-negative endometrial cancer

Table III. Cytoplasmic expression of PARP-1 in endometrial cancer patients

Parameters		H-SCORE < 75					H-SCORE < 50					Allred scale				
		N = 265	Mean	SD	Median	*p*	*N* = 265	Mean	*SD*	Median	*p*	*N* = 265	Mean	*SD*	Median	*p*
All cells with positive cytoplasmic PARP-1 expression	NTNEC	225	92.84	8.28	95.41	0.8826	236	93.08	8.17	95.93	0.1325	238	93.02	8.14	95.9	0.2064
	TNEC	39	92.62	9.94	96.6		28	90.51	11.04	95.5		26	90.8	11.51	95.75	
Cells with weak positive cytoplasmic PARP-1 expression (1+)	NTNEC	225	26.05	14.18	23.03	0.3785	236	25.64	14.31	22.59	0.019	238	25.93	14.36	22.74	0.1333
	TNEC	39	28.36	19.72	21.77		28	32.71	19.84	31.3		26	30.62	20.62	29.61	
Cells with moderate positive cytoplasmic PARP-1 expression (2+)	NTNEC	225	25.12	8.07	25.02	0.366	236	24.97	8.03	24.32	0.8102	238	25.04	8.07	24.32	0.4996
	TNEC	39	23.77	11.05	22.39		28	24.55	12.41	22.56		26	23.84	12.35	22.76	
Cells with strong positive cytoplasmic PARP-1 expression (3+)	NTNEC	225	41.67	21.9	43.13	0.7661	236	42.47	22.11	44.6	0.044	238	42.05	21.98	43.94	0.2276
	TNEC	39	40.48	28.38	43.7		28	33.25	27.93	28.41		26	36.34	30.23	28.41	

NTNEC – non-triple-negative endometrial cancer, TNEC – triple-negative endometrial cancer

Table IV. The relationship between PARP-1 nuclear expression and overall survival according to different score systems (Cox regression analysis)

Parameters	H-score < 75				H-score < 50				Allred scale			
	HZ	95%	CI	p	HZ	95%	CI	p	HZ	95%	CI	p
All cells with positive expression	1	0.93	1.07	0.98	1.02	0.94	1.1	0.69	0.99	0.92	1.06	0.74
Cells with strong positive expression 3+	1.02	0.99	1.05	0.19	1.02	0.99	1.05	0.16	1.02	0.98	1.05	0.42
Cells with moderate positive expression 2+	0.94	0.88	1	0.04	0.94	0.89	1.01	0.08	0.93	0.87	1	0.06
Cells with weak positive expression 1+	0.98	0.91	1.05	0.49	0.97	0.89	1.06	0.51	1.01	0.91	1.12	0.82
Cells without expression	1	0.93	1.07	0.98	0.98	0.91	1.07	0.69	1.01	0.94	1.09	0.74

HZ – hazard ratio

Table V. The relationship between PARP-1 cytoplasmatic expression and overall survival according to different score systems (Cox regression analysis)

Parameters	H-score < 75				H-score < 50				Allred scale			
	HZ	95%	CI	p	HZ	95%	CI	p	HZ	95%	CI	p
All cells with positive expression	0.97	0.92	1.03	0.36	0.98	0.94	1.02	0.23	0.97	0.91	1.03	0.29
Cells with strong positive expression 3+	0.98	0.96	1.01	0.25	1	0.94	1.06	0.95	0.97	0.92	1.01	0.16
Cells with moderate positive expression 2+	1	0.94	1.06	0.99	1	0.94	1.06	0.99	1	0.93	1.07	0.9
Cells with weak positive expression 1+	1.02	0.99	1.06	0.25	1.03	0.98	1.07	0.22	1.04	0.99	1.09	0.13
Cells without expression	1.03	0.97	1.09	0.36	1.02	0.96	1.09	0.45	1.03	0.97	1.1	0.29

HZ – hazard ratio

after using PARP-1 inhibitors on cell lines, the percentage of cells responding to paclitaxel therapy increased [23]. Both doxorubicin and platinum derivatives are used in the treatment of advanced endometrial cancer. Other authors noted the influence of oestrogen levels on the therapeutic effect of olaparib in cells lacking the PTEN protein. In mice deprived of appendages, the tumour volume was reduced by a factor of 6 following PARP-1 inhibitor testing. This relationship has not been observed in the case of normal oestrogen levels [6]. According to our study, TNEC was characterized by a lower percentage of cells with positive nuclear expression of PARP-1 receptor. It should be emphasized that this relationship occurred only in the case of TNP diagnosis according to H-SCORE criteria < 50. According to H-SCORE < 75 criteria, there was a higher PARP-1 nuclear expression at the level of 1+ in TNEC. It is noteworthy that according to H-SCORE < 50 criteria and the Allred scale there was a greater percentage of cells with PARP-1 nuclear expression at the 1+ level and a reduced percentage at the level of 3+ in TNEC. A similar relationship was observed in relation to the cytoplasmic expression of PARP-1, but only in relation to the criterion according to H-SCORE < 50. This result shows that PARP-1 expression is variable. According to Ghabreau *et al*., in endometrial cancer, there was a correlation between the percentage of cells showing positive PARP-1 expression and the histological degree of differentiation. The higher grading was associated with a lower percentage of PARP-1 expressing cells. The same authors found a linear relationship between PARP-1 and PR expression. It should be noted that the cited study found no differences in the expression of PARP-1 depending on the histopathological type [5]. The conclusions from the cited studies confirm the lower PARP-1 expression in TNP (+) endometrial cancers. According to Barret *et al*., over-expression of PARP-1 was associated with shorter survival and faster relapse of the neoplastic disease [24]. This fact has not been

confirmed in this study. On the other hand, it was found that the expression of the PARP-1 receptor at the 2+ level is a factor that increases the risk of death.

Considering the above data, when assessing the response to treatment with PARP-1 inhibitors, individual grades and the total expression of PARP-1 should be taken into account. This would allow the selection of a group of patients who can show the best response to therapy with PARP-1 inhibitors. PARP-1 expression did not affect the survival in the group of TNP (+) neoplasms. In this study, a digital image analysis system was used to analyse the degree of expression of individual receptors or proteins. The only exception was the evaluation of HER-2 receptor expression, which was performed manually according to the applicable criteria [25]. Computer analysis of the microscopic image has been introduced into clinical trials to optimize the results. Considerable subjectivism was noticed in the assessment of receptor expression in relation to the same histopathological preparation assessed by several pathologists. To eliminate the above-mentioned phenomenon, programs for counting cells showing a positive response to the immunohistochemical staining were developed and algorithms were introduced into the programs, which also define the individual levels of protein and receptor expression. It should be mentioned that the literature describes the limitations of the computer method of microscopic image analysis as well as the limitations of subjective assessment by pathomorphologists [26]. The digital image analysis system allows the degree of expression of each individual cell to be assessed. The software used in this dissertation allowed for the assessment of both nuclear and cytoplasmic expression. Thanks to the use of this type of software, it was possible to evaluate each preparation according to the same criteria. Digital image analysis has already been used in endometrial cancer research [27–29]. Besides the evaluation of the receptor expression itself, it is possible to evaluate the surface area, optical density, or the shape of the cell nuclei. Over the years, many research teams have been researching the variables relating to the morphology of cell nuclei in relation to endometrial cancer [28, 30]. Currently, research is carried out on computer image analysis, which can help in the event of doubts in the histopathological diagnosis, including in relation to the pathology of the endometrium [31]. In this study, the TMA method was used. It allows us to reduce the costs and time of the analyses, because it allows us to evaluate many cases at the same time [32–34]. In a single so-called multiple fragments from different histopathological slides can be placed in the "recipient block". The literature describes the capacity of the so-called "recipient's block" with up to 1000 samples taken (cylindrical fragments) from the so-called "donor blocks" [35]. It should be emphasized that this method is particularly useful in examining a large number of cases with the use of various antibodies [36]. Due to the collection of individual tissue fragments, it is not suitable for presenting the final histopathological results in individual cases. The heterogeneity of tumours is a significant limitation for the application of the TMA method in single cases. The consistency of the results at the level of 95% in the case of analyses of large numbers of samples in experimental studies is acceptable; however, in the case of an individual diagnosis, it is insufficient. The usefulness of the TMA method for the determination of HER-2 expression has been demonstrated in studies [37]. The TMA method is used more and more often, and the number of studies with its participation is growing. The literature provides examples of combining the TMA method with digital image analysis [38]. The TMA method is used in relation to endometrial cancer [39, 40]. Most of the previous studies on endometrial cancer showing TNP (+) used the TMA technique [7–9, 11].

Conclusions

Total and individual levels of nuclear or cytoplasmic PARP-1 expression varied with the presence or absence of TNP.

PARP-1 nuclear expression at the 2+ level had a significant effect on the increased risk of death (according to H-SCORE < 75).

The authors declare no conflict of interest.

References

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: globocan Estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Cancer J Clin 2021; 0: 1-41.
2. Kiliańska ZM, Żołnierczyk J, Węsierska-Gądek J. Biologiczna aktywność polimerazy poli(ADP-rybozy)-1. Post Hig Med Dosw 2010; 64: 344-363.
3. Bian X, Gao J, Luo F, et al. PTEN deficiency sensitizes endometrioid endometrial cancer to compound PARP-PI3K inhibition but not PARP inhibition as monotherapy. Oncogene 2018; 37: 341-351.
4. Philip CA, Laskov I, Beauchamp MC, et al. Inhibition of PI3K-AKT-mTOR pathway sensitizes endometrial cancer cell lines to PARP inhibitors. BMC Cancer 2017; 8: 1-11.
5. Ghabreau L, Roux JP, Frappart PO, et al. Poly(adp-ribose) polymerase-1, a novel partner of progesteron receptors in endometrial cancer and its precursors. Int J Cancer 2004; 109: 317-321.
6. Janzen DM, Paik DY, Rosales MA, et al. Low levels of circulating estrogen sensitize PTEN-null endometrial tumors to poly(ADP-ribose) polymerase (PARP) inhibition in vivo. Mol Cancer Ther 2013; 12: 2917-2928.
7. Kothari R, Morrison C, Richardson D, et al. The prognostic significance of the triple negative phenotype in endometrial cancer. Gynecol Oncol 2010; 118: 172-175.

8. Voss MA, Gordon N, Maloney S, et al. Tetraspanin CD151 in a novel prognostic marker in poor outcome endometrial cancer. Br J Cancer 2011; 104: 1611-1618.
9. Xue-Feng J, Qiong-Ian T, Xi-ming S, et al. Tumor-associated macrophages, epidermal growth factor receptor correlated with the triple negative phenotype in endometrial endometrioid adenocarcinoma. Pathol Res Pract 2012; 208: 730-735.
10. Altundag O, Dursun P, Roach EC, et al. Triple negative endometrial cancer may be more sensitive to platinum based chemotherapy. J Buon 2013; 18: 289-295.
11. Porzio R, Cordini C, Rodolfi AM, et al. Triple negative endometrial cancer: incidence and prognosis in a monoistitutional series of 220 patients. Oncol Lett 2020; 19: 2522-2526.
12. Bulsa M, Urasińska E. Triple negative endometrial cancer. Ginekol Pol 2017; 88: 212-214.
13. McCarty KS, Miller LS, Cox EB, et al. Estrogen receptor analyses. Correlation of biochemical and immunohistochemical methods using monoclonal antireceptor antibodies. Arch Pathol Lab Med 1985; 109: 716-721.
14. Singh M, Zaino RJ, Filiaci VJ, et al. Relationship of estrogen and progesterone receptors to clinical outcome in metastatic endometrial carcinoma: a gynecologic oncology group study. Gynecol Oncol 2007; 106: 325-333.
15. Thinke AA, Chang MJ, Fook-Chong S, et al. Immunohistochemical expression of hormone receptors in invasive breast carcinoma: correlation of results of H-SCORE with pathological parameters. Pathology 2001; 33: 21-25.
16. Allred D, Harvey JM, Berardo M, et al. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol 1998; 11: 155-168.
17. Gottwald L, Kubiak R, Pasz-Walczak G, et al. The value of progesterone and estrogen receptors expression in tissue microarray method in prognosis of patients with endometrioid endometrial cancer. Ginekol Pol 2013; 84: 95-101.
18. Wolff AC, Hammond MEH, Allison KH, et al. Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. J Clin Oncol 2018; 36: 2105-2122.
19. Bulsa M, Urasińska E. Triple negative phenomenon in endometrial cancer: recognition criteria and impact on survival. Ginekol Pol 2021.
20. Bryant HE, Schiltz N, Thomas HD, et al. Specific killing of BRCA2-deficient tumours with inhibitors of polu(ADP-ribose) polymerase. Nature 2005; 434: 913-917.
21. Mendes-Pereira AM, Martin SA, Brough R, et al. Sunthetic lethal targeting of PTEN mutant cells with PARP inhibitors. EMBO Mol Med 2009; 1: 315-322.
22. Magan N, Isaacs RJ, Stowell KM. Treatmet with the PARP-inhibitor PJ34 causes enhanced doxorubicin-mediated cell death in HeLa cells. Anticancer Drugs 2012; 23: 627-637.
23. Dinkic C, Jahn F, Zygmunt M, et al. PARP inhibition sensitizes endometrial cancer cells to paclitaxel-induced apoptosis. Oncol Lett 2017; 13: 2847-2851.
24. Barreta A, Sarian LO, Ferracini AC, et al. Immunohistochemistry expression of targeted therapies biomarkers in ovarian clear cell and endometrioid carcinomas (type I) and endometriosis. Hum Pathol 2019; 85: 72-81.
25. Wolff AC, Hammond MEH, Schwartz JN, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J Clin Oncol 2007; 25: 118-145.
26. Lewington S, Clarke R, Qizilbash N, et al. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet 2002: 360: 1903-1913.
27. Jagielski L, Jeleń M, Kobierzycki C, et al. Increase of nuclear expresion of metallothionein I/II in neoplastic transformation of the endometrium. Ginekol Pol 2015; 28: 182-187.
28. El-Sharkawy SL, Abbas NF, El-Henawy AM, et al. Morphometric and DNA image analysis of endometrial hyperplasia and carcinoma. Appl Immunohistochem Mol Morphol 2017; 25: 32-38.
29. Elhafey AS, Papadimitriou JC, El-Hakim MS. Computerized image analysis of p53 and proliferating cell nuclear antigen expression in benign, Hyperplastic, and malignant endometrium. Arch Pathol Lab Med 2001; 125: 872-879.
30. Miller J, Geisler JP, Manahan KJ, et al. Nuclear size, shape, and density in endometrial carcinoma: relationship to survival at over 5 years of follow-up. Does analyzing only cells occupying the G0-G1 peak add useful information? Int J Gynecol Cancer 2004; 14: 138-144.
31. Sun H, Zeng X, Xu T, et al. Computer-aided diagnosis in histopathological images of the endometrium using a convolutional neural network and attention mechanisms. J Biomed Health Inform 2019; 1: 1-10.
32. Graham A, Faratian D, Rae F, et al. Tissue microarray technology in the routine assessment of HER-2 status in invasive breast cancer: a prospective study of the use of immunohistochemistry and fluorescence in situ hybridization. Histopathology 2008; 52: 847-855.
33. Chen W, Foran D. Advances in cancer tissue microarray technology: towards improved understanding and diagnostics. Anal Chim Acta 2006; 30: 74-81.
34. Gulmann C, O'Grady A. Tissue microarrays: an overview. Curr Diagn Pathol 2003; 9: 149-154.
35. Kononen J, Budendorf L, Kallioniemi OP, et al. Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med 1998; 4: 844-847.
36. Lugli A, Tornillo L, Mirlacher M, et al. Hepatocyte paraffin 1 expression in human normal and neoplastic tissues: tissue microarray analysis on 3,940 tissue samples. Am J Clin Pathol 2004; 122: 721-727.
37. Ganceberg D, Di Leo A, Rouas G, et al. Reliability of the tissue microarray based FOSH for evaluation of the HER-2 oncogene in vreast carcinoma. J Clin Pathol 2002; 55: 315-317.
38. Chen W, Foran DJ. Advances in cancer tissue microarray technology: towards improved understanding and diagnostics. Anal Chim Acta 2006; 30: 74-81.
39. Visser N, van der Wurff A, Pijnenborg J, et al. Tissue microarray is suitable for scientific biomarkers studies in endometrial cancer. Virchows Arch 2018; 472: 407-413.
40. Peres G, Spadoto-Dias D, Bueloni-Dias F, et al. Immunohistochemical expression of hormone receptors, Ki-67, endoglin (CD105), claudins 3 and 4, MMP-2 and -9 in endometrial polyps and endometrial cancer type I. Onco Targets Ther 2018; 11: 3949-3958.

Address for correspondence

Sylwia Romana Jankowska-Szabłowska, PhD
Department of Pathology
Pomeranian Medical University
Szczecin, Poland
e-mail: sylwia_jankowska@interia.eu