Histol Histopathol (2018) 33: 171-179

http://www.hh.um.es

Histology and Histopathology

From Cell Biology to Tissue Engineering

Expression of p53 and selected proliferative markers (Ki-67, MCM3, PCNA, and topoisomerase IIα) in borderline ovarian tumors: Correlation with clinicopathological features

Klaudiusz Ciepliński[1], Maciej Jóźwik[2], Anna Semczuk-Sikora[3], Marek Gogacz[4], Dorota Lewkowicz[5], Atanas Ignatov[6] and Andrzej Semczuk[4]

[1]Department of Obstetrics and Gynecology, Municipal Hospital, Płock, [2]Department of Gynecology and Gynecologic Oncology, Medical University of Białystok, Białystok, [3]Department of Pathology of Pregnancy, [4]2nd Department of Gynecology, [5]Department of Clinical Pathology, Lublin Medical University, Lublin, Poland and [6]Department of Obstetrics and Gynecology, Otto-von-Guericke University, Magdeburg, Germany

Summary. Background. The expression of p53 has been studied not only in primary human ovarian carcinomas, but also in borderline ovarian tumors, however, the results were discordant. Expression patterns of proteins involved in cell proliferation and apoptosis have been investigated in various human neoplasms, including female genital tract neoplasms.

Objective. The aim of this investigation was to assess the staining pattern and immunolocalization of p53 and selected proliferative markers (Ki-67, MCM3, PCNA, and topoisomerase IIα) in borderline ovarian tumors (BOTs).

Design. The study group consisted of 42 women who underwent pelvic surgery between 2006-2015. The median patients' age was 46 years. The immunoperoxidase technique was employed using antibodies against p53, Ki-67, MCM3, PCNA, and topoisomerase IIα.

Results. For p53, nuclear expression was observed in BOTs, however, cytoplasmatic immunoreactivity was also detected. Altogether, 25 (60%) tumors demonstrated positive p53 immunostaining, including overexpression found in 6 (14%). There were no significant differences in p53 expression between subgroups of clinicopathological variables. Immunoexpression of Ki-67, MCM3, PCNA, and topoisomerase IIα was nuclear. Ki-67 expression was positive in 12 (29%) cases and there was a trend towards a relationship between patients' age and Ki-67 staining (P=0.08). Interestingly, a significantly higher Ki-67 expression was found in tumors of ≥10 cm in diameter compared to smaller tumors (P=0.008). MCM3 expression was detected in 38 (90%) tumors, and PCNA expression in 28 (67%), yet none of clinico-pathological factors was related to them. Topoisomerase IIα expression was present in 14 (33%) cases and, interestingly, its significantly higher expression was observed in BOTs of ≥10 cm in diameter compared to smaller tumors (P=0.008). Moreover, Spearman's correlation revealed highly significant positive associations between Ki-67 and topoisomerase IIα (R=0.403, P=0.008) and Ki-67 and MCM3 (R=0.469, P=0.001).

Conclusions. We report a high positive immunostaining rate for p53, suggesting a role of TP53 alterations in the development of BOTs in humans. The new finding of higher topoisomerase IIα immunostaining positivity in BOTs of ≥10 cm may be clinically relevant and requires further studies on larger patient groups.

Key words: p53, Ki-63, MCM3, PCNA, Topoisomerase

Introduction

Borderline ovarian tumors (BOTs) were first described by Taylor in 1929 and were named "semimalignant tumors of the ovary" or ovarian tumors of low malignant potential (Taylor, 1929; Prat, 1999;

Offprint requests to: Andrzej Semczuk, MD, PhD, 2nd Department of Gynecology, Lublin Medical University, 8 Jaczewski Street, 20-954 Lublin, Poland. e-mail: andrzej.semczuk@umlub.pl

DOI: 10.14670/HH-11-902

Morice et al., 2012). In general, approximately 15-20% of all primary ovarian malignancies are BOTs and, based on literature review, their incidence has been gradually increasing during the last decades compared with a slightly decreasing incidence of primary human ovarian carcinomas (Bjørge et al., 1997; Skirmisdottir et al., 2008). BOTs markedly differ from ovarian carcinomas in several clinico-pathological variables, including proportional distribution of different histological subtypes, patients' age, rate of implicated infertility, as well as frequency of *BRCA* mutations (Fischerova et al., 2012; Seong et al., 2015). Although the overall survival of women affected by BOTs is excellent, there is a limited number of patients suffering from more aggressive forms of the disease (with invasive implants) whose outcome has not been satisfactory yet (Hart, 2005; McCluggage, 2010; Fischerova et al., 2012).

The tumor suppressor gene *TP53*, named "the guardian of the genome", is located on the short arm of human chromosome 17 in region 17p13.1 and encodes a 53 kDa protein, known as p53 (Balint and Vousden, 2001; Hayat, 2002). This gene has been extensively studied in a variety of female genital tract neoplasms, and its prognostic impact on patients' survival has been reported (Skirmisdottir and Seidal, 2001; Matias-Guiu and Davidson, 2014; Semczuk et al., 2014). Mutations of *TP53* as well as p53 expression have been previously investigated in primary human BOTs, but the results are inconsistent (Kupryjanczyk et al., 1995; Lee et al., 1995; Gershenson et al., 1999; Miliaras, 1999; Nielsen et al., 2004; Vereczkey et al. 2011; Giurgea et al., 2012; Ozer et al., 2012; Sylvia et al., 2012; Gajewska et al., 2014). For example, Gershenson et al. (1999) reported that p53 overexpression was significantly associated with an increased probability of progression/recurrence and a decreased overall survival of patients affected by the advanced-stage serous BOTs. On the contrary, in another study, p53 overexpression was not associated with decreased survival of BOT patients (Nielsen et al., 2004).

Assessment of tumor proliferative activity is widely applied to determine growth fraction of investigated cell populations (Yaziji and Gown, 2001; McCluggage, 2006; Deavers, 2008). In gynecologic pathology, Ki-67 protein and Proliferative Nuclear Cell Antigen (PCNA) are harnessed most frequently for this purpose (Hayat, 2002; McCluggage, 2006). Generally, employment of immunohistochemical methods is a simple and reliable procedure for studying cell proliferation, especially in archival, paraffin-embedded slides. Few reports evaluated the expression of various proliferative markers, especially Ki-67, in BOTs (Kohlberger et al., 1997; Kuhn et al., 1998; Liu et al., 2002; Gursan et al., 2009; Giurgea et al., 2012; Sylvia et al., 2012).

This situation prompted us to evaluate the immunostaining pattern of p53 and selected proliferative markers /Ki-67, DNA replication licensing factor: minichromosome maintenance 3 (MCM3), PCNA, and topoisomerase IIα/ in a group of women affected by BOT, and to relate their expression to clinical and pathological variables. Our working hypothesis was that proliferative markers other than Ki-67 may also be applied in the histochemical evaluation of primary human BOTs.

Materials and methods

Patients

The study group consisted of a retrospective cohort of 42 unselected Caucasian women undergoing pelvic surgery for ovarian mass at the Lublin Medical University, Lublin, Poland in years 2006-2015, whose postoperative anatomopathological result was BOT. The median patient age was 46 years (range: 27-80 years). Thirty (71%) patients were premenopausal and 12 (29%) were postmenopausal. Three women (7%) were pregnant at the time of surgery (their median age: 30 years; range 28-37 years). Premenopausal patients with unilateral tumors wishing to preserve their fertility underwent salpingo-oophorectomy. Other patients underwent explorative laparotomy with the goal of maximum surgical debulking. The clinical stage of the disease was determined based on modified FIGO classification (Berek et al., 2015). The clinico-pathological characteristic of the study group is presented in Table 1. None of these patients received chemotherapy,

Table 1. Clinico-pathological characteristics of 42 women with BOTs.

Characteristics	n	%
Age (years)		
< 30	6	14
30-50	24	57
> 50	12	29
Hormonal status		
Premenopausal	30	71
Postmenopausal	12	29
FIGO stage		
I	40	95
II-IV	2	5
Histopathologic type		
Serous	22	52
Mucinous	18	43
Endometrioid	2	5
Tumor structure		
Unilocular cystic	23	55
Multilocular septate	19	45
Tumor size (cm)		
<10	27	64
≥10	15	36
Coexistence of cancer		
No	35	83
Yes	7	17
Tumor infiltration		
No	31	74
Yes	11	26

radiotherapy or any hormonal treatment before surgery. The histologic type of the tumor was confirmed by reviewing the hematoxylin/eosin stained slides by a highly-experienced pathologist (D.L.) and the diagnosis criteria of the World Health Organization classification were applied (Tavassoli and Devilee, 2003).

Immunohistochemistry

Serial 4-μm sections were cut from paraffin-embedded slides. Formalin-fixed, paraffin-embedded slides were dewaxed and rehydrated through a graded series of ethanol solutions, followed by antigen retrieval in EnVision® Target Retrieval Solution (DAKO, North America Inc., CA, USA) at low (p53, Ki-67, PCNA, and topoisomerase IIα) or high (MCM3) pH. To block the endogenous peroxidase activity, the sections were incubated with 0.3% hydrogen peroxide for 5 min at room temperature. After washing in a DAKO buffer, the sections were immunostained with monoclonal antibodies, applying the conditions given in Table 2. The immunoperoxidase technique was employed based on the DAKO procedure (DAKO, North America Inc., CA, USA). Binding was visualized by incubating sections with DAB (3,3''-diaminobenzidine) (DAKO, Glostrup, Denmark). A hematoxylin counterstain was used, and coverslips were applied. Appropriate controls were included in each experiment.

Evaluation of immunostaining

All the slides were independently evaluated as a

Table 2. Immunostaining procedures applied.

Antibody	Clone	Antibody retrieval	Dilution	Incubation
p53	DO-7	EnVision Target Retrieval Solution, low Ph	1:25	room temperature
Ki-67	MIB-1	EnVision Target Retrieval Solution, low pH	1:50	room temperature
MCM3	101	EnVision Target Retrieval Solution, high pH	1:25	room temperature
PCNA	PC10	EnVision Target Retrieval Solution, low pH	1:1000	room temperature
Topoizomerase IIα	Ki-S1	EnVision Target Retrieval Solution, low pH	1:50	room temperature

Table 3. Ki-67 expression pattern in BOTs.

Characteristics	n	Ki-67 expression		P-value
		Negative n (%)	Positive n (%)	
Age (years)				
< 30	6	2 (33)	4 (67)	
30–50	24	19 (79)	5 (21)	P=0.08
> 50	12	6 (75)	3 (25)	
Hormonal status				
Premenopausal	30	21 (70)	9 (30)	
Postmenopausal	12	9 (75)	3 (25)	P=1.0
FIGO stage				
I	40	28 (70)	12 (30)	
II-IV	2	0	2 (100)	P=1.0
Histopathologic type				
Serous	22	19 (86)	3 (14)	
Mucinous	18	10 (56)	8 (44)	P=0.079
Endometrioid	2	1 (50)	1 (50)	
Tumor structure				
Unilocular cystic	23	16 (69.5)	7 (30.5)	
Multilocular septate	19	14 (74)	5 (26)	P=1.0
Tumor size (cm)				
< 10	27	22 (81)	5 (19)	
≥ 10	15	8 (53)	7 (47)	P=0.008
Coexistence of cancer with BOT				
No	35	25 (71)	10 (29)	
Yes	7	5 (71)	2 (29)	P=1.0
Tumor infiltration				
No	31	21 (68)	10 (32)	
Yes	11	9 (82)	2 (18)	P=0.464

Table 4. Topoizomerase IIα expression pattern in BOTs.

Characteristics	n	Topoizomerase IIα expression n (%)	P-value
Age (years)			
< 30	6	1 (17)	
30-50	24	7 (29)	P=0.047
>50	12	8 (67)	
Hormonal status			
Premenopausal	30	8 (27)	
Postmenopausal	12	8 (67)	P=0.032
FIGO stage			
I	40	15 (37)	
II-IV	2	1 (50)	P=1.0
Histopathologic type			
Serous	22	6 (27)	
Mucinous	18	9 (50)	P=0.318
Endometrioid	2	1 (50)	
Tumor structure			
Unilocular cystic	23	11 (48)	
Multilocular septate	19	5 (26)	P=0.208
Tumor size (cm)			
<10	27	6 (22)	
≥10	15	10 (67)	P=0.008
Coexistence of cancer with BOT			
No	35	9 (26)	
Yes	7	2 (29)	P=1.0
Tumor infiltration			
No	31	11 (35)	
Yes	11	5 (45)	P=0.720

consensus score by two scientists (D.L. and A.S.), who were blinded to the clinical data during scoring. For p53 immunoreactivity, the interpretation of the staining was based on the score by Gershenson et al. (1999) where sections were considered negative when the percentage of tumor cell nuclei staining was ≤5%. Ki-67 was analyzed independently in each tumor slide as previously reported by Semczuk et al. (2001). A given case was considered positive when tumor cells showed nuclear immunoreactivity of >10%. For the other proliferative markers, we applied previously introduced staining scores: by Nodin et al. (2012) for MCM3, by Guo et al. (1993) for PCNA, and by Bar et al. (2012) for topoisomerase IIα.

Statistical analysis

In the analysis for statistical differences, Pearson's χ^2 and Fisher's exact tests were applied. Moreover, Kolmogorow-Smirnow and Shapiro-Wilk tests were used to test for the normalcy of distribution of the variables. The majority of them demonstrated distributions different from a normal distribution. Consequently, to verify possible relationships, Spearman's rank correlation coefficient was used. Data were analyzed using the software package SPSS, version 12.0 for Windows (StatSoft Inc., Tulsa, OK, USA). P-values <0.05 were considered statistically significant.

Results

In the current study, 42 cases of BOTs were analyzed for the relationships between clinico-pathological features and protein (p53, Ki-67, MCM3, PCNA, and topoisomerase IIα) immunoreactivity.

p53 expression in BOTs

Most of the time, nuclear p53 expression was observed (Fig. 1A,B), however, cytoplasmatic immunoreactivity was also detected in some cases (data not shown). Altogether, 25 (60%) cases showed positive (>5% of positively-stained tumor cell nuclei) immunostaining for p53, including 6 (14%) cases demonstrating protein overexpression (>75% of positively-stained tumor cell nuclei). There were no significant correlations between the p53 expression and clinico-pathological variables, including patients' age, clinical stage, histological type, or tumor size. There was no difference in BOT p53 expression between pregnant and non-pregnant women (P>0.05).

Expression of selected proliferative markers in BOTs

Nuclear Ki-67 expression was present in 12 (29%) cases. Examples of the staining pattern of Ki-67 antigen are shown in Fig. 1C,D. A negative correlation was found between Ki-67 immunoreactivity and patients' age (P=0.048, R=-0.307). Interestingly, a significantly higher Ki-67 expression was found in tumors of ≥10 cm in diameter compared to smaller tumors (P=0.008). Nuclear MCM3 expression was detected in 38 (90%) cases (Fig. 1E,F). There was a trend towards a higher MCM3 expression in women of ≤50 years of age when compared to older patients (P=0.063). No significant correlation between MCM3 expression and other clinico-pathological features of BOTs was found. PCNA expression was demonstrated in 28 (67%) tumors and the immunostaining was solely nuclear; examples of the PCNA expression in BOTs are shown in Fig. 1G,H. None of clinico-pathological parameters were related to the expression of this marker. Nuclear topoisomerase IIα immunoreactivity was analyzed according to the criteria by Bar et al. (2012), where expression present in >10% of the cells was considered positive. Sixteen cases (38%) showed such increased immunoreactivity (Fig. 1I,J). Numerical relationships between the protein's reactivity and clinico-pathological parameters are summarized in Table 4. Interestingly, a significant association existed between the marker's expression and patients' age and hormonal status. Moreover, a significantly higher

Table 5. Correlations between p53 expression and selected proliferative markers immunoreactivity in 42 cases of BOTs.

	p53	Topoizomerase Iiα	MCM3	Ki-67	PCNA
p53		R=0.046 P=0.772	R=-0.066 P=0.676	R=-0.087 P=0.579	R=0.082 P=0.605
Topoizomerase IIα	R=0.046 P=0.772		R=0.295 P=0.058	R=0.403 P=0.008	R=-0.259 P=0.098
MCM3	R=-0.066 P=0.676	R=0.295 P=0.058		R=0.469 P=0.002	R=0.117 P=0.461
Ki-67	R=-0.087 P=0.579	R=0.403 P=0.008	R=0.469 P=0.002		R=0.203 P=0.197
PCNA	R=0.082 P=0.605	R=-0.259 P=0.098	R=0.117 P=0.461	R=0.203 P=0.197	

Spearman's rank correlation coefficient.

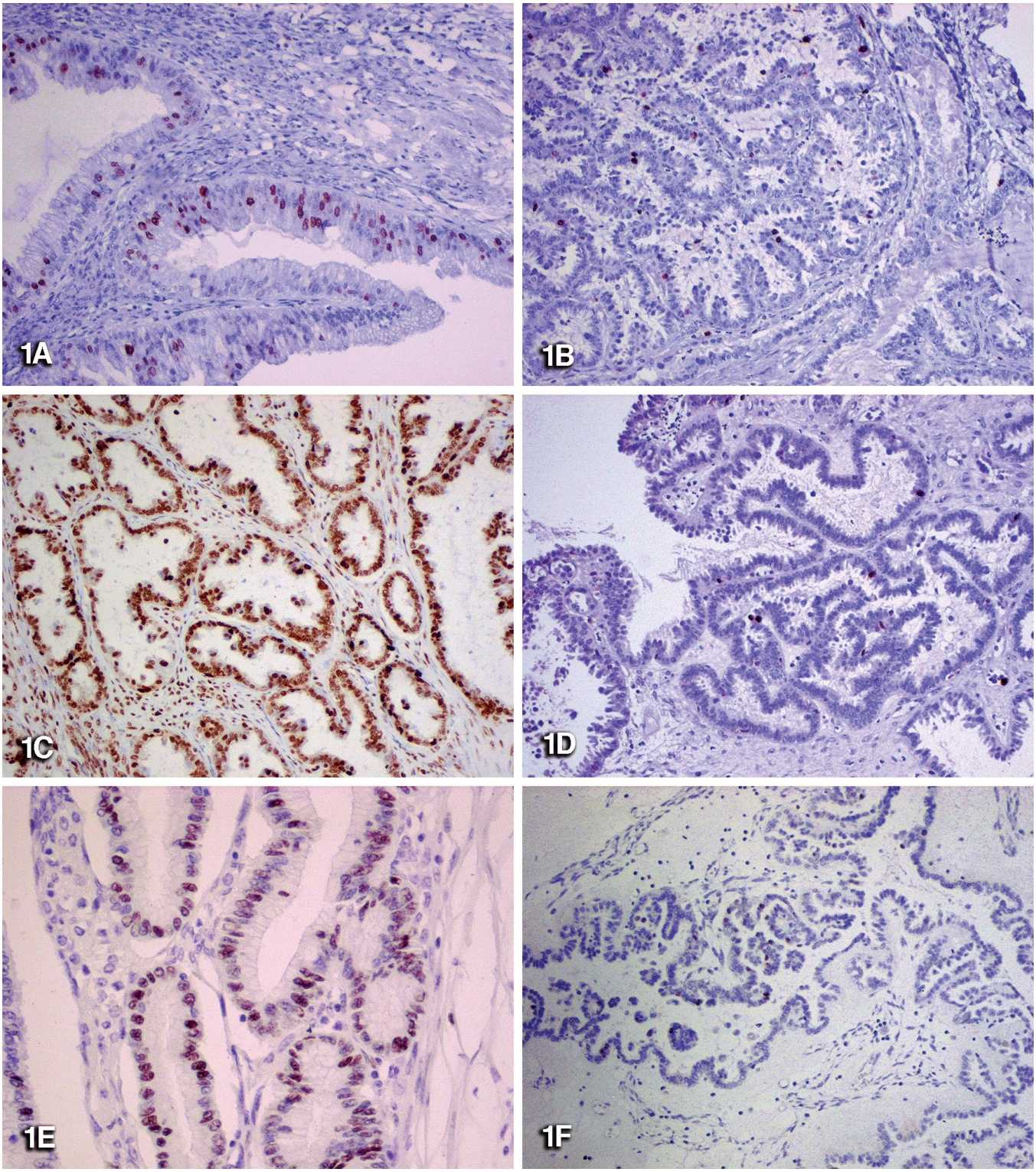

Fig. 1. Examples of the expression patterns of p53 (**A,** positive staining; **B,** negative staining) and proliferative markers: Ki-67 (**C,** positive staining; **D**, negative staining), MCM3 (**E,** positive staining; **F,** negative staining). A, C, E, x 200; B, D, F, x 100.

expression was observed in BOTs of ≥10 cm in diameter compared to smaller tumors. There was no difference observed in immunoreactivity of the proliferative markers between pregnant and non-pregnant women (P>0.05).

Correlation between p53 and selected proliferative markers in BOTs

Table 5 presents the correlation study between p53 and selected proliferative markers in the whole group of 42 patients. Highly significant positive correlations between Ki-67 and topoisomerase IIα and Ki-67 and MCM3 were established. The association between topoisomerase IIα and MCM3 was of marginal significance (P=0.058).

Discussion

Expression patterns of proteins involved in cell proliferation and apoptosis have been widely studied in various human neoplasms, including female genital tract carcinomas (McCluggage and Young, 2005; Deavers, 2008). The expression of p53 has been studied not only in ovarian carcinomas, but also in BOTs, however, the results were discordant. Furthermore, it is commonly accepted that BOTs exhibit an intermediate p53 expression pattern between benign and malignant ovarian tumors (Klemi et al., 1994; Halperin et al., 2001; Giordano et al., 2008; Gursan et al., 2009; Marinas et al., 2012; Sylvia et al., 2012).

In the current work, we aimed at investigating the simultaneous expressions of p53 and selected

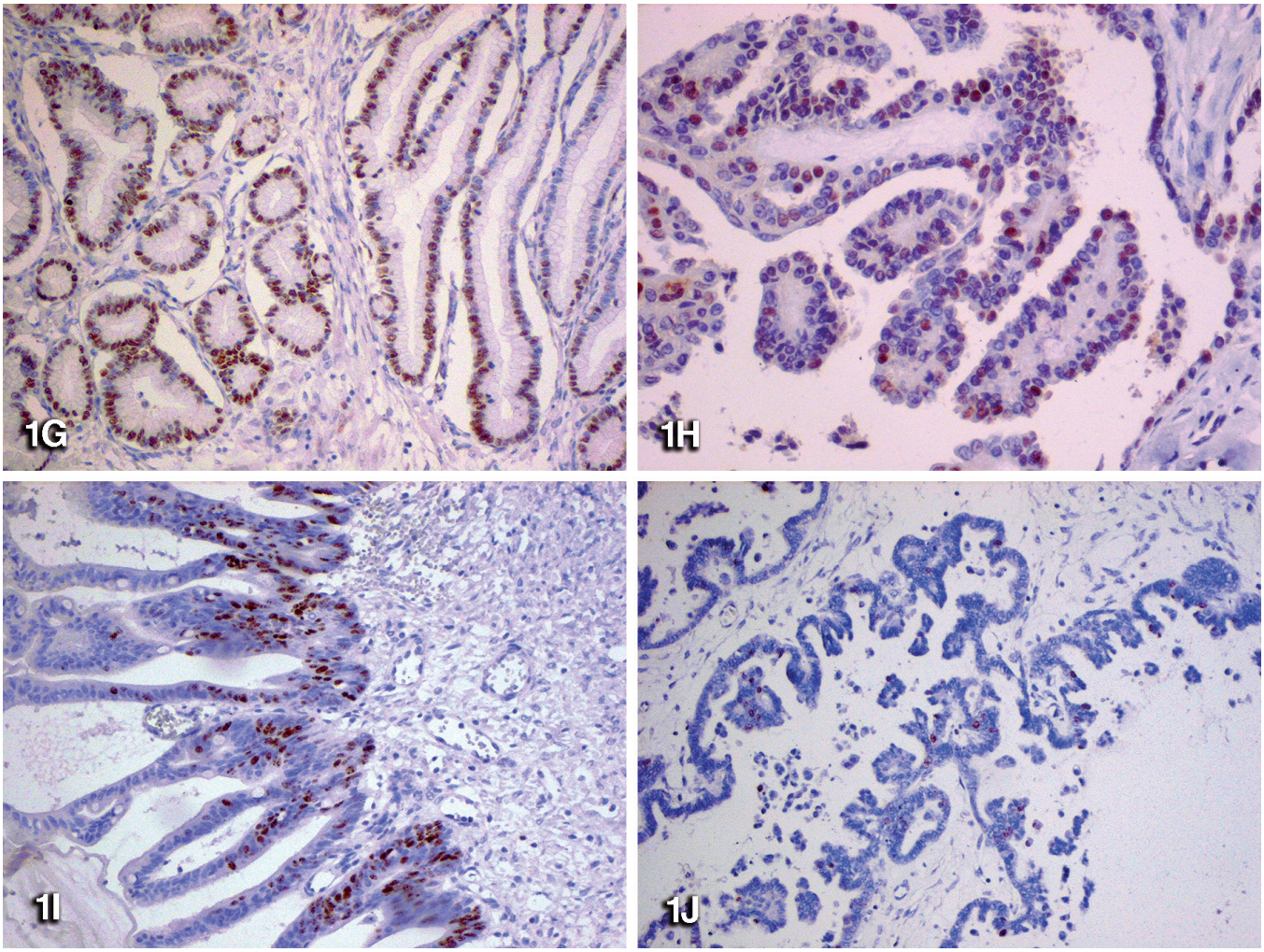

Fig. 1. Examples of the expression patterns of p53. PCNA (**G,** positive staining; **H,** negative staining) and topoisomerase IIα (**I,** positive staining; **J,** negative staining) in BOTs. G-I, x 200; J, x 100.

Table 6. Expression of p53 in BOTs reported in the literature (chronological order).

Authors	No of patients	No of p53-positive cases (%)
Berchuck et al., 1994	49	2 (4)
Klemi et al., 1994	10	1 (10)
Kupryjanczyk et al., 1995	12	8 (66)
Lee et al., 1995	17	3 (18)
Kohlberger et al., 1997	46	0
Kuhn et al., 1998	54	5 (9)
Gershenson et al., 1999	68	13 (19)
Miliaras, 1999	13	0
Halperin et al., 2001	20	0
Nielsen et al., 2004	85	17 (20)
Fauvet et al., 2005	34	9 (26)
Gursan et al., 2009	10	0
Aktas et al., 2012	44	30 (68)
Giurgea et al., 2012	15	1 (7)
Ozer et al., 2012	16	not reported
Marinas et al., 2012	4	1 (25)
Sylvia et al., 2012	10	2 (20)
Gajewska et al., 2014	16	15 (94)
Present study	42	25 (60)

proliferative markers /Ki-67, MCM3, PCNA, and topoisomerase IIα/ in a cohort of women affected by BOTs. Our data revealed a high (60%) expression pattern of p53 in BOTs, although only 14% of cases showed overexpression. In the literature, divergent results are reported (Table 6). Due to their relative rarity, research on BOTs suffers from a limited number of observations, the largest one consisting of 85 cases (Nielsen et al., 2004). On the one hand, several papers reported on the lack of p53 expression in BOTs (Kohlberger et al., 1997; Halperin et al., 2001; Gursan et al., 2009). p53 expression has not been considered a prominent feature of early-stage BOTs (Berchuck et al., 1994). In the Nielsen study (2004), 20% of BOT samples expressed p53, and similar percentages were also published by Gershenson et al. (1999), Fauvet et al. (2005), and by Sylvia et al. (2012). On the other hand, results in line with ours were previously published by Turkish authors who reported a p53-positive staining ratio of 68% (Aktas et al., 2012). In one study, as many as 94% of cases investigated were reported as p53-positive (Gajewska et al., 2014). These apparent divergences may be attributed to tumor heterogeneity, marked differences in immunohistochemistry methods, as well as interpretative data. Moreover, as suggested by Nielsen et al. (2004), prolonged storage of paraffin-embedded slides may result in obtaining different scores compared to slides evaluated immediately after preparation. Finally, differences in semi-quantification of p53 expression (assessment of both percentage of positive cells and signal intensity, or only one of the two) may explain varied outcomes.

Presently, no significant differences between subgroups of clinico-pathological parameters with regard to the p53 immunostaining were observed, in agreement with other authors (Kupryjanczyk et al., 1995; Darai et al., 1998; Marinas et al., 2012). Similarly to our results, no substantial difference in p53 expression between serous and mucinous BOTs was observed by Fauvet et al. (2005). Interestingly, these investigators finally suggested that "p53 could be a common feature of both mucinous and serous borderline ovarian tumours" (Fauvet et al., 2005).

As for the data on the expression of proliferative markers, a significant negative correlation was found between Ki-67 immunoreactivity and patients' age, suggesting a higher biological potential of BOTs in younger patients. Earlier, marked differences in Ki-67 expression were found between primary human ovarian carcinomas and BOTs, and between BOTs and benign ovarian tumors (Garzetti et al., 1995; Halperin et al., 2001; Gursan et al., 2009; Giurgea et al., 2012; Sylvia et al., 2012). Based on immunohistochemistry, Halperin et al. (2001) suggested that BOTs had much more in common with benign serous ovarian tumors than serous papillary ovarian carcinomas. It is assumed that assessment of Ki-67 and PCNA expression patterns may be one of the immunohistochemical methods differentiating benign/borderline tumors from malignant ovarian neoplasms (Frigerio et al., 1997; Nakayama et al., 2003; Zagorianakou et al., 2004).

There are also data showing that serous BOTs have a significantly lower apoptotic index and higher proliferative index than mucinous ones (Liu et al., 2002). Somewhat similar results were presented for ovarian carcinomas, where serous tumors revealed a high Ki-67 proliferation index (55%), followed by endometrioid (50%) and mucinous histotypes (26%) (Sylvia et al., 2012). Two of 15 (13%) BOTs expressed Ki-67, and both of these tumors were of serous type, in the work by Giurgea et al. (2012). In our study, however, there were no differences in Ki-67 expression between histologic types of BOTs.

In the present work, topoisomerase IIα staining increased with patients' age and, interestingly, a higher expression was noted for BOTs of ≥10 cm in diameter compared to smaller tumors. These observations are entirely new. The association of topoisomerase IIα expression with advanced clinical stage may reflect a highly progressive growth of platinum-resistant ovarian cancers and unfavorable prognosis for the patient (Ferrandina et al., 2008). Moreover, topoisomerase IIα/p53 immunopositive ovarian carcinomas tend to be associated with advanced stage and histologic grade (Bar et al., 2012). In the van der Zee study (van der Zee et al., 1991), topoisomerase IIα expression was higher in malignant ovarian tumors compared to benign and borderline ones. These authors suggested that "topo IIα activity might help in the future to select the proper treatment for the individual patient" (van der Zee et al., 1991). Significant correlations between the expression of proliferative markers Ki-67, topoisomerase IIα and MCM3 found in the current study support the temporary conclusion of interchangeable application of these

markers for BOT evaluation and are in line with our working hypothesis. A question to be addressed by future research is: which of the three markers is best suited for this role?

In conclusion, we report a high p53 expression in BOTs, suggesting a role of *TP53* alterations in the development of these tumors in humans. p53 staining was not related to clinical or pathological variables of BOTs. Significant correlations between Ki-67 and MCM3 and PCNA expression patterns in BOTs were established. A substantially higher expression of topoisomerase IIα was noted in large (>10 cm in diameter) BOTs compared to smaller ones, an observation which may be clinically relevant and requires further studies on larger patient populations.

Acknowledgements. We would like to thank Mrs Mariola Bigas and Mrs Maria Nowak for their immunostaining analysis and Mr Robert Klepacz for his expertise in high-quality photographs. This study was supported by a grant from the Lublin Medical University, Lublin, Poland (Dz. St. 326/16 to AS).

The authors declare no conflict of interest

References

Aktas I.Y., Bugdayci M. and Usubutun A. (2012). Expression of p16, p53, CD24, EpCAM and calretinin in serous borderline tumors of the ovary. Turk. Patoloji. Derg. 28, 220-230.

Balint E.E. and Vousden K.H. (2001). Activation and activities of the p53 tumour suppressor protein. Br. J. Cancer 85, 1813-1823.

Bar J.K., Grelewski P., Noga L., Rabczyński J., Gryboś M. and Jeleń M. (2012). The association between p53/topoisomerase I and p53/topoisomerase IIα immunophenotypes and the progression of ovarian carcinomas. Adv. Clin. Exp. Med. 21, 35-42.

Berchuck A., Kohler M.F., Hopkins M.P., Humphrey P.A., Robboy S.J., Rodriquez G.C., Soper J.T., Clarce-Pearson D.L. and Bast R.C. (1994). Overexpression of p53 is not a feature of early-stage borderline epithelial ovarian tumors. Gynecol. Oncol. 52, 232-236.

Bjørge T., Engeland A., Hansen S. and Trope C.G. (1997). Trends in the incidence of ovarian cancer and borderline tumours in Norway, 1954-1993. Int. J. Cancer 71, 780-786.

Berek J.S., Crum C. and Friedlander M. (2015). Cancer of the ovary, fallopian tube, and peritoneum. Int. J. Gynecol. Obstet. 131 (Suppl. 2), S111-S122.

Darai E., Walker-Combrouze F., Mlika-Cabanne N., Madelenat P., Potet F. and Scoazec J.Y. (1998). Expression of p53 protein in borderline epithelial ovarian tumors: a clinicopathologic study of 39 cases. Eur. J. Gynaecol. Oncol. 19, 144-149.

Deavers M.T. (2008). Immunohistochemistry in gynecologic pathology. Arch. Pathol. Lab. Med. 132, 175-180.

Fauvet R., Dufournet C., Poncelet C., Uzan C., Hugol D. and Darai E. (2005). Expression of pro-apoptotic (p53, p21, bax, bak and fas) and anti-apoptotic (bcl-2 and bcl-x) proteins in serous versus mucinous borderline ovarian tumours. J. Surg. Oncol. 92, 337-343.

Ferrandina G., Petrillo M., Carbone A., Zannoni G., Martinelli E., Prisco M., Pignata S., Breda E., Savaraese A. and Scambia G. (2008). Prognostic role of topoisomerase-IIα in advanced ovarian cancer patients. Br. J. Cancer 98, 1910-1915.

Fischerova D., Zikan M., Dundr P. and Cibula D. (2012). Diagnosis, treatment, and follow-up of borderline ovarian tumors. Oncologist 17, 1515-1533.

Frigerio L., Agnello A., Sassi I., Mangili F., Taccagni G., Mariani A. and Ferrari A. (1997). Adjuvant role of MIB1 in differentiating serous ovarian tumors – preliminary report. Anticancer Res. 17, 1287-1292.

Gajewska M., Wielgoś M., Panek G. and Marczewska J. (2014). Incidence of proapoptotic proteins p53 and p21 in epithelial ovarian tumors. Ginekol. Pol. 85, 111-116 (In Polish).

Garzetti GC, Ciavattini A., Goteri C., De Nictolis M., Stramazzotti D., Lucarini G. and Biagini G. (1995). Ki67 antigen immunostaining (MIB1 monoclonal antibody) in serous ovarian tumors: index of proliferative activity with prognostic significance. Gynecol. Oncol. 56, 169-174.

Gershenson D.M., Deavers M., Diaz S., Tortolero-Luna G., Miller B.E., Bast Jr R.C., Mills G.B. and Silva E.G. (1999). Prognostic significance of p53 expression in advanced-stage ovarian serous borderline tumors. Clin. Cancer Res. 5, 4053-4058.

Giordano G., Azzoni C., D'Adda T., Rocco A., Gnetti L., Froio E., Merisio C. and Melpignano M. (2008). Human papilloma virus (HPV) status, p16INK4a, and p53 overexpression in epithelial malignant and borderline ovarian neoplasms. Pathol. Res. Pract. 204, 163-174.

Giurgea L.N., Ungureanu C. and Mihailovici M.S. (2012). The immunohistochemical expression of p53 and Ki67 in ovarian epithelial borderline tumors. Correlation with clinicopathological factors. Rom. J. Morphol. Embryol. 53, 967-973.

Guo L.N., Wilkinson N., Buckey C.H., Fox H., Hale R.J. and Chawner L. (1993). Proliferating cell nuclear antigen (PCNA) immunoreactivity in ovarian serous and mucinous neoplasms: diagnostic and prognostic value. Int. J. Gynecol. Cancer 3, 391-394.

Gursan N., Sipal S., Calik M. and Gundogdu C. (2009). P53, Bcl-2, Ki-67 Li (Labeling Index) status in benign, proliferative, and malignant ovarian surface epithelial neoplasms. Eurasian J. Med. 41, 10-14.

Halperin R., Zehavi S., Dar P., Habler L., Hadas E., Bukovski I. and Schneider D. (2001). Clinical and molecular comparison between borderline serous ovarian tumors and advanced serous papillary ovarian carcinomas. Eur. J. Gynaecol. Oncol. 22, 292-296.

Hart W.R. (2005). Borderline epithelial tumors of the ovary. Mod. Pathol. 18 (Suppl.2), S33-S50.

Hayat M.A. (2002). Microscopy, immunohistochemistry, and antigen retrieval methods for light and electron microscopy. New York, Kluver Academic Press/Plenum.

Klemi P.J., Takahashi S., Joensuu H., Kiiholma P., Narimatsu E. and Mori M. (1994). Immunohistochemical detection of p53 protein in borderline and malignant serous ovarian tumors. Int. J. Gynecol. Pathol. 13, 228-233.

Kohlberger P.D., Kieback D.G., Mian C., Wiener H., Kainz C., Gitsch G. and Breitenecker G. (1997). Numerical chromosomal aberrations in borderline, benign, and malignant epithelial tumors of the ovary: correlation with p53 protein overexpression and Ki-67. J. Soc. Gynecol. Investig. 4, 262-264.

Kuhn W., Marx D., Meidel A., Fattahi-Meibodi A., Korabiowska M., Ruschenburg I., Droese M., Schauer A. and Meden H. (1998). Borderline tumors of the ovary: A clinico-pathologic and immunohistochemical study of 54 cases. J. Obstet. Gynaecol. Res. 24, 437-445.

Kupryjanczyk J., Bell D.A., Dimeo D., Beauchamp R., Thor A.D. and Yandell D.W. (1995). p53 gene analysis of ovarian borderline tumors and stage I carcinomas. Hum. Pathol. 26, 387-392.

Lee J.H., Kang Y.S., Park S.Y., Kim B.G., Lee E.D., Lee K.H., Park K.B., Kavanagh J.J. and Wharton J.T. (1995). p53 mutation in epithelial ovarian carcinoma and borderline ovarian tumor. Cancer Genet. Cytogenet. 85, 43-50.

Liu A., Chen L., Ngan H.Y., Khoo U.S., Zhao Y. and Cheung A.N. (2002). Apoptotic and proliferative activity in ovarian benign, borderline and malignant tumors. Chin. Med. Sci. J. 17, 106-111.

Marinas M.C., Mogos D.G., Simionescu C.E., Stepan A. and Tanase F. (2012). The study of p53 and p16 immunoexpression in serous borderline and malignant ovarian tumors. Rom. J. Morphol. Embriol. 53, 1021-1025.

Matias-Guiu X. and Davidson B. (2014). Prognostic biomarkers in endometrial and ovarian carcinoma. Virchows Arch. 464, 315-331.

McCluggage W.G. (2006). Immunohistochemical and functional biomarkers of value in female genital tract lesions. Int. J. Gynecol. Pathol. 25, 101-120.

McCluggage W.G. (2010). The pathology of and controversial aspects of ovarian borderline tumours. Curr. Opin. Oncol. 22, 462-472.

McCluggage W.G. and Young R.H. (2005). Immunohistochemistry as a diagnostic aid in the evaluation of ovarian tumors. Semin. Diagn. Pathol. 22, 3-32.

Miliaras D. (1999). Volume-corrected mitotic index and p53 immunoreactivity in borderline and malignant epithelial ovarian tumors. Anal. Quant. Cytol. Histol. 21, 425-429.

Morice P., Uzan C., Fauvet R., Gouy S., Duvillard P. and Darai E. (2012). Borderline ovarian tumour: pathologic diagnostic dilemma and risk factors for invasive or lethal recurrence. Lancet Oncol. 13, e103-e115.

Nakayama K., Takebayashi Y., Hata K., Fujiwaki R., Iida K., Fukumoto M. and Miyazaki K. (2003). The proliferative activity of ovarian tumors of low malignant potential differs from that of ovarian carcinoma. Anticancer Res. 23, 4657-4662.

Nielsen J.S., Jakobsen E., Holund B., Bertelsen K. and Jakobsen A. (2004). Prognostic significance of p53, Her-2, and EGFR overexpression in borderline and epithelial ovarian cancer. Int. J. Gynecol. Cancer 14, 1086-1096.

Nodin B., Fridberg M., Jonsson L., Bergman J., Uhlen M. and Jirstrom K. (2012). High MCM3 is an independent biomarker of poor prognosis and correlates with reduced RBM3 expression in a prospective cohort of malignant melanoma. Diagn. Pathol. 7, 82.

Ozer H., Yenicesu G., Arici S., Cetin M., Tuncer E. and Cetin A. (2012). Immunohistochemistry with apoptotic-antiapoptotic proteins (p53, p21, bax, bcl-2), c-kit, telomerase, and metallothionein as a diagnostic aid in benign, borderline, and malignant serous and mucinous ovarian tumors. Diagn. Pathol. 7, 124.

Prat J. (1999). Ovarian tumors of borderline malignancy (tumors of low malignant potential): a critical appraisal. Adv. Anat. Pathol. 6, 247-274.

Semczuk A., Skomra D., Cybulski M. and Jakowicki J.A. (2001). Immunohistochemical analysis of MIB-1 proliferative activity in human endometrial cancer. Correlation with clinicopathological parameters, patient outcome, retinoblastoma immunoreactivity and K-ras codon 12 point mutations. Histochem. J. 33, 193-200.

Semczuk A., Ignatov A., Obrzut B., Reventos J. and Rechberger T. (2014). Role of p53 pathway alterations in uterine carcinosarcoma (Malignant Mixed Mullerian Tumors). Oncology 87, 193-204.

Seong S.J., Kim D.H., Kim M.K. and Song T. (2015). Controversies in borderline ovarian tumors. J. Gynecol. Oncol. 26, 343-349.

Skirmisdottir I. and Seidal T. (2001). Prognostic impact of concomitant p53 and PTEN on outcome in early stage (FIGO I-II) epithelial ovarian cancer. Int. J. Gynecol. Cancer 21, 1024-1031.

Skirmisdottir I., Garmo H., Wilander E. and Holmberg L. (2008). Borderline ovarian tumors in Sweden 1960-2005: trends in incidence and age at diagnosis compared to ovarian cancer. Int. J. Cancer 123, 1897-1901.

Sylvia M.T., Kumar S. and Dasari P. (2012). The expression of immunohistochemical markers estrogen receptor, progesterone receptor, Her-2-neu, p53 and Ki-67 in epithelial ovarian tumors and its correlation with clinicopathologic variables. Indian J. Pathol. Microbiol. 55, 33-37.

Tavassoli F.A. and Devilee P. (2003). WHO Classification of Tumours. Pathology and Genetics of Tumors of the Breast and Female Genital Organs. IARC Press, Lyon.

Taylor H.C. (1929). Malignant and semimalignant tumors of the ovary. Surg. Gynecol. Obstet. 48, 204-230.

van der Zee A.G., Hollema H., de Jong S., Boonstra H., Gouw A., Willemse P.H., Zijlstra J.G. and de Vries E.G. (1991). P-glycoprotein expression and DNA topoisomerase I and II activity in benign tumors of the ovary and in malignant tumors of the ovary, before and after platinum/cyclophosphamide chemotherapy. Cancer Res. 51, 5915-5920.

Vereczkey I., Sevester O., Dobos J., Gallai M., Szakacs O., Szentirmay Z. and Toth E. (2011). Molecular characterization of 103 ovarian serous and mucinous tumors. Pathol. Oncol. Res. 17, 551-559.

Yaziji H. and Gown A.M. (2001). Immunohistochemical analysis of gynecologic tumors. Int. J. Gynecol. Pathol. 20, 64-78.

Zagorianakou N., Stefanou D., Makrydimas G., Zagorianakou P., Briasoulis E., Karavasilis B. and Agnantis N.J. (2004). CD44 expression in benign, borderline, and malignant tumors of ovarian surface epithelium. Correlation with p53, steroid receptor status, proliferative indices (PCNA, MIB1) and survival. Anticancer Res. 24, 1665-1670.

Accepted May 11, 2017