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Clinico-imaging and morphological aspects of the benign serous ovarian epithelial tumors in children and adolescents

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Abstract

Benign serous ovarian epithelial tumors represent a major area of interest in pediatric pathology through the incidence and the hormonal and reproductive implications that they induce. In this study, we analyzed 24 tumors diagnosed and surgically operated in children and adolescents, in relation to clinical, histological and immunohistochemical parameters, which can provide information on the potential for growth of lesions. The average age of diagnosis was 13.2 years, the majority of tumors being present in patients over 10 years (75%), with accompanying symptoms (83.3%), unilateral (91.7%) and dimensions of maximum 10 cm (66.7%). The histopathological aspect indicated a cystic growth pattern, sometimes papillary, and in three cases, the presence of atypical focal areas of the tumor epithelium. The Ki67 proliferation index values were higher in the case of tumors larger than 10 cm, those with papillary pattern, and in those with atypical areas, while p53 reactions were present only in cases with atypical proliferation areas. The parameters investigated in this study are useful both for assessing the risk of tumor growth and progression, as well as for stratifying patients for active clinical surveillance.

Keywords: pediatric ovarian benign tumors, growth potential, Ki67, p53.

Introduction

Ovarian epithelial tumors represent over 60% of ovarian tumors, of which over 50% are benign, 5% borderline and approximately 35% malignant [1, 2]. In pediatric pathology, ovarian tumors are more common after the age of 14, when hormonal activity is intense and are the most common gynecological neoplasias [3–5]. However, for children ovarian tumors represent only about 2–3% of all neoplasms for this age category [3, 6–8]. Of the ovarian epithelial tumors, the serous type is the most common [9].

In some cases, especially for tumors less than 8 cm in size, the therapeutic behavior is one of follow-up the tumor growth rate, in order to avoid radical surgery or the implicit complications [10]. On the contrary, other studies report cases of benign ovarian serous tumors in children and adolescents who have become giant in a relatively short time or have been treated incorrectly [7, 11]. There are numerous studies that have addressed the clinical, paraclinical and therapeutic aspects of pediatric ovarian epithelial tumors, including those of serous tumors, the investigations having in most cases only the profile of some particular aspects reports. In this context, it is important to identify clinicopathological parameters that help to assess the risk of tumor growth and to impose an active surveillance of patients. This aspect is extremely important in order to make therapeutic decisions that must take into account the preservation of fertility and the hormonal status that are influenced by the radical treatment, especially in the case of children and adolescents [1].

In the same time, serous cystadenomas (CAs) or cystadenofibroids with focal borderline changes are described, which have benign behavior, but which can be considered controversial lesions if we refer to the theory that CAs, borderline tumors and ovarian carcinomas are considered a lesional continuum [1, 12]. Ki67 and p53 are faithful markers for the assessment of cell proliferation and cell cycle disruption in ovarian neoplasia, and p53 alteration is characteristic for high-grade malignant epithelial tumor lesions [12]. Such markers, in addition to the clinical and histological aspects of benign ovarian serous tumors in children and adolescents, may be useful for establishing an effective therapeutic management.

In this study, we analyzed the clinico-imaging, histopathological (HP) and immunohistochemical (IHC) aspects of benign ovarian serous epithelial tumors in pediatric pathology, in order to identify particular aspects that may support criteria for the assessment of tumor growth potential.

Materials and Methods

This retrospective study included 24 benign ovarian serous tumors, which were diagnosed in the Department

3of Pathology, Emergency County Hospital, Craiova, Romania. The tumors came from children and adolescents who were hospitalized with suspicion of ovarian tumor and subsequently treated surgically in the Clinics of Pediatrics and Pediatric Surgery of the same Hospital, respectively, between years 2013–2018.

The clinical, epidemiological, imaging and morphological data were identified in the Hospital's electronic databases. The biological material was represented by cystectomy or salpingo-oophorectomy surgical specimens, which were fixed in 10% neutral buffered formalin. HP analysis of cases was performed by the classical method of paraffin embedding and Hematoxylin–Eosin (HE) staining. The cases included in the study were reviewed histopathologically, the classification of lesions being enhanced according to the criteria established by the working group for ovarian tumors within *World Health Organization* (WHO) [13].

For all cases, serial sections of 4 μm were made, which were attached to the poly-L-lysine slides and which were subjected to the IHC technique, consisting in deparaffinization in xylene, hydration in alcohol, endogenous peroxidase blocking using 0.3% hydrogen peroxide and blocking of non-specific antigenic sites with 5% bovine serum albumin (BSA) in phosphate-buffered saline (PBS). For the antigen retrieval, heat-induced epitope retrieval (HIER) technique was used. The antibodies used in this study were anti-Ki67 and anti-p53, which were incubated overnight at 4°C (Table 1).

Table 1 – ***Antibodies used: clone, dilution, antigen retrieval and external positive controls***

Antibody	Clone/ Manufacturer	Dilution	Antigen retrieval	External control
Anti-Ki67	MIB-1/Dako	1:100	Citrate buffer, pH 6	Tonsil
Anti-p53	DO-7/Dako	1:100	Tris-EDTA buffer, pH 9	Tonsil

EDTA: Ethylenediaminetetraacetic acid.

The IHC reactions were developed using Labeled Streptavidin–Biotin (LSAB) 2 system (Dako, Redox, Romania, code K0675) and for the signal detection, we used 3,3'-diaminobenzidine (DAB) tetrahydrochloride (Dako, Redox, Romania, code K3468) as chromogen. External positive and negative controls were used for the validation of reactions.

The assessment of reactions was done by using a positivity index (PI), which resulted by reporting the average number of labeled cells to the total number of tumor cells counted at 20× microscope objective, using 10 microscopic fields/case.

The statistical analysis used mean values, standard deviations and comparison tests [Student's *t*-test, unifactorial analysis of variance (ANOVA) test, Pearson's test] within Statistical Package for the Social Sciences (SPSS) 10 software, the $p<0.05$ values being considered significant. For the images acquisition, we used the Nikon Eclipse E600 microscope equipped with Lucia 5 software.

The local Ethical Committee approved the study and informed consent was obtained for all cases.

Results

The analysis of the clinical-epidemiological data for the 24 cases indicated an age of the patients between 6–18 years, with a diagnosis average of 13.2 years and with the prevalence of the cases over 10 years (75%) (Table 2).

Table 2 – ***The distribution of cases in relation to the analyzed parameters***

Parameters		No. of cases
Age [years]	≤10	6
	>10	18
Symptoms	absent	4
	present	20
Location	unilateral	22
	bilateral	2
Size [cm]	≤10	16
	>10	8
Histological type	CA	8
	PCA	2
	CAF	7
	PCAF	7

CA: Cystadenoma; PCA: Papillary cystadenoma; CAF: Cystadenofibroma; PCAF: Papillary cystadenofibroma; Size – largest dimension.

Tumors were identified incidentally in four (16.7%) cases, in most cases there were accompanying symptoms represented by pain and abdominal distension (12 cases), palpable mass (one case), digestive disturbances, such as loss of appetite, transit disorders, vomiting (three cases), or mixed aspects (four cases) (Table 2).

Anamnesis indicated the absence of a significant heredocolateral history, and the general external and external genital examinations were normal. However, in three cases, the symptomatology persisted for more than one year until the presentation to the doctor. Patients were normoponderal in 17 (70.8%) cases and overweight in seven (29.2%) cases, of which three cases had an irregular menstrual cycle. We did not find other personal pathological aspects for the analyzed cases. The usual laboratory tests (including complete hemoleucogram, coagulation status, renal function, exclusion of pregnancy), tumor and inflammatory markers were normal and cancer antigen-125 (CA-125) values were also normal.

In most cases, the tumors were unilateral (91.7%), the bilateral presence of the cysts being identified in two cases. The tumors had dimensions between 3.5–24 cm, most of them having dimensions of maximum 10 cm (66.7%) (Table 2).

Ultrasound (US) and computed tomography (CT) scans indicated the suspicion of cystic formations, respectively the presence of fluid in the cysts, as well as the relationship with adjacent structures. For the analyzed cases, laparoscopic treatment of the removal of the cysts or of the affected ovarian and salpinx was practiced, depending on the size, location and age of the patients (Figure 1A).

The macroscopic morphological analysis indicated the presence of single- or multi-locular cysts, with smooth outer surface, thin and glossy wall and clear or yellow content, sometimes with whitish areas or prominent intracystic papillary areas, and in two cases, the appearance was that of a cystic torsion (Figure 1, B–D).

The microscopic HP analysis indicated the presence of serous CAs in eight (33.4%) cases, with cysts covered by a layer of high ciliary columnar cells or non-ciliated epithelium (Figure 2A).

These were followed as frequency of cystadenofibromas (CAFs) and papillary cystadenofibromas (PCAFs) with seven (29.7%) cases characterized by the presence of poor cellular fibrous stroma and respectively by the addition of simple papillae (Figure 2B). Papillary cystadenomas (PCAs) were present in two cases, accounting for 8.3% (Figure 2C). The presence of calcifications was especially noted in the case of CAs. The presence of focal necrosis areas was observed in the case of twisted cysts. In papillary forms, the papillae lacked architectural complexity, and the covering epithelium was in most cases without atypia. In the case of a PCA and for two cases of PCAFs, focal proliferation and atypia were identified, with stratified epithelia and the tendency to form more complex papillae (Figure 2D).

The IHC analysis indicated the presence of Ki67 immunostaining in all cases analyzed at the nuclear level of cystic epithelium, with mean PI values of 4±2.6. Ki67 PI values ranged from 1–9%, being higher for patients over 10 years, for tumors over 10 cm in size, and for PCAs and PCAFs, the maximum values being identified in the atypical focal areas (Table 3) (Figure 3, A and B).

The p53 immunostaining was identified only in the case of tumors presenting atypical focal areas, with PI values ranging from 7–10 and with an average value of 8.6±1.5. All the other cases were p53 negative (Figure 3C). The p53 positive cases presented ages over 10 years, dimensions over 10 cm and areas of focal atypia.

Table 3 – ***Ki67 PI values in relation to the analyzed parameters***

Parameter/ PI	Age [years]		Size [cm]		Histological type			
	≤10	*>10*	*≤10*	*>10*	*CA*	*PCA*	*CAF*	*PCAF*
Ki67	2.5± 1.8	4.6± 2.7	2.7± 2.1	6.7± 1.2	1.6± 0.7	7	3.4± 2.2	6.7± 1.2

PI: Positivity index; CA: Cystadenoma; PCA: Papillary cystadenoma; CAF: Cystadenofibroma; PCAF: Papillary cystadenofibroma.

The statistical analysis indicated significantly higher differences in mean Ki67 PI values for tumors over 10 cm (p<0.001, Student's t-test) and in cases with papillary growth pattern (p<0.001, ANOVA test).

Discussions

Serous ovarian tumors account for over 50% of all tumors with this localization, over half of them being benign, which represents 20–30% of ovarian epithelial tumors [1, 14]. About 15% of serous tumors are classified as borderline and account for 30–40% of all ovarian borderline tumors, while carcinomas represent 35% of serous tumors and account for 65–70% of all ovarian epithelial tumors [1, 14–16].

The most common ovarian tumors in pediatric pathology are germ cell neoplasms (over 60%), followed by epithelial ones (approximately 15–20%) and sex cord-stromal tumors (over 15%) [6, 7, 9]. Other studies indicate that epithelial tumors are by far the most common in this age category [4, 17].

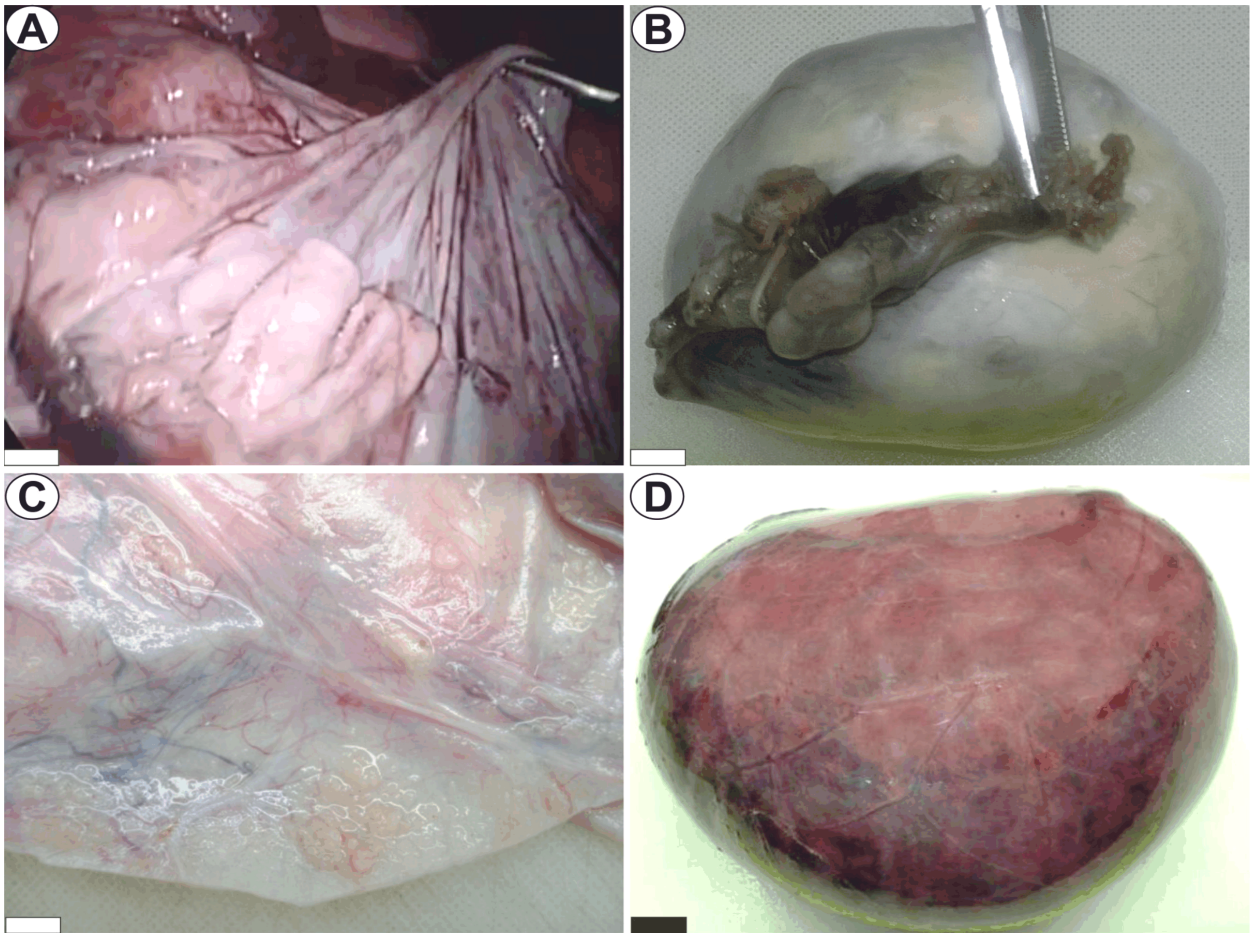

Figure 1 – ***(A) Ovarian serous CA: intraoperative appearance; (B) Ovarian serous CA after fixation; (C) Ovarian serous PCA after evacuation; (D) Ovarian CA with torsion. CA: Cystadenoma; PCA: Papillary cystadenoma.***

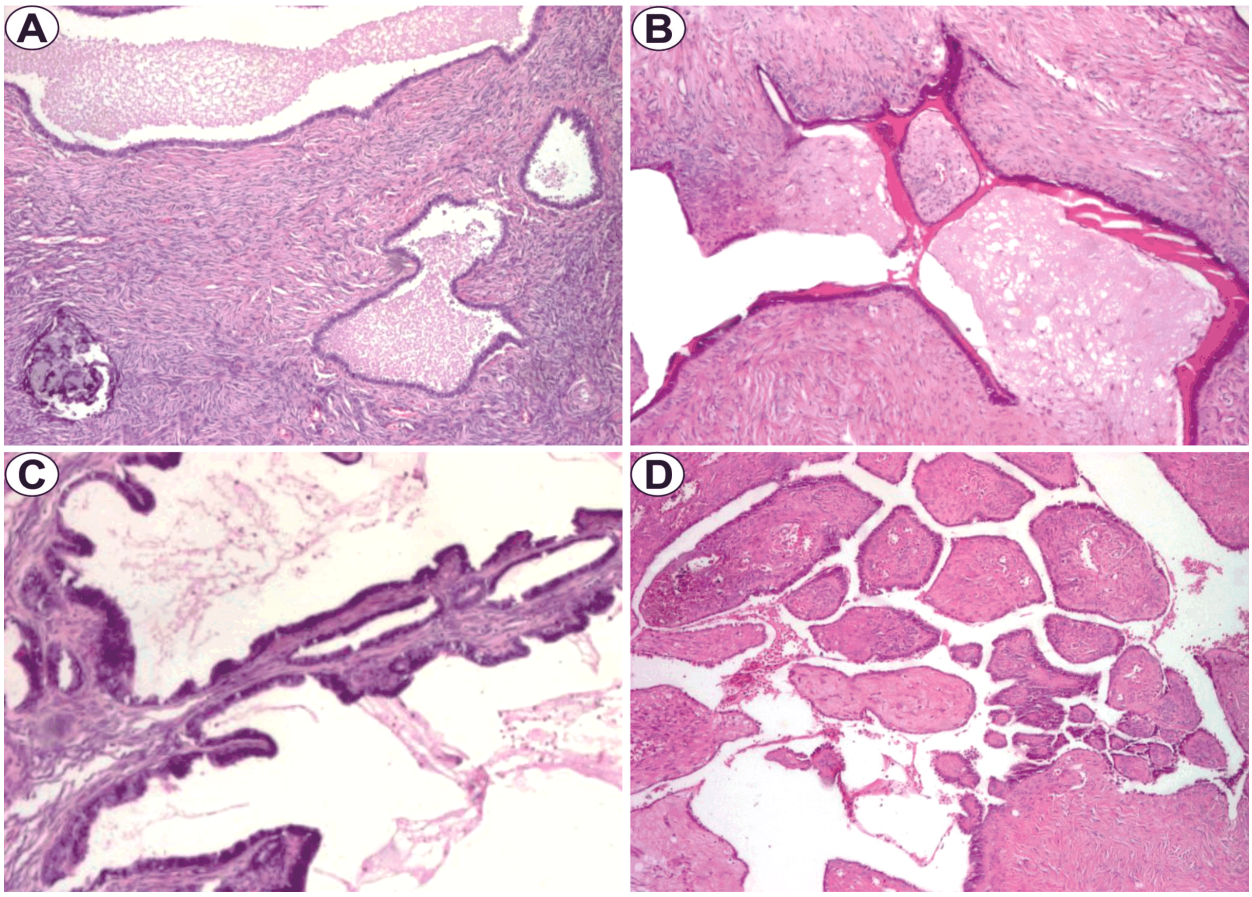

Figure 2 – *(A) Serous CA with calcifications; (B) Serous PCAF; (C) Serous PCA; (D) Serous PCAF with focal atypia. HE staining: (A and B) ×100; (C) ×200; (D) ×40. CA: Cystadenoma; PCAF: Papillary cystadenofibroma; PCA: Papillary cystadenoma.*

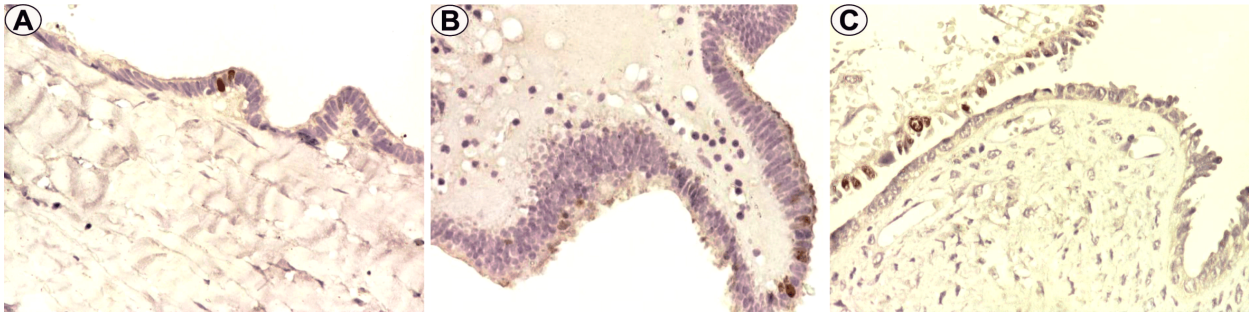

Figure 3 – *(A) PCAF; (B) PCA with focal atypia; (C) PCAF with focal atypia. Anti-Ki67 antibody immunostaining: (A and B) ×100. Anti-p53 antibody immunostaining: (C) ×100. PCAF: Papillary cystadenofibroma; PCA: Papillary cystadenoma.*

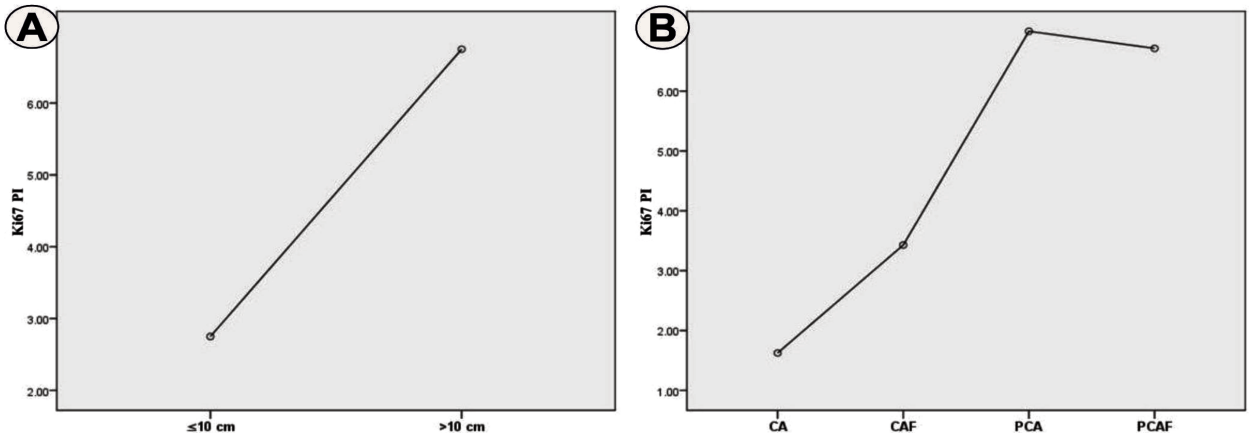

Figure 4 – *Distribution of Ki67 PI values depending on: (A) Tumor size; (B) Tumor type. PI: Positivity index; CA: Cystadenoma; CAF: Cystadenofibroma; PCA: Papillary cystadenoma; PCAF: Papillary cystadenofibroma.*

Of the epithelial tumors, serous CAs are the most common in pediatric pathology, with almost 50% of cases [9]. The average age of presentation at diagnosis is approximately 13 years, with ovarian tumors generally having a close relationship with the hormonal storm associated with adolescence [7]. In our study, the mean age of diagnosis was 13.2 years, 75% of the lesions being diagnosed in ages of more than 10 years. Serous ovarian CAs are reported in newborns, for whom radical treatment was performed due to the high torsion risk [18]. Also, Basirat *et al.* reported the presence of an ovarian cyst at fetal age (37 weeks), with a diameter of 30 mm, which after birth by Caesarean section showed a regression in the sense of disappearance [19]. The aspect is not unique, in a study that analyzed 38 ovarian tumors, Chu *et al.* indicating the prenatal diagnosis of lesions in 21.6% of cases [20].

In this study, for 87.5% of the analyzed cases, the patients presented symptoms specific to an ovarian tumor mass. Abdominal pain, nausea and repeated vomiting and subfebrility are the most common symptoms in patients with cystic structures of ovarian origin [3, 4]. In the case of serous CAs, the anemia caused by massive menstrual bleeding (menorrhagia) is one of the causes for presentation to pediatrician [7]. In this study, the paraclinical investigations were within normal limits, including markers regarding anemia, coagulation, inflammation or tumor presence. Also, the most common symptoms were pain and abdominal distension, loss of appetite, transit disorders, vomiting or mixed issues, sometimes with their persistence for a long period of time. Data from the literature indicate the importance of paraclinical investigations that are indicated for establishing the type of treatment, for excluding cancerous lesions or for establishing associated functional disorders [1].

Also, in order to establish an appropriate curative or conservative surgical treatment, to confirm the lesions and to reduce the risk of injury of adjacent structures, the imaging analysis of ovarian tumors in children and adolescents is of the maximum importance [10]. Also, the imaging analysis must differentiate ovarian tumors from other lesions, such as tube-ovarian abscesses, tubal pregnancy, other cysts (omental, mesenteric, retroperitoneal, pancreatic, choledochal, splenic), but also from renal or digestive malformations [4, 7, 21]. In our study the location, size and relationship with the neighboring structures was established by US and CT examinations, 91.7% of the lesions being unilateral and 66.7% being of maximum 10 cm in the largest dimension. Data from the literature indicate dimensions of benign serous ovarian tumors at pediatric age with dimensions between 3–10 cm, but there are also case reports with tumors over 30 cm and up to 4 kg in weight [4, 7]. In our study, the largest tumor size was of 24 cm.

HP aspects of ovarian serous tumors are variable and depending on the cystic and papillary architecture, are described simple or PCAs, simple or PCAFs, adenofibromas [1]. In this study, CAs predominated, present in 33.4% of cases, followed by PCAFs and CAFs, with 29.7% cases and PCAs with 8.3% of cases, the histological aspects being the classic ones, including the presence of calcifications and of hemorrhagic necrosis areas. Many of the ovarian cysts are functional non-neoplastic like the simple, follicular or luteal body cysts and differential diagnosis must be made with these [4]. At the same time, serous ovarian tumors must be differentiated by other types of epithelial tumors or by ovarian torsion [22].

A particular aspect was the identification of tumor focal areas of epithelial architectural complexity (three cases), with atypical appearance and which were present especially in the case of tumors with papillary pattern. Such tumors are described in the literature in reproductive age, as tumors with borderline areas and benign biological behavior, if atypical areas represent less than 10% of the tumor, bearing the name of cystadenomas or cystadenofibromas with focal proliferation or focal atypia [1, 15].

Data from the literature that analyzed Ki67 and p53 immunoexpression in benign ovarian serous tumors are relatively few and controversial. Ki67, which is a non-histone protein expressed in the nuclei in most phases of the cell cycle, is a useful marker for assessing cell proliferation, respectively the growth potential of a tumor, including in the ovarian level [23]. The PI for Ki67 in the case of benign and borderline serous ovarian tumors is described as being generally low, less than 2% but can reach up to almost 15% for benign epithelial tumors, respectively up to almost 23% for the borderline ones [1, 23, 24]. In our study, Ki67 PI did not exceed 9%, the highest values being identified in the case of tumors with papillary pattern, with atypical areas and with dimensions over 10 cm, the patients having generally over 10 years.

p53 is considered to be the guardian of the genome, with multiple functions, including deoxyribonucleic acid (DNA) repair, differentiation, induction of apoptosis, the literature data indicating the negativity in case of benign ovarian epithelial tumors [1, 24, 25]. Regarding the serous borderline tumors, the data are controversial, the percentages of positivity ranging from 0–30% [25]. In our study, p53 was positive only in the atypical areas of PCAs/PCAFs, the maximum value of PI being 10%.

The results of the study indicate the relations between the age of the patients, the size and tumor type histology and the tandem Ki67/p53 immunoexpression that support the possibility of elaborating criteria for the assessment of growth potential for benign ovarian serous tumors.

Conclusions

The study indicates the importance of investigating the clinicopathological parameters of benign ovarian serous tumors in the context of assessing the risk of tumor growth and progression. Age over 10 years, size over 10 cm, papillary tumor pattern and focal atypia are associated with the analyzed tumor proliferation and growth markers, an aspect that may be useful for stratifying patients for active clinical surveillance. Further studies are needed to assess the relationships described in the study on large groups of benign ovarian tumors, including other tumor types.

Conflict of interests

The authors declare that they have no conflict of interests.

Authors' contribution

Mioara Desdemona Stepan and Cristina Adriana Becheanu equally contributed to this article.

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