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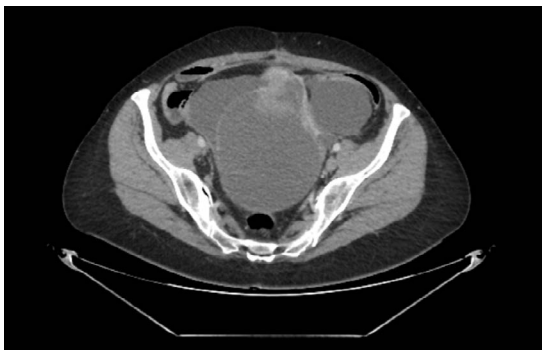

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CONTENTS

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Long non-coding RNA H19 correlates with unfavorable prognosis and promotes cell migration and invasion in ovarian cancer

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ABSTRACT

Objectives: The purpose of this study is to investigate the expression pattern of lncRNA H19 in OC tissues and to detect the ability of H19 to influence OC cell migration and invasion *in vitro*.

Material and methods: We quantified the levels of H19 within the obtained cancerous and adjacent noncancerous tissues from 258 OC patients. H19 association with patient progression-free survival (PFS) was analyzed by a Kaplan-Meier plot. Expression levels of H19 were reduced by small interfering RNA transfection against H19 or restored by a H19 overexpression plasmid transfection in OC cells. H19 effects on OC cell migration and invasion *in vitro* were evaluated using wound-healing assay and transwell invasion assay. Wound healing assay and transwell invasion assay were used to evaluate the effects of H19 on OC cell migration and invasion *in vitro*.

Results : H19 is upregulated remarkably in primary OC tissues and human OC cell lines (OVCAR3, SKOV3, A2780, and Caov-3). We found that the median PFS was longer in patients with lower levels of H19 than in those with high levels, suggesting that overexpres- sion of H19 was linked to poor prognosis in OC patients. Intriguingly, the depletion of H19 expression induced by small interfering RNA inhibited the capability of migration and invasion of OC cell lines. Restoration of H19 in OC cell lines significantly increased cell migration and invasion.

Conclusions: The key finding of the present study suggests that overexpression of H19 may be associated with an unfavorable prognosis for OC and is likely to be a possible contributory force involved in OC cell migration and invasion. H19 may provide a new and attractive target for future prognostic and therapeutic intervention of OC patients.

Key words: ovarian cancer; long non-coding RNA; H19; prognosis; migration

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INTRODUCTION

The continuing increase in the incidence and prevalence of ovarian cancer (OC) is a cause for concern [1]. Up until now, OC represented the second most common gynecological malignancies, accounting for 5% of all cancers in females [2]. On an annual basis, an estimated 220,000 newly diagnosed OC cases worldwide [3]. Due to its late presentation and often being diagnosed at an advanced stage, most patients succumb to recurrence and wide metastasis, explaining the high mortality rate [4]. OC is heterogeneous in nature and encompasses a collection of distinct histologic types, all with characteristic differences, which is one of the major obstacles to improvement in this disease [5]. Early stage OC symptoms of peritoneal metastasis are generally nonspecific, which subsequently allows for frequent misdiagnoses as well as underdiagnoses. Expression patterns of a recently identified biomarker family, long non-coding RNA (lncRNA), seem to be characteristic of tumor type and developmental origin, including OC [6, 7].

Understanding of expression pattern and imprinting of H19 has progressed considerably in recent years. The function of H19 in cancers remains to be elucidated due to its dual roles acting either as a tumor suppressor or an oncogene. In a recent study, H19 is shown to inhibit cancer progression [8, 9]. However, increasing evidence showed that H19 expression was increased in several cancers such as breast cancer, lung cancer, gastric cancer, and bladder cancer [10–13], highlighting its oncogenic properties. In-

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triguingly, several gain- or loss-of-function studies demonstrated that H19 knockdown suppressed cell migration and invasion in clear cell renal carcinoma and glioma [14, 15]. Few data support the functional role of H19 in OC, and only a study reported by Zhu *et al.* proposed the contributory effect of H19 in OC [16]. However, measurement of H19 in 70 pairs of OC tissue samples and normal controls may limit the validity of results.

To investigate the expression and functional role of H19 in human ovarian cancer, in this study we quantified the levels of H19 within the obtained cancerous and adjacent noncancerous tissues from OC patients. Progression-free survival (PFS) was calculated by following up with the patients. In addition, functional assays were applied in OC cells. Our research may provide evidence for the diagnosis and treatment of ovarian cancer.

MATERIAL AND METHODS

Study subjects

We collected cancerous and adjacent noncancerous tissues from 258 patients with OC who were admitted into our hospital between January 2010 and January 2014. These patients had a mean age of 48.5 ± 6.3 (ranging from 29 to 68 years old). The inclusion criteria for patient enrollment: (1) an ovary biopsy with pathological and imaging diagnosis of OC; (2) an initial treatment in our hospital for OC; (3) no standard therapies, such as chemotherapy, surgery, or radiation therapy; (4) no previous history of the malignant tumors; (5) no distant metastases or second primary tumor. The study protocol was approved by the Institutional Ethics Committees at our hospital, and signed written informed consent was received from all patients prior to enrollment.

Follow-up

The follow-up was scheduled at discharge, ranging from 3 to 36 months and consisting of a telephone interview, a medical record review, or a hospital visit. The follow-up period ended on December 2016. Among 258 cases, 6 cases were censored. A follow-up rate of 93.0% was achieved. The primary endpoint was progression-free survival (PFS) was defined as the time from diagnosis to either disease progression or relapse, or to death as a result of any cause.

Cell preparation

Human normal ovarian surface epithelial cell lines were purchased from Shanghai Huiying Bio-technology Co., Ltd., China and human OC cell lines, OVCAR3, SKOV3, A2780, and Caov-3, from Cell Bank of Chinese Academy of Sciences, Shanghai, China. SKOV3 cells were cultured with McCoy's 5A Medium Modified (Sigma, St. Louis, MO, USA), in addition,RPMI-1640 medium (Gibco Company, Grand Island, NY, USA) for OVCAR3 and A2780 cells, and DMEM-H (Dulbecco's Modified Eagle's Medium, High Glucose; Hyclone Laboratories, Logan, Utah, USA) for Caov-3 cells. All of the media were supplemented with 10% fetal bovine serum (FBS, Gibco Company, Grand Island, NY, USA), and all cells were incubated with 5% CO_2 at 37°C. In order to evaluate the regulatory effects of H19 on OC cells, SKOV3 cells showing the highest expression level of H19 were treated by small interfering RNA (siRNA) against H19 and a H19 overexpression plasmid, respectively. An ineffective scramble of siRNA, a siRNA against H19, and a H19 overexpression plasmid was purchased from Shanghai GenePharma Co., Ltd. (Shanghai, China). The Lipofectamine 2000 (Invitrogen Corp., Carlsbad, CA, USA) was used to transfection operations according to the manufacturer's instructions.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Total RNA from tissues was isolated by TRIzol kits (Invitrogen, Carlsbad, CA, USA). High-molecular-weight RNA was identified on a denaturing formaldehyde gel, then l μg of RNA was reversely transcripted into cDNA using AMV-reverse transcriptase. Primers were obtained from Invitrogen Inc., Carlsbad, CA, USA (Tab. 1). GAPDH was used as a loading control. The PCR cycling reaction conditions: pre-denaturation at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 40 s, annealing at 60°C for 40 s and extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min. PCR products were then subject to agarose gel electrophoresis and analyzed by Opticon Monitor™ version 3.0 software (Bio-Rad, Inc., Hercules, CA, USA). The Opticon monitor software (MJ Research, San Francisco, CA, USA) was used to set the cycle threshold or Ct line manually. Data were analyzed by $2^{-\Delta\Delta Ct}$ method. ΔCt = Ct (target gene)-Ct (loading control), $\Delta\Delta Ct = \Delta Ct$ (experimental group)-ΔCt (control group). The experiment was repeated three times independently, with the average obtained.

Transwell assay

Forty eight hours after transfection, 1×10^5 cells were counted and inoculated in Matrigel-coated (80 μl of at a ratio of 1:8) transwell chambers containing 100 μl serum-free DMEM medium. The Matrigel and cells that remained on the upper side were wiped off following 24 hours incubation. Then, the passed cells were fixed in 4% paraformaldehyde for 15 min and subsequently stained with 0.2% crystal violet for 10 min. Lastly, an inverted light microscope (Olympus IX70, Tokyo, Japan, at × 200 magnification) was applied to count the number of invading cells in five predetermined fields to evaluate cell invasion. All experiments were independently performed at least three times.

Table 1. primer sequences of OPN for reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Gene	Sequences
H19	F: 5′-GTCCGGCCTTCCTGAACACCTT-3′
	R: 5′-GCTTCACCTTCCAGAGCCGAT-3′
GAPDH	F: 5′-GACAACTTTGGCArCGTGGA-3′
	R: 5′-ATGCAGGGATGATGTTCTGG-3′

Scratch test

On the back of the 6-well plate, use a marker pen to scratch an even with an interval width of 0.8 cm. Each well was required to be crossed by more than five lines and added with 5×10^5 wells. When the cells grew to 100%, a uniform scratch was made in the center of the well using a sterile micropipette tip, followed by washing with phosphate-buffered saline. After 58 hours incubation with 5% CO_2 at 37°C, the wound healing was photographed for the record. The rate of cell migration was assessed by the wound closure assay. All experiment was also independently performed at least three times.

Statistical analysis

Statistical analysis was done using SPSS software (IBM SPSS Statistics, version 21.0, Armonk, NY, USA). Measurement data were expressed as the mean ± standard deviation (SD); Fisher's least significant difference (LSD) was conducted for pairwise comparisons, one-way analysis of variance (ANOVA) test for comparisons among multiple groups, and *t*-test for comparisons between two groups when demonstrating normal distribution. The association with survival was analyzed initially by Kaplan-Meier plot and log-rank test. Differences were accepted as significant if p-values less than 0.05.

RESULTS

Increased expression levels of H19 in OC primary tissues

Firstly, in order to evaluate the expression pattern of H19 in OC, we quantified the expression levels of H19 within the obtained cancerous and adjacent noncancerous tissues from 258 OC patients using RT-qPCR. We found that the expression levels of H19 in cancer tissues (5.82 ± 0.67) were higher than those in adjacent noncancerous tissues (2.46 ± 0.29, $P < 0.01$).

Increased expression levels of H19 associated with poor survival of OC patients

Next, we classified 258 OC patients into low- and high-level groups in terms of expression levels of H19 in OC primary tissues to evaluate the association between the H19 expression levels and patient survival. We regarded patient PFS as the end point event. The Kaplan Meier survival analysis (Fig. 1) showed the median PFS was longer in OC tissues with low expression levels of H19 than in those with high expression levels (30.00 months *vs.* 24 months, $P < 0.01$). The data reveal that increased expression levels of H19 associated with poor survival of OC patients.

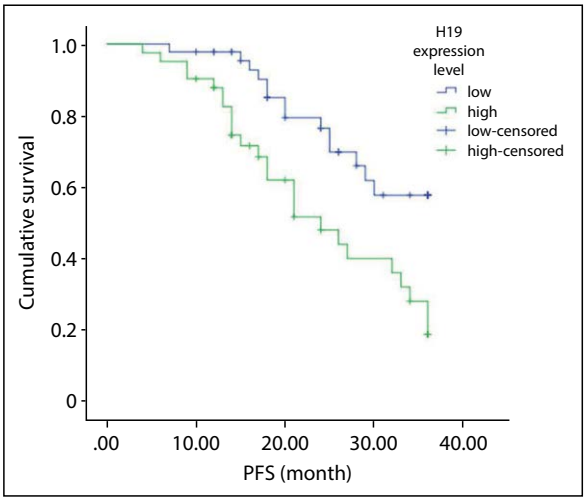

Figure 1. The Kaplan-Meier plot showed that increased expression levels of H19 are associated with poor survival of OC patients

Increased expression levels of H19 in OC cell lines

In this study, we chose OC cell lines that have been widely used in OC research, especially in functional studies. We examined the expression levels of H19 in OVCAR3, SKOV3, A2780, and Caov-3 cell lines by RT-qPCR. The result showed that H19 was increased in these four OC cell lines ($P < 0.05$, Fig. 2) in which SKOV3 cell lines were highest.

Silencing or restoring H19 in OC cells

Furthermore, we treated SKOV3 cells with an ineffective scramble of siRNA, siRNA against H19, and a H19 overexpression plasmid, respectively. We quantified the expression levels of H19 in SKOV3 cells after different treatments by RT-qPCR. Indeed, H19 expression was restored by H19 overexpression plasmids, while H19 expression was suppressed by siRNA against H19 ($P < 0.05$). There was no significant difference in H19 expression levels when untreated SKOV3 cells were compared to SKOV3 cells treated with ineffective scramble of siRNA. The data are shown in Figure 3.

The contributory effects of H19 on OC cell invasion and migration in vitro

To know the function of H19 in OC, we tested the effects of H19 on cell invasion and migration *in vitro* by transwell invasion assay and wound-healingassay. To determine the

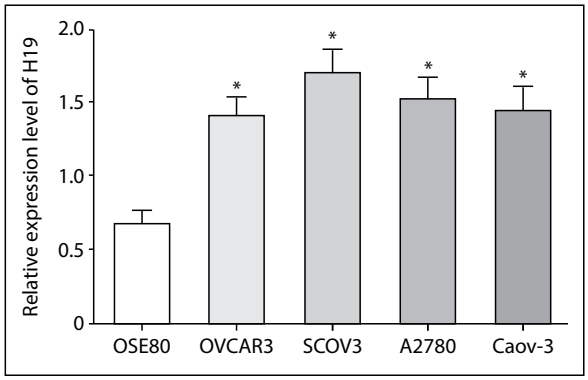

Figure 2. Increased expression levels of H19 in OC cell lines, determined by RT-qPCR. Compared with IOSE80 cells, OVCAR3, SKOV3, A2780, and Caov-3 cells showed higher expression levels of H19 (* — $P < 0.05$). Among these four OC cell lines, SKOV3 cell lines exhibited the highest expression levels of H19

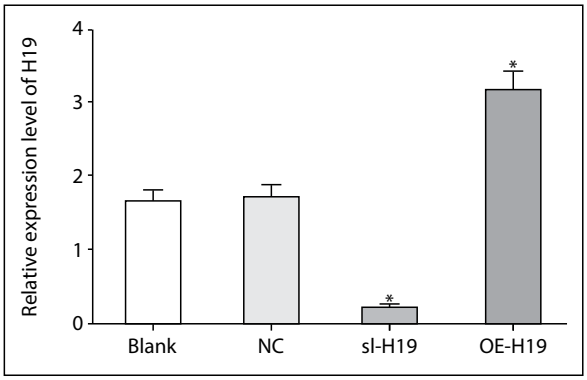

Figure 3. H19 expression was suppressed or restored in SKOV3 cells, verified by RT-qPCR; * — $P < 0.05$ compared with untreated SKOV3 cells

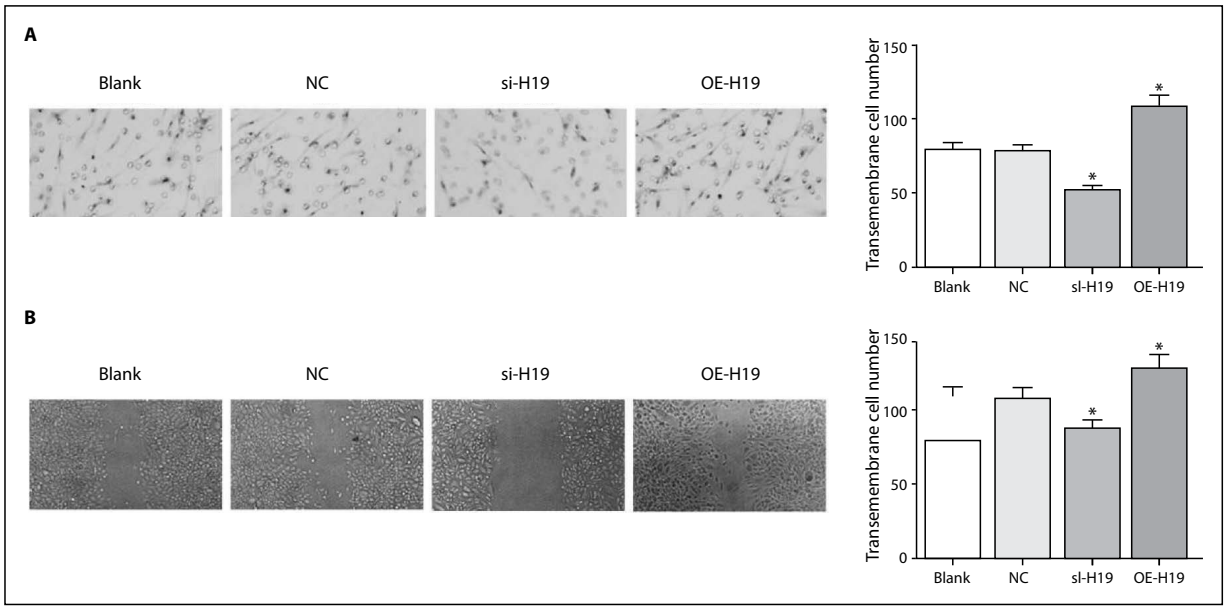

Figure 4. H19 could promote OC cell invasion and migration; **A.** wound-healing assay was used to determine the effect of H19 on the invasion potential of SKOV3 cells (× 200); **B.** transwell invasion assay was used to determine the impact of H19 on migration potential of SKOV3 cells (×200); * — $P < 0.05$ compared with untreated SKOV3 cells

effect of H19 on the invasion potential of SKOV3 cells, we first carried out Matrigel invasion assays (Fig. 4A). After harvesting for 48 h, we found fewer SKOV3 cells treated with siRNA against H19 passed through Matrigel, but more SKOV3 cells treated with H19 overexpression plasmids passed through Matrigel ($P < 0.05$). An ineffective scramble of siRNA did not significantly affect the invasion of SKOV3 cells ($P > 0.05$). Next, wound-healing assay was used to determine the effect of H19 on the migration potential of SKOV3 cells (Fig. 4B). After harvesting for 48 h, we found SKOV3 cells treated with siRNA against H19 were distinctively less migrated, but SKOV3 cells treated with H19 overexpression plasmids healed the wound area faster ($P < 0.05$). An ineffective scramble of siRNA did not significantly affect the migration of SKOV3 cells ($P > 0.05$). These findings indicated that H19 could promote OC cell invasion and migration *in vitro*.

DISCUSSION

LncRNAs are becoming new candidates for diagnosing cancer disease, explaining the mechanism of the pathogenesis and development of malignant tumors, predicting prognosis and treating disease as targets in recent years [17]. In the present study, we demonstrate the role of H19 as an oncogene in OC. Significantly, H19 was found to be increased in primary OC tissues and cell lines compared with adjacent normal tissues and normal cell lines,

and overexpression of H19 was linked to poor prognosis in OC patients. In addition, in order to detect the ability of H19 to influence OC cell migration and invasion *in vitro*, the expression levels of H19 were reduced by small interfering RNA transfection against H19, or restored by a H19 overexpression plasmid transfection in OC cells. The contributory effects of this lncRNA on cell migration and invasion indicate that H19 promotes tumorigenesis in OC.

Recently, H19 is highlighted for its association and involvement with many cancers, as it plays an important role in regulating the expression of many genes that are essentials for numerous cellular processes [18]. Yang *et al.* reported that ectopic expression of H19 increased cell proliferation, and cell apoptosis was induced in gastric cancer cell lines while siRNA-mediated down-regulation of H19 [12]. They also found H19 affected the activity of p53, and that this effect was leading to partial p53 inactivation. Suppression of H19 induces invasion of serous borderline ovarian tumor cells *via* reducing PI3K/Akt-mediated inhibition E-cadherin [19]. He *et al.* reported the mechanism that overexpression of H19 was sufficient to increase the expression of E2F1 by which H19 promotes OC migration and invasion [14]. E2F1 exerts an anti-proliferative effect in OC cells, becoming a target for preventing OC [20]. Luo and his team found that upregulated H19 promotes bladder cancer cell migration associating with enhancer of zeste homolog 2 (EZH2), and that this association was leading to Wnt/β-catenin activation and subsequent inhibition of E-cadherin [13]. EZH2 is found to be upregulated in malignant tumors and is involved in metastasis, including OC, and overexpression of EZH2 facilitates OC cell invasion and migration [21]. An unexpected mode of action of H19 can antagonize the let-7 family of microRNAs [22]. An increase of microRNA let-7i expression induces OC cell apoptosis, which is the mechanism of propofol, can effectively inhibit proliferation and induce apoptosis in OC cells [23]. Inhibited let-7i expression remarkably reduced the resistance of OC cells to the chemotherapy drug, and decreased let-7i expression was associated with the shorter PFS of OC patients, which may explain the fact that overexpression of H19 was linked to poor prognosis in OC patients [24]. A high H19 expression contributes to poor overall survival and can be served as an independent predictor of the overall survival of gastric cancer patients [25], which may provide evidence for the prognostic role of H19 in OC, as reflected in our study.

CONCLUSIONS

Based on the key findings obtained from our study, we believe that H19 could potentially act as atherapeutic target. The reasons were as follows: (1) H19 expression levels are significantly increased in tissues and cell lines of OC; (2) H19 in relation to OC prognosis; (3) inhibiting H19 suppresses OC cell migration and invasion.

Taken together, our findings indicate that H19 plays a vital role in the development and progression of OC. The development of downregulation of this oncogenic lncRNAs based on H19-based therapeutic strategies may provide new and promising alternative therapeutics for future OC treatment. However, a larger sample size and longer follow-up period are required to confirm the correlation between H19 expression level and overall 5-year survival rate of OC patients. Meanwhile, more attention should be paid to exploring the mechanism of H19 and its interaction with oncogenes, and a new target should be discovered to cope with the highly migratory and invasive OC.

Ethical approval

The study protocol was approved by the Institutional Ethics Committees of Haimei Hospital, University Of Chinese Academy Of Sciences, and signed written informed consent was received from all patients prior to enrollment.

Consent for publication

Informed consent was obtained from all individual participants included in the study.

Availability of data and material

The datasets used or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of interests

None.

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Endocervical polyps in high risk human papillomavirus infections

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ABSTRACT

Objectives: Human papillomavirus (HPV) positive patients with and without endocervical polyps is compared with respect to HPV genotypes and presence of pre-invasive diseases. To our knowledge, this is the first and largest report in the literature examining the endocervical polyps in HPV positive cases.

Material and methods: Clinicopathological data for the first one million screening patients (n = 1 060 992) from around the entire country during 2015 and 2016 were targeted for this research. Colposcopy, colposcopic surgical diagnostic procedures and final pathology results of 3499 patients with high-risk (HR) HPV-positive were obtained from reference colposcopy centers. Patients with endocervical polyps (n = 243 [6.9 %]) were accepted as experimental arm while patients without any endocervical polyp (n = 3256 [93.1%]) were regarded as the control group. Age, HPV genotype, Pap smear abnormality, and final pathological results were compared between two groups using Student's t-test and cross-tabulation chi-square test.

Results: The incidence of endocervical polyp was found to be 6.9 % in HR HPV-positive women. The most common HPV genotypes observed in both groups were HPV 16 or 18. Abnormal cytology reports (≥ ASC-US) were not significantly different between both groups. However, with respect to final pathological diagnosis, patients with endocervical polyp had significantly lower numbers of pre-invasive diseases (31.3% vs 44.2%; $p < 0.10$).

Conclusions: Endocervical polyps may be more common in patients with HR HPV infections. HPV 18 is observed significantly more, in the HR HPV positive endocervical polyp group. Patients with endocervical polyps do not have increased risk for preinvasive cervical diseases.

Key words: cervical cancer; colposcopy; human papillomavirus; endocervical polyp; HPV 18; cervical cancer screening

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INTRODUCTION

Endocervical polyps can be described as benign polypoid epithelial overgrowths that arise from the endocervical canal. Their prevalence ranges between 2% and 5% among women of reproductive age. With a peak incidence between the ages of 40 and 60 years, they are more commonly seen in the postmenopausal period [1]. With almost all endocervical polyps being benign in character, and the incidence of malignancy varying between 0.1% and 0.2%, approximately two-thirds of endocervical polyps are asymptomatic, but the most common symptom is abnormal vaginal bleeding [1, 2].

Although the endocervical polyps are common, there are few studies in the published literature. The etiology still has not gained clarity, although chronic inflammation (chronic cervicitis, foreign body reaction), and abnormal local response to estrogen stimulation are the main hypotheses responsible for the formation of cervical polyps [2, 3]. Up to now, there are no reports in the literature evaluating these polyps in high-risk (HR) human papillomavirus (HPV) infections. To our knowledge, this is the first and largest report in the literature examining the endocervical polyps in HPV positive cases.

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MATERIAL AND METHODS

This study was approved by the Institutional Review Board and all participants gave written general informed consent for use of personal information in health research (Turkish Public Health Institution No: 23776858-825.03). The experimental arm of the study group consisted of women with a positive HPV test in a national cervical cancer screening program conducted by the Department of Cancer Control and the Public Health Institute of Turkey. Since 2014, Turkey has used HPV DNA for primary screening with extended genotyping and conventional Pap smear to triage HPV-positive cases. Women between the ages of 30 and 65 years are invited for screening by primary health care personnel. Conventional cytology and HPV DNA samples are taken together from each woman at the initial visit to enable cytology testing in those found to be HPV-positive without the need for another visit. For women who are found to be HPV-positive by Hybrid Capture 2 (Qiagen), genotyping is performed using the CLART kit (Genomica). HPV-positive women with abnormal cytology or those positive for HPV 16 or 18 are referred for colposcopy, which is performed free of charge in a post screening diagnostic center (colposcopy centers).

Clinicopathological data for the first one million screening patients (n = 1 060 992) from around the entire country during 2015 and 2016 were targeted for this research. Among this screening population, information from patients who were screened and referred to colposcopy centers was retrospectively collected Colposcopy, colposcopic surgical diagnostic procedures (punch biopsy, loop electrosurgical excision procedure, endocervical curette, conization, etc.) and final pathology results of 3499 patients with HR HPV-positive could be obtained from reference colposcopy centers. Patients with endocervical polyps (n = 243 [6.9 %]) were accepted as experimental arm, patients without any endocervical polyp (n = 3256 [93.1%]) were regarded as the control group. The parameters collected for comparison of the groups were age, HPV genotype, Pap smear abnormality, and final pathological results. Descriptive epidemiological data and clinicopathological comparisons were evaluated using the Student *t*-test and chi-square test with cross tables. The binary logistic regression test was used for the multivariate analysis. All data were collected and evaluated using Microsoft Excel (Microsoft Corp., Redmond, WA, US) and SPSS 23.0 (IBM Corp., Armonk, NY, US). A p-value less than 0.05 was accepted as significant for statistical comparisons.

RESULTS

The median age of the patients was 43 years (standard deviation [SD] ± 7.58) in the endocervical polyp group and 43 years (SD ± 8.53) in the control arm (p > 0.5). Polyps were most commonly seen in women between the ages of 30 to 44 (n = 138). There was no significant difference in patients with and without endocervical polyps with respect to the different age groups (30–44, 45–54, and 55–65 years) (Tab. 1). With respect to HPV genotypes, the most commonly encountered HPV in the endocervical polyp group was HPV 16 (n = 148 [60.9%]), followed by HPV 18 (n = 46 [18.9%]), HPV 51 (n = 22 [9.1%]), HPV 31 (n = 21 [8.6%]), and HPV 39 (n = 19 [7.8%]). For patients with no endocervical polyp, the most common were HPV 16 (n = 2011 [61.8%]), followed by HPV 18 (n = 458 [14.1%]), HPV 51 (n = 382 [11.7%]), HPV 31 (n = 300 [9.2%]), and HPV 52 (n = 258 [7.9%]). Among patients with endocervical polyps, 76.1% (n = 185) had HPV 16 or 18, whereas the remaining 23.9% (n = 58) had HPV types other than HPV 16 and 18. Among patients with no cervical polyp, 72.9% (n = 2372) and 27.1% (n = 884) had HPV 16 or 18 (p = 0.26).

Among the 243 patients with endocervical polyps, cytology results were normal or infection in 49.8%, insufficient in 8.2%, and abnormal in 42% (≥ atypical squamous cells

Table 1. Comparison of HR HPV-positive patients with and without endometrial polyps

	Patients with endocervical polyp (243 patients) (n, %)	Patients without endocervical polyp (3256 patients) (n, %)	p value
Age, median	43	43	
Age intervals			
30–44	138 (56.8%)	1864 (57.2%)	0.880
45–54	78 (32.1%)	932 (28.6%)	0.240
55–65	27 (11.1%)	460 (14.1%)	0.190
HR HPV genotypes			
16 or 18	185 (76.1%)	2372 (72.9%)	0.260
Other genotypes	58 (23.9%)	884 (27.1%)	
Pap smear			
Normal	22 (9.1%)	237 (7.3%)	0.300
ASC-US	27 (11.1%)	411 (12.6%)	0.490
LSIL	64 (26.3%)	983 (30.2%)	0.200
HSIL	7 (2.9%)	86 (2.6%)	0.820
Final pathology			
Normal	167 (68.7%)	1818(55.8%)	< 0.001
CIN1	33 (13.6%)	675 (20.7%)	0.007
CIN2	16 (6.6%)	269 (8.3%)	0.350
CIN3	27 (11.1%)	409 (12.6%)	0.500
Cancer	0	85 (2.6%)	0.011
Total ≥ CIN1	76 (31.3%)	1438(44.2%)	< 0.001

ASC-US — atypical squamous cells of undetermined significance; CIN — cervical intraepithelial lesions; HPV— human papillomavirus; HR — high-risk; HSIL — high-grade squamous intraepithelial lesion; LSIL — low-grade squamous intraepithelial lesion

Table 2. Comparison of HPV genotype distributions within different age groups between patients with and without an endocervical polyp

	Ages 30–44			Ages 45–54			Ages 55–65			Total		
Genotypes	**With polyp, n (%)**	**Without polyp, n (%)**	**p value**	**With polyp, n (%)**	**Without polyp, n (%)**	**p value**	**With polyp, n (%)**	**Without polyp, n (%)**	**p value**	**With polyp, n (%)**	**Without polyp, n (%)**	**p value**
HPV 16	91 (65.9%)	1147 (61.5%)	0.30	43 (55.1%)	551 (59.1%)	0.490	14 (51.9%)	313 (68%)	0.080	148 (60.9%)	2011 (61.8%)	0.79
HPV 18	21 (15.2%)	246 (13.2%)	0.50	18 (23.1%)	134 (14.4%)	0.040	7 (25.9%)	78 (17%)	0.230	46 (18.9%)	458 (14.1%)	0.04
HPV 31	15 (10.9%)	163 (8.7)	0.39	6 (7.7%)	93 (10%)	0.51	0 (0%)	44 (9.6%)	0.09	21 (8.6%)	300 (9.2%)	0.76
HPV 33	2 (1.4%)	50 (2.7%)	0.38	4 (5.1%)	17 (1.8%)	0.049	1 (3.7%)	11 (2.4%)	0.67	7 (2.9%)	78 (2.4%)	0.64
HPV 35	9 (6.5%)	70 (3.8%)	0.10	1 (1.3%)	45 (4.8%)	0.15	0 (0%)	32 (7%)	0.16	10 (4.1%)	147 (4.5%)	0.77
HPV 39	13 (9.4%)	127 (6.8%)	0.25	2 (2.6%)	64 (6.9%)	0.14	4 (14.8%)	19 (4.1%)	0.01	19 (7.8)	210 (6.4)	0.40
HPV 45	2 (1.4%)	54 (2.9%)	0.32	3 (3.8%)	22 (2.4%)	0.41	0 (0%)	9 (2%)	0.46	5 (2.1%)	85 (2.6%)	0.60
HPV 51	13 (9.4%)	223 (12%)	0.37	6 (7.7%)	107 (11.5%)	0.30	3 (11.1%)	52 (11.3%)	0.97	22 (9.1%)	382 (11.7%)	0.21
HPV 52	7 (5.1%)	156 (8.4%)	0.17	1 (1.3%)	59 (6.3%)	0.07	1 (3.7%)	43 (9.3%)	0.32	9 (3.7%)	258 (7.9%)	0.02
HPV 56	7 (5.1%)	111 (6%)	0.99	6 (7.7%)	59 (6.3%)	0.20	3 (11.1%)	34 (7.4%)	0.41	16 (6.6%)	204 (6.3%)	0.78
HPV 59	5 (3.6%)	87 (4.7%)	0.57	1 (1.3%)	32 (3.4%)	0.30	1 (3.7%)	31 (6.7%)	0.53	7(2.9%)	150 (4.6%)	0.21
HPV 58	7 (5.1%)	95 (5.1%)	0.99	6 (7.7%)	42 (4.5%)	0.20	1 (3.7%)	37 (8%)	0.41	14 (5.8%)	174 (5.3%)	0.78
HPV 68	10 (7.2%)	87 (4.7%)	0.170	4 (5.1%)	42 (4.5%)	0.800	2 (7.4%)	24 (5.2%)	0.620	16 (6.6%)	153 (4.7%)	0.190
Total	202	2625		101	1267		37	727		340	4619	

of undetermined significance [ASC-US], n = 102). Of those with abnormal cytology findings, 27 (11.1%) patients had ASC-US, 64 (26.3%) had low-grade squamous intraepithelial lesion (LSIL), 1 (0.4%) had ASC-H, 7 (2.9%) had high-grade squamous intraepithelial lesion (HSIL), and the remaining 3 (1.2%) had atypical glandular cells. These figures were not significantly different when compared with the control arm (Tab. 1). However, when final pathology reports were compared between both groups, patients with endocervical polyps had significantly higher normal pathologies (68.7% vs 55.8%; $p < 0.001$) (Tab. 1). Patients with no endocervical polyp had significantly higher numbers of cervical intraepithelial lesions (CIN) grade 1 or greater lesions (44.2% vs 31.3%; $p < 0.001$).

A separate subgroup analysis was performed comparing HPV genotypes with respect to different age intervals among both groups (Tab. 2). In all age groups, HPV 16 was the dominant and most commonly seen genotype, showing a decreasing prevalence with age and a peak among patients aged between 30 and 44 years old. HPV 18 had a similar pattern of decreasing prevalence with age. There was no significant difference within the different age groups with respect to HPV 16, despite a higher ratio of HPV 16 among patients without an endocervical polyp. HPV 18 was more common in patients with endocervical polyps than in those without endocervical polyps (18.9% vs 14.1%; $p = 0.04$). HPV 52 was more common in patients without an endocervical polyp than in the group with an endocervical polyp (7.9% vs 3.7%; $p = 0.02$). HPV 18 was significantly more common in patients with endocervical polyps between the ages of 45 and 54 (23.1% vs 14.4%; 5.1% vs 1.8%). HPV 39 was also more common in patients with endocervical polyp among those aged between 55 and 65 (14.8% vs 4.1%; $p = 0.01$).

DISCUSSION

The prevalence of endocervical polyps ranges between 2% and 5% among women of reproductive age, with a peak incidence between the ages of 40 and 60 years. In this study, the incidence of endocervical polyp was found to be 6.9% in HR HPV-positive women, with a peak incidence between the ages of 30 and 44 years. This is 1.2 to 3 times more compared with the general population. Accordingly, we can hypothesize that endocervical polyps may be more common in patients with HR HPV infections.

When the HR HPV-positive women with endocervical polyps were examined, we found that HPV 16 was the most common, followed by HPV 18, HPV 51, HPV 31, and HPV 39, respectively. For patients without an endocervical polyp, the most common were HPV 16, HPV 18, HPV 51, HPV 31, and HPV 52, respectively. Our findings show that there was no relationship detected between the presence of endocervical polyp and HPV genotypes in women between the ages of 30 and 44 years old. With respect to HPV genotypes, with HPV 18 being observed significantly more, and HPV 52 be-

ing significantly low in the endocervical polyp group, these 2 HPV types were found to be significantly different in the endocervical polyp group. It is also well known that HPV 18 is commonly detected in adenocarcinomas that usually develop from the endocervical canal [4–6].

With cervical cancer not being detected in any of the women with endocervical polyps, when all HR HPV-positive patients were compared, an endocervical polyp was not found to be significantly associated with a higher risk for preinvasive cervical diseases. Similar to our findings, in a study evaluating 228 women with asymptomatic cervical polyps, invasive cancer was not detected in any of the polyps despite a high rate of cervical cytology abnormalities (29.8%) [7]. Based on the results of our study, some may hypothesize that endocervical polyps are not associated with increased risk of preinvasive or invasive cervical diseases. However, in contrast to our findings, Chin et al. [8] published a study of nine cases of squamous intraepithelial lesions (SIL) arising in endocervical polyps. They reported the highest incidence of SIL in endocervical polyps to be around 1 in 200. The authors examined 12 smear samples obtained from nine women and found them to be normal in 58.8% of the patients, ASC-US in 25% of the patients, and LSIL in 16.7% of the patients. All patients had a biopsy, which, within the polyps detected, revealed HSIL in four patients and LSIL in the remaining five patients. Based on their results, the authors highlighted the importance of removing the endocervical polyps because of the significant incidence of SIL in endocervical polyps [8]. However, their findings were based on only nine patients. Also, in contrast to their findings, this incidence has been reported in much lower rates (0.025–0.2%) in 2 other previous studies [1, 9]. Our study is the largest series in the published literature and revealed no additional increased risk for preinvasive diseases even in HR HPV positive cases. While patients with an endocervical polyp had significantly lower numbers of CIN1 or greater lesions, none of the patients had cervical cancer. Again similar to our findings, most studies support the notion that there is no clear indication to excise asymptomatic polyps if cytological and colposcopic findings are normal [7, 10, 11].

Although this study is the first report in the literature investigating the coexistance of HPV positivity and presence of endocervical polyps, the study has some limitations. This study includes a cross-sectional time frame. Long-term follow-up results of the patients with HR HPV positive endocervical polyp are required. In this way, the relationship between endocervical polyps and preinvasive cervical lesions can be further clarified. Molecular and immunohistochemical studies are needed to verify the relationship between HR HPV and endocervical polyps. The adenocarcinoma arising from endocervical polyp background is an important challenge to solve the clinical impression of our data. On the other hand, the retrospective nature of our study is also a definite bias factor. Nevertheless, this study is the first of its kind, and provides important clues regarding endocervical polyps, and may have a particular impact on future researches.

Conflict of interest

The authors report no conflict of interest.

Financial disclosure

Authors have no financial interests about the research.

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Human papillomavirus genotyping in high-grade squamous intraepithelial lesion

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ABSTRACT

Objectives: Human papillomavirus infection is one of the most common sexually transmitted infections. Long-term exposure to the HPV leads to high-grade squamous intraepithelial lesions affecting cervical cancer. Knowledge about the distribution of HPV genotypes is crucial to guide the introduction of prophylactic vaccines. We aimed the genotype distribution in patients reporting due to abnormal Pap — smear tests.

Material and methods: We provide a prospective observational cohort study. We obtained material from 428 women registered to Provincial Hospital in Poznan and Specialist Medical Practice in 2018–2021. In the current study, we analyze results from the first 110 inclusions with the diagnosis of HSIL from a cervical biopsy.

The probe for the molecular test was collected with a combi brush and passed to an independent, standardized laboratory. HPV detection was done using PCR followed by DNA enzyme immunoassay and genotyping with a reverse hybridization line probe assay. Sequence analysis was performed to characterize HPV-positive samples with unknown HPV genotypes. The molecular test detected DNA of 41 HPV genotypes. We performed statistical analyzes using the STATISTICA package 13.3.

Results: We found that 98.2% of patients received HPV-positive test results. The most frequent HPV genotype was 16, which assumed for 54.1%. In patients negative for HPV 16, the percentage decreased with increasing age. We detected that the following HPV types are next most common: HPV 31 (16.2 %), HPV 52 (11.7%), HPV 51 (9.9%), HPV 18 (9.0%), HPV 33 (9%). Moreover, thyroid diseases were the most common comorbidities and occurred in 15 patients.

Conclusions: To our knowledge, this study is the most extensive assessment of HPV genotypes in HSIL diagnoses in Poland.

Key words: HPV genotyping; HSIL; high-grade lesion; cervix biopsy

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INTRODUCTION

Cervical cancer remains the fourth most common cancer in women worldwide, causing about 275,000 deaths annually [1, 2]. Cervical cancer mortality has decreased significantly in recent decades, especially in developed countries. Since the '90s, we have observed the same tendency in Poland, reflecting the improvement in the epidemiological situation. However, to achieve tremendous success, both preventive and therapeutic activities must be continued step by step. In Poland in 2014, cervical cancer was diagnosed in nine women a day, and nearly 50% died from it [3]. This direction of change is undoubtedly related to thriving preventive programs. In most countries, it is based on a typical Pap smear. The American College of Obstetricians and Gynecologists included the hrHPV DNA test in the screening guidelines in 2003 for the first time. Since 2013, subsequent reports, including current European guidelines, have shown the advantage of human papillomavirus (HPV) tests over traditional Pap-smear in women aged 30–65 [4, 5].

An abnormal cytology, a positive HPV test, or a suspicious cervical image indicate extended diagnostics. It comprises a colposcopy with a biopsy to look for precan-

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cerous lesions. A biopsy diagnosis of high grade squamous intraepithelial lesion (HSIL) is the current threshold for excision of a precancerous lesion [6]. However, most HSIL will not progress to invasive cervical cancer (ICC) if not treated [7]. HSIL are heterogeneous and include both productive hrHPV infections and early and advanced transforming hrHPV lesions. Productive infections and early transforming infections may regress spontaneously [8]. In contrast, we should treat advanced transforming lesions without delay because of the risk of progression in a short time. Therefore, it is essential to select among HSIL patients those at increased risk so as not to expose everyone to invasive treatment. It especially concerns women in productive age, whose interventions may impact the future pregnancy.

The factor that has a significant impact on the development of cervical cancer is primarily persistent infection with high-risk HPV (hrHPV), leading to an uncontrolled course of the disease. The oncogenic potential of particular HPV genotypes has already been acknowledged. HPV is the indubitable etiological agent for SIL development and cervical cancer. Over 40 years ago, the role of human papillomavirus (HPV) in cervical cancer was established [9, 10]. Persistent infection with hrHPV is the direct cause of the majority of cervical intraepithelial neoplasia and invasive cervical cancers. Moreover, current data indicate that genotypes 16 and 18 are assumed to be responsible for about 70% of cervical cancer cases [11, 12].

This paper summarizes the results of HPV DNA genotyping in women diagnosed with HSIL in Poznan, Poland. So far, we do not have reliable data on the contribution of selected oncogenic HPV types in the formation of cervical pathology in the Polish population. To our knowledge, it is the most extensive analysis that has been described in Poland to date. We aim to provide distribution of particular HPV genotypes concerning age groups in women diagnosed with HSIL. This knowledge might enable estimating the potential effectiveness of primary prevention, which is HPV vaccination. Additionally, we aim to analyze the risk factors for developing HSIL lesions and the variables that may affect its more frequent occurrence.

MATERIAL AND METHODS

We present a prospective observational cohort study conducted in Provincial Hospital in Poznan and Specialist Medical Practice, Poland. Inclusion criteria were: 1) an abnormal cytological test result (≥ ASC-US), positive HPV test result or abnormal cervix image, 2) 18 years of age or older. The exclusion criteria were: 1) the previous diagnosis of ICC, 2) current pregnancy or pregnancy in the previous three months, 3) insufficient material for HPV genotyping.

We collected the data on relevant medical history, the number of sexual partners, parity and living status, using a standardized questionnaire from each subject. In the current study, we analyze results from the first 110 inclusions with the diagnosis of HSIL from a cervical biopsy.

We recruited 428 patients registered to Provincial Hospital in Poznan and Specialist Medical Practice between 2018 and 2021 because of either an abnormal Pap-smear result (≥ ASC-US) and positive HPV test result or abnormal cervix image resulting in the collection of material for histopathological examination. From all women, we distinguished those diagnosed with high-grade squamous intraepithelial lesions.

The follow-up schedule for all women included cytology every six months — close supervision for two years, then return to the routine screening program. Following colposcopy and LEEP conization was done in most cases. Women diagnosed with either negative for intraepithelial lesion (NILM), a negative result for HPV infection, or a typical cervix image did not require extended diagnosis and returned to the regular screening program.

Pap-smear and HPV genotyping

Parallel to the Pap-smear, we tested those women for the presence of HPV and determined their genotypes. The test material was obtained using a cervex-brush from the external os of the cervix and vaginal wall. The obtained specimen was placed into a liquid-based medium, ThinPrep PreserCyt Solution. An HPV test is a quality test. It serves to identify high- risk HPV DNA of the following genotypes: 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 68a, 68b, 69, 70, 71, 72, 73, 81, 82, 83, 84, 87, CP6108, 90 in vitro. A positive test in molecular test confirms the presence of DNA of at least one of the abovementioned oncogenic types of human papillomavirus in the collected specimens.

The probe for a molecular test (Linear Array HPV Genotyping-Roche Diagnostics) was collected with a combi brush and passed to an independent standardized laboratory. HPV detection was done using PCR followed by DNA enzyme immunoassay with a reverse hybridization line probe assay. Sequence analysis was performed to characterize HPV-positive samples with unknown HPV genotypes. The molecular test detected DNA of 41 HPV genotypes.

Colposcopy and LEEP-conization

If needed, following colposcopy and LEEP-conization were performed. A specialist in gynecologic oncology with 10-year experience examined colposcopy with SmartOPTIC colposcope. In all cases, a trial with a 5% aqueous solution of acetic acid was performed, as well as Schiller's test with Lugol's iodine. According to Reid's Colposcopic Index, the colposcopic images were evaluated, assessing the colour, lesion boundaries and surface, blood vessels, and iodine

Table 1. Descriptive characteristics of the study groups, means or n (%)

n	110
Age [yrs]	33.3
Living status	
City > 100,000 inh.	51 (46.4%)
Town or city < 100,000 inh.	59 (53.6%)
Parity	
0	50 (45.5%)
1–2	49 (44.5%)
≥ 3	11 (10.0%)
Comorbidities	40 (36.4%)
Thyroid disease	15 (13.6%)
PCOS/ prediabetes/DM type 1	6 (5.5%)
Fertility issues	5 (4.6%)
CVD	3 (2.8%)
Endometriosis	3 (2.8%)
HPV status	
(+)	108 (98.2%)
(–)	2 (1.8%)

PCOS — polycystic ovary syndrome; DM — diabetes mellitus; CVD — cardiovascular disease; HPV — human papillomavirus infection

test. All colposcopic images were archived. We used the International Federation of Cervical Pathology and Colposcopy classification and recommended by the Polish Society of Colposcopy and Cervical Pathophysiology.

Excisions were done with colposcopic guidance after application of acetic acid 5% and Lugol's iodine. The sizes of the loops were selected according to the size of the lesions. Finally, the curettage of the cervical canal was performed to obtain endocervix material. 12 to 16 paraffin blocks were created from each cervical specimen, and four to five sections were examined from each block. Histopathological analysis was performed in an independent laboratory by experienced pathologists.

All patients gave informed consent to participate in the study. The Bioethics Committee approved the study of the Medical Chamber of Wielkopolska on the 17th of March 2021 (95/2021).

Statistical analysis

We performed calculations using the statistical package Statistica (ver. 13.3) and graphs — using Excel. Statistical hypotheses were verified at the level of significance of 0.05. We performed the Pearson's Chi-square test to analyze the correlation between individual genotypes and age groups. We searched for other correlations between risk factors, and the occurrence of individual diagnoses using Pearson's Chi-square or Yates corrected Chi-square tests.

Table 2. The quantitative and percentage distribution of individual genotypes

HPV genotype	Presence n	Presence %	Deficiency n	Deficiency %
16	60	54.1	51	45.9
31	18	16.2	93	83.8
52	13	11.7	98	88.3
51	11	9.9	100	90.1
18	10	9.0	101	90.0
33	10	9.0	101	90.0
53	7	6.3	104	93.7
56	6	5.4	105	94.6
59	6	5.4	105	94.6
45	5	4.5	106	95.5
66	5	4.5	106	95.5
6	5	4.5	106	95.5
58	4	3.6	107	96.4
73	4	3.6	107	96.4
82	4	3.6	107	96.4
39	3	2.7	108	97.3
67	3	2.7	108	97.3
42	3	2.7	108	97.3
54	3	2.7	108	97.3
62	3	2.7	108	97.3
35	2	1.8	109	98.2
68	2	1.8	109	98.2
61	1	0.9	110	99.1
70	1	0.9	110	99.1
81	1	0.9	110	99.1
83	1	0.9	110	99.1

HPV — human papillomavirus

RESULTS

The mean age of the entire population was 33. Most patients had less than three children, and more than half lived in the town or city with less than 100,000 inhabitants. More than one-third of patients had comorbidities. The most frequent were the thyroid diseases, comprising hypothyroidism, Hashimoto's disease and Graves' disease. Thyroid diseases were the most common comorbidities and occurred in 15 patients, although we did not find statistical significance. We also observed cases of fertility issues, polycystic ovaries syndrome and Diabetes Mellitus type 1. Table 1 presents descriptive characteristics of the study group.

A total of 108 patients (98.2%) were positive for HPV DNA. The quantitative and percentage distribution of individual genotypes is presented in Table 2. Six genotypes were the most frequent in the study group- 16, 31, 52, 51, 18 and 33. They all belong to high-risk oncogenic HPV types.

Table 3. Relation between presence of HPV genotype 16 and age groups

Age groups	HPV genotype 16 presence					
	no		yes		all	
	n	%	n	%	n	%
< 30	21	43.7%	13	21.7%	34	31.5%
30–40	18	37.5%	37	61.7%	55	50.9%
> 40	9	18.8%	10	16.6%	19	17.6%
All	48	100.0%	60	100.0%	108	100.0%

HPV — human papillomavirus

The HPV genotype 16 accounted for 54.1% of all HPV-positive patients, and the result turned out to be statistically significant for the age groups (p = 0.027). Table 3. presents the relation between the presence of HPV genotype 16 and age groups. Among women who do not have the HPV genotype 16, the percentage of women under 30 years of age is twice as high as those of patients with HPV 16. In contrast, there were more women with the HPV genotype 16 than people without it in the group between 30 and 40 years of age.

The most frequent HPV genotypes in women below 30 years of age were: 16 (47%), 31 (26.5%), 51 (14.7%), 52 (14.7%), 53 (11.8%).

The most frequent HPV genotypes in women between 30 and 40 years of age were: 16 (67.3%), 31 (12.7%), 33 (10.9%), 51 (10.9%).

The most frequent HPV genotypes in women above 40 were: 16 (47.6%), 52 (14.3%).

We have found that single HPV infections are more common in women over 30 than infections with multiple HPV types. Only the youngest patients are more likely to be infected with more than one type of virus. In the youngest patients, infections with a single genotype of the virus occurred in 30.3% of HPV-positive patients and infections with at least two types- in 69.7% of patients. In women between 30 and 40, infection with a single virus type occurred in 57.4% of HPV-positive patients and multiple infections in 42.6% of patients. 76.2% of HPV-positive women were infected with a single HPV type in the oldest age group, and 23.8% had multiple infections.

The most frequent Pap-smear results in women below 30 years of age were: LSIL, HSIL and ASC-H. The most frequent Pap-smear results in women above 30 years of age were: ASC-H, HSIL and LSIL. The percentage distribution of Pap-smear diagnoses of HSIL, LSIL and ASC-H was similar (29%, 29% and 28%, respectively). Moreover, 12% of women administered to the hospital with a diagnosis of atypical squamous cells of undetermined significance and 2% — AGC. We have compiled all Pap-smears in Figure 1.

Figure 2 presents the scheme of recruiting patients for the study and the follow-up results. As far as follow up is concerned, we performed LEEP-conization in most cases. In 11.8% of patients, it was not possible to perform LEEP-conization due to various reasons. We present the results of excised lesions in Figure 3. Two-thirds of cases of the high-grade squamous intraepithelial lesion in the biopsy was confirmed in LEEP-conization. Three women heard the diagnosis of invasive cervical cancer — one of them — squamous cell carcinoma and two — adenocarcinoma. In 30% of patients, the final diagnosis was milder — in 8%, LSIL and 22% of women had a correct result of a histopathological examination.

The most frequent LEEP-conization result in women below 30 years of age was HSIL (16/34), accounting for 47.1%. In patients between 30 and 40 years of age, HSIL accounted for 69.1% (38/55). It was also the most common final diagnosis in the oldest women — 52.4% (11/21).

Among the diagnoses of NILM and LSIL in LEEP-conization 96.6% (28/29) of patients were HPV (+), including 37.9% (11/29) HPV 16 (+). The most common Pap-smear result was LSIL 41.4% (12/29) and HSIL 27.6% (8/29). Among the diagnoses of HSIL and ICC in LEEP-conization 98.5%

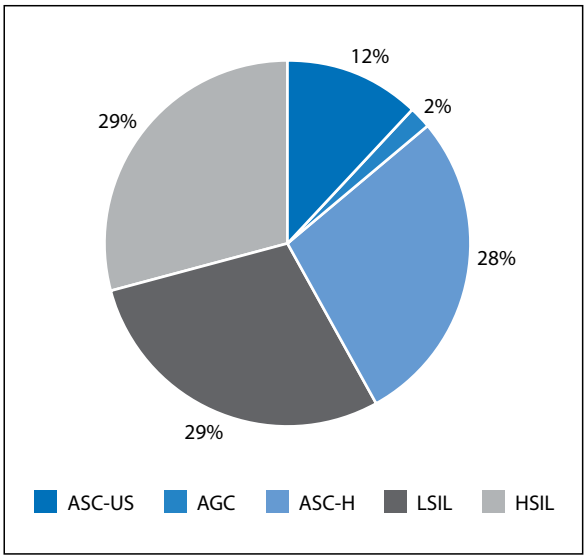

Figure 1. Pap-smear results; ASC-US — atypical squamous cells of undetermined significance; AGC — AGC — atypical glandular cells; ASC-H — atypical squamous cells cannot exclude HSIL; LSIL — low-grade squamous intraepithelial lesion; HSIL — high-grade squamous intraepithelial lesion

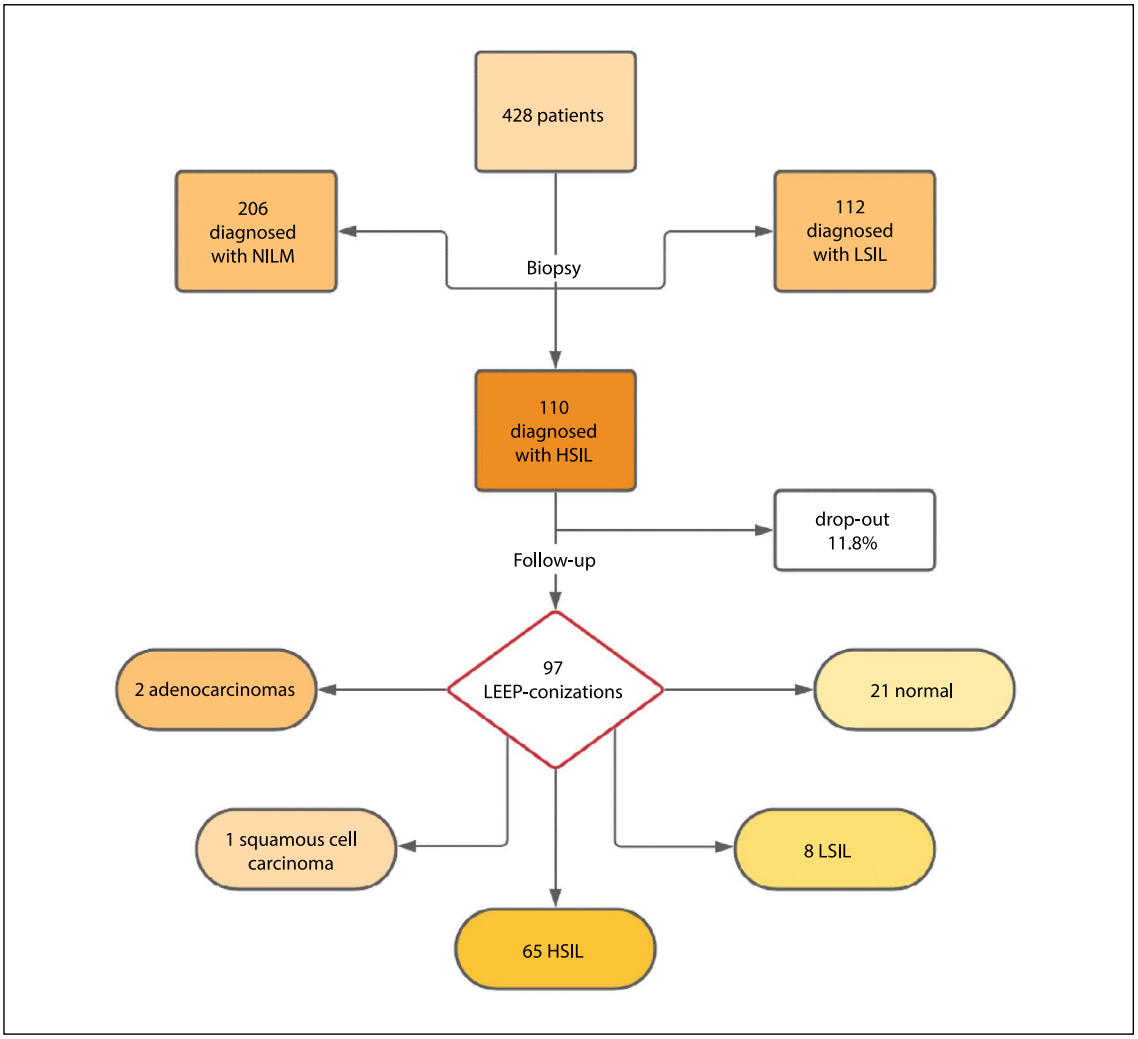

Figure 2. The scheme of recruiting patients for the study

(67/68) of patients were HPV (+), including 60.3% (41/68) HPV 16 (+). The most common Pap-smear result was ASC-H 32.4% (22/68) and HSIL 29.4% (20/68).

DISCUSSION

We designed this study to define the distribution of HPV genotypes within HSIL. To our knowledge, it is the most extensive assessment of HPV genotype in HSIL in Poland to date. Additionally, we have not found such a database of one roof patients.

There were twenty-six HPV genotypes present in our study group out of 40 possible. Of these, 16 belonged to high-risk HPV, 2 to low-risk HPV, and eight had unknown oncogenic potential. The analysis confirmed a significant relationship between HPV 16 genotype and age groups. Six genotypes were the most frequent in the study group — 16, 31, 52, 51, 18 and 33. They all belong to high-risk oncogenic HPV types. The HPV genotype 16 accounted for 54.1% of all HPV-positive patients. Interestingly, in the age group

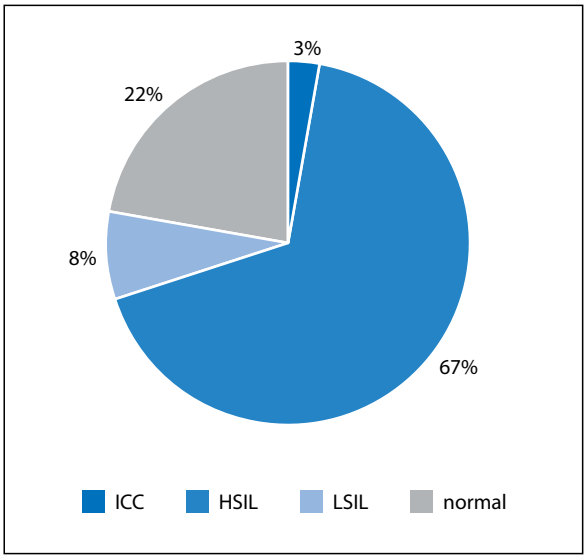

Figure 3. LEEP-conization results; ICC — invasive cervical cancer; HSIL — high-grade squamous intraepithelial lesion; LSIL — low-grade squamous intraepithelial lesion

between 30 and 40 years of age, genotype 16 accounted for 67.3%.

A study conducted over a decade ago in the same region of Poland showed that the most common viruses detected in women diagnosed with HSIL are: 16, 33, 18, 31, 56. They accounted for 75.86% (88/116) of all detected HPV genotypes [13].

According to Clifford GM et al. [14] of 15 HPV types that are considered oncogenic, HPV type 16 and 18 accounts for about 70% of cervical cancers while HPV types 31, 33, 35, 45, 52 and 58 are associated with an additional 15% of cervical cancer. In the study group, 16 and 18 HPV genotypes constituted 59% (65/110). We noticed that the HPV 18 genotype is not detected as often compared to other databases. The abovementioned differences may result from the fact that only 3 of our patients had finally been diagnosed with ICC. Most women were finally diagnosed with HSIL. However, the next common genotypes were mainly similar to the results of other researchers. In our study, HPV types 31, 33, 35, 45, 52 and 58 occurred in 38% of all HPV-positive patients.

It was beyond the scope of our study, but we noticed a high incidence of autoimmune diseases and, in particular, thyroid diseases. All autoimmune diseases accounted for 37.5% (15/40) of all possible comorbidities. Interestingly, we observed that thyroid diseases, including hypothyroidism, Graves' disease, and Hashimoto's disease, occurred in 15 patients. This finding may contribute to new research and extended observations. So far, various meta-analyses and prospective studies have focused on demonstrating the safety of vaccines against HPV in people suffering from autoimmune diseases. These studies did not show a relationship between the increased number of cases, exacerbations, or progression of autoimmune diseases [15, 16]. Perhaps now is the time to look for the relationship between the increasing incidence of autoimmune diseases, especially autoimmune thyroiditis (AT), and greater exposure to HPV infections. So far, we have not been able to find such studies in the available databases.

The paper presented by Aubin F. et al. [17] is the only one relating to the incidence of HPV infections in patients suffering from autoimmune diseases. Different studies have demonstrated a link between genital HPV infection and systemic lupus erythematosus (SLE). The prevalence of genital HPV infection was increased in SLE women compared to the general population (12 to 20% vs 7%) [18, 19], and the increase was statistically significant for multiple HPV infections and infections with some of the HR-HPV types such as HPV16 [20]. In a prospective observational study of 144 SLE patients, the prevalence of HPV infection increased significantly, from 12.5% at baseline to 25% after three years [21]. In patients with Sjögren's syndrome and rheumatoid arthritis, no significant differences were found regarding either Pap smear results or HPV status [22, 23]. Researchers from Canada reported an increased prevalence of abnormal Pap smears in 320 women with systemic sclerosis than the general population (25.4% vs 13.8%) [24]. We did not find in the literature data describing the relationship between other autoimmune diseases and HPV infection. Future studies should take into the account abovementioned relation.

Of all the patients diagnosed by biopsy as HSIL, 70% at the final diagnosis of LEEP-conization resulted from HSIL or ICC. In the other patients, the result was typical, or they had LSIL. Proper identification of women with confirmed HSIL among those with abnormal Pap smear results before colposcopy and biopsy is essential to avoid over investigation and over-treatment. Not every cytologically diagnosed HSIL will progress to histological HSIL or ICC. From follow-up and LEEP-conization results, we see that even with biopsy-confirmed HSIL, only 68/97 women still had high-grade lesions or developed cancer. hrHPV testing has shown high sensitivity for HSIL detection. More and more Western countries use hrHPV testing either as a stand-alone screening test or in combination with cytology or triage.

The large meta-analysis compiled by Smith JS et al. [12] proves that HPV16/18 prevalence was 70% overall and varied from 65% in South/Central America to 76% in North America. Combined HPV16/18 prevalence in all HSIL cases constituted 52%. HPV16 was the predominant type in HSIL from all continents. This meta-analysis concluded that more than two-thirds of cervical cancers and half of HSIL could be prevented after using HPV 16/18 vaccines.

Analyzing the obtained data, we noticed that the percentage of multiple HPV infections decreases with increasing age, and the incidence of only one HPV genotype rises significantly. Referring to Sukasem C. et al. [25], the presence of a single HPV-genotype infection was more common than multiple infections (77.27% and 22.73%, respectively). The hrHPV infections were eight times more frequent than lrHPV. The most common genotypes by decreasing order of frequency were HPV16, 58, 18, 33, 68, 31 and 66 (7/167; 4.19%).

Persistent HPV infections may progress into high-grade cervical lesions and ICC. Therefore, the analysis of the frequency of HPV genotypes in large groups of patients may contribute to the development of new preventive strategies. Information on the occurrence of genotypes in different countries will help implement protective vaccinations against specific, most virulent genotypes of HPV [26–28]. Nonetheless, these data on the prevalence and distribution of HPV genotypes in the population may raise the understanding of the HPV molecular epidemiology in Poland. Lastly, the limitation of our publication is the relatively small number of patients with ICC examined.

CONCLUSIONS

To our knowledge, this study is the most extensive assessment of HPV genotypes in HSIL diagnoses in Poland.

Funding

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Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Bioethics Committee of the Medical Chamber of Wielkopolska (protocol code 95/2021, date of approval: 17.03.2021).

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Data availability statement

The data presented in this study are available on request from the first and second author. The data are not publicly available due to sensitive information regarding both the health and epidemiological status of the study group.

Conflict of interest

The authors declare no conflict of interest.

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Human papillomavirus genotyping in low-grade squamous intraepithelial lesions

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ABSTRACT

Objectives: Human papillomavirus infection is one of the most common sexually transmitted infections. Histological LSIL in 70–80% of cases will regress spontaneously, while a subset is associated with residual risk for a future precancerous lesion. This study evaluates the performance of HPV genotypes for LSIL preceded by normal or mildly abnormal Pap smear.

Material and methods: We provide a prospective observational cohort study. We obtained material from 428 women registered to Specialist Medical Practice and Provincial Hospital in Poznan in 2018–2021. In the current study, we analyze results from the first 112 inclusions with the diagnosis of LSIL from a cervical biopsy.
The probe for the molecular test was collected with a combi brush and passed to the independent, standardized laboratory. HPV detection was done using PCR followed by DNA enzyme immunoassay and genotyping with a reverse hybridization line probe assay. Sequence analysis was performed to characterize HPV — positive samples with unknown HPV genotypes. The molecular test detected DNA of 41 HPV genotypes. We performed statistical analyzes using the STATISTICA package 13.3.

Results: We found that 77.7% of patients received HPV-positive test results. The most frequent HPV genotype was 16, which was assumed for 22.3%. We detected that following HPV types are next most common: HPV 56 (11.6%), HPV 52 (8.9%), HPV 31 (8.0%) and HPV 51 (8.0%). Among HPV 16-negative women, the vast majority are those living in the town ($p = 0.048$). Moreover, thyroid diseases were the most common comorbidities.

Conclusions: To our knowledge, this study is the most extensive assessment of HPV genotypes in LSIL diagnoses in Poland.

Key words: HPV genotyping; LSIL; low-grade lesion; cervix biopsy

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INTRODUCTION

Human papillomavirus (HPV) infection is a common sexually transmitted disease. A vast majority of the infections resolve within one or two years; however, if not controlled immunologically or by screening, some genotypes may lead to persistent infection resulting in cervical cancer. The carcinogenicity of these HPV types results primarily from the oncoproteins E6 and E7, which impair growth regulatory pathways. It is still unclear which precancerous lesions progress, and which do not [1, 2]. Infections with oncogenic HPV genotypes can cause cancer in both women and men. However, cervical tissue is more prone to HPV-dependent cancer development. Thus, human papillomaviruses cause over eight times more cancers in women than in men [3, 4].

Cervical cancer remains the fourth most frequent cancer in women worldwide, causing about 275,000 deaths annually [5, 6]. Moreover, depending on the screening and treatment programs proposed by different countries, the incidence of cervical cancer varies by geographic region. In most countries, prevention programs are based on typical Pap-smear. In 2003, the American College of Obstetricians and Gynecologists first proposed the HR HPV DNA test as screening. Since then, other international societies have

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started to indicate greater effectiveness of this method in middle-aged patients compared to classical Pap-smear [7, 8].

Lesions classified as low grade squamous intraepithelial lesions (LSIL) show some heterogeneity. The microscopic image is usually more viral than in inconspicuous infections, and lesions are characterized by cell proliferation through the lower layers of infected cervical tissue.

Randomized trials and systematic reviews show that, compared with repeated Pap-smear, hrHPV testing has higher sensitivity and similar specificity in identifying underlying or incipient cervical precancer in women with ASC-US [9, 10]. Accordingly, triage by hrHPV testing has become standard practice [7, 11, 12]. Low-grade squamous intraepithelial lesions are associated with a risk for precancer like that among hrHPV-positive women with ASC-US [13]. Because most women with LSIL test positive for hrHPV [14], triage by hrHPV testing is inefficient [15]. The widespread practice of referring all women with hrHPV infection and ASC-US or LSIL to colposcopy carries a considerable burden and cost. Because HPV types 16 and 18 cause about 70% of cervical cancer cases [12], genotyping for these types have been proposed as an additional tool to allow more fine-tuned management.

This paper summarizes the results of HPV DNA genotyping in women diagnosed with LSIL in Poznań, Poland. So far, we do not have reliable data on the contribution of selected oncogenic HPV types in the formation of cervical pathology in the Polish population. To our knowledge, it is the most extensive analysis that has been described in Poland to date. We aim to provide distribution of particular HPV genotypes concerning age groups in women diagnosed with LSIL.

MATERIAL AND METHODS

We present a prospective observational cohort study conducted in Specialist Medical Practice and Provincial Hospital in Poznan, Poland. Inclusion criteria were: 1) an abnormal cytological test result (≥ ASC-US), positive HPV test result or abnormal cervix image, 2) 18 years of age or older. The exclusion criteria were: 1) current pregnancy or pregnancy in the previous three months, 2) insufficient material for HPV genotyping.

We collected the data on relevant medical history, number of sexual partners, parity and living status, using a standardized questionnaire from each subject. In the current study, we analyze results from the first 112 inclusions with the diagnosis of LSIL from a cervical biopsy.

We recruited 428 patients registered to Provincial Hospital in Poznan and Specialist Medical Practice between 2018 and 2021 because of either an abnormal Pap-smear result (≥ ASC-US) and positive HPV test result or abnormal cervix image resulting in the collection of material for histopathological examination. From all women, we distinguished those diagnosed with low-grade squamous intraepithelial lesions.

The follow-up schedule for all women included cytology every six months — close supervision for two years, then return to the routine screening program. If needed, following colposcopy and LEEP conization was performed. Women diagnosed with either negative for intraepithelial lesion (NILM), a negative result for HPV infection, or a typical cervix image did not require extended diagnosis and returned to the regular screening program.

Pap-smear and HPV genotyping

Parallel to the Pap-smear, we tested those women for the presence of HPV and determined their genotypes. We obtained material with a cervex-brush from the external os of the cervix and vaginal wall. Then, we placed it into a liquid-based medium, ThinPrep PreserCyt Solution. A quality test, identifies high- risk HPV DNA of the following genotypes: 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 68a, 68b, 69, 70, 71, 72, 73, 81, 82, 83, 84, 87, CP6108, 90 in vitro. A positive test result confirms the presence of DNA from at least one oncogenic HPV virus.

The probe for a molecular test (Linear Array HPV Genotyping-Roche Diagnostics) was collected with a combi brush and passed to an independent standardized laboratory. HPV detection was done using PCR followed by DNA enzyme immunoassay with a reverse hybridization line probe assay. Sequence analysis was performed to characterize HPV-positive samples with unknown HPV genotypes. The molecular test detected DNA of 41 HPV genotypes.

Colposcopy and LEEP-conization

If needed, following colposcopy and LEEP-conization were performed. A specialist in gynecologic oncology with 10 years of experience examined colposcopy with SmartOPTIC colposcope. The doctor performed an acetic acid test and a Schiller test in each patient. According to Reid's Colposcopic Index, the colposcopic images were evaluated, assessing the color, lesion boundaries and surface, blood vessels, and iodine test. All colposcopic images were archived. We used the International Federation of Cervical Pathology and Colposcopy classification and recommended by the Polish Society of Colposcopy and Cervical Pathophysiology.

Excisions were done with colposcopic guidance after application of acetic acid 5% and Lugol's iodine. The sizes of the loops were adequate to the size of the lesions. Finally, the curettage of the cervical canal was performed to obtain endocervix material. Twelve to sixteen paraffin blocks were prepared from each cervical specimen, and four to five sections were examined from each block. Histopathological analysis was performed in an independent laboratory by experienced pathologists.

All patients gave informed consent to participate in the study. The Bioethics Committee approved the study of the Medical Chamber of Wielkopolska on the 17th of March 2021 (95/2021).

Statistical analysis

We performed calculations using the statistical package Statistica (ver. 13.3) and graphs - using Excel. Statistical hypotheses were verified at the level of significance of 0.05. We performed the Pearson's Chi-square test to analyze the correlation between individual genotypes and age groups. We searched for other correlations between risk factors and the occurrence of individual diagnoses using Pearson's Chi-square or Yates corrected Chi-square tests.

RESULTS

The mean age of the entire population was 33. The majority of patients had less than three children, and more than half lived in the town or city with less than 100,000 inhabitants. About one-third of patients had comorbidities. The most frequent were the thyroid diseases, comprising both hypothyroidism and Hashimoto's disease. Thyroid diseases were the most common comorbidities and occurred in 14 patients, although we did not find statistical significance. We also observed cases of fertility issues, polycystic ovaries syndrome and prediabetes. Table 1 presents the descriptive characteristics of the study group.

A total of 87 patients (77.7%) were positive for HPV DNA. The quantitative and percentage distribution of individual genotypes is presented in Table 2. Five genotypes were the most frequent in the study group — 16 (22.3%), 56 (11.6%), 52 (8.9%), 31(8.0%), 51 (8.0%). They all belong to high-risk oncogenic HPV types. The HPV genotype 16 accounted for 28.7% of all HPV-positive patients.

In all age groups, the number of HPV-negative patients was lower than those infected with HPV. We analyzed most frequent genotypes — HPV 16 (p = 0.691), HPV 31 (p = 0.201), HPV 52 (p = 0.363) and HPV 56 (p = 0.785).

The relationship between the presence of HPV genotype 16 and the living status turned out to be statistically significant (p = 0.038). Among HPV 16-negative women, the vast majority are those living in the town (63.2%). Most peo-

Table 1. Descriptive characteristics of the study groups, means or n (%)

n	112
Age [yrs]	33.4
Living status	
City > 100,000 inh.	47 (42.0%)
Town or city < 100,000 inh.	65 (58.0%)
Parity	
0	60 (53.6%)
1–2	45 (40.2%)
≥ 3	7 (6.2%)
Comorbidities	38 (33.9%)
Thyroid disease	14 (12.5%)
PCOS/prediabetes	9 (8.0%)
Fertility issues	7 (6.3%)
HPV status	
(+)	87 (77.7%)
(–)	25 (22.3%)

PCOS — polycystic ovarian syndrome; HPV — human papillomavirus

Table 2. Distribution of individual HPV genotypes

HPV genotype	Presence n	Presence % (n = 112)	% of genotypes (n = 152)
16	25	22.3	16.4
56	13	11.6	8.6
52	10	8.9	6.6
31	9	8.0	5.9
51	9	8.0	5.9
53	8	7.1	5.3
54	7	6.3	4.6
66	7	6.3	4.6
84	7	6.3	4.6
18	6	5.4	3.9
6	5	4.5	3.3
42	5	4.5	3.3
33	4	3.6	2.6
39	4	3.6	2.6
61	4	3.6	2.6
89	4	3.6	2.6
73	3	2.7	2.0
35	2	1.8	1.3
40	2	1.8	1.3
58	2	1.8	1.3
59	2	1.8	1.3
67	2	1.8	1.3
68	2	1.8	1.3
81	2	1.8	1.3
CP6108	2	1.8	1.3
7	1	0.9	0.7
11	1	0.9	0.7
43	1	0.9	0.7
45	1	0.9	0.7
70	1	0.9	0.7
87	1	0.9	0.7

HPV — human papillomavirus

ple living in the city were infected with the HPV genotype 16 (60.0%). We present this correlation in Table 3.

The most frequent HPV genotypes in HPV-positive women below 30 years of age were: 16 (25.0%), 31(14.3%) and 56 (14.3%). The most frequent HPV genotypes in HPV-positive women between 30 and 40 years of age were: 16 (30.6%), 52 (14.3%) and 56 (12.2%). The most frequent HPV genotypes in HPV-positive women above 40 were: 16 (30.0%) and 56 (30.0%).

We have found that single HPV infections are more common in women over 30 than infections with multiple HPV types. Only in the youngest group of patients, there was the same frequency of single and multiple infections. In women between 30 and 40, infection with a single virus type occurred in 59.2% of HPV-positive patients and multiple infections in 40.8% of patients. 60% of HPV-positive women were infected with a single HPV type in the oldest age group, and 40.0% had multiple infections.

The most frequent Pap-smear results in women below 30 years of age were: LSIL and ASC-US. The most frequent Pap-smear results in women above 30 years of age were: LSIL, ASC-US and ASC-H. We have compiled all Pap-smears in Figure 1.

The biopsy in all patients was preceded by colposcopy. We present the results of colposcopy in Figure 2. What draws attention is the diversity of results according to the Reid Colposcopy Index. As far as follow up is concerned, we performed LEEP-conization in 28 cases. In over half of the patients (15/28), the final diagnosis was milder than one from biopsy- either no pathology was found, or koilocytes were present. Low squamous intraepithelial lesions were detected in one-third of the patients and HSIL changes- in 5/28 of the women. Almost 18% of cases of the LSIL in biopsy turned out to be HSIL in LEEP-conization. Among the diagnoses of NILM in LEEP-conization, 76.9% (10/13) of patients were HPV (+). Eighty percent of patients finally diagnosed with LSIL were HPV (+). Among the diagnoses of HSIL in LEEP-conization, all patients were HPV (+). The most common Pap-smear result was LSIL 41.4% (12/29) and HSIL 27.6% (8/29). Apart from LEEP-conization, nine patients required another biopsy, of which five had confirmed LSIL lesions, and four showed no pathological changes. Additionally, twenty-two patients, after biopsy, decided to be vaccinated with a 9-valent HPV vaccine. In control, 77.3% of the women (17/22) were HPV-negative. In contrast, 4/22 post-vaccination patients had recurrent infection with the high-oncogenic HPV genotypes.

Table 3. Dependence of HPV 16 on place of residence

Living status	HPV genotype 16 presence						χ^2	p
	no		yes		all			
	n	%	n	%	n	%		
City	32	36.8	15	60.0	47	42.0		
Town	55	63.2	10	40.0	65	58.0	4.30	0.038
All	87	100.0	25	100.0	112	100.0		

HPV — human papillomavirus

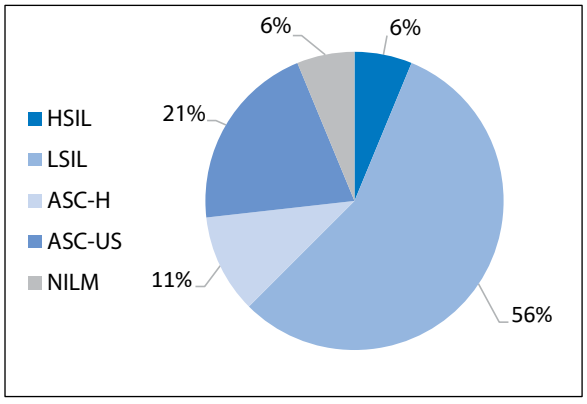

Figure 1. Pap-smear results; HSIL — high-grade squamous intraepithelial lesion; LSIL — low-grade squamous intraepithelial lesion; ASC-H — atypical squamous cells cannot exclude HSIL; ASC-US — atypical squamous cells of undetermined significance; NILM — negative for intraepithelial lesion or malignancy

DISCUSSION

The main goal of our study was to determine the distribution of HPV genotypes in patients with LSIL lesions. To our knowledge, it is the most extensive assessment of HPV genotype in LSIL in Poland to date. Additionally, we have not found such a database of one roof patients.

There were 31 HPV genotypes detected in our study group. Our analysis confirmed that HPV genotype 16 was most common. The most frequent HPV genotypes in HPV-positive women below 30 years of age were: 16, 31 and 56, in the group of women between 30 and 40 years of age: 16, 52 and 56 and in women above 40: 16 and 56.

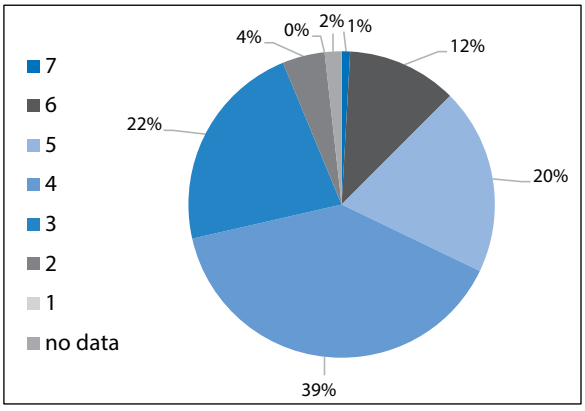

Figure 2. Colposcopy results using Reid Colposcopic index (RCI)

We have found that single HPV infections are more common in women over 30 than infections with multiple HPV types. Only in the youngest group of patients was there the same frequency of single and multiple infections. In women between 30 and 40, infection with a single virus type occurred in 59.2% of HPV-positive patients and multiple infections in 40.8% of patients. 60.0% of HPV-positive women were infected with a single HPV type in the oldest age group, and 40.0% had multiple infections.

The discrepancy in the colposcopy results relating to the same biopsy result may indicate that it is worth taking biopsies and not limited to visual assessment. It is a slightly invasive study because HSIL lesions were detected in five patients in the subsequent LEEP conization. Some researchers say that the diagnosis of LSIL associates with a significant increase in the level of stress in patients [16]. The untreated LSIL lesions go into spontaneous remission in the majority of women. Only a fraction of them will contribute to the development of more malignant lesions [17].

A study conducted about a decade ago in the same region of Poland showed that one-third of patients experienced disease regression during the year of follow-up. In 41% of patients, the LSIL lesion remained at the same level for one year. However, although none of the women developed ICC, a quarter of the study group in the control biopsy progressed to HSIL [18].

High-risk HPV genotypes are closely related to the development of cervical cancer and its precursors [19]. There are different ways of transforming from LSIL lesion to malignancy. Because of that, knowledge about the HPV genotype could be used in separating HPV-positive women at a higher risk of cancer from those that can be observed without intervention over longer intervals. Widespread use of HPV genotyping would improve the efficiency of screening programs while reducing the tendency to over-treatment. [20–22].

HPV as a known carcinogen has led to the development of effective preventive vaccines and sensitive HPV DNA and RNA tests. The analysis of genotypes occurring in patients before and after HPV vaccination may improve their quality in the future [23]. Although screening tests and preventive vaccination programs can significantly reduce the rate of cancer development, their systematic implementation has been a great challenge all over the world for decades [1].

A meta-analysis conducted by Clifford G. et al. [24] on 8308 patients showed that HPV genotype distribution was assessed by geographic region. Almost half of the data (49%) came from European databases to compare the results of this meta-analysis with our observations. However, Poland did not participate in data transmission to the abovementioned publication, which may constitute a certain limitation. A total of 71.1% of patients diagnosed with LSIL turned out to be HPV-positive compared to 77.7% of those observed in our research group. The most common HPV genotype were: HPV16 (present in 26.3% of all HPV-positive LSILs), HPV 31 (11.5%), HPV 51 (10.6%), HPV 53 (10.2%), HPV 56 (9.5%), HPV 52 (9.0%), HPV 18 (8.6%), HPV 66 (8.6%), HPV 58 (8.4%), HPV 6 (8.0%), HPV 39 (7.6%), HPV 33 (7.4%), HPV 59 (6.1%), HPV 35 (5.7%), and HPV 45. HPV genotypes occurring less than 5% were not considered.

The development of a diagnostic and therapeutic strategy in patients with abnormal cytology may in the future depend on the knowledge of the most common HPV genotypes in each region [24–26]. Disseminating this information in different parts of the world may, firstly, provide important epidemiological information. In addition, and more importantly, data collected from large research groups could directly translate into the design of multivalent prophylactic vaccines. Nonetheless, these data on the prevalence and distribution of HPV genotypes in the population may raise the understanding of the HPV molecular epidemiology in Poland.

CONCLUSIONS

To our knowledge, this study is the most extensive assessment of HPV genotypes in LSIL diagnoses in Poland. Genotyping of human papillomaviruses in the population of women diagnosed with LSIL may help in the future to predict whether LEEP-conization and further invasive diagnostics will be necessary. Our research will make possible to show which viruses are the most common in the Polish population.

Funding

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Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Bioethics Committee of the Medical Chamber of Wielkopolska (protocol code 95/2021, date of approval: 17.03.2021).

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Data availability statement

The data presented in this study are available on request from the first and second author. The data are not publicly available due to sensitive information regarding both the health and epidemiological status of the study group.

Conflict of interest

The authors declare no conflict of interest.

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Investigation of awareness and anxiety levels of pregnant women during pandemic process

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ABSTRACT

Objectives: It is currently unknown that how pregnant women deal with the Coronavirus disease and its results. The aim of this study is to evaluate the psychological impact of the coronavirus pandemic on pregnant women and to determine whether pregnant women have sufficient knowledge and awareness for a healthy antenatal process.

Material and methods: This study was conducted at two centers. Regardless of the gestational age, a questionnaire was distributed to 1003 pregnant women in total, from which 51 original questions we prepared. Five hundred twenty-six participants were included in the study. The questionnaire was delivered using the QR code method. The questionnaires were answered online by participants via SurveyMonkey.

Results: The period when anxiety was highest was the 2nd trimester, whereas women in the 1st trimester had the lowest level of anxiety. High levels of awareness were observed in patients with heart disease, but patients with diabetes mellitus had a high level of anxiety.

Conclusions: It is important to maintain the mental and physical health of pregnant women, who are in a more delicate condition than other individuals in the society. In this regard, healthcare professionals have important duties such as taking necessary precautions and explaining the seriousness of the situation to pregnant women.

Key words: anxiety; awareness; COVID-19; pregnancy

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INTRODUCTION

The coronavirus disease (COVID-19) pandemic started on December 31, 2019, in the Chinese city of Wuhan, the capital of Hubei province [1]. On January 30, 2020, the World Health Organization reported that the COVID-19 outbreak was a public health emergency of international concern.

As of November 18, 2020, the virus, which has rapidly spread across the country and around the world, has become a very serious issue and public health problem, with 56,237,909 cases, 1,349,116 deaths, and 39,155,994 recovered cases in approximately 11 months.

In Turkey, the first case was reported on March 11, 2020, which was later than in other developing countries owing to the precautions taken prior to the emergence of the first case. As of November 18, 2020, 425,628 cases, 11,820 deaths, and 361,655 recovered cases have been reported in Turkey [2].

Pregnant women are known to be predisposed to the complications and the severe outcomes of a COVID-19 infection, as declared from SARS and MERS [3, 4].

The panic caused by the virus across the world coupled with the thought of staying home under quarantine, fear of death, protecting loved ones, and the mothering instinct has

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caused significant anxiety problems in particularly sensitive populations. While the effect of the pandemic on pregnant women is not yet known and only a few studies available, the Royal College of Obstetrics and Gynecology reported that the COVID-19 pandemic increases the risk of perinatal anxiety, depression, and domestic violence [5]. The importance of mentally and emotionally supporting women has been emphasized in COVID-19 guidelines. Necessary measures should be taken to offer psychological support for pregnant women suffering from anxiety caused by this dramatic outbreak.

Pregnancy is related to increased risk for severe psychological issues such as depression, anxiety and postpartum psychosis due to physiological, immunological and hormonal alterations [6].

MATERIAL AND METHODS

This multi-centric prospective study, conducted between March 30 and May 30, 2020, was designed as a descriptive and cross-sectional study. The questionnaires were planned to be administered in the outpatient clinic for pregnant women and the obstetric emergency services at the Umraniye and Kanuni Sultan Suleyman Training and Research Hospitals (see Supplement 1).

The questionnaire was designed to identify awareness levels of COVID-19, identify anxiety levels in the admitted pregnant women, and evaluate their attitudes in terms of prevention measures.

The questionnaire comprises of 51 original questions that investigate the pregnant women's demographic data, history of contact with COVID-19-positive patients, knowledge and concerns about COVID-19, precautionary measures, and approach to outbreak.

A link to the online questionnaire (SurveyMonkey) was sent to all pregnant participants, who were admitted to the obstetric outpatient clinics at Umraniye and Kanuni Sultan Suleyman Training Research Hospitals. The questionnaire was delivered using the QR code method and filled online to avoid contamination during this risky time.

The study was approved by the institutional ethics committee. Before starting the study, all volunteers received an informed consent form.

Questionnaires were distributed to a total of 1003 pregnant women aged 18–48 years who volunteered to participate in the survey study. Adolescent pregnant women and pregnant women with diagnosed depression, anxiety, and psychosis were excluded from the study. Illiterate pregnant women and pregnant women with diagnosed or suspected COVID-19 were not included in the study.

Five hundred twenty-nine of 1003 participants were included in the study, 526 of them answered all questions. Comparisons were made in terms of age, educational background, number of children, contact history, pregnancy week, risk factors, and anxiety and awareness levels.

According to responses to the 15 questions asked to measure anxiety regarding COVID-19 in pregnant women, based on the responses, we created a scoring system to determine the level of anxiety in pregnant women. Every correct response measuring anxiety was converted into a hundred-point scale for standardization and then assessed. Percentile values ranked at the 33.3 and is categorized as average, and anxiety was classified (Tab. 1).

According to the distribution of the responses given by the pregnant women to the 19 questions asked to assess their awareness of COVID-19, a scoring system of 19 questions to determine the level of awareness in pregnant women was created. Every correct answer indicating awareness was converted into a hundred-point scale for standardization. Percentile values ranked at the 33.3 and is categorized as average, and awareness was classified (Tab. 2).

In order to assess the level of anxiety that commonly used in Turkey and can be applied easy which has been benefited from Beck Anxiety Scala [7].

Statistical Analysis

Power analysis was performed using the G * Power (v3.1.7) program to determine the number of samples. The statistical analyses were performed using the Statistical Package for the Social Sciences, version 25 (SPSS Inc.). Along with the descriptive statistical methods for data analysis, the Shapiro–Wilk test and boxplot graphs were used to test the normality of variables in terms of normal distribution. One-way ANOVA analysis of variance was used in intergroup comparisons of normally distributed variables, while the Bonferroni test was used to determine groups with differences. The Kruskal-Wallis test was used for intergroup comparisons of non-normally distributed parameters, while Dunn's test was used to determine the groups with differences. The Mann-Whitney U test was used to compare the parameters between two groups. $P < 0.05$ was considered statistically significant.

RESULTS

Distribution of demographic characteristics, distribution of Coronavirus (COVID-19) according to other characteristics, distribution of the answers given to the questions asked about the measuring the anxiety of pregnant women about Coronavirus (COVID-19) and distribution of the answers given to the questions asked about measuring the awareness of pregnant women about Coronavirus (COVID-19) are listed in Tables 1–4, respectively.

According to the responses given to 19 questions asked to determine the level of awareness, the scores of awareness of COVID-19 were 5.26–100, with a mean score of

Table 1. Distribution of the answers given to the questions asked about the measuring the anxiety of pregnant women about Coronavirus (COVID-19)

		n	%
Do you think that you are vulnerable to Coronavirus (COVID-19) because you are pregnant?	No	195	37.1%
	Not sure	138	26.2%
	Yes	193	36.7%
Do you think that mother-to-child transmission of Coronavirus (COVID-19) infection is possible during pregnancy?	Not sure	200	38.0%
	No	163	31.0%
	Yes	163	31.0%
Do you think that Coronavirus (COVID-19) can transmit through breastmilk or breastfeeding?	Not sure	210	39.9%
	No	131	24.9%
	Yes	185	35.2%
Which of the following do you think that could happen to you because of the anxiety of getting infected by Coronavirus (COVID-19)?	Labor pain	77	14.6%
	Bleeding	38	7.2%
	Water break	22	4.2%
	Preterm labor	78	14.8%
	Abortus/Stillbirth	69	13.1%
	None	315	59.9%
Do you take vitamins to boost your immune system against Coronavirus (COVID-19) infection?	No. I don't	269	51.1%
	Sometimes, I do	103	19.6%
	Regularly, I do	154	29.3%
Are you anxious for the family members in case they may get infected by Coronavirus (COVID-19)?	I am not anxious	47	8.9%
	I am a little bit anxious	120	22.8%
	I am anxious	208	39.5%
	I am very anxious	151	28.7%
Does the thought of getting infected by Coronavirus (COVID-19) cause any of the followings?	Numbness/Tingling	19	3.6%
	Hot flushes	74	14.1%
	Weakness/Shivering	43	8.2%
	The idea of something bad is going to happen	161	30.6%
	Tachycardia	56	10.6%
	Dizziness	18	3.4%
	Feeling like drowning	55	10.5%
	Feeling like you can't breathe	73	13.9%
	Fear of death	87	16.5%
	Stomach pain	63	12.0%
	No. I don't feel any of them	171	32.5%
If you had get infected by the Coronavirus (COVID-19), what is your possibility of getting well?	I don't think that I could get well	92	17.5%
	I am not sure that I could get well	148	28.1%
	I may get well	225	42.8%
	I would definitely get well	61	11.6%
Do you think that the doctors can make the right diagnosis of Coronavirus (COVID-19) infection and then treat you in the right way?	No	39	7.4%
	Not sure	196	37.3%
	Yes	291	55.3%
Do you think of terminating your pregnancy because of the risk of mother-to-child transmission of Coronavirus (COVID-19) infection?	No	467	88.8%
	Not sure	46	8.7%
	Yes	13	2.5%

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Table 1. cont. Distribution of the answers given to the questions asked about the measuring the anxiety of pregnant women about Coronavirus (COVID-19)

		n	%
Do you think that you wash your hands after coughing, sneezing or touching your nose more frequently than before the Coronavirus (COVID-19) pandemic started?	No	35	6.7%
	Not sure	79	15.0%
	Yes	412	78.3%
Do you avoid going out because of the Coronavirus (COVID-19) pandemic?	No	30	6.1%
	Sometimes	0	0.0%
	Yes	460	93.9%
What would you do if a doctor advised a treatment at home to your housemate due to the Coronavirus (COVID-19) infection? (n: 283)	I do nothing	4	1.4%
	I would take the precautions like putting on a mask, wearing gloves, etc. and I would continue to live in the same house	54	19.1%
	I would live in another room in the house	165	58.3%
	I would move to another house	60	21.2%
What would you do if your doctor advised you to get tested for Coronavirus (COVID-19) after exposure to a person who is suspected of having Coronavirus (COVID-19) infection? (n: 285)	I would refuse	37	13.0%
	I am not sure about it	82	28.8%
	I would accept	166	58.2%
If you thought that you had some of the signs/symptoms of Coronavirus (COVID-19) infection, what would you do? (n: 282)	I would immediately go to a hospital	148	52.5%
	I would observe the signs/ /symptoms and wouldn't go to the hospital unless I get worse	24	8.5%
	I would call my healthcare professional friends and consult about going to the hospital	20	7.1%
	I would call 184 and consult the Ministry of Health	64	22.7%
	I would not go to a hospital and quarantine myself at home	14	5.0%
	I don't think that I should do something about it	12	4.3%

Table 2. Distribution of the answers given to the questions asked about measuring the awareness of pregnant women about Coronavirus (COVID-19)

		n	%
Do you think that you have enough information about the transmission route of Coronavirus (COVID-19) infection?	No	57	10.8%
	Not sure	62	11.8%
	Yes	407	77.4%
Do you think that you have enough information about the signs/symptoms of Coronavirus (COVID-19) infection?	No	51	9.7%
	Not sure	68	12.9%
	Yes	407	77.4%
What are the most important signs/symptoms of Coronavirus (COVID-19) infection in your opinion?	High fever	453	86.1%
	Cough	329	62.5%
	Shortness of breath	372	70.7%
	Other	32	6.1%
Would you get vaccinated with Coronavirus (COVID-19) vaccine?	No	66	12.5%
	Not sure	164	31.2%
	Yes	296	56.3%

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Table 2. cont. Distribution of the answers given to the questions asked about measuring the awareness of pregnant women about Coronavirus (COVID-19)

		n	%
Do you think that you and your family get well-informed about Coronavirus (COVID-19) infection?	No	94	17.9%
	Not sure	337	64.1%
	Yes	95	18.1%
Do you think that Coronavirus (COVID-19) infection is preventable?	No	43	8.2%
	Not sure	127	24.1%
	Yes	356	67.7%
Do you think that Coronavirus (COVID-19) infection is lethal?	No	34	6.5%
	Not sure	69	13.1%
	Yes	423	80.4%
Do you think that the precautions that the Ministry of Health take against Coronavirus (COVID-19) are sufficient?	No	114	21.7%
	Not sure	105	20.0%
	Yes	307	58.4%
Have you ever get informed about Coronavirus (COVID-19) infection by the healthcare professionals?	No	311	59.1%
	I don't remember	41	7.8%
	Yes	174	33.1%
Do you try to protect yourself from getting infected by Coronavirus (COVID-19) and if you do, what do you do for it?	Washing hands frequently	466	88.6%
	Handsanitizer/Cologne	315	59.9%
	Staying away from crowded places	438	83.3%
	Mask	324	61.6%
	Gloves	139	26.4%
	Staying at home	27	5.1%
	Other	17	3.2%
	None	5	1.0%
Which of the following can provide reducing the number of new Coronavirus (COVID-19) cases and deaths from this infection in Turkey?	Good personal hygiene	85	16.2%
	The precautions that the Ministry of Health takes	121	23.0%
	Healthy eating habits/Good genetics	17	3.2%
	Staying at home except for essential needs	101	19.2%
	None	122	23.2%
	All	80	15.2%
Would you quarantine yourself at home for 14 days if you exposed to a person who is infected with Coronavirus (COVID-19)?	No	16	3.0%
	Not sure	18	3.4%
	Yes	492	93.5%
Would you see a friend/relative in 14 days since he/she came from a foreign country?	No	507	96.4%
	Not sure	14	2.7%
	Yes	5	1.0%
Do you follow the daily news about the Coronavirus (COVID-19) in Turkey and worldwide?	No	16	3.0%
	Sometimes	53	10.1%
	Yes	457	86.9%
Do you think that the Coronavirus (COVID-19) pandemic has been exaggerated?	No	326	62.0%
	Not sure	70	13.3%
	Yes	130	24.7%
Do you think that you need to get informed more about the Coronavirus (COVID-19) infection?	No	186	35.4%
	Not sure	71	13.5%
	Yes	269	51.1%

→

Table 2. cont. Distribution of the answers given to the questions asked about measuring the awareness of pregnant women about Coronavirus (COVID-19)

		n	%
What would you do if your doctor advised you hospitalization due to the Coronavirus (COVID-19) infection? (n: 283)	I would not accept the treatment	21	7.4%
	I am not sure about it	39	13.8%
	I would accept the treatment	223	78.8%
What would you do if your doctor advised you to get tested for Coronavirus (COVID-19) after exposure to a person who is diagnosed with Coronavirus (COVID-19) infection? (n: 282)	I would refuse	29	10.3%
	I am not sure about it	57	20.2%
	I would accept	196	69.5%
Who would you share it with if you were diagnosed with Coronavirus (COVID-19) infection? (n: 286)	I wouldn't share it with anyone	18	6.3%
	I would share it only with my spouse	53	18.5%
	I would share it with my first degree relatives and friends	46	16.1%
	I would share it with the doctors who are following me for pregnancy	47	16.4%
	I would share with everybody	122	42.7%

Table 3. Distribution of demographic characteristics

		n	%
Health-care worker (n: 416)	Yes	29	7.0%
	No	387	93.0%
How many children?	None	241	45.8%
	1	172	32.7%
	2	67	12.7%
	3 ≤	46	8.7%
Marital status	Married	513	97.5%
	Single	9	1.7%
	Widowed/Divorced	4	0.8%
Educational status	Primary school	74	14.1%
	Middle school	94	17.9%
	High school	130	24.7%
	Associate's degree	67	12.7%
	Postgraduate and more	161	30.6%
Do you smoke?	Never	459	87.3%
	Less than 10 cigarettes	45	8.6%
	10 to 20 cigarettes	18	3.4%
	More than 20 cigarettes	4	0.8%
Gestational age	< 14 weeks	123	23.8%
	14–28 weeks	152	29.5%
	> 28 weeks	241	46.7%
How many people do live in your house?	1–2	226	43.0%
	3 to 5	262	49.8%
	6 or more	38	7.2%
How many times have you been to the Emergency Room in the last year?	Never	187	36.3%
	1 to 5 times	253	49.1%
	More than 5 times	75	14.6%
Have you exposed to a person who is suspected of Coronavirus (COVID-19) infection?	No	500	95.1%
	Yes	8	1.5%
	Not sure	18	3.4%

→

Table 3. cont. Distribution of demographic characteristics

		n	%
Have you ever used public transportation in the last month?	Yes	121	23.0%
	No	405	77.0%
How many times do you touch your face in a daytime?	Never	55	10.5%
	1 to 5 times	258	49.0%
	More than 5 times	213	40.5%
Do you go to the hospital for your routine prenatal visits?	No. I don't go	106	20.2%
	Sometimes, I go	99	18.8%
	Regularly, I go	321	61.0%
Which risk factors do you have related to Coronavirus (COVID-19) infection?	Diabetes	16	3.0%
	Hypertension	12	2.3%
	Lung Diseases	28	5.3%
	Cancer	7	1.3%
	Heart Diseases	21	4.0%
	Liver Diseases	8	1.5%
	Renal Diseases	6	1.1%
	Thyroid Diseases	20	3.8%
	Other Risk Factors	15	2.9%
	None	420	79.8%

Table 4. Distribution of Coronavirus (COVID-19) according to other characteristics

		n	%
Do you think that antibiotics are effective against Coronavirus (COVID-19)?	No	321	61.0%
	Not sure	159	30.2%
	Yes	46	8.7%
Do you think that social media is taken advantage for giving information about the Coronavirus (COVID-19) infection?	No	126	24.0%
	Not sure	121	23.0%
	Yes	279	53.0%
Have you ever get vaccinated against influenza?	No	449	85.4%
	I don't remember	51	9.7%
	Yes	26	4.9%

60.13 ± 14.81. Of the participants, 3.6% who scored 33.3 and below had low awareness, 58.7% who scored 33.3–66.6 had moderate awareness, and 37.6% who scored ≥ 67 had high awareness (Tab. 5).

In terms of number of children, however, there was a significant difference among the scores of COVID-19 awareness ($p < 0.05$): the awareness increased as the number of children increased. The awareness score of women who had no children was significantly lower than that of those who had three children ($p = 0{,}016$; $p < 0.05$). There was a significant difference among the scores of COVID-19 awareness in terms of the number of people living in the household ($p < 0.05$); the awareness score of those with a household of 1–2 people was significantly lower than that of those with a household of 3–5 people (p: 0,012; $p < 0.05$). There was a significant difference among the scores of COVID-19 awareness in terms of visiting the healthcare facility

Table 5. Distribution of COVID-19 Awareness Scores

	COVID-19 Awareness Scores
Min–Max	5.26–100
Avr ± SD	60.13 ± 14.81
Low Awareness Levels	19 (3.6)
Moderate Awareness Levels	309 (58.7)
High Awareness Levels	198 (37.6)

SD — standard deviation

for routine pregnancy check-ups ($p < 0.05$); the awareness score of those continuing routine pregnancy check-ups was significantly higher than that of those not undergoing or occasionally undergoing routine pregnancy check-ups (p: 0,001; $p < 0.01$). (Tab. 6)

The awareness score of patients with heart disease was significantly higher than that in those without heart disease ($p < 0.05$) (Tab. 7).

According to the responses given to 15 questions asked to determine the level of anxiety, the scores of COVID-19 anxiety ranged from 13.33 to 86.67, with a mean score of 53.49 ± 13.63. Those with a score of ≤ 33.3 were classified as having low anxiety and accounted for 11.4% of the respondents; those with scores of 33.3–66.6 had a moderate level of anxiety and accounted for 66.2% of the respondents, and those with a score of ≥ 67 had a high level of anxiety and accounted for 22.4% of the respondents (Tab. 8).

In terms of contact with an individual with suspected COVID-19 in the past 14 days, there was a significant difference among the scores of COVID-19 anxiety ($p < 0.05$). It was found that those with a history of contact had significantly higher anxiety scores than those without a history of contact ($p = 0,014$; $p < 0.05$). There was a significant difference among the scores of COVID-19 anxiety in terms of gestation week ($p < 0.05$), with the highest level of anxiety in the 2nd trimester and lowest level of anxiety in the 1st trimester ($p = 0,014$; $p < 0.05$). There was a significant difference among the scores of COVID-19 anxiety in terms of visiting the healthcare institution for routine pregnancy check-ups ($p < 0.05$). Those visiting regularly had significantly higher anxiety scores than those not visiting at all or visiting occasionally for routine pregnancy check-ups (p: 0.008; $p < 0.01$) (Tab. 9).

Those with DM had significantly higher anxiety scores than those without DM ($p < 0.05$) (Tab. 10).

There was a statistically significant positive correlation between the scores of COVID-19 anxiety and the scores of COVID-19 awareness ($r = 0.252$; $p = 0.001$; $p < 0.01$). As the

Table 6. Evaluations based on COVID-19 Awareness Scores

		COVID-19 Awareness Scores			p
		Average	SD	Median	
Educational Status	Primary School	60.88	17.73	63.16	[a]0.152
	Middle School	63.38	14.76	63.16	
	High School	59.72	16.64	57.89	
	Associate's degree	58.52	14.24	57.89	
	From Bachelor's degree to Doctorate	58.91	11.58	57.89	
Health-care worker	Yes	58.80	13.72	57.89	[b]0.280
	No	62.00	15.49	63.16	
Number of children	Zero	58.62	13.95	57.89	[a]0.025*
	1 child	60.86	14.59	63.16	
	2 children	60.57	17.08	63.16	
	3 and more	64.76	15.80	63.16	
Use of public transportation	Yes	60.77	13.78	63.16	[b]0.595
	No	59.95	15.12	57.89	
Suspected COVID-19 exposure	No	60.24	14.77	57.89	[c]0.531
	Yes	63.16	12.89	63.16	
	Not sure	55.85	16.95	60.53	
Gestational age	< 14 gw	60.76	13.93	63.16	[a]0.380
	14–28 gw	59.14	14.23	57.89	
	> 28 gw	60.36	15.16	57.89	
How many people do live in your house?	1–2 people	58.59	13.63	57.89	0.011*
	3–5 people	61.91	15.06	63.16	
	> 6 people	57.06	18.38	57.89	
Routine prenatal visits	Never	57.69	15.87	57.89	0.001**
	Irregular	59.62	14.18	57.89	
	Regular	64.43	14.94	68.42	

a — Oneway Anova test, b — Student t test, c — Kruskal Wallis test, * — $p < 0.05$; SD — standard deviation

Table 7. Evaluation of COVID-19 Awareness Scores according to the risk factors of pregnant women

		COVID-19 Awareness Scores			p
		Average	SD	Median	
Diabetes mellitus	No	60.20	14.76	57.89	0.571
	Yes	58.22	16.81	60.53	
Hypertension	No	60.30	14.65	57.89	0.312
	Yes	53.07	20.38	57.89	
Lung diseases	No	60.24	15.01	57.89	0.364
	Yes	58.27	10.90	57.89	
Cancer diseases	No	60.14	14.77	57.89	0.699
	Yes	60.15	19.66	63.16	
Heart diseases	No	53.13	12.88	52.63	0.016*
	Yes	60.43	14.83	63.16	
Liver diseases	No	60.08	14.86	57.89	0.475
	Yes	63.82	12.08	65.79	
Renal diseases	No	60.12	14.79	57.89	0.830
	Yes	61.40	18.13	63.16	
Thyroid diseases	No	59.95	14.77	57.89	0.094
	Yes	64.74	15.75	68.42	
Other diseases	No	60.05	14.78	57.89	0.399
	Yes	63.16	16.40	68.42	
None	No	59.93	15.23	63.16	0.988
	Yes	60.19	14.73	57.89	

Mann Whitney U test, * — $p < 0.05$; SD — standard deviation

Table 8. Distribution of COVID-19 Anxiety Scores

	COVID-19 Anxiety Scores
Min–Max	13.33–86.67
Avr ± SD	53.49 ± 13.63
Low Anxiety Levels	60 (11.4)
Moderate Anxiety Levels	348 (66.2)
High Anxiety Levels	118 (22.4)

SD — standard deviation

awareness level of pregnant women increased, the anxiety level increased as well (Fig. 1).

DISCUSSION

A total of 526 pregnant women from all three trimesters who were admitted to two centers in the Asian and European sides of Istanbul with the highest patient admissions were surveyed. Based on the data, 58.7% of the pregnant women were found to have moderate awareness. When the awareness rates of pregnant women with comorbidities were examined, it was found that the awareness levels of pregnant women with heart disease were higher. In total, 81.9% of the participants believed that they and their relatives were not sufficiently informed about COVID-19, and 59.1% thought that healthcare workers did not inform them about the COVID-19 properly. In addition, 64.6% of the participants thought they needed information about COVID-19. These data suggest that the impact of COVID-19 on prenatal and postnatal periods is yet to be proven [8].

Although 58.7% of the pregnant women thought that the measures implemented by the Ministry of Health from the beginning of the pandemic in Turkey were satisfactory, only 18.1% thought that they were informed about how to protect and isolate themselves from the COVID-19 pandemic. These results are noteworthy and can encourage the Ministry of Health and healthcare workers to conduct more projects on informing people about the modes of transmission and protection against the virus.

While the COVID-19 awareness level is expected to increase as the level of education increases, there was no significant difference in the present study. This is consistent with the results obtained by Wang et al. [9]. According to their data, the anxiety rate was increased in patients with low educational background, whereas in our study, there was no positive correlation between educational background and anxiety.

Table 9. Evaluations based on COVID-19 Anxiety Scores

		COVID-19 Anxiety Scores			p
		Average	SD	Median	
Educational Status	Primary School	53.15	16.22	53.33	[a]0.992
	Middle School	54.11	12.36	53.33	
	High School	54.26	12.75	53.33	
	Associate's degree	53.63	15.11	53.33	
	From Bachelor's degree to Doctorate	52.63	13.20	53.33	
Health-care worker	Yes	54.94	13.88	53.33	[b]0.284
	No	54.38	13.95	53.33	
Number of children	Zero	53.44	12.85	53.33	[a]0.611
	1 child	53.06	13.69	53.33	
	2 children	53.43	16.84	53.33	
	3 and more	55.51	12.42	60.00	
Use of public transportation	Yes	52.78	13.92	53.33	[b]0.511
	No	53.71	13.55	53.33	
Suspected COVID-19 exposure	No	53.25	13.67	53.33	[c]0.044*
	Yes	62.17	13.06	59.67	
	Not sure	57.78	12.10	56.67	
Gestational age	< 14 gw	51.60	13.14	53.33	[a]0.047*
	14–28 gw	55.13	12.61	53.33	
	> 28 gw	53.31	14.19	53.33	
How many people do live in your house?	1–2 people	53.22	12.60	53.33	0.830
	3–5 people	53.82	14.22	53.33	
	> 6 people	52.98	15.57	53.33	
Routine prenatal visits	Never	51.13	15.27	53.33	0.010*
	Irregular	53.25	12.90	53.33	
	Regular	56.83	13.54	60.00	

a — Oneway Anova test, b — Student t test, c — Kruskal Wallis test, * — $p < 0.05$; SD — standard deviation

Even though awareness levels increased as the number of children and the household size increased in the present study, there was no significant increase in the anxiety levels. In the study by Wang et al. [9], a higher number of children and increased household size were not associated with increased awareness and anxiety. Remarkably, based on data obtained in the current study, there was no significant difference between the awareness levels of healthcare worker women and non-healthcare worker women. Forty point nine percent of the pregnant women expressed hesitation to report infection with COVID-19 to their physician and the Ministry of Health. This indicates that almost 50% of the pregnant women suffering from the disease try to avoid quarantine and do not understand the gravity of the situation. The concealment of a diagnosis puts the person's immediate environment and public health and healthcare workers at risk. It can be concluded that during the management of this process, it is necessary to provide the necessary psychosocial support and increase awareness of the COVID-19 pandemic among the public.

In terms of both anxiety and awareness scoring, those who visited the healthcare institution regularly for routine pregnancy check-ups had high levels of awareness and high anxiety scores. In addition, there was a statistically significant correlation between the scores of COVID-19 anxiety and awareness.

In our study, 66.2% of the participants were moderately concerned, while Saccone et al. [10], found that 53% of respondents had been psychologically affected at a high level.

In terms of comorbidities, those with DM were more concerned about getting infected by the virus, while patients with cancer and patients with chronic lung disease, who are thought to be more prone to stress and depression, did not have a significantly increased anxiety.

Table 10. Evaluation of COVID-19 Anxiety Scores according to the risk factors of pregnant women

		COVID-19 Anxiety Scores			p
		Average	SD	Median	
Diabetes mellitus	No	52.31	13.68	2	0.046*
	Yes	59.58	10.74	60.00	
Hypertension	No	53.46	13.51	53.33	0.757
	Yes	55.00	18.88	53.33	
Lung diseases	No	53.71	13.74	53.33	0.107
	Yes	49.76	11.11	46.67	
Cancer	No	53.47	13.63	53.33	0.484
	Yes	55.24	14.25	60.00	
Heart diseases	No	53.48	13.71	53.33	0.640
	Yes	53.97	11.72	60.00	
Liver diseases	No	53.55	13.63	53.33	0.456
	Yes	50.00	14.25	53.33	
Renal diseases	No	53.46	13.66	53.33	0.607
	Yes	56.66	11.74	53.33	
Thyroid diseases	No	53.32	13.54	53.33	0.101
	Yes	58.00	15.46	60.00	
Other diseases	No	53.50	13.73	53.33	0.972
	Yes	53.33	9.76	53.33	
None	No	53.96	13.48	53.33	0.591
	Yes	53.38	13.68	53.33	

Mann Whitney U test,* — $p < 0.05$; SD — standard deviation

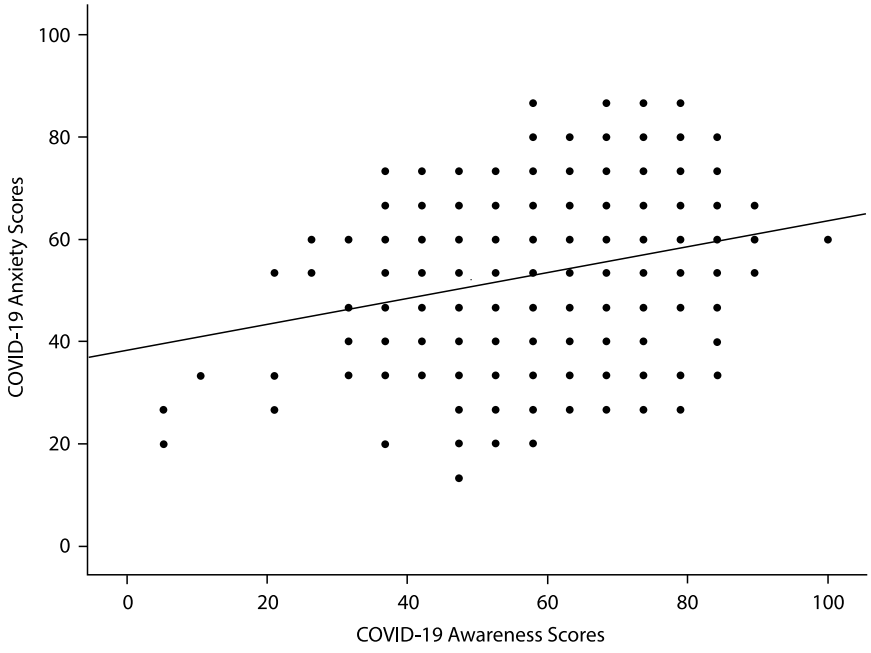

Figure 1. Relationship between COVID-19's anxiety scores and awareness scores on pregnant women

The period when anxiety was observed at the highest level was the 2nd trimester, whereas the lowest level was seen in the 1st trimester. On the contrary, in the study by Tang et al. [11], the anxiety levels of pregnant women in the 1st trimester were higher.

The study by Durankuş and Aksu included 260 participants, and the study by Yassa et al., included 172 participants. Both reported negative psychological effects of COVID-19 on pregnant women [12, 13]. In the present study, 44.7% of the participants from all trimesters thought that physicians could not diagnose COVID-19 in a timely manner and treat COVID-19 properly, while only 7.5% of participants thought so in the study by Yassa et al. [13].

In the present study, 47% of participants thought that social media was not being used efficiently for informing the society. Wu Y et al. [14], reported apart from the benefits of social media, it causes extreme fear, isolation, fear of death, and proneness to depression among the society, especially during the time of lockdown.

Mirzadeh and Khedmat particularly stressed that pregnant women need psychological support during this crisis [15].

Previous studies showed that there is an increased predisposition to emotional state disorders in pregnancy and childhood [16–18]. Pregnant women experience more anxiety and suffer from fears that arise as the delivery date approaches during advanced gestation weeks [16]. It is also obvious that if the concerns that pregnant women suffering from infectious diseases have about the health of their babies are added to this, their mental health can be affected even more.

During the pandemic, women are under stress and may therefore complain about many psychological symptoms or nonspecific symptoms that can be confused with those of COVID-19. They can also face many problems, such as emergence of unintended pregnancies.

In the present study, for example, it is noteworthy that 39% of the pregnant women avoided visiting the healthcare institution for routine pregnancy check-ups.

According to all these results, healthcare workers should further inform pregnant women about COVID-19.

Limitations

A significant number of individuals who had been followed up or treated as outpatients or inpatients for confirmed or suspected cases of COVID-19 could not be surveyed as part of the present study since our hospital served as a major hospital during this pandemic. The duration of the study was prolonged to reach the targeted number of pregnant women due to the decrease in the number of patients admitted to outpatient clinics due to fear and anxiety. Patients who had difficulty reading and understanding Turkish were not surveyed to avoid incorrect results. Owing to the high number of questions asked, some of the patients filled out the questionnaire by skipping some of the questions and some were unable to complete the questionnaire. Of all the pregnant women admitted to the emergency service, those who needed urgent diagnosis and treatment were not surveyed. Due to socioeconomic reasons such as not having access to internet or phone, some pregnant women could not be surveyed online.

CONCLUSIONS

This study shows that pregnant women have insufficient knowledge about this important health problem, their anxiety is high, and their awareness is insufficient.

Healthcare workers have an important duty to ensure early identification of the negative outcomes that may arise due to COVID-19 in pregnant women so that pregnant women are provided with the necessary psychological support.

This study presents significant clues that might constitute a ground for future studies. In addition, this study offers guidance for family physicians, obstetricians, midwives, and other healthcare workers for developing measures to protect maternal and newborn health at an advanced level.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethics approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee (The ethics committee of University of Health Sciences Umraniye Training and Research Hospital, date: April 14, 2020; approval number: B.10.1.TKH.4.34.H.GP.0.01/84-15/04/2020-54132726-000-8582/00116578941) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Our scientific research application No. 15916306-604.01.01 was approved by Istanbul Provincial Health Directorate and No. 2020-05-04T23_03_03 was approved by the Ministry of Health of the Republic of Turkey.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Consent for publication

Patients signed informed consent regarding publishing their data.

This article does not contain any studies with animals performed by any of the authors.

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Perinatal outcomes of the antenatally diagnosed omphalocele cases: a single tertiary center experience

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ABSTRACT

Objectives: To evaluate the perinatal outcomes of antenatally diagnosed omphalocele cases.

Material and methods: This was a retrospective study conducted between July 2014 and February 2020 at the prenatal diagnosis center of a university clinic. Gestational week of diagnosis, associated anomalies, karyotype analysis results, complications during pregnancy, termination/delivery characteristics, and postnatal results were evaluated.

Results: The analysis was performed on 58 patients. The median diagnosis time was 14.5 weeks of gestation. Thirty-three cases (57%) were defined in the first trimester. 33 (57%) of 58 patients had one or more concomitant anomalies, while 25 patients (43%) had isolated omphalocele. The most common associated anomaly was a cardiac anomaly which was observed in 17 fetuses (30% of all omphalocele cases). Karyotype analysis was performed in forty-five patients (41 in the prenatal period, 4 in the postnatal period). A normal karyotype was detected in 27 cases (60%). Trisomy 18 was the most common chromosomal anomaly (n = 15, 33.3%). Thirty of 58 patients (52%) requested termination of pregnancy (TOP) in the early pregnancy period. Thirteen of the cases died in-utero (22%). Fifteen pregnancies resulted in live births (26%), of those eight were lost in the first year of life (six of them had additional anomalies, while two of them had isolated omphalocele but the omphalocele pouch was containing the liver in those two babies).

Conclusions: Most of the cases with an omphalocele can be diagnosed in the first trimester. Cardiac anomalies were the most common associated anomalies, while trisomy 18 is the most common chromosomal anomaly. Thus, earlier and effective counseling can be made about the prognosis of pregnancy.

Key words: exomphalos; perinatal outcomes; omphalocele

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INTRODUCTION

Omphalocele (*exomphalos*) is one of the most common anterior abdominal wall defect and its frequency is reported to be 1 in 4000–7000 live births [1]. It is defined as herniation of intra-abdominal organs covered with peritoneal membrane and umbilical cord due to midline defect of the anterior abdominal wall. The peritoneal membrane consists of the wharton gel between the inner layer of the peritoneum and the outer layer of the amnion [2]. Abdominal wall herniation is considered physiological before the 12th gestational week. For this reason, the diagnosis of omphalocele is made more during the first trimester screening [3, 4] especially during the nuchal translucency (NT) measurement as part of first trimester screening [5]. This is the main reason for the high misdiagnosis rates in the first trimester.

Detailed ultrasonographic examination including fetal echocardiography and karyotype analysis should be requested in terms of searching for the concomitant anomalies that have an increased rate in omphalocele cases. The frequency of chromosomal anomalies, especially trisomy 18, was higher in omphalocele cases. Trisomy 18 is present in 80% of cases if other anomalies accompanied to omphalocele, whereas the rate is 54% in the omphalocele cases accompanied with only increased NT [6]. Since omphalocele may be associated by many structural anomalies (Pentalogy of Cantrell, Beckwith-Wiedeman syndrom, bladder exstrophy, imperforate anus, spina bifida complex/OEIS complex, neural tube defects, diaphragmatic herniation, single gene disorders and many other syndromes) tar-

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geting detailed ultrasonography in the second trimester and if necessary cyto-genetic analysis such as comparative genetic hybridization should be performed even if it is thought to be isolated in the first trimester [7]. Cardiac anomalies especially secundum atrial septal defect (ASD) and muscular ventricular septal defect (VSD) are the most common concomitant structural anomalies in omphalocele cases. The most common extracardiac anomalies are in the genitourinary system (Pyelectasis, Hydronephrosis, Cloacal exstrophy, Multicystic kidney) and gastrointestinal system (Beckwith-Wiedeman syndrome). Pulmonary hypoplasia is also common, especially if the defect is large [8, 9]. Therefore, detailed cardiac evaluation is essential in these cases.

The prognosis of the omphalocele is usually severe in non-isolated cases. Omphalocele sac can include liver, stomach and intestinal. There is a high termination rate and in-utero mortality rate for omphalocele because of the associated structural and chromosomal anomalies. Chromosomal anomaly and/or related structural anomaly incidence rate can be high as 88% for omphalocele. Therefore, live birth rate is reported to be 25–40% [1, 10]. But prognosis is better in isolated omphalocele cases. The most difficult question is to reveal whether the case is really isolated omphalocele.

In this study, we aimed to evaluate the prenatal results of patients with omphalocele diagnosed and followed up in our clinic.

MATERIAL AND METHODS

This retrospective study was conducted between July 2014 and February 2020 at the Maternal-Fetal Unit of a University Hospital. This study was approved by the Faculty of Medicine Ethics Committee. This center is the tertiary referral center for the perinatal diagnosis. Women admitted for routine ultrasound examination or referred to our hospital for a suspected fetal anomaly underwent detailed fetal anomaly ultrasonography scan. For those detected with omphalocele and continued pregnancy, a detailed ultrasonographic evaluation including fetal cardiography was performed to determine associated anomalies by using VolusonE6 (GE Medical Systems, Zipf, Austria) with a transabdominal 4–8--MHz probe. The data were scanned retrospectively from the viewpoint recording system and the neonatal registry system. Maternal age, gravidity, parity, gestational age at diagnosis, associated structural malformations, whether invasive diagnostic procedures were performed or not, the results of karyotype analysis and fetal echocardiography results were determined. Each patient was evaluated in a council of clinical geneticists, pediatric surgeons and perinatologists. Each family was informed in detail about the current situation by the council. TOP were performed if the family requested the termination and the fetus was evaluated postmortem if the family approved this examination. The ratio of TOP was determined. During the follow-up, whether intrauterine growth restriction (IUGR), intrauterine fetal death, preterm labor were evaluated**.** IUGR was defined as fetal ultrasonographic measurements being below 10% percentile according to gestational age. Small gestational age (SGA), IUGR distinction was not made. Doppler studies were evaluated after the diagnosis of IUGR. Delivery before completing the 37th gestational weeks were defined as preterm delivery. Gestational age at delivery, route of delivery, low APGAR score (10th minute ≤ 5), birth weight, size and content of defect and postnatal information were recorded by using the labor unit registry and neonatal intensive care (NICU) registry. Neonatal care was performed by the neonatologist and the pediatric surgery team in same hospital.

Statistically analysis was performed by using SPSS® (SPSS Inc., Chicago, IL, USA, version 20). Descriptive analysis was performed for this study. Continuous data was presented as mean ± SD, median (min–max.). Categorical data was presented as n (%).

RESULTS

Seventy-four patients with omphalocele were detected during the study period. Sixteen of them were excluded from the study because follow-up data were not available. Analyzes were performed on 58 cases. Mean maternal age was 29.0 ± 6.7, primigravidity was present in 18 (31%) cases. The median of diagnosis time was 14.5 weeks of gestation. Thirty-three cases (57%) were defined in the first trimester. Twenty-five cases did not have any associated anomalies (43%), 33 of total cases (57%) had one or more associated anomalies. Thirty of 58 cases (52%) performed termination of pregnancy (TOP) in the early pregnancy period. Thirteen of the cases (22%) died in-utero. Fifteen pregnancies (26%) resulted in live births. Clinical and demographic variables and results were presented in Table 1.

The most common associated anomaly is cardiac anomaly and it was observed in 17 fetuses. Among the cardiac anomalies, atrioventricular septal defect (AVSD) was the most common anomaly. Central nervous system (CNS) anomalies were observed in 15 cases. Urogenital system anomalies were the third most common anomalies with eight cases. Other system anomalies most frequently belong to the skeletal system (short femur length and short humerus length) and were seen in six cases. Thirteen cases had multiple anomalies associated omphalocele. Associated anomalies and their properties are shown in Table 2.

Karyotype analysis was performed in 41 cases in the pre-natal period and in four cases in the post-natal period (78%). Normal karyotype was detected in 27 cases (60%). Trisomy 18 was the most common karyotype anomaly with 15 cases (83%). Trisomy 13 was seen in two cases and Turner

Table 1. Demographic and clinical properties of the cases

	Omphalocele n = 58 mean ± SD n % median (min–max)
Maternal age	29.0 ± 6.7 28 (17–44)
Primigravidity	18 (31%)
Twin pregnancy	4 (6.8%)
Gestational week at diagnosis	15.8 ± 3.7 14.5 (12–25)
First trimester diagnosis	33 (57%)
Fetal karyotype N/A Normal (n = 45) Abnormal (n = 45)	 13 (22%) 27 (60%) 18 (40%)
Associated anomalies None Present	 25 (43%) 33 (57%)
Pregnancy outcomes Termination of pregnancy In-utero exitus Delivery	 30 (52%) 13 (22%) 15 (26%)

SD — standard deviation; N/A — not-available

Table 2. Associated anomalies and characteristics

Associated anomalies	(n %) in total cases (n = 58)	% in non-isolated cases (n = 33)
Cardiac AVSD VSD Hypoplastic left heart Hypoplastic left heart double outlet right ventricle Fallot Tetralogy	17 (29%) 10 2 2 1 2	52%
CNS Cerebellar hypoplasia Holoprosencephaly Encephalocele Spina bifida Acrania Ventriculomegaly Mega sisterna magna	15 (26%) 2 3 3 1 2 2 2	45%
Urogenital system Pyelectasis Polycystic kidney Multicystic dysplastic kidney Increased renal echogenicity	8 (14%) 3 2 2 1	24%
Others Skeletal system (short FL, short HL) Single umbilical artery	6 (10%) 3 3	18%
Multiple anomalies in different systems	13 (22%)	39%
Isolated omphalocele	25 (43%)	

AVSD — atrioventricular septal defect; VSD — ventricular septal defect; FL — femur length; HL — humerus length

Table 3. Karyotype analysis and results

	(n = 45)
Normal	27 (60%)
Aneuploidy Trisomy 18 Trisomy 13 Turner (45, X0)	18 (40%) 15 (83%) 2 (11%) 1 (6%)

Table 4. Pregnancy and delivery outcomes of the omphalocele cases

	Omphalocele n = 58 mean ± SD, median (min–max.); n %
Preterm delivery	7/15 (46%)
IUGR	7/28 (25%)
Gestational week at delivery	34.3 ± 4.5 36 (24–39)
Route of delivery (live) Vaginal C/S	 5 (33%) 10 (67%)
Birth Weight [gr]	2688 ± 586 2700 (375–3700)
Apgar score (10^{th} minute ≤ 5)	3 (20%)
Size of defect [cm] < 5 cm ≥ 5 cm	 8 (53%) 7 (47%)
Content of the defect Bowel Bowel + Liver Bowel + Liver + Stomach	 7 (47%) 7 (47%) 1 (6%)
Outcome of neonate Discharged Death	 7 8

SD — standard deviation; IUGR — intrauterine growth restriction; C/S — cesarean section

(45, X0) was determined in one case. Results of the karyotype analysis were presented as Table 3.

Preterm delivery was determined in seven cases (46%). IUGR was determined in seven cases (25%). Fifteen live births were performed. Mean gestational week at the delivery was 34.3 week. One-third of the cases have been delivered by the vaginal route. Omphalocele alone was not considered a cesarean indication, and the decision for cesarean was determined according to general obstetric indications. 10^{th} minute low APGAR score (≤ 5) was present in the 3 cases. Eight of them were lost in the first year (six of them had additional anomalies, two cases were isolated omphalocele, but liver was also present in the pouch). There were no additional major organ anomalies or karyotype abnormalities of seven babies who were born alive and continued their lives, and the youngest was seven months old and the eldest was three years old. Obstetrics and neonatal outcomes of the cases were presented in the Table 4.

DISCUSSION

In our series of 58 cases, we evaluated obstetric and neonatal outcomes in cases with omphalocele. Due to the liberal use of ultrasonography in the antenatal period and increased evaluation experience and knowledge, omphalocele diagnostic accuracy is close to 100% [1]. In the intrauterine period, the differential diagnosis of omphalocele and gastroschisis can be made almost 100%. Gastroschisis was not ever misdiagnosed as omphalocele in our series. The sensitivity for omphalocele diagnosis in the first trimester is reported to be 90% [10, 11]. In our study, 33 of the 58 cases (57%) were diagnosed omphalocele correctly in the first trimester. The diagnosis of omphalocele in the first trimester is very important in terms of detecting structural and chromosomal anomalies and enabling earlier decisions about the pregnancy. In omphalocele cases, live birth rates are as low as 25–40% because elective TOP rates and in-utero exitus rates are high [10]. In our series, the TOP rate was high as 52% (n = 30) and live birth rate was 25% (n = 15). According to a study conducted in 11 countries in Europe, the live birth rate for omphalocele (n = 137) was given as 41%, fetal death rate was 22% and TOP rate was 37% [12].

The prognosis of omphalocele depends on concomitant structural and/or chromosomal anomalies [1, 2, 6–10]. Structural anomalies were associated in 57% of our cases while chromosomal anomalies were found in 40%. In another series of 90 cases, 69% of central omphalocele cases had chromosomal anomaly, while in epigastric omphalocele, this rate was 12% [13]. They concluded that the types of the omphalocele may be different entities but as a result, 22% of cases live and omphalocele has poor prognosis irrespective of the types. Thus in a study evaluating 79 isolated omphalocele cases diagnosed in the first trimester and without structural and chromosomal anomalies, live birth rate was 68% and the mortality rate was 33% [10]. In another series of 67 cases, the rate of chromosomal anomaly was reported as 39% like our results [14]. In another study evaluating 98 cases diagnosed in the first trimester, it was found that 45.9% of the cases were associated by major structural anomalies and 53.8% had chromosomal anomalies [6]. Fleurke-Rozema H et al. [15], reported that 141 cases with omphalocele 83% had additional anomalies of which 57% had a chromosomal anomaly. Similarly, in the presence of increased NT (> 3.5 mm), chromosomal anomaly was detected in 40.8% of cases with omphalocele [16]. A normal NT is therefore a reassuring sign, but the residual risk of aneuploidy may still be as high as 28% [17]. In another series, the rate of related structural anomaly was reported as 78.7% (26/33), and the rate of chromosomal anomaly was 27.6% (8/29) [18]. The most common accompanying chromosomal anomaly is trisomy 18 [1, 2, 8]. Trisomy 18 was the most common karyotype anomaly (15/18) in our series. The most common associated structural anomaly in our series was cardiac anomalies (17/58). It is stated that the most frequently observed structural anomaly is cardiac [9, 19]. However, CNS anomalies were most frequently accompanied in another case series [20]. The general recommendation is that omphalocele cases detected in the first trimester and thought to be isolated should definitely be evaluated in the second trimester. Because it may be accompanied by syndromic structural conditions like Pentalogy of Cantrel; bladder exstrophy, imperforate anus, spina bifida complex, Beckwith–Wiedemann syndrome (2). Isolated omphaloceles are related to Beckwith–Wiedemann syndrome with a 10–20% probability, appropriate prenatal cytogenetic testing should be discussed with patients [7]. The fact that the negative results of molecular genetic tests do not exclude this diagnosis.

This study has some limitations due to its retrospective structure. In addition, the lack of molecular cytogenetic methods is another limitation. But we have a good number of cases for a single center. Follow-up data is also a positive aspect in this study, because newborn care and surgery of cases are performed in the same hospital.

CONCLUSIONS

In conclusion, it is important to diagnose these cases in the first trimester. Intense efforts should be made to recognize concomitant structural and chromosomal anomalies because they determine the prognosis. If structural and/or chromosomal anomalies are present, the prognosis is poor. Genetic counseling should also be recommended in cases considered to be isolated.

Conflict of interest

The authors report no conflicts of interest.

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The pregnancy rate among Romanian adolescents: an eleven years (2009–2020) observational, retrospective study from a single center

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ABSTRACT

Objectives: It has been recently documented that Romania has the highest prevalence of adolescent pregnancy in Europe.

Material and methods: Therefore, the present study aims to offer a conclusive view of the current situation by assessing a series of parameters in the last 11 years.

Results: Throughout the present manuscript, we showed that 1788 pregnancies occurred in the last 11 years in just one center from the northeastern region of Romania. The Kolmogorov–Smirnov test ($p < 0.05$) performed suggests that gestational age does not follow a normal distribution; an interval during which 899 (50.27%) male and 889 (49.72%) female babies were born. There were a total of 1383 (86.00%) deliveries at full-term and 225 (13.99%) were under 37 weeks. Of 1788 teenage girls, 1467 (82.04%) were from the rural area, whereas 321 (17.95%) from the urban area.

Conclusions: Fortunately, one common feature that we observed was that starting from 2017 there was a significant reduction within the last 2 studied parameters, the situation being much more fluctuating until 2014.

Key words: adolescent pregnancy; birth under 37 weeks; full-term birth; gestational age; prevalence; rural; urban

Ginekologia Polska 2022; 93, 1: 42–46

INTRODUCTION

Congruent with the World Health Organization's (WHO) guidelines, "adolescent pregnancy" is by definition the process of child-bearing in which the mother's age is under twenty years old before the ending of the current gestation. The latest figures issued indicate that more than 20 million girls remain pregnant during the transition period from adolescence to early adulthood. Around 12 million give birth, whereas approximately 800 000 births occur when girls are under the age of 15 [1].

Even though the tendency of unintended pregnancies is growing in developed countries, the governments possess all the resources necessary to mediate this phenomenon. Unfortunately, this is not the case for lower or middle-class countries [2]. It was previously documented that various factors are responsible [3].

One example of a middle-class country with no strong knowledge in terms of sexual education is Romania, which currently has the highest number of cases of teenage pregnancies in Europe; around 34 700 pregnancies in 2011 according to the United Nations Statistics Division's Demographic Yearbook data [4]. By analyzing all evidence, only on two previous occasions, it has been discussed by the authors the critical phase encountered in Romania [5, 6].

The repercussions are pronounced, starting from the method of delivery which, imperatively, affects the fetus [7, 8] and may culminate in death [9]. On the other hand, among 21 countries with liberal abortion laws, the percentages regarding the number of abortions is 61% [4], this further indicating a poor family planning [10].

Based on the aforementioned, the main objectives that defined the present study are the following: (I) to establish

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the prevalence of adolescent pregnancy; (II) gestational age, (III) full-term births versus those under 37 weeks, and (IV) the percentage of minors from the urban (U) and rural (R) area that gave birth.

MATERIAL AND METHODS

Data

The data used in this manuscript correspond to the period 2009–2020. All information needed have been extracted from the archive of the Clinical Hospital of Obstetrics and Gynecology "Cuza Voda" from Iasi, a reference unit from the northeastern region of Romania.

Study participants

A total of 1788 (mean = 16.32; confidence interval [CI] 95% = 0.04) teenage girls were included in this study and subsequently divided into groups based on their age. From the total, (n = 1) was 11, (n = 2) 12, (n = 13) 13, (n = 54) 14, (n = 221) 15, (n = 539) 16, and (n = 958) 17. As we mentioned above, this manuscript describes the situation of 1788 teenage girls that have been hospitalized in "Cuza Voda" from Iasi in the last 11 years. The main procedures that girls underwent were as follows: 580 had an emergency lower segment caesarean section (C-section); 537 had episiotomy; 239 had postpartum evacuation of uterus by dilatation and curettage; 114 had suture of 1st or 2nd degree tear of perineum; 86 had suture of current obstetric laceration of cervix; 67 had medical and surgical induction of labour; 56 had suture of current obstetric laceration of vagina; 30 had surgical augmentation of labour; 28 had suture of current obstetric laceration of bladder and/or urethra without perineal involvement; 15 had spontaneous vertex delivery; 8 had other invasive procedures on female genital organs; 8 had surgical induction of labour by artificial rupture of membranes (ARM); 8 had postpartum manual exploration of uterine cavity; 7 had suture of 3rd or 4th degree tear of perineum; one had emergency classical caesarean section; one had postpartum evacuation of uterus by suction curettage; one had vacuum extraction; one had spontaneous breech delivery, and one had medical and surgical augmentation of labour.

Inclusion/exclusion criteria and limitations

The main exclusion criterion was over 18 years of age. We implied this method because it is considered that the person already has discernment. There were no restrictions regarding religion, ethnicity, social status, or medical status. It should be also mentioned that data presented were centralized per year. Additionally, data regarding the number of vaginal versus caesarean sections (C-sections), and complications that occurred during intervention have been already discussed by other teams [5, 6].

Ethical approval

The design of this study was approved by the ethical committee of the Clinical Hospital of Obstetrics and Gynecology "Cuza Voda" from Iasi (no 116/565/January/25/2021). It must be stated that the present study respected the Helsinki Declaration on Human Rights, concomitantly with National and European legislation regarding the Biomedical Research.

Statistical Analysis

An MS Excel was used to build a database. We used Microsoft Excel 2010 and then exported the files into Minitab 19 software (Minitab Inc., 2019). Due to heterogeneity between groups, we were unable to perform any standard statistical tests either for equal or unequal groups.

RESULTS

As can be seen in Figure 1, there was a fluctuating tendency in the past 11 years. Specifically, the peak was reached in 2015 with 189 (10.57%) adolescent pregnancies, with slight differences between 2014 (n = 180 — 10.06%) and 2016 (n = 182 — 10.17%). A significant decrease was noted starting with 2017 (n = 153 — 8.55%) followed by 2018 (n = 127 — 7.10%), 2019 (n = 118 — 6.59%), and 2020 (n = 109 — 6.09%). The situation in 2018 differed from that in 2009 with only one case (n = 128 — 7.15%). Even though in 2010 only 154 cases were registered (8.61%), for 4 years, the number of adolescent pregnancies was varying — 2011 (n = 142 — 7.94%), 2012 (n = 169 — 9.45%), and 2013 (n = 137 — 7.66%).

The predetermined interval associated with the gestational age was between 24 and 42 weeks (mean = 38.09, CI 95% = 0.09). Teenage girls gave birth to 899 male (50.27%) and 889 female (49.72%) babies, respectively. After we perform a Kolmogorov–Smirnov test, we noted that there are significant differences (p = 0.010; KS — 0.250) by showing that the data do not follow a normal distribution (Fig. 2).

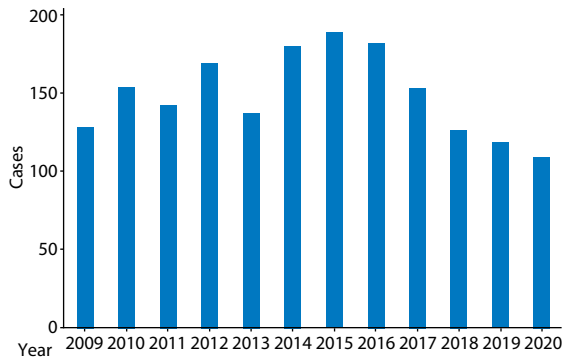

Figure 1. Overall prevalence among teenage girls within "Cuza Voda" in the last 11 years

We observed that most of the teenage girls gave birth after a full-term pregnancy (n = 1383 — 86.00%) in contrast to those that gave birth under 37 weeks (n = 225 — 13.99%) (Fig. 3). In 2015 and 2016 was noted the same number (n = 157 — 9.76% per year), followed by 2012 (n = 148 — 9.20%). We also noted the same number of full-term deliveries in 2010 and 2017 (n = 128 — 7.96% per year), one less than in 2011 (n = 129 — 8.02%). Even if there is a difference of 10 cases between 2013 (n = 125 — 7.77%) and 2018 (n = 115 — 7.15%), the tendency decreased significantly in 2019 (n = 103 — 6.40%), and 2020 (n = 97 — 6.03%). The lowest rate of full-term pregnancies has been noted in 2009 (n = 96 — 5.97%). On the other hand, the peak of under 37 weeks deliveries was reached in 2015 and 2009 (n = 32 — 1.99%), followed by 2010 (n = 26 — 1.61%) and 2012 (n = 21 — 1.30%). In 2016 and 2017 we observed the same number (n = 25 — 1.55% per year) of under 37 weeks deliveries. Analogous, in 2013, 2018, and 2020 we also noted the same number (n = 12 — 0.74% per year), with the mention that in 2011 and 2019 there was a difference of only 2 cases; 2011 (n = 13 — 0.80%) and 2019 (n = 15 — 0.93%). In 2014 there was no full-term, nor under 37 weeks delivery reported (0.00%).

As expected, there were significant differences among adolescent depending on the environmental origin. As indicated in Figure 4, the number of adolescents from the rural area in 2014 and 2016 was identical (n = 145 — 8.10% per year), followed by 2012 (n = 141 — 7.88%) and 2010 (n = 138 — 7.71%). There was also a small difference in 2011 (n = 120 — 6.71%) and 2017 (n = 125 — 6.99%) of 5 cases, the peak being reached in 2015 (n = 162 — 9.06%). Although there was a difference of only several cases between 2009 (n = 101 — 5.64%), 2013 (n = 106 — 5.92%), and 2018 (n = 109 — 6.09%), from that specific point the tendency started to decrease the following 2 years; in 2019 (n = 95 — 5.31%), the lowest ratio being registered last year (n = 80 — 4.47%). If we refer to urban girls, it can be observed a gradual increase per year starting from 2010 until 2014; in 2010 were registered 16 cases (0.89%), whereas in 2011 (n = 22 — 1.23%), in 2012 (n = 28 — 1.56%), in 2013 (n = 31 — 1.73%), and in 2014 (n = 35 — 1.95%). The highest point reached was in 2016 with 37 hospitalizations (2.06%), with a slight difference of several (< 15 cases) in 2018 (n = 18 — 1.00%), between 2009/2015 (n = 27 — 1.51% per year), 2019 (n = 23 — 1.28%), 2017 (n = 28 — 1.56%), and 2020 (n = 29 — 1.62%).

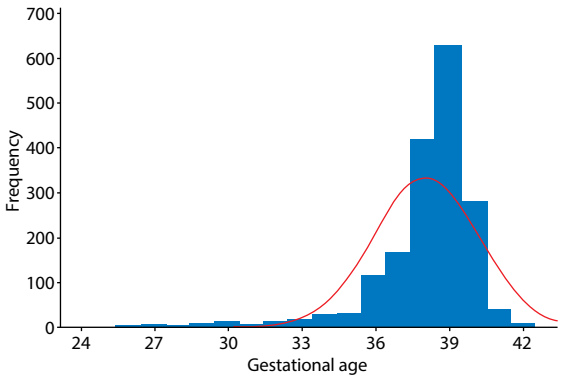

Figure 2. Kolmogorov–Smirnov histogram of "GESTATIONAL AGE" within "Cuza Voda" in the last 11 years (mean — 38.09; SD — 2.143; KS — 0.250; p < 0.010)

DISCUSSION

The most numerous adolescent pregnancies were registered between 2014 to 2016. More precisely, it accounted for 30.08% (n = 551). In this context, we support the conclusions of Iorga and his collaborators [6]. The number of pregnancies is dependent on age, concomitantly with a decrease of abortions; only 23 abortions on request were performed between 2007 and 2014. Another argument that strengthens our results is that both Iorga and our group clearly

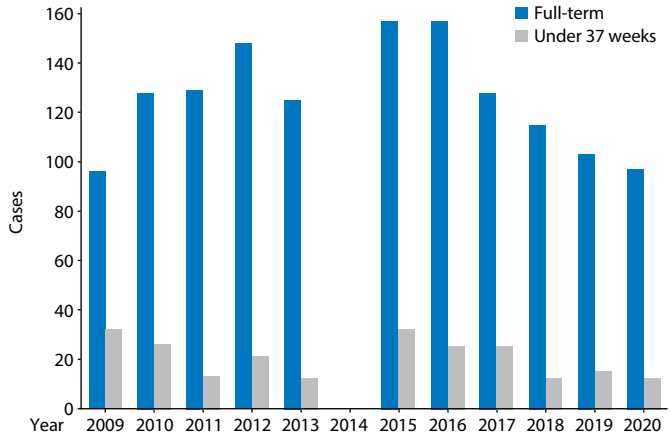

Figure 3. Ratio of full-term versus under 37 weeks deliveries within "Cuza Voda" in the last eleven years

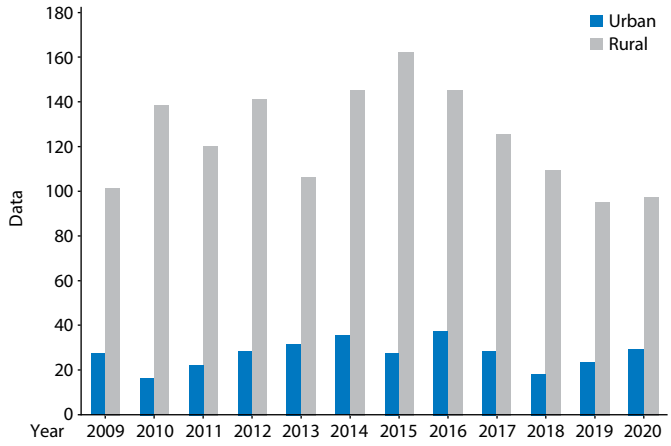

Figure 4. Environment origin ratio (U) versus (R) among teenage girls within "Cuza Voda" in the last 11 years

demonstrated the predominance of adolescent pregnancies among teenage girls from the rural area. While they reported disproportionality up to 5 times, in our case was 2, up to 8 times (1467 — 82.04% from the rural versus 321 — 17.95%) from the urban area).

While the rates of adolescent pregnancy have elevated in Romania, Vaz et al. [11] demonstrated that live births (LB) from teenage mothers (10–19 years) have reduced in Brazil (23.5% in 2000 to 19.2% in 2011). While LB increased by 5.0% among teenage girls aged 10–14 years, those between 15–19 sustain those four main themes influenced their sexual status: sexual knowledge and access to sexual health resources, alcohol use and relationships with others and the own person. Another Nigerian study emphasizes the results of Lys et al. [12], since in 50.1% of the cases, the first childbirth occurred within 15–19 years, and 38.1% within 20–29 years [13]. Such a phenomenon was also observed in Nepal, according to another team of researchers [14].

Dimitriu et al. [5] enrolled 74 female patients aged 14 to 20 and disseminated a questionnaire consisting of 15-items regarding their social, educational, and medical background. According to their results, 71.6% (n = 53) gave birth following a C-section and 28.3% (n = 21) through vaginal delivery. Noteworthy is that patients aged 14 to 16 years had a lower rate of C-sections in contrast with those between 17 to 20 years. They showed that 83.0% of the C-sections and 76.1% of the vaginal deliveries were at term, but there was a risk that women could give birth through C-sections. Although Dimitriu and co-authors enrolled a small cohort, Iorga reported in his study almost 300 C-section interventions and 992 natural births.

In our case, 1383 (86.00%) deliveries were at full-term and 225 (13.99%) were under 37 weeks. This aspect could be of significant interest since Murray et al. [15] discussed in a recent systematic review in which were included 41 344 children the possible long-term cognitive outcomes on both early term and late preterm births. Even though Dimitriu and his collaborators [5] concluded that there is a higher frequency in primiparae who gave birth under 37 weeks, our results contradict these findings.

Rada [16] performed a study between 2011 and 2012 in which 1215 participants of both sexes were enrolled and showed that 7.2% engaged in sexual intercourse for the first time at an early age (for example 15 years) or even earlier. Compared with the findings of Rada, we had only one case in which an eleven years old girl gave birth.

It has been discussed on 3 different occasions about volunteering termination of a pregnancy. Ganatra and Hirve [17] showed that 13.1% of the 17 171 married women had an abortion, the most taking place in the private sector. They also argued that adequate counseling, spacing and lack used of contraceptive were the main reasons for abortions. Insufficient financial support from the state in severe cases (81%), despite the fact that mother had a medium standard life (75%) is another cause of abortion. Polish women do not take into consideration society's opinion (95%), but 97% informed their partner, 82% the family members, whereas 32% tell friends and 31% did not inform the gynecologist about this decision [18].

On the other hand, 74% of the Greek adolescents stated that they had acquired information on contraception (friends, doctors, and media — 64%, 47%, and 36%, respectively). Mavroforou et al. [19] further demonstrated that withdrawal (49%) and condom use (28.5%) were also amongst the popular contraceptive precautions. Interestingly, adolescent's decision towards abortion was 65%, the partner's influence accounting for 73%. Even though 91% of them knew about the risks from doctors (87%) and socio-economic reasons (89%) were mainly invoked, their parents were rarely aware (28%) about the pregnancy and possible abortion (28%). Even though most were at the first abortion (78%), a significant proportion was aware of

the Greek Church's opposition (89%) or the existence of an abortion law (86%).

Given the fact that Romania is a former communist state, the repercussions exercised by this regime remain notable even after 31 years since its decline. This could be the reason for the poor education of the general population. It is known that abortions or any other method of contraception was forbidden. Kaestle et al. [20] conducted a study 16 years ago which reunited post-Communist countries from Eastern and Central Europe, the authors concluding that the highest pregnancy rate among adolescents was in Romania (34% in 1000 women aged between 15 and 20 years). Impressed by this study, it is one reason that we wanted to offer a conclusive overview of the current situation in Romania. If it is to take into account all consequences associated with adolescent pregnancy, 11 countries including Romania successfully reduced the mortality rate up to 75% from 1990 to 2015 [21].

Even in today's society where women's health and the phenomenon of globalization are two topics of great interest the church, and the government exert important influences in this context, often being contradictory. Romanian legislation allows elective abortion until 14 weeks of pregnancy, but it is difficult to achieve because of discrimination and the limited number of clinics that perform such interventions [5].

It can be concluded that in Romania the situation is critical since these data are reported from a single-center. Therefore, this event is the consequence of the poor organization of the government, in parallel with the persistence of communist principles even after more than three decades. Teenage girls do not possess a stable spectrum of knowledge concerning the risks to which they expose and the child at the same time.

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Data availability statement

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgments

Not applicable.

Conflicts of interest

The authors declare no conflict of interest.

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COVID-19 in pregnancy, management and outcomes among pregnant women and neonates — results from tertiary care center in Wroclaw

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ABSTRACT

Objectives: A novel coronavirus — SARS-CoV-2 — outbreak has, for sure, been the greatest medical challenge in recent years. The maternal and neonatal consequences of the infection are still largely unknown.

Material and methods: This prospective study aims to describe the perinatal care and outcomes of SARS-CoV-2 positive pregnant women and their newborn infants during the third wave of the pandemic, in a large tertiary university center in Wroclaw/Poland from 15 February to 1 May 2021.

Results: The paper describes a group of 83 women with confirmed SARS-CoV-2 infection during delivery, as well as their newborn infants (n = 84). The course of COVID-19 disease in pregnant patients was mostly asymptomatic (54.2%) but 31% women manifested mild to moderate symptoms and 14% had severe infection. The median gestational age at the delivery was 39 weeks. On average, 16.7% of mothers were separated from their newborns at birth, 83.3% practiced skin-to-skin, and roomed in with their babies, and 84.5% of the infants received any mother's milk. Preterm infants were more often born by mothers with symptomatic course of COVID-19 infection. Need for neonatal treatment was only due to prematurity. Neonates with acquired infection (after 14th day of life) had to be treated symptomatically with fever and loose stools, only 28.5% had symptoms of respiratory failure.

Conclusions: Despite the confirmed SARS-CoV-2 infection, the majority of mother- infant dyads were in a good health condition. The data on perinatal care reported in the paper could be helpful contribution supporting childbirth physiology protection during the COVID-19 pandemic.

Key words: COVID-19; perinatal care; SARS-CoV-2; neonate; pregnancy outcomes

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INTRODUCTION

Identification of the novel coronavirus known as severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2] has led to concerns about obstetrical patients and neonates as the data regarding COVID-19 infection in these populations were limited. Both groups represent a unique at-risk population, where pregnant women could be at increased risk for severe illness from COVID-19 compared to non-pregnant women, and newborns are potentially exposed to infection in utero, intrapartum, and postpartum [1]. Leading international health agencies and professional societies recommendations concerning the management of suspected or confirmed SARS-CoV-2 pregnant women and their infants were changing over time. Firstly, strict infection control measures were taken to reduce the perinatal transmission [2]. Clinical practices included elective cesarean delivery, separation of newborn and temporary suspension of breastfeeding [3]. Subsequent recommendations have appeared in April 2020 pointing the mode of delivery should be individualized due to obstetric and fetal indications and must not be impacted by COVID-19 infection unless worsening COVID-19 symptoms require urgent delivery. Neither Caesarean nor vaginal birth should be favored as there is no evidence of superiority of one mode of

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delivery over another [4]. The presence of a support person during active labor should not be contraindicated as it could improve birth outcomes for both mothers and infants. Hospitals should ensure universal testing as much as possible, not only for the woman but also for her birth partner [5]. The usage of personal protective equipment by patients and health workers during all procedure is obligatory. In the postpartum period, practices like skin-to-skin contact, rooming-in, and breastfeeding are all recommended unless the mother or neonate require additional medical care. These practices also do not increase the risk of neonatal infection with SARS-CoV-2. Mothers with COVID-19 should follow hand hygiene rules and wear a surgical mask to minimize any risk of virus transmission, especially while breastfeeding when close contact is maintained [4, 6]. Long term benefits of breastfeeding outweigh any potential risks of transmission of the virus through breast milk. Therefore, women should be encouraged to breastfeed or in cases when direct breastfeeding is impossible, the collection of the milk by using breast pumps should be suggested [7].

Our hospital followed the statements of National Consultants in the field of Obstetrics and Gynecology, Neonatology, as well as President of the Polish Society of Obstetricians and Gynecologists and President of the Polish Neonatology Society. Subsequently pregnancies with confirmed SARS-CoV-2 infection for epidemiological indications were mostly managed by performing a caesarean section, with exception for situations of advanced, dynamic deliveries [8]. In Poland, the presence of a birth support person depends on the decision made by the head of hospital in consultation with the head of Obstetrics and Gynecology Ward and in our hospital was not permitted.

According to the recommendations of the Polish Neonatal Society, the procedures after birth in a mother diagnosed with COVID-19 depends on factors such as the mother's health, the health of the newborn, the mother's decision, as well as the type of departments and the resulting human resources and the availability of protective measures. Following the recommendations newborns of SARS-CoV-2 positive mothers were bathed immediately after delivery to reduce the risk of virus transmission. If the child was clinically in good condition, a decision was made to delay cord severance, irrespective of the result of the mother's SARS-CoV-2 test. Skin-to-skin contact lasting two hours, or more was practiced unless the health of the mother and child required immediate intervention. Depending on the clinical condition of the mother and baby, newborn infants, stayed with their mothers in rooming-in system and were fed naturally [9].

Objectives

Despite publication of a consistent international recommendations which significantly helped making medical decisions, management of COVID-19 in pregnant patients and their newborns differ among hospitals. Also, the data regarding perinatal consequences and infection's impact on pregnant women and neonates born to SARS-CoV-2 positive mothers are limited. The study aims to describe how the 2nd Department of Gynecology and Obstetrics and Department of Neonatology in Wroclaw Medical University Teaching Hospital were providing perinatal care to pregnant women with COVID-19 and their neonates and present the mothers' and newborns' health evaluation and related outcomes.

MATERIAL AND METHODS

Between 15 February and 01 May 2021 Wroclaw Medical University Teaching Hospital was classified as a hybrid hospital for all Lower Silesia region for both COVID-19 and non- COVID-19 patients. Data regarding all pregnant women and their newborns analyzed in this study came from that time frame. In this study only pregnant women with confirmed SARS-CoV-2 infection were included. Based on regulations given by Polish Ministry of Health universal testing of pregnant women for SARS-CoV-2 was performed at hospital admission office using RT-qPCR test. Infants born to SARS-CoV-2 positive mothers were universally screened within 24 hours at birth. If the test results were inconclusive the next nasopharyngeal swab was taken at least 24 hours after the first test. Electronic hospital records were used to collect the data on maternal characteristics, maternal and perinatal outcomes, and information about procedures after birth e.g., mother–newborn separation, skin-to-skin contact, breastfeeding, and rooming-in and the need for exacerbating treatment in the Intensive Care Unit. All patient's data were analyzed anonymously. Approval was obtained from the Bioethics Committee of the Wrocław Medical University (KB-338/2021) for the retrospective analysis of all data concerning patients with COVID-19. The collected data was processed using the STATISTICA 13 package (license number JPZ903B482814ARACD-5).

RESULTS

Maternal characteristics

The data analysis included 83 women who gave birth with confirmed SARS-CoV-2 infection, of which 54.2% were asymptomatic. Thirty-eight women (45.8%) had symptomatic COVID-19 infection; 26 of women manifested mild to moderate symptoms and 12 had severe infection symptoms. The most commonly reported symptoms were cough- 63.2%, fever — 50%, dyspnea — 39.5%, weakness — 21.1%, rhinitis — 13.2%, headache, loss of taste and smell — 10.5%, muscle aches — 7.9%, sore throat, shivers, tightness of the chest, vomiting, diarrhea, nausea, thrombocytopenia — 2.6% (Fig. 1).

Data regarding obstetric patients are presented in Table 1. The median age for all women analyzed in the study was

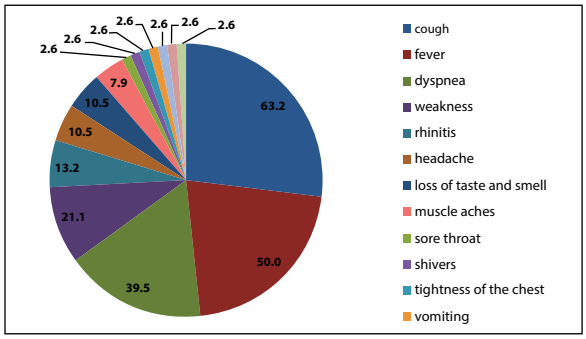

Figure 1. The most reported symptoms of COVID-19 among infected women

Table 1. Obstetric characteristics

Feature	n = 83 no. [%]
Age (y)	
≤ 29	31 (37.3)
30–34	36 (43.4)
≥ 35	16 (19.3)
Median, range	31, 23-44
Pre-existing comorbidities	
Yes	21 (25.3)
No	62 (74.7)
Pregnancy morbidities	
Yes	25 (30.1)
No	58 (69.9)
Symptoms of COVID	
Yes	38 (45.8)
Mild/moderate	26 (68,4)
Severe	12 (31.6)
No	45 (54.2)
Gravida	
1	29 (34.9)
2	39 (47.0)
> 3	15 (18.1)
Median, range	2, 1–5
Multiple pregnancies	
Yes	1 (1.2)
No	82 (98.8)

31 years (23–44). Sixteen pregnant patients admitted to the department due to COVID-19 infection were over 35 years of age, which constituted 19.3% of the women participating in the study. Pre-existing disease was found in 21 (25.3%) patients positive to COVID-19. The most reported chronic disorders were hypothyroidism, hypertension, cardiac diseases, diabetes mellitus, insulin resistance, thrombophilia, Hashimoto, Graves-Basedow disorder. Pregnancy related pathologies were marked in 30.1% of the patients, out of which gestational diabetes — 24%, pregnancy induced hypertension — 20%, hypothyroidism — 28%, anaemia — 20%, insulin resistance and pregnancy cholestasis — 2%. One woman was suspected of a preeclampsia, but eventually no pharmacotherapy was needed and implemented. Women participating in the study were mainly in their second pregnancy (47.0%).

Maternal and perinatal outcomes

During labor women wore surgical masks and health workers used personal protective equipment. Considering the pandemic situation and decision made by the head of the hospital and principal epidemiologist of hospital the presence of a birth support partner was not permitted. The mode of delivery was mostly caesarean section. The average caesarean section (CS) rate was 68.7%, of which elective CS was 10.5%; urgent/emergency CS due to maternal and fetal indications was performed in 89.5%; CS solely due to COVID-19 was performed in 19.3%.

Median gestational age at the delivery was 39 weeks. 81.9% deliveries occurred at term and 18.1% were preterm births. Among 15 preterm births, 9 (60%) came from symptomatic COVID-19 mothers – six premature babies were born to mothers with severe COVID-19 symptoms and three were born to mothers with mild/moderate symptoms of infection. 11 pregnancies were delivered due to an exacerbation of COVID-19 infection.

Six mothers (7.2%) were admitted to the Intensive Care Unit. Four mothers required mechanical ventilation and two were qualified to Extra Corporeal Membrane Oxygenation (ECMO). Among adverse maternal outcomes premature rupture of membrane was reported in 11 cases (13.1%), three cases occurred among symptomatic and eight cases among asymptomatic pregnant women. Most women were discharged home without complications. Two maternal deaths due to COVID-19 infection were reported (2.4%). The data analysis is presented in Table 2.

Neonatal characteristics

The study describes outcomes of 84 neonates born to SARS-CoV-2 positive mothers and 7 newborns with confirmed SARS-CoV-2 COVID-19 infection admitted to Department and Clinic of Neonatology. Sixty-eight infants were born at term and 15 were preterm. Two newborns come from twin pregnancy. All the positive mother's neonates were born alive. Infants born to SARS-CoV-2 positive mothers were universally screened within 24 hours at birth. If the RT-qPCR test result were inconclusive the next nasopharyngeal swab was taken at least 24 hours after the first test. No positive SARS-CoV-2 test results were reported among infants born in our hospital. Initially, seven neonates tested positive after

Table 2. Delivery outcomes

Feature	n = 83 no. [%]
Deliveries	
Total term birth	68 (81.9)
Total preterm birth	15 (18.1)
Asymptomatic women:	
Term birth	39 (86.7)
Preterm birth	6 (13.3)
Symptomatic women:	
Mild/moderate	
Term birth	24 (63.1)
Preterm birth	3 (7.9)
Severe	
Term birth	5 (13.2)
Preterm birth	6 (15.8)
Mode of Delivery	
Cesarean section	57 (68.7)
Vaginal	26 (31.3)
Indications for caesarean section	
Elective	6 (10.5)
Urgent/emergency:	51(89.5)
Maternal[1]	10 (17.5)
Obstetric[2]	27 (47.4)
Fetal[3]	9 (15.8)
COVID[4]	11 (24.1)
ICU admission	6 (7.2)
Mother's death	
Yes	2 (2.4)
No	81 (97.6)

[1]cholestasis gravidarum, hypertension, PIH, vaginal septum, cardiological indication; [2]labor dystocia, the risk of scar dehiscence after previous cesarean section, intrauterine infection, bleeding; [3]abnormal fetal heart rate tracing, the risk of intrauterine fetal asphyxia, fetal malpresentation, multiple gestation; [4]severe course of COVID infections

birth, but second tests repeated after 24 h were all negative. One of the infants was re-hospitalized on the 15th day of life due to COVID-19 infection and it is likely the infection was acquired postpartum from mother. NICU admission was necessary in 18 cases (21.4%). In all newborn infants, respiratory support was required. In order to enable efficient breathing of (16.7%) newborns, non-invasive ventilation (nCPAP) was used. 9.5% of newborns required support for the adaptation period through lung inflation. For 10.7% newborns, oxygen therapy was needed. One neonate required resuscitation. Due to clinical state and respiratory failure, 4.8% of neonates required intubation and invasive mechanical ventilation. During hospitalization, 13 (15.5%) neonates were observed and monitored for vital functions in incubators. (Tab. 3).

Table 3. Neonatal characteristics and outcomes

Feature	n = 84 no. [%]
Gestational age at birth [w]	
< 28	1 (1.2)
28–32	5 (6.0)
33–36	9 (10.7)
≥ 37	69 (82.1)
Median, range	39, 26–41
Birth weight [g]	
< 1500	4 (4.8)
1500–2499	8 (9.5)
≥ 2500	72 (85.7)
Median, range	3360, 850–4460
Birth weight for gestational age	
LGA	9 (10.7)
AGA	64 (76.2)
SGA: LBW VLBW ELBW	11 (13.1) 7 (63.6) 3 (27.2) 1 (9.1)
Sex	
Male	47 (56.0)
Female	37 (44.0)
Apgar at minute 1	
1–3	1 (1.2)
4–6	6 (7.1)
7–10	77 (91.7)
Median	10
Apgar at minute 5	
1–3	1 (1.2)
4–6	2 (2.4)
7–10	81 (96.4)
Median	10
NICU admission	
Yes	18 (21.4)
No	66 (78.6)
Ventilation	
Yes	18 (20.2)
No	66 (79.8)
Non-invasive ventilation	14 (77.8)
Invasive ventilation	4 (22.2)
Oxygen	9 (50.0)
Respiratory morbidities	
None	75 (89.3)
Tachypnea	2 (2.4)
Virus RSV	1 (1.2)
Respiratory distress syndrome	5 (6.0)
Pneumothorax	1 (1.2)
Separated from the mother	
Yes	14 (16.7)
No	70 (8.3)
Any Breast-feeding	
Yes	71 (84.5)
No	12 (14.3)
Missing data	1 (1.2)
Donor Milk (Human Milk Bank)	6 (7.1)

Average length of infant's hospitalization reached 7.4 days (medium 4) and included preterm as well. Majority of neonates were discharged home without any complications, one negative neonate from a positive mother, was still admitted at the time of this analysis (born April 04, 2021). During the hospital stay, 14/84 of newborns (16.7%) were separated from their mothers as the infants or mothers required additional medical care. The rest of infants practiced skin-to-skin contact and stayed with their mother in the rooming-in system (Tab. 3).

Out of 84 newborns, 71 were breastfed and 61 of neonates were additionally fed with milk formula or milk from Human Milk Bank were used (6 premature). The median age of pregnancy was 39 (26-41) weeks. The median birth weight of newborns was 3360 g (850–4460 g). In the case of mothers with COVID-19, most newborns were born appropriate for gestational age (75.0%). Eleven newborns were born too small and nine too big for gestational age. Most newborns scored 7–10 on the Apgar scale at 1 and 5 minutes after delivery (Tab. 3).

Neonates with acquired COVID-19 infection

Eight infants were admitted to the Department and Clinic of Neonatology due to COVID-19 symptoms or positive rapid antigen test results. RT-qPCR made after hospital admission tested positive in seven cases. One of the neonates was born in our hospital and re-hospitalized on the 15th day of life due to COVID-19 infection. Two infants came from COVID-19 positive mothers, two with COVID-19 symptoms but without test, two from quarantine parents and one infant with hospital acquired infection. Five infants were admitted to NICU, and two infants were asymptomatic SARS-CoV-2 infection roomed-in with their infected mother. Symptoms and treatment of infected infants are shown in Table 4.

Table 4. Data of newborns with confirmed SARS-CoV-2 infection

Features	Infected newborns n = 7 no. [%]
Gestational age (weeks)	
Median, range	37, 27–39
Preterm birth	2 (28.6)
Caesarean section	3 (42.8)
Birth weight [grams]	
Median, range	2872.5; 750–3800
Apgar score, median	
1′	10
5′	10
Symptoms	
Fever	5 (71.4)
Respiratory failure	2 (28.6)
Apathy	2 (28.6)
Rhinitis	1 (14.3)
Inflammatory changes in chest X-ray	1 (14.3)
Loose stools	7 (100)
Treatment	
Oxygen	3 (42.9)
Non-invasive ventilation	1 (14.3)
Antibiotics	2 (28.6)
Paracetamol	4 (57.1)

DISCUSSION

This paper describes the management of mothers and newborns during the COVID-19 pandemic at the Second Department of Gynecology and Obstetrics and Department of Neonatology at Wroclaw Medical University Hospital.

The majority of reported worldwide pregnant SARS-CoV-2 positive women have asymptomatic course of infection. Study from the USA estimated that over 86% of pregnant women who tested positive for SARS-CoV-2 during labour were asymptomatic [10]. Another study, Preg-CoV-19 Living Systematic Review that include almost 65,000 pregnant women reported 74% of asymptomatic infection among this group of patients basing on universal screening and testing [11]. In our study 54.2% pregnant women were asymptomatic. Among symptomatic women 31.6 % manifested severe symptoms- the cases we present confirm that most of the symptomatic women manifest only mild to moderate symptoms of infection [12]. The Allotey review reported that fever (40%), cough (39%) and dyspnea (19%) were the most common symptoms [11]. Similar results were observed in our hospital: cough (63.2%), fever (50 %) and dyspnea (39.5%).

Women with symptomatic COVID-19 infection are at increased risk of caesarean birth and fetal distress during active labour [13, 14]. The average caesarean section rate in Italian study (Donati et al. [15]) was 33.7%; elective CS was performed in 15.4% of the cases; urgent/emergency CS due to maternal or fetal indications was performed in 15% and due to COVID-19 was performed in 3.3% of the cases. In another study conducted in Mexico City (Cardona- Perez et al. [16]) CS rate reached 81% among SARS-CoV-2 positive women comparing to non-infected pregnant women (73%). Indications for CS among COVID-19 positive women were firstly, solely maternal (40%) then fetal (31%) and lastly obstetrical (18%). CS rate in our hospital reached 68.7%, among which elective CS was performed in 10,5%, urgent/emergency due to maternal or fetal indications 89,5%; due to worsening COVID-19 disease 19.3% [16].

Pregnant women (especially in the third semester) may be at increased risk of more severe course of the COVID-19 disease compared with non-pregnant women. An increase in ICU admission and mechanical ventilation among pregnant woman with COVID-19 comparing to non-pregnant women is notable (respectively OR 1.62, 95%

CI 1.33–1.96 OR 1.88, 95% CI 1.36–2.60) [17]. The Allotey review reported that admission to an intensive care unit was 4% and 3% required invasive ventilation. ICU admission in our hospital was necessary in 7,2% and invasive ventilation was required in 4.8% cases [11].

In a large retrospective US study comparing the outcomes for pregnant women with and without COVID-19 maternal death was rare, but rates were significantly higher for women with COVID-19 (141 deaths per 100 000 women) than for women without COVID-19 (5 deaths per 100 000 women) [18]. Another study from the Washington State reported 13.6 fold higher SARS-CoV-2 case-fatality rate for pregnant women with COVID-19 (maternal mortality rate of 1250 of 100,000 pregnancies) than for non-pregnant controls (91.7 of 100,000 rate) [19]. In our hospital we reported 2 maternal deaths (2.4%) due to COVID-19.

There are several studies that look for the relationship between COVID-19 disease and pregnancy or neonatal outcomes. SARS-CoV-2 infection in pregnancy seems to be associated with increased risks of preeclampsia, stillbirth, preterm birth, premature rupture of membrane and NICU admission. In the Cardona-Pérez et al. study, an increased risk of preeclampsia in women with SARS-CoV-2 infection was found [16]. In our study group, only one woman (0.01% of respondents) was suspected of having pre-eclampsia, which does not allow us to confirm the conclusion of quoted Cardona-Pérez et al. [16] the thesis that SARS-CoV-2 infection increases the risk of pre-eclampsia. In this study, also increased risk of premature rupture of membrane (PROM) was reported. The prevalence of those adverse outcomes among positive pregnant women was 12%. Another study reported the prevalence of PROM at the level of 15.4 % [20]. In our study PROM was observed in 13.1% cases.

Chen et al. [21] reported a 20% preterm birth incidence (out of 118 pregnancies) in SARS-CoV-2 positive patients, but no vertical transmission. Another study by Allotey et al. showed that the rate of preterm birth reported among pregnant women diagnosed with COVID-19 was 17% [11]. The frequency of preterm birth in our hospital was 18,1%. Six newborns were born by mothers with severe symptoms of infections and three from mothers with mild/moderate symptoms.

In Italy, it was found that appropriate, physiologically compatible treatment of a pregnant woman infected with SARS-CoV-2 may lead to reducing the rate of unnecessary Caesarean sections, separation of mothers from newborns and delayed breastfeeding. For this reason, the authors encourage that both during the current pandemic and similar situations in the future, emphasis should be placed on creating a protective mother-newborn relationship [15].

Potential routes of perinatal SARS-CoV-2 infection include infection through placenta, contact with maternal secretions during delivery, through droplets after delivery, and during breastfeeding. However, perinatal infections are rare. According to current research, the percentage of infected newborns ranges from 2.2% to 9.1% [1, 15, 23]. In our hospital no positive SARS-CoV-2 test result among infants just after delivery was reported.

Researchers found a statistical correlation between a positive COVID-19 test in the mother and/or newborn and the birth weight to gestational age ratio, the need to stay in the neonatal intensive care unit, the occurrence of respiratory diseases and the length of hospitalization [16]. In our hospital 85.7 % of newborns weighed ≥ 2500 and median Apgar score at 1. and 5. minutes was 10. Similarly in another study 86% newborns weighed ≥ 2500 and the medium Apgar index was 9 at 1 minute and 10 at minute 5 (15). Also, in a study conducted in Mexico City 81% newborns weighed ≥ 2500 [16].

One of the adverse neonatal outcomes observed among neonates born to SARS-CoV-2 positive mother is a necessity of NICU admission. Such a necessity in our hospital occurred in 18 cases (21.4 %), which is comparable to another studies e.g., Nayak et al. [11] — 23%, Allotey et al. [20] — 25%.

Donati et al. [15] reported that among neonates born to SARS-CoV-2 positive mothers 2.2 % developed severe morbidity, acute respiratory distress syndrome and interstitial pneumonia. Zhu et al. [23] reported that newborns (n = 10) born to mothers with confirmed COVID-19 infection developed shortness of breath (n = 6), fever (n = 2), thrombocytopenia accompanied by disrupted liver function (n = 2), rapid heart rate (n = 1), vomiting (n = 1), and pneumothorax (n = 1). In our hospital in all newborns admitted to NICU, respiratory support was required. Among adverse outcomes respiratory distress syndrome — 6%, pneumothorax — 1.2% and one neonate required resuscitation. Current evidence suggest that development of severe COVID-19 in neonates and children is rare, and to date there is no evidence for congenital abnormalities associated with maternal infection.

In Nayak et al. [20] study, out of 165 neonates 138 (83.6%) roomed-in with mother and initiated breast feeding. Next study describing 525 SARS-CoV-2 positive women, reported that 39% of them were separated from their newborns at birth; 26.6% practiced skin-to-skin contact; 72.1% were able to room in with their babies; and 79.6% of the infants received their mother's milk, 69% by direct breast-feeding and 10.6% by pumping or expressing breastmilk [15]. Results from our hospital were as follows: 14/84 of newborns (16.7%) were separated from their mothers as the infants or mothers required additional

medical care. The rest of the infants practiced skin-to-skin contact and stayed with their mother in the rooming-in system. 71 (84.5%) neonates were any breastfed, 6 preterm neonates received donor milk from Human Milk Bank (2 of them over 2 months).

CONCLUSIONS

At the beginning of the pandemic, the limited evidence regarding treatment of pregnant women and newborns with a confirmed or even suspected COVID-19 led to a "better safe than sorry" care choices. Today, however, the evidence shows that respecting physiology in women with confirmed or suspected SARS-CoV-2 infection during labour and delivery avoids unnecessary cesarean section, early mother-infant separation, and formula feeding unless the severity of the women's clinical conditions requires such decisions. The data on management of pregnant woman and early newborn care reported in the paper could be helpful contribution supporting childbirth physiology protection during the COVID-19 pandemic. These data, together with the available evidence on COVID-19, will support health professionals in their daily work and provide an opportunity for decision makers to properly manage the next waves of the COVID-19 pandemic.

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Not applicable.

Conflict of interest

The authors declare no conflict of interest.

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Diagnosing antenatal fetal distress

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ABSTRACT

Objectives: The values of acceleration capacity and deceleration capacity are known to capture fetal neurological development. The fetal growth restriction was found to be featured by decreased variables of phase rectified signal averaging. We have speculated that acceleration capacity and deceleration capacity could be of use in the detection of antenatal fetal distress during fetal growth restriction. The study was focused on the detection of the accuracy of acceleration capacity and deceleration capacity in diagnosing fetal distress.

Material and methods: In total, 124 pregnant women at 26–36 weeks of gestation were included in the study. The patients with appropriate to gestational age fetuses (n = 32) were enrolled in Group I. The patients with fetal growth restriction and an absence of fetal distress (n = 48) were observed in Group II. Lastly, the patients with fetal growth restriction and fetal distress (n = 44) were included in Group III. Fetal cardiosignals were obtained via non-invasive fetal electrocardiography. The maximally decreased acceleration capacity and deceleration capacity values were found in Group III.

Results: A correlation was found between umbilical artery resistance index and acceleration capacity and deceleration capacity variables in all study groups. We have found that the application of phase rectified signal averaging in the antenatal period showed high sensitivity and specificity in fetal distress detection.

Conclusions: Fetal acceleration capacity and deceleration capacity is a prospective option for the detection of fetal compromise during fetal growth restriction.

Key words: fetal growth restriction; fetal non-invasive electrocardiography; acceleration capacity and deceleration capacity; fetal distress

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INTRODUCTION

Fetal neurological development and maturation change cardiovascular response to its intrauterine activity. The investigation of heart rate variability (HRV) provides a piece of objective information about fetal health. The variations of the cardiocycles duration are a "window" into fetal life. Fetal non-invasive electrocardiography is a challenging technique for the detection of fetal cardiosignals. The variables of fetal HRV are known to reflect its status [1].

The conventional biophysical marker of fetal well-being is the reactivity of fetal heart rate in the non-stress test (NST) [2]. The absence of the accelerations on the fetal heart rate tracing could be associated with fetal compromise or fetal "sleep". Thus, NST is not specific in diagnosing fetal distress [3].

The values of phase rectified signal averaging are known to capture fetal neurological development. The fetal growth restriction (FGR) was found to be featured by decreased variables of acceleration capacity and deceleration capacity (AC/DC) [4–6]. FGR is known to be associated with an increased rate of fetal deterioration. We have speculated that AC/DC could be of use in the detection of antenatal fetal distress during FGR.

The study was focused on the detection of the accuracy of AC/DC in diagnosing fetal distress.

MATERIAL AND METHODS

In total, 124 pregnant women at 26–36 weeks of gestation were enrolled in the investigation. Only those who met the inclusion criteria and gave informed consent were included in the study (Tab. 1). The idiopathic FGR was detected by ultrasound. The population was divided into three groups. The patients with appropri-

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Table 1. The inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
FGR diagnosed via ultrasound. The fetal weight parameters were lower than 10th percentile	Multiple gestation, any prodrome of maternal internal disease (cardiovascular disease, renal diseases, endocrine disorders, etc.) before pregnancy

FGR — fetal growth restriction

ate to gestational age fetuses (n = 32) were enrolled in Group I (control). The patients with FGR and an absence of fetal distress (n = 48) were observed in Group II. Lastly, the patients with FGR and fetal distress (n = 44) were included in Group III. Fetal cardiosignals were obtained via non-invasive fetal electrocardiography (NI-FECG) from the maternal abdominal wall. The Cardiolab Babycard equipment (Ukraine) was used in this study. The diagnosis of fetal distress was performed via Doppler ultrasonography according to the abnormal umbilical and ductus venosus hemodynamic variables.

The results obtained were analyzed with the chi-square test to compare data between groups. For the assessment of the difference between non-parametric variables, the Mann-Whitney test was used. The significance was set at p-value < 0.05. For the statistical analysis of the relationship between X and Y, the correlations coefficients were estimated with Spearman's test. SPSS for Windows Release 25.0 (SPSS Inc. Chicago, Illinois), the software was used for statistical analysis. The use of fetal HRV variables in diagnosing fetal distress was investigated. The sensitivity (Se) and specificity (Sp) of NST and AC/DC were calculated. The relative risk (RR) of NST and AC/DC in fetal compromise prediction were also checked.

RESULTS

The average values of maternal age, body mass index, and parity were not different in Group I, Group II, and Group III (Tab. 2). The observed Group II and Group III patients had a higher manifestation of gestational hypertensive disorders and early onset FGR.

The maximally decreased AC/DC values were found in Group III (Tab. 3). The variables of phase rectified signal averaging were lower in Group II than in Group I, but higher than in Group III. Thus, the gradual decline of AC/DC was found amongst all study groups.

The investigation of the possible coupling between the AC/DC and fetal umbilical artery resistance index (RI) values in the study population revealed certain regularity. A significant relationship was found in Group I ($R = 0.64$, $p < 0.05$). A similar correlation was detected in growth-retarded fetuses. The values of the Spearmen correlation were almost equal in Group II and Group III (respectively, $R = 0.62$, $p < 0.05$; $R = 0.68$, $p < 0.05$). Therefore, AC/DC could be speculated as a marker for fetal deterioration. The detected correlation between AC/DC and umbilical blood pH in all study groups supported this thesis. The values of correlation coefficients ($R = 0.70$, $p < 0.05$; $R = 0.68$, $p < 0.05$; $R = 0.72$, $p < 0.05$ in Group I, Group II, and Group III, respectively) reflected the possible use of AC/DC in fetal monitoring.

The Se and Sp of nonreactive NST in diagnosing fetal distress were 65.22% (95% CI, 49.75%–78.65%) and 60.87% (95% CI, 45.37% –74.91%). The Se and Sp of the reduced AC/DC were 97.73% (95% CI, 87.98%–99.94%) and 95.83% (95 CI, 85.75% – 99.49%). The RR for fetal during FGR in the case of nonreactive NST was 0.59 (95% CI, 0.38–0.90; $p = 0.02$). The same RR in the case of the reduced AC/DC was 0.04 (95% CI, 0.01–0.16; $p < 0.001$).

DISCUSSION

Fetal electronic monitoring is known to have some serious restrictions in diagnosing fetal compromise. Only bradycardia is an evident sign of fetal deterioration [2, 3]. Several techniques were proposed for early detection of fetal distress. Our work has supported the opinion about the prospect of NI-FECG in fetal status detection [7, 8].

Since the main problem of NI-FECG is a low signal-to-noise ratio, the use of the AC/DC variable is the most convenient tool for the assessment of HRV. AC/DC could be calculated even in case of prolonged episodes of signal loss. The high-quality tracing for 30 minutes is an issue for the obstetrician. Therefore, the use of STV and LTV is not obvious [8, 9]. But the assessment of NST is of insufficient accuracy [3].

Table 2. Subject characteristics in the observed women

Clinical feature, units	Group I	Group II	Group III
Maternal age, years	22.0 ± 4.2	22.8 ± 3.9	21.9 ± 4.6
Body mass index	25.6 ± 4.4	25.8 ± 5.1	25.5 ± 5.4
Parity	1.5 ± 0.4	1.6 ± 0.3	1.6 ± 0.4
Pre-eclampsia or gestational hypertension, number of cases (%)	–	14 (29.1%)	26 (59.1%)
Early-oset FGR (before 32 weeks), number of cases (%)	–	19 (39.6%)	30 (68.2%)

Table 3. The values of AC/DC in growth-retarded fetuses

Variable, units	Group I	Group II	Group III
AC, ms	2.18 ± 0.36	1.84 ± 0.23*	1.58 ± 0.32*/**
DC, ms	2.11 ± 0.25	1.75 ± 0.22*	1.52 ± 0.28*/**

*the differences were statistically significant compared to control (Group I) ($p < 0.05$); **the differences were statistically significant compared to Group II ($p < 0.05$); AC — acceleration capacity; DC — deceleration capacity

AC/DC is known to reflect the ability to increase or decrease heart rate [2, 5, 7]. The process of regulation captures the autonomic modulations. The lost autonomic function is a marker of a fatal event. The value of AC/DC is linked both to sympathetic and vagal activity. The disturbed AC/DC was found in myocardial infarction, heart failure, dilated cardiomyopathy, etc [5].

We have found that the application of phase rectified signal averaging in the antenatal period showed high sensitivity and specificity in fetal distress detection. Since the main problem of NST assessment is a dependence on fetal stationary condition ("sleep" or awake), we could speculate that AC/DC has a universal ability to reflect fetal deterioration.

The fetal HRV parameters are known to be associated with the process of neurological maturation. Therefore, the delay in neurological development has a negative projection on the fetal cardiovascular system [10]. The dysautonomia could be a reason for fetal compromise in growth-retarded fetuses.

CONCLUSIONS

Fetal AC/DC is a prospective option for the detection of fetal compromise during fetal growth restriction.

Conflict of interest

The authors declare no conflict of interest.

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Relationship between Apgar score and umbilical cord blood acid-base balance in full-term and late preterm newborns born in medium and severe conditions

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ABSTRACT

Objectives: Application of Apgar scores (AS) and umbilical cord blood acid-base analysis is a base for the prediction of future neurological development in children. In clinical practice we often observe huge discrepancy between clinical and biochemical status of newborn. Because many obstetricians consider both assessments as substitute and measure of their proceeding's outcome, we decided to scientifically measure actual correlation between them among newborns born with Apgar less than 8 points.

Material and methods: This was an observational retrospective study. The study included 141 newborns born in general medium and severe condition (Apgar < 8 points in first minute of life). Acid-base analysis of umbilical cord vein blood immediately after birth was performed. We correlated gasometric parameters with Apgar scores of newborns.

Results: The clinical condition of a newborn at 1, 5, and 10 minutes after birth correlates positively and significantly with pH values (0.25; 0.24; and 0.26; respectively) and bicarbonate levels (0.21; 0.27; 0.28; respectively) in the umbilical cord vein, however correlation was low. Subsequently we qualified newborns to four groups depending on the degree of invasiveness of respiratory support after delivery, and the groups were compared in terms of parameters of acid-based balance. No significant differences were observed between groups in terms of acid-base balance parameters.

Conclusions: There is low, but significant correlation between clinical condition of a newborn after birth with most of acid-base parameters from umbilical vein blood. The assessment of the newborn's condition after birth using the Apgar score, (but not acid-base parameters) determines the degree of invasiveness of respiratory support activities for newborns after birth.

Key words: Apgar score; umbilical cord; gasometric parameters; moderate condition newborn; severe condition newborn

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INTRODUCTION

The Apgar score is a versatile and widely used assessment of the clinical condition of a newborn after birth. The Apgar score helps to identify newborns requiring further resuscitation and allows the assessment of a newborn's reflexes in a simple and fast manner [1]. However, the subjectivity of this assessment makes the Apgar score an unreliable predictive tool and assessing of the general condition of the newborn immediately after birth is burdened with high error probability [2–4]. Umbilical cord blood gasometry (UCBG) is a test that should objectively assess the condition of a baby after birth. The accuracy and reliability of tests assessing the acid-base balance in the fetus depends on the methodology of blood collection and the puncture of the appropriate umbilical cord vessel. Currently, there is no consensus among leading scientific societies in which precise clinical situations the UCBG should be performed. However, the majority recommends this study be performed in cases of increased risk of fetal metabolic acidosis, e.g., operative delivery, abnormal cardiotocography (CTG) tracing, decreased Apgar scores in newborns, prolonged second stage of labour, and uterine tachysystole during delivery [5, 6]. The literature data indicate that the method of taking blood samples from the umbilical cord is important in achieving reliable gasometry

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results. Therefore, more dependable results are acquired by centers taking this test routinely in all newborns instead of only specific indications [7]. In our center, blood sampling from the umbilical cord vein for blood gas analysis testing is standard in all newborns due to easy identification of the vessel and its easy collection. In the literature, in most studies assessing the newborn's morbidity and mortality depending on the pH, blood gas analysis was determined from arterial blood. This method has a strong physiological basis, due to blood flows in this vessel before gas exchange in the placenta from the newborn's internal iliac vessels, which better reflects the condition of the fetus. The blood flowing in the opposite direction from the placenta to the fetus through the umbilical cord vein reflects the mixed state/performance of the placenta, e.g., the acid-base balance of mother and child.

The placenta is responsible for equalizing acid-base imbalance in fetal life. Its efficiency in the case of fetal hypoxia depends on many factors (e.g., gestational age, anemia, presence of a heart defect). During an uncomplicated physiological delivery, the average value of base excess (BE) is no more than –5 mmol/L and a change of 3 mmol/L requires regular uterine contractions for several hours. In the case of complicated labor, the situation is different. In repeated moderate decelerations of the fetal pulse rate, the 1 mmol/L reduction of BE takes place after approximately 30 minutes. In the case of severe bradycardia, the 1 mmol/L reduction of BE takes place after two to three minutes [8]. During the interpretation of UCBG both values are important: pH and BE value, which allow to distinguish respiratory and metabolic acidosis.

Apgar scores, as well as UCBG are commonly considered as distinct tools for prediction of future neurological development in children, which carry the same important clinical information about the condition of the newborn after birth. Obstetricians, considering the subjectivity of the Apgar scale, often use cord blood gas analysis as measures of their proceeding's outcome, marginalizing the assessment of the fetal clinical condition. The aim of this study was to investigate the actual correlation between Apgar scores and umbilical cord blood gas analysis results in the group of newborns born in moderate and severe clinical conditions. The analysis of the clinical condition of this newborns' group is often the subject of discussion and disagreement between obstetricians and neonatologists.

MATERIAL AND METHODS

This observational retrospective study included 141 newborns from single pregnancies at the gestational age of 35 weeks and above born with ≤ 7 points of Apgar score at 1 minute of life, what means born in moderate and severe condition, in whom no malformation was found. The Apgar test was done at 1, 5 and 10 minutes by neonatologists of the unit. All births took place in the Department of Obstetrics and Gynecology of the Provincial Combined Hospital in Kielce from 01.01.2018 to 30.06.2019. Number of patients included in study were estimated a priori based on power calculation of estimated correlation (80%). We retrieved preliminary data from similar study results [9]. The study was retrospective and medical records were analyzed. Gestation period was confirmed by embryo biometry in the first trimester of pregnancy, and in case of discrepancies > 7 days corrected. In all cases, blood from the umbilical cord vein was collected immediately after the umbilical cord was clamped for gas analysis, using the ABL800FLEX. In order to limit the influence of immaturity on the clinical condition of the newborn after birth, all newborn babies born before 35 weeks gestation were excluded from the study. Also, we excluded newborns with birth defects and those where we did not obtain a complete blood gas analysis from the umbilical cord vein. The analysis of Apgar score correlation at 1, 5, and 10 minutes of life with the parameters of acid-base balance of newborns was performed. Next, newborns were divided into one of four groups depending on the degree of invasiveness of management supporting the respiratory process in newborns within the first 15 minutes after birth: Group I — children not requiring respiratory support, in whom only dry and wrap management was used, Group II — children requiring only continuous positive airway pressure (CPAP), Group III — children requiring lung recruitment, positive pressure ventilation or intubation with mechanical ventilation, and Group IV — children requiring cardiopulmonary resuscitation. Acid-base balance parameters were then compared among the groups. Statistical analyses were performed using Statistica 13.1 (Tibco Software Inc). Arithmetic means and standard deviations were used to describe the examined groups, in the case of skewness distributions the median and 25^{th} and 75^{th} percentile or interquartile ranges were used as a measure of central tendency. Qualitative data was presented as a percentage. Because the assumption of normal distribution was not met Spearman's rank correlation was used to investigate if a relationship existed. Continuous variables between groups were compared using Kruskal-Wallis test. Differences were considered significant in case of $p < 0.05$.

RESULTS

Data from one hundred and forty-one newborns was analyzed. Tables 1 and 2 present the characteristics of the study group. An analysis of the correlation of Apgar score at 1, 5 and 10 minutes of life with the acid-base balance parameters of the newborn was performed. Table 3 presents correlation coefficients with probability values. A significant positive correlation of pH [0.25 ($p = 0.002$); 0.24 ($p = 0.002$);

Table 1. Baseline characteristics of study group

Parameter			
Delivery (n; %)	1st (87; 61.7%)	2nd (41; 29%)	3rd and more (13; 9.2%)
Mode of delivery	vaginal delivery (60; 42.5%)	vacum extraction (5; 3.5%)	cesarean section (76; 53.9%)
Gestational age	< 37 weeks (16; 11.3%)	≥ 37 weeks (125; 88.7%)	
1st minute Apgar score	4–7 points (138; 97.8%)	0–3 points (3; 2.2%)	
5th minute Apgar score	8–10 points (100; 70.9%)	4–7 points (40; 28.3 %)	0–3 points (1; 0.7%)
10th minute Apgar score	8–10 points (128; 90.7%)	4–7 points (12; 8.5%)	0–3 points (1; 0.7%)
pH (n; %)	< 7.0 (1; 0.7%)	< 7.1 (6; 4.25%)	< 7.2 (29; 20.5%)
BE (n; %)	< –12 (8; 5.67%)	< –16 (1; 0.7%)	

BE — base excess

Table 2. Quantitative characteristics of study group

Parameter	Mean (+/–SD)
Mother's age	32.4 y (6.2 y)
Newborn weight	3327 g (577 g)
	median (25th–75th percentile)
pH	7.28 (7.22–7.36)
pCO2 [mmHg]	45.97 (38.3–51.7)
BE [mmol/L]	–4.49 (-6.8–(-1.8))
HCO3- [mmol/L]	18.84 (16.3–21.4)

0.26 (p = 0.001); respectively] and bicarbonate concentration [0.21 (p = 0.009); 0.27 (p = 0.001); 0.28 (p = 0.000); respectively] with clinical status (Apgar scores) at 1, 5, and 10 minutes of life was observed. A positive correlation of base excess [0.26 (p = 0.001); 0.26 (p = 0.001); respectively] and Apgar scores at 5 and 10 minutes of life and a negative correlation of carbon dioxide partial pressure [–0.18 (p = 0.03)] with an Apgar score at 1 minute of life was also seen. In every case, correlation was low. The management with each newborn after birth was consistent with the ERC 2015 recommendations [10]. Eighty-two newborns were qualified to Group I (58%), 27 were qualified to Group II (19%), 32 newborns in Group III (22%), and Group IV (1%) included 2 children. The parameters of acid-base balance between particular groups of newborns were compared. No statistically significant differences between these groups were found. The results of the study are presented in Table 4.

DISCUSSION

Clinical assessment of a newborn after birth using the Apgar score is very important because it identifies those that require the implementation of resuscitation procedures. As shown in this study, it is the clinical condition,

Table 3. Correlation coefficients of Apgar score with the acid-base balance parameters (with probability values)

Parametars	Apgar 1st minute	Apgar 5th minute	Apgar 10th minute
pH	0.25 (p = 0.002)	0.24 (p = 0.002)	0.26 (p = 0.001)
pCO2 [mmHg]	–0,18 (p = 0.03)	–0.07 (p = 0.4)	–0.11 (p = 0.18)
BE [mmol/L]	0.16 (p = 0.05)	0.26 (p = 0.001)	0.26 (p = 0.001)
HCO3–[mmol/L]	0.21 (p = 0.009)	0.27 (p = 0.001)	0.28 (p = 0.000)

BE — base excess

Table 4. Comparison the parameters of acid-base balance between particular groups of newborns

ERC group	1	2	3	4	
Number of cases	37 (26%)	22 (16%)	80 (57%)	2 (1%)	
pH (IQR)	7.27 (0.17)	7.3 (0.11)	7.32 (0.14)	7.28 (N/A)	p = 0.24
pCO2 [mmHg] (IQR)	43.7 (18.9)	42.25 (11.8)	44.75 (11.3)	48.55 (N/A)	p = 0.72
BE [mmol/L] (IQR)	–5.8 (5.3)	–4.35 (6.9)	–2.9 (4.27)	–2.8 (N/A)	p = 0.28
HCO3- [mmol/L] (IQR)	18.4 (4.3)	19.05 (4.5)	20.2 (4.1)	20.25 (N/A)	p = 0.08

IQR — interquartile range

not the results of the UCBG, which determines the degree of invasiveness of intervention in newborns immediately after birth. The results of the study showed that the groups of patients with different degrees of invasive procedures did not differ in terms of the values of umbilical cord blood analysis parameters (Tab. 4). In literature there are many studies demonstrating association of low Apgar scores with increased risks of neonatal and infant death and with neurologic disability, however, the absolute risks for Apgar score < 7, are low (< 5% in for most neurologic conditions) and majority of surviving babies with low Apgar scores grow up without disability [11]. A population-based cohort study of the 150 081 children shows continuously increasing risks of developmental vulnerability and special needs at 5 years of age with decreasing 1 min and 5 min Apgar scores [12]. The Risks of cerebral palsy and epilepsy are inversely associated with 5 minute and 10 minutes Apgar scores across the entire range of Apgar scores [13]. On the other hand the decreased Apgar score should not confirm serious abnormalities in the fetus, but may be a result, for example, of the temporary action of analgesic drugs administered to the mother during labour. The correct Apgar score does not exclude the possibility of adverse outcome in the newborn. In literature there are reports of higher morbidity and mortality in children born with an Apgar score of 7, 8 or 9 versus 10 points [14] and a higher risk of physical disorders [adjusted odds ratio (aOR) for Apgar 9 = 1.23, 95% CI 1.05–1.44] and emotional disorders (aOR for Apgar 9 = 1.20, 95% CI 1.03–1.41) in 5 years of children's age full-term neonates born at the time of delivery with an Apgar score $\leq$ 9 points at 5 minutes of life in comparison with children born at 10 points [15]. As a result of the above findings, The American College of Obstetricians and Gynecologists states, the Apgar scale cannot be used to predict neonatal mortality and neurological consequences in the future. The *subjective* Apgar score cannot be the only marker of clinical condition used to diagnose perinatal asphyxia [2–4].

UBCG is widely recognized as a source of objective information of fetal well-being. A key role in obtaining a reliable pH result for a cord blood sample is acquired by the method of sampling, the location of the umbilical cord puncture site, and the correct operation of the blood gas analyzer. According to some authors, in certain clinical situations, the probability of incomplete (UCBG) may increase, e.g., situations with Apgar scores < 7 [odds ratio (OR) 1.68, 95% CI 1.29–2.19], cesarean section (OR 1.31, 95% CI 1.11–1.55) or multiple pregnancies (OR 2.02, 95% CI 1.69–2.43) [16]. The causes mentioned above may be the reason for discrepancies between the clinical condition and blood gas parameters of the acid-base balance. In our center, blood sampling from umbilical cord is being taken in each newborn. Due to easy identification of the vessel and its easy collection we assessed blood gas analysis from the umbilical cord vein. In the systematic review and meta-analysis of 2010, five studies assessing venous blood gas analysis met the inclusion criteria. The meta-analysis showed significant association with neonatal morbidity (OR 4.0, 1.2–13.3, I2 = 44.5%) for the assessment of the pH of blood collected from the umbilical cord vein [17]. The median difference between the vein and the umbilical artery is 0.09. In the case of simultaneous vascular puncture, the difference ranged from 0.02–0.45, median difference BE was 0.8 mmol/L [18]. In 18% of the cases in the study mentioned above, the sample was taken from a different vessel than intended, which was secondary to the blood gas analysis results from individual samples. Having such a high percentage of cases proves that the puncture of the umbilical cord vessel, particularly the artery, is a procedure with a high error probability. Due to the large diameter of the vessel, blood collection from the umbilical cord vein is technically easier than from the umbilical artery. The blood gas parameters of venous cord blood are characterized by high predictive capacity in the detection of arterial pH < 7 [area under the curve (AUC) 0.955 95% CI 0.946–0.964] [19]. BE from the vein was also characterized by high predictive capacity of BE $\leq$ –12 in the arterial vessel (AUC = 0.961, 95% CI 0.963–0.971). If the pH value of blood collected from the umbilical cord vein is $\geq$ 7.16, the risk of neonatal acidosis is lower than 5% [19].

The results of published studies unequivocally indicate the case of an uncomplicated physiological delivery, the pH of blood collected from the umbilical cord vein compared to blood collected from the umbilical cord artery is 7.35 +/– 0.05 vs 7.28 +/– 0.05, respectively (arithmetic mean +/– standard deviation) [20]. If the dispersion at the level of two standard deviations is considered normal in laboratory tests, the normal pH (reference) values can be 7.25–7.45 for venous blood and 7.18–7.38 for arterial blood.

The pH value alone (determining the level of acidosis) is not a good prognostic factor in relation to further child development; for the distinction is crucial how long the hypoxia lasted and whether we are dealing with only respiratory acidosis (BE within normal range) or metabolic acidosis (BE $\leq$ –12 mmol/L), which is more severe in consequences [21]. The value of base deficit, increasing with the accumulation of organic acids, correlates with increased mortality and morbidity in the future [22]. BE in the range of –12 to –16 mmol/L is associated with an increased risk of mortality, moderate to severe encephalopathy, multi-organ failure and future neurological deficits. Such consequences are involved in 10% of newborns with umbilical artery base deficit (BD) in the range of 12 to 16 mmol/L and in 40% of those with a BD > 16 mmol/L [23, 24]. Metabolic acidosis, according to the International Cerebral Palsy Task Force, is

defined as a pH in umbilical cord blood gas analysis < 7 and BE < –12 mmol/L [25]. The cut-off points for diagnosis were originally identified based on the consensus of a working group supported by scientific pediatric and obstetric societies from seven countries rather than on high quality scientific evidence. However, the subsequent meta-analysis of 51 studies [17] which combined the result of arterial umbilical cord blood gas analysis with measures of compromise of neonatal or childhood well-being indicated an increased risk of morbidity and mortality at higher pH values (pH < 7.2) [17]. A pH < 7.2 increased both infant mortality (OR = 4.3, 95% CI; 2.2–8.7, in 5 studies) and morbidity (hypo-ischemic encephalopathy, seizures, paraventricular leukomalacia, mechanical ventilation, intraventricular hemorrhage, and abnormal neurological examination) (OR = 2.2, 95% CI; 1.3–3.7) [26]. Many children born with acidosis may not develop neurological complications in the future. On the other hand, most children with cerebral palsy are born with umbilical cord blood gas analysis within a normal range, as the etiology of this disease is very complex [27].

Acidosis in newborns has complicated about 1–2% of births, but it is believed that it should not be of much importance in newborns with good clinical standing. However, in one retrospective study, which recruited children with scores of 7 or more on the Apgar scale at 5 minutes of life, it was shown that in the group of children with acidosis (pH $\leq$ 7.1 from the umbilical artery) the complications are more frequent than in children with a pH > 7.1 in the blood gas analysis. Statistically significant differences were found in the incidence of respiratory distress syndrome (RDS) [aOR 4.6, (95% CI 3.1–6.85)], meconium aspiration syndrome (MAS) [aOR 2.43, (95% CI 1.3–4.53)], and hospitalization in neonatal intensive care unit (NICU) [aOR 3.68 (95% CI 2.81–4.82)]. Additionally, in the group of examined children, obtaining a BE value $\leq$ 10 mmol/L from the umbilical cord artery compared to a BE value > –10 mmol/L was also associated with a statistically increased number of complications: MAS [aOR 2.67, (95% CI 1.57–4.53)], necessity of hospitalization in NICU [aOR 2.33, (95% CI 1.77–3.07)], and development of neonatal sepsis [aOR 3.13, (95% CI 1.56–6.25)] [7]. In our group of patients, only one neonate fulfilled the arbitrary criteria of acidosis, which was associated with the moderate clinical condition according to the Apgar scale (6 points). The vast majority of newborns in our study group (79.5%) had a pH higher than 7.2. In our group of patients there are low but statistically significant correlation between clinical condition of a newborn after birth with most of acid-base parameters from umbilical vein blood. This low correlation emphasizes that these both methods are not substitutes and the combined interpretation of Apgar score results and umbilical cord venous blood gas sample is more useful in the assessment of the condition of the newborn after birth than the interpretation of the results of these tests separately.

The limitation of our study may be due to the lack of lactate concentration determination in the obtained samples. This study is not routinely performed in our center, as the available literature points to poor predictive value of this determination in relation to the further occurrence of complications in newborns.

CONCLUSIONS

1. The combined interpretation of Apgar score results and umbilical cord venous blood gas sample is more useful in the assessment of the condition of the newborn after birth than the interpretation of the results of these tests independently.
2. There is low correlation between clinical condition of a newborn after birth with most of acid-base parameters from umbilical vein blood.
3. The evaluation of a newborn after birth using the Apgar score is a main factor, which determines the degree of invasiveness of respiratory support activities for newborns after birth.

Data Availability

The datasets generated and/or analysed during the current study are available in the public repository, DOI 10.17605/OSF.IO/HYNPK.

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Conflict of interest

All authors declare no conflict of interest.

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Diagnostic imaging in gynecology

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ABSTRACT

Ultrasound (US), computed tomography (CT) and magnetic resonance imaging (MRI) are the "gold standard" among gynecological imaging methods. They are important diagnostic tools used to determine the site of origin of a pelvic mass and to characterize the adnexal lesions. This paper summarizes the diagnostic performance of ultrasound, computed tomography, and magnetic resonance imaging in various gynecological diseases and tumours diagnostics.

Key words: oncology, cancer, computed tomography, magnetic resonance imaging

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INTRODUCTION

Pathological lesions within the female reproductive system organs are classified by clinical, surgical and histopathological criteria. The American College of Radiology (ACR), National Comprehensive Cancer Network (NCCN) and the International Federation of Gynecology and Obstetrics (FIGO) revised the recommendations for diagnostic imaging in cancer detection and staging [1, 2]. European recommendations are also commonly used [3].

Nowadays there are several diagnostic methods used before and after treatment in gynecology, all of them having some advantages and limitations. Accurate diagnosis requires comparison of clinical data and results achieved in diagnostic imaging, and depend on the diagnostic tool and radiologist's level of experience. The gynecological imaging "gold standards" are ultrasound (US), computed tomography (CT) and magnetic resonance imaging (MRI). These methods are useful for determining the site of origin of a pelvic mass and to characterize the adnexal lesions [3–10].

Ultrasonography is the primary imaging technique in patients with clinical symptoms like abnormal uterine bleeding, endocrine disorders, pelvic pain, gynecologic infections and female pelvic lesions. In many cases US has important role in the primary diagnosis of ovarian cancer, the assessment of the tumor in the pelvis and abdominal cavity. For proper diagnosis of pelvic and abdominal organs combination of trans-abdominal (TAUS) and trans-vaginal US (TVUS) is needed. Those techniques may be successfully applied for assessment of an origin of different adnexal masses using IOTA Simple Rules or, in more experienced hands, ADNEX models [11]. It should be remembered that the range of the probe in TVUS is specific and in some cases it does not allow for a full assessment of pelvic pathology, neither for the assessment of pelvic lymph nodes. According to ACR recommendations (2016), if there is a large lesion in pelvis that is outside the ultrasound probe range, transabdominal ultrasound should be performed. For an accurate assessment of both the abdominal cavity and pelvis, we should perform both TAUS and TVUS examinations. TAUS should be performed in two phases, with a full bladder first, to evaluate the uterus and bladder. When the bladder is full, the reproductive organ can be examined, and if we find a tumor, we can possibly assess its relation to adjacent organs, bladder infiltration, ureter infiltration, and find the cause of stagnation in the kidneys (differentiation between infiltration and urolithiasis). Next, the post-voiding examination should be performed to evaluate the parenchymal organs of the abdominal cavity, the aortic space and lymph nodes around the iliac vessels and possible peritoneal tumor cells.

Computed Tomography (CT) is the primary method used to assess the stage of cancer. A CT should be performed before and after intravenous administration of the contrast medium and after oral administration of the contrast medium. Positive oral contrast agents are useful for intestinal and peritoneal tumor cells detection and differentiation of lesions and lymph nodes in the aortic space with intestinal

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loops. Examination after oral administration of contrast medium is particularly useful in debilitated patients. Negative oral contrast agents are useful for calcified tumor detection. Lesions in the peritoneum with more than 10 mm of width in the short axis are visible on CT scan. Peritoneal tumor implants smaller than 10 mm may be difficult to detect, especially if they are not accompanied by ascites. If lymph nodes short axis is more than 10 mm, or in case of morphological changes — such as rounded node shape or necrosis in the node, lymph node metastases are also suspected. The disadvantages of CT are the patient's exposure to ionic radiation, possible allergic reaction to iodine contrast agents and worse soft tissue visualisation in comparison to MRI.

Magnetic Resonance Imaging (MRI) is a diagnostic modality that provides much better images of soft tissue than CT scans. Due to the possibility of tissue differentiation, MRI allows for better estimation of the primary tumor extent and adjacent structures infiltration assessment. Local infiltration assessment on MRI should be performed in planes — transverse, coronal and sagittal to the long axis of the uterus. Dynamic Contrast Enhancement (DCE) MRI imaging after intravenous contrast injection is useful in detecting well vascularized tumors. Chemical shift imaging or sequences with fat suppression are excellent for detecting fat within lesions. Post-contrast T1 weighted images with fat saturation enable peritoneal lesions detection. Diffusion Weighted Imaging (DWI) is sensitive to the movement of water molecules at the cellular level. Malignant tumors, for example, are more likely to show a high intensity signal on DWI at a high b (1000) value and are visible as dark on ADC (Apparent Diffusion Coefficient) maps. MRI with dynamic contrast enhancement combined with DWI allows for better characterization and assessment of tumor stage and extent including detection of subtle peritoneal disease, as well as assessment of tumor response and recurrence. DWI and the corresponding ADC maps improve tumor characteristics and stage estimation in patients with endometrial and cervical cancer, but are less specific in distinguishing between benign and malignant myometrial and ovarian tumors. In case of kidney dysfunction, when using gadolinium based contrast agent is not possible, DWI constitutes a very important part of MRI examination.

Despite the advantages (no ionic radiation, better soft tissue visualization) MRI has some disadvantages — such as low availability, higher cost, long image acquisition time leading to motion artefacts, reduced patient cooperation and MRI safety problems [12].

OVARIAN CANCER, FALLOPIAN TUBES, PRIMARY PERITONEAL CANCERS

Ultrasound (US) is the first choice examination performed in patients with suspected ovarian cancer. Specific features of ovarian tumors visible on US are: enlargement of ovarian dimensions above 5 cm, presence of internal partitions in the tumor, papillary growths on the inner surface of the tumor, complex structure (solid-cystic), free fluid in Pouch of Douglas (max 5 mm), poor tumor mobility, infiltration of adjacent organs. In some cases, TAUS allows for visualization of solid hypoechoic peritoneal lesions and infiltrations to the adjacent tissue.

In case of ovarian cancer, NCCN set the following recommendations: CT with iodine contrast agent of the chest, abdomen and pelvis as well as abdominal and pelvic MRI for primary diagnosis and follow-up imaging. According to the ACRAC the most important examination is CT with contrast enhancement of the abdomen and pelvis for both pre-treatment assessment and follow-up (Fig. 1). MRI has great value in assessing the staging of ovarian cancer. According to the recommendations of ESUR (European Society of Urogenital Radiology) MRI is recommended in case of ambiguous ultrasound results. The IOTA (International Ovarian Tumor Analysis) guideline suggests the malignant character of the lesion visualized in MRI if its size is more than 4 cm, its morphology is complex (solid and cystic) with solid enhancing component, contains thick septations > 3 mm, has papillary projections or central necrosis. Malignant lesions usually have Type 3 dynamic contrast curve and possible additional findings, like ascites, lymph node enlargement, peritoneal carcinomatosis or organ invasion.

The characteristic symptoms strongly suggestive of malignancy on CT scan in the first stage of ovarian cancer include: wall irregularities or thickening > 3 mm, enhancing solid components, abdominal wall tumors and papillae. Serous cystic adenocarcinomas may contain microcalcifications. Serous cystic adenomas are usually small and single-celled, whereas mucous cystic adenomas are larger multicellular clusters [13]. Mucinous tumors are attenuated on CT and have heterogeneous signal intensity on

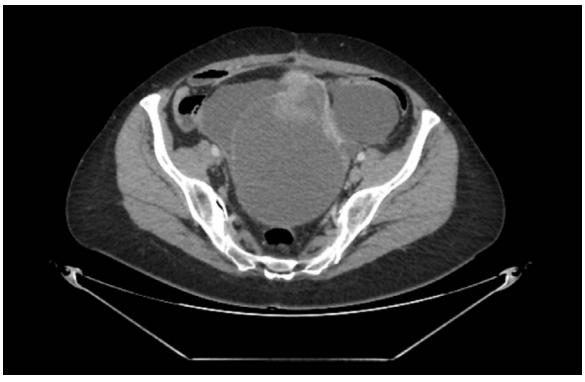

Figure 1. CT image of ovarian cancer. The tumor is seen to consist of both solid and cystic components. The solid component enhanced after intravenous administration of contrast medium

T1-weighted and T2-weighted MRI images, which results in the appearance of "stained glass". Primary malignant tumors of the fallopian tubes may have the appearance of solid, mixed solid / cystic masses or papillary projections in dilated fallopian tubes that enhance less than myometrium on CT and MRI. The adnexa of uterus masses with the appearance of "sausages" associated with hydrosalpinx and the presence of intrauterine fluid strongly indicate malignant fallopian tube cancer.

MRI is a very good diagnostic method for assessing whether the ovarian cancer is suitable for surgery. Its sensitivity is 92%, specificity 85% [14]. It appears to be a much better method than CT in evaluating the local stage of ovarian cancer, with sensitivity of 55%, specificity 88% and accuracy 89%. At higher stages of ovarian cancer, peritoneal metastasis is manifested in the form of nodules or "plaques" in the peritoneum, fluid in the peritoneal cavity, mesenteric fat infiltration and mesenteric lymph or node enlargement in para-aortic area.

In grade II and III of the disease, DCE-MRI with DWI improves the detection of pelvic and peritoneal tumor cells. Local tumor involvement can be suspected if there is a lack of adipose tissue between the tumor and adjacent pelvic structures, irregular contact surface between the tumor and the adjacent structure, less than 3 mm distance between the tumor and the pelvic wall and ureteral obstruction and dislocation/closure of the iliac vessels.

MRI outperforms CT in cancer infiltration assessment. Symptoms of bowel infiltration are wall thickening and its irregular shape. Metastatic retroperitoneal, pelvic and inguinal lymph nodes are also possible. Peritoneal tumor manifests as peritoneal wall enhancement and thickening, peritoneal tuberosity/mesentery, omental caking, and/or mesenteric infiltration.

Presence of ascites should be treated as suggestive of peritoneal disease. Oral administration of the contrast agent may improve detection. CT has limitations in the visualization of peritoneal tumor implants smaller than 1 cm, especially if ascites is not present. The correct path of peritoneal fluid circulation involves the pelvis and the diaphragm. The role of MDCT is limited in detecting small peritoneal metastases. If the peritoneal metastasis is greater than 1 cm, the MDCT sensitivity is 85 to 93% and the specificity is 91–96%. For metastases with a diameter less than 1 cm, the MDCT sensitivity decreases to 25–50%.

In stage IV of the disease, cancer is likely to spread in certain ways including transperitoneal, lymphatic or hematogenous way. The most common sites of ovarian cancer distant metastases include lungs and liver. Cytological evaluation is necessary to confirm malignant pulmonary effusion, however, the presence of thickening, tuberosity or masses suggests malignancy.

Another imaging modality useful in ovarian cancer detection is PET (Positron-Emission Tomography). This examination is performed if recurrence is suspected, with growing marker values observed with no corresponding pathological process visible on diagnostic images. PET/CT is particularly useful in distinguishing between patients in stage IIIc-IV and I-IIIb and in this case the specificity is 91%, sensitivity 100% and accuracy 98% compared to 64%, 97% and 88% of CT alone. PET/CT has high sensitivity in detecting peritoneal metastases larger than 1 cm and lymph nodes larger than 7 mm [14] .

ENDOMETRIAL CANCER

TVUS is mainly applied for evaluation of endometrium among women with symptoms of bleeding in post-menopausal period. The screening diagnosis obtained on TVUS is based on the measurement of endometrium thickness. There is a notable thickening of endometrium visible on US examination in case of endometrial cancers [15]. Main limitations of TVUS are: operator dependence, limited vision field, danger of overestimation of infiltration extent and lymphatic vessels area, low efficiency of the method in predicting cancer extent, evaluating cervix infiltration and lymph nodes assessment. However, there are literature data confirming high accuracy of US. Recently performed study conducted in Sweden involved a large group of patients (850 women with endometrioid endometrial cancer). The examinations performed by ultrasound experts revealed high accuracy of myometrial invasion ultrasound assessment.

ACR and NCCN definitely recommend MRI examination of pelvis with and without intravenously administered contrast agent to assess the depth of myometrial invasion and tumor extent in order to establish treatment plan for individual patients (Fig. 2) [16]. Overall accuracy of MRI in cancer staging is 83–92%, while sensitivity, specificity and accuracy of MRI in evaluation of cervical infiltration on T2-weighted images are 100%, 87% and 90% and on T1-weighted images after contrast administration — 100%, 95% and 96% [17].

The depth of myometrial invasion in endometrial cancer is an important morphological prognostic factor correlating with tumor grade, presence of lymph node metastases, and overall patient survival. MRI with contrast medium administration is superior to CT with contrast enhancement, US and MRI without contrast administration in assessment of local stage of disease. It is possible to perform pelvic CT with contrast instead of MRI in case of lack of MRI equipment availability. ACR does not recommend tomography without contrast administration. Sensitivity and specificity of CT in myometrium infiltration assessment ranges from 40 to 83% and respectively 42 to 75%, while accuracy in myometrium and cervix infiltration assessment is 81 to 95% [1, 18].

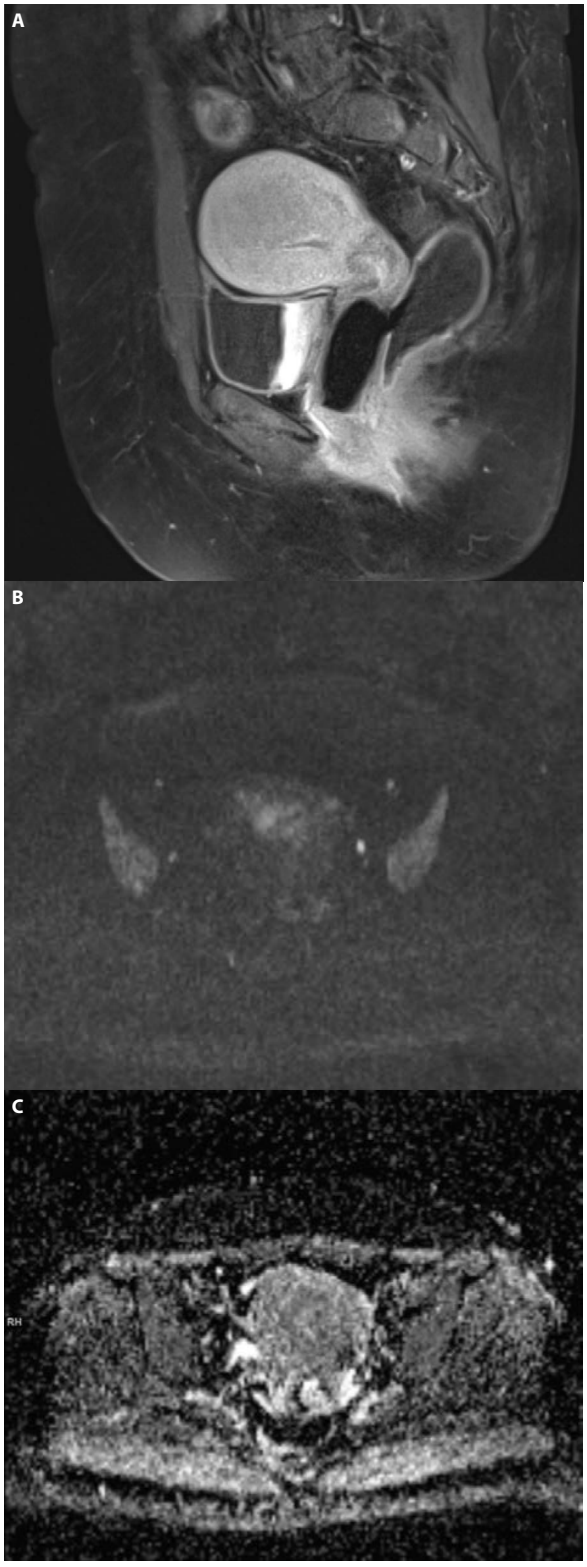

Figure 2. MR images of uterine cancer; **A.** Example MR image in transversal plane, enlarged uterus, thickened endometrium; **B.** Sagittal MR image of the pelvis, thickened endometrium is visible; there is no border between endometrium and myometrium. Tumor infiltrates more than 50% myometrium; **C.** DWI and corresponding ADC map, the area of restricted diffusion is visible

Both CT with contrast enhancement and abdomen and pelvis MRI are useful in lymph nodes evaluation. For highly malignant tumors it is recommended to perform chest CT for possible metastases assessment.

In case of tumors classified as stage I to IIIb MRI of the pelvis is superior to CT or PET in evaluation of local extent of disease. According to the American Joint Committee on Cancer (AJCC) and FIGO staging system, stage I tumors are divided into: stage I — cancer is found only in the uterus, and it has not spread to other parts of the body; stage IA — cancer is found only in the endometrium or less than one-half of the myometrium; stage IB — spread to one-half or more of the myometrium. Stage II tumors involve the cervical stroma causing widening of the internal os. Stage IIIA and B tumors comprise local invasion of the serosa, adnexa, vagina, and parametrium, while stage IIIC1 involves cancer spread to the regional pelvic lymph nodes and stage IIIC2 — spread to the para-aortic lymph nodes with or without spread to the regional pelvic lymph nodes. Stage IV, also divided into A and B means that cancer has metastasized to the rectum, bladder, and/or distant organs beyond the true pelvis. The accuracy of determining myometrial and cervical invasion increases with the addition of dynamic contrast imaging and is the most advantageous when performed 2 to 4 minutes after contrast medium administration. In case of contraindications for contrast administration, DWI is applied with similar sensitivity and specificity to enhanced imaging in myometrium invasion detection.

At present, for assessment of lymph nodes invasion and distant extent of the disease MRI and abdominal CT with contrast enhancement is recommended for initial management planning. Invasion of pelvic, para-aortic, or inguinal lymph nodes has an impact on prognosis. Accuracy of DWI in lymph nodes evaluation is similar to MRI with contrast enhancement.

Endometrial cancer is characterized by intensive FDG uptake, while PET/CT are not significant enough to be taken into consideration in initial staging of early endometrial cancer due to limited spatial resolution and physiological uptake in pre-menopausal women. Sensitivity, specificity and accuracy of PET/CT in lymph nodes metastases detection regardless of lymph node size is 53%, 99% and 98 %, respectively [19] maximum standardized uptake value (SUVmax).

UTERINE SARCOMA

In case of uterine sarcoma CT or MRI of the abdomen and pelvis is recommended, although MR imaging is preferred for local extension evaluation or in patients with incidentally detected tumors with incomplete resections after myomectomy or morcellation. Chest CT is also recommended due to frequent cases of lung metastases. Recommendations

after therapy include chest, abdomen and pelvis CT. Pelvis MRI is also suggested if recurrence of the disease is suspected. Due to the fact that MRI enables visualisation of tissue differentiation and is performed in three planes, it allows for distinguishing between recurrence and normal pelvic structures.

MRI examination also facilitates differentiating between sarcoma subtypes. It enables determining tumor location and contour, as well as presence of hemorrhagic, necrotic and cystic components. It is difficult to differentiate leiomyosarcomas from leiomyomas with imaging examination, if rapid growth or visible metastases are not present. Leiomyosarcoma is typically visible as infiltrating myometrial mass with irregular or ill-defined margins. On imaging after contrast administration, peripheral enhancement with central heterogeneous signal intensity from necrosis and haemorrhage is present.

Endometrial stromal sarcoma (ESS) and undifferentiated sarcoma (UES) most often appear as polypoid endometrial masses with wormlike bands of low T2-weighted signal intensity corresponding to regions of preserved myometrium. Sometimes they have more marginal nodularity resulting from their tendency to infiltrate lymph vessels. Adenosarcomas are mixed tumors, including benign and malignant components, frequently appearing as complex, multiseptated, cystic, and solid polypoid endometrial masses.

The usefulness of DWI is limited due to significant overlap of ADC values between benign leiomyomas and sarcomas. However, if patients are unable to receive contrast, this examination should be applied to support diagnosis.

CERVICAL CANCER

In 2018 an updated FIGO staging system for uterine cervical cancer was introduced [20]. Imaging examinations became an important part of staging process, being a reliable source of information pertaining to cancer spread complementary to this obtained in clinical examination. Imaging examinations are indicated in patients with FIGO stage IB disease or greater. Due to important prognostic factors obtained in imaging, such as tumor size, parametrial spread, and lymph node metastases it is considered as highly valuable. Contrast enhanced pelvic MRI is performed in patients who need preoperative planning. For FIGO stage II disease or higher, chest, abdomen, and pelvis CT is recommended in order to detect possible metastases and contrast-enhanced pelvic MRI to assess local extent of disease (Fig. 3) [21].

To evaluate the primary tumor on MRI imaging, both T2-weighted large field-of-view sequences and the small field-of-view sections obtained perpendicular to the endocervical canal are recommended. To enable better evaluation of the vaginal fornices for local spread, application

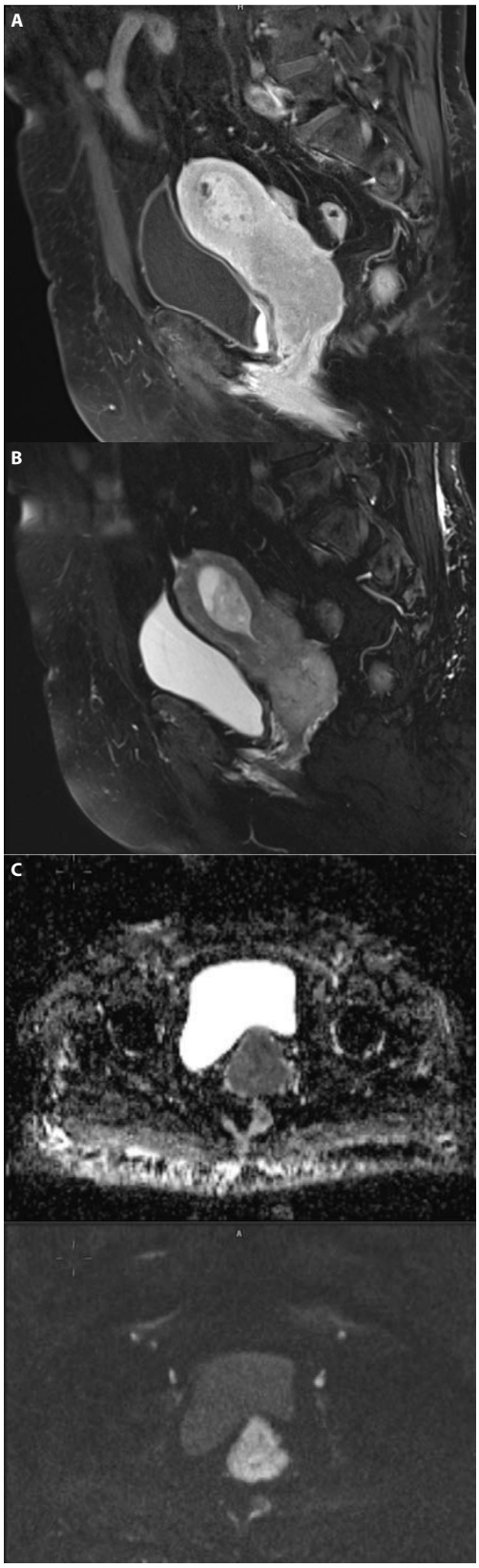

Figure 3. Example MR images of cervical cancer; **A.** T1-weighted image in sagittal plane after intravenous contrast injection, visible infiltration of uterus and vagina; **B.** T2 sagittal image of cervical cancer; **C.** DWI and corresponding ADC map — the area of restricted diffusion is visible

of vaginal gel is recommended. Contrast administration improves detection of small tumors, depth of stromal invasion, and bladder or rectal wall invasion. As cervical cancer normally exhibits lower ADC values than the normal cervix, DWI can also be helpful in tumor detection.

Stage I disease means that carcinoma is strictly confined to the cervix. It is further subdivided into: stage IA which represents invasive carcinoma that can be diagnosed only with microscopy, with maximum depth of invasion < 5 mm (IA1 — stromal invasion < 3 mm and IA2 — stromal invasion ≥ 3 mm and < 5 mm in depth) and IB — invasive carcinoma confined to the uterine cervix, with measured deepest invasion ≥ 5 mm (also divided in new staging system into: IB1 — tumor measures < 2 cm, IB2 ≥ 2 cm and < 4 cm and IB3 ≥ 4 cm in greatest dimension).

On MR imaging, the normal cervix has a trilaminar appearance on T2-weighted imaging with endocervical mucosa of a high signal intensity, a low signal intensity fibromuscular stroma, and an intermediate signal intensity outer smooth muscle layer. On T2-weighted sequences tumors appear intermediate to high signal intensity relative to the hypointense middle stromal layer and have variable enhancement pattern on sequences received after contrast administration.

Stage II disease extends beyond the uterus but does not involve the pelvic sidewalls or the lower one-third of the vagina.

Stage IIA disease extends into the upper two-thirds of the vagina or to the pelvic wall. Tumors are subdivided into stage IIA 1 and IIA 2 regarding their size greater or smaller than 4 cm. IIB disease means parametrial invasion, but not up to the pelvic wall. MRI findings highly indicative of parametrial invasion include disruption of the low T2-weighted signal intensity inner cervical stromal ring and/or presence of nodular or irregular tumor extending into the parametrium. It is possible to exclude parametrial invasion if the inner cervical stromal rim is thicker than 3 mm. Such a finding is called a hypointense rim sign. In case of parametrial invasion surgical procedure is not taken into consideration.

Accuracy of MRI in cervical cancer staging (operable or inoperable lesion) equals 75–96%. MRI is highly accurate in evaluating tumor size in correlation with histopathological examination, 70–90%. MRI has high NPV in parametrial invasion assessment, 94–100% [22]. DWI with ADC sequences may be helpful to distinguish benign reactive changes or stromal edema from true tumor invasion. On CT findings suggestive of parametrial invasion include encasement of the ureters, presence of vasculature, and thickening of the uterosacral ligaments.

Findings present in stage III involves the lower third of the vagina and/or extends to the pelvic wall and/or causes hydronephrosis or non-functioning kidney and/or involves pelvic and/or para-aortic lymph nodes. In stage IIIA, the tumor extends to the lower one-third of the vagina. Stage IIIB disease involves extension to the pelvic sidewall or to the ureters. When the tumor is within 3 mm of the pelvic sidewall musculature, it can be suggestive of pelvic sidewall involvement. In 2018 system another category appeared: IIIC — meaning involvement of pelvic and/or para-aortic lymph nodes, irrespective of tumor size and extent (IIIC1 — pelvic lymph node metastasis only and IIIC2 — para-aortic lymph node metastasis).

Stage IV disease includes invasion of the urinary bladder, rectum, or tumor extension beyond the true pelvis. Stage IVA means invasion of the bladder or rectum and is suggested by following findings: loss of the perivesical or perirectal fat plane, disruption of the normal muscle low T2-weighted signal intensity, asymmetric nodular wall thickening, intraluminal mass, or fistula. If tumor extends beyond the pelvis and spreads to distant organs, is classified as stage IVB Lymph node metastasis is a significant prognostic factor in cervical cancer staging and is associated with a reduction in survival. It has been added to FIGO classification (2018).

At the beginning, cervical lymph nodes drain to the parametrial nodes and then spread to the external iliac, internal iliac, presacral, common iliac, and para-aortic lymph nodes. Specificity of CT and MRI in the detection of lymph node metastasis is greater than 90% but sensitivity is less than 60%.

Surgical excision of the lymph nodes, either elective or selective still remains the standard procedure in diagnosing lymph nodes metastasis. Short-axis diameter larger than 1 cm is the main criterion used for identification of abnormal nodes. It is impossible to reliably detect micrometastases with CT or MR imaging [23]. Additional characteristics such as a rounded shape, irregular margins, clusters of multiple small lymph nodes, signal intensity similar to the primary tumor, and necrosis are suggestive of metastases. What is more, lymph nodes positive for metastasis show increased tracer uptake on PET/CT examination being localised on the drainage pathway from uterine cervical cancer.

PET/CT and PET/MRI are highly accurate in lymph nodes metastases diagnostics, 85–95% [24]. Another advantage is sensitivity of PET/CT in lymphadenopathy detection, with PET having higher sensitivity than MRI (75% to 56%) and CT (58%). It was proven, that while CT and MRI demonstrate similar diagnostic performance, PET/CT outperforms both in lymphadenopathy detection.

PET/MRI is a chosen method for primary tumor evaluation with sensitivity of 99% [25]. This examination, according to the recent literature data and updated FIGO system is a vital method in local and general cervical cancer staging.

SUMMARY

Ultrasounds, especially TVUS as a widespread and fundamental skill among gynecology specialists is considered

as the initial examination in pelvis viscera diagnostics. It may also have a substantial role in staging while performed by a US expert in dedicated cancer units. For local staging of a uterine body and a cervix cancer MR imaging is the best choice, while for assessment of retroperitoneal space of above mentioned malignancies and for an ovarian cancer staging the method of choice is CT. Additionally, combined imaging modalities, such as PET/CT or PET/MRI are promising tools improving staging accuracy.

Conflict of interest

All authors declare no conflict of interest.

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Alpha-fetoprotein (AFP) — new aspects of a well-known marker in perinatology

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ABSTRACT

Alpha-fetoprotein (AFP) is a serum protein, which is characteristic of the fetal development period and a well-known oncological marker. The predominance of AFP among serum proteins is common in fetal life, whereas after birthing its functions are gradually taken over by albumins. An understanding of the mechanism of AFP transfer between fetus and mother has led to the development of screening tests for identifying neural tube defects and Down's syndrome. Currently, the knowledge on patophysiology and the possible importance of AFP in perinatology and fetal medicine extends far beyond those 2 disease states. Throughout the 50 years of research on AFP, there has been dynamic progress of diagnostic techniques, from the qualitative ones that are used solely for scientific studies to the widely used radioimmunoassays and immunoenzymatic assays (enzyme-linked immunosorbent assay, chemiluminescence immunoassay, time-resolved fluorescence immunoassay).

Some genetic mutations cause complete inhibition of AFP production by the fetus. This affects the results of screening tests during pregnancy, and also leads to constantly high levels of AFP in adults, which are not linked to oncogenesis.

Key words: fetal defects; alpha-fetoprotein; isoforms; AFP-L3

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INTRODUCTION

Alpha-fetoprotein (AFP) was isolated for the first time in 1956 from the serum of human fetuses by C. Bergstrand and B. Czar during the electrophoresis of serum proteins of neonates as a fraction between albumins and alpha1-globulin [1, 2]. The oncological association of this protein with hepatocellular carcinoma was discovered first in the 1960s by G.I. Abelev and Y.S. Tatarinow [3]. Since the discovery of this relationship, over 24 000 scientific works have been devoted to the biology of AFP, its role in fetal physiology, and use in prenatal and oncological diagnostics. In the 1970s, it was considered that AFP can be highly useful in the diagnosis of neural tube defects. Consequently, the protein has been used in the triple test since the early 1990s, and in the quadruple test for the diagnosis of Down's syndrome (DS) since 1996 [4].

AFP MOLECULAR STRUCTURE AND ITS ISOFORMS

The molecular structure

AFP is a glycoprotein (contains 4.5% carbohydrates) consisting of 590 amino acids. It has a molecular mass of 69–70 kDa and a half-life of 5–6 days [4, 5]. The structure of human AFP is similar to that of other mammals and avian albumin forms [6]. Together with the genes that encode albumins, alpha-albumins, and vitamin D3-binding protein, the AFP-encoding gene forms a common family, which is located in the tandem system on chromosome 4 in locus 4q11-4q13 [5, 7, 8]. All the members of this multigene family bear similar structure and physicochemical properties. Apart from their capacity to transport different particles, they exhibit free radical-scavenging functions, esterase activity, and the ability of chemotaxis, leukocyte adhesion, and

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lipid peroxidation [8]. The proteins of the multigene family commonly have a two-dimensional alpha-helical U-form secondary structure (65–70% of the alpha-helical structure in AFP and 50% in albumins is compliant) and lack the beta-helical structure [3]. In addition, their spatial conformation is similar and comprises 3 (I–III) homologous domains, each consisting of 3 spherical subdomains conjugated by 15 regularly distributed disulphide bonds. AFP penetrates fetal and tumor cells via AFP receptors (AFP-R) and binds with the cytoplasmic proteins present in the cells. Binding of AFP with AFP-R takes place through the C-end domain (CD) in the tertiary structure. Following the binding, the AFP-R complex is internalized in the cell through endocytosis [11, 12].

Transformed AFP and growth-inhibitory peptide

Domain III of an AFP molecule contains a 34-amino-acid sequence found deep in the primary, tightly wrinkled structure. During fetal stress/shock and exposure to high concentrations of estrogen, the tertiary structure of an AFP molecule undergoes changes, resulting in „transformed AFP" and the deeply hidden 34-amino-acid sequence becomes exposed [9, 10]. This sequence has been synthesized in laboratory conditions as a growth-inhibitory peptide (GIP) and was thoroughly investigated for its biological activity. It is known that the sequence exhibits growth-inhibitory functions in fetal cells and tumor cells, in contrast to the typical primary AFP molecule [10]. GIP also prevents the local distribution and metastasis of cancers by blocking the adhesion of tumor cells to the extracellular matrix and preventing platelet aggregation. In recent years, the use of GIP to deliver chemotherapy drugs such as doxorubicin or tamoxifen into tumor cells has been extensively studied [11].

mRNA variants and isoforms of AFP

So far, numerous genetic variants of mRNA matrix have been discovered for AFP: 1.6 kb, 1.7 kb, and 2.2 kb (1 kb = 1000 base pairs). In humans, the 2.2-kb mRNA molecule is predominant (AFP matrix with a molecular weight of 69–70 kDa), while the remaining variants are found at trace levels. Interestingly, the majority of radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) test sets detect AFP molecules with a molecular weight of 69–70 kDa (for 2.2 kb mRNA). Therefore, the rare isoforms are synthesized on shorter mRNA matrices and are not detected in a standard test at all. Over the last 20 years, 3 AFP isoforms — L1, L2, and L3 — have been used in scientific research, and their inclusion in clinical diagnostics has also been postulated. These isoforms were distinguished based on AFP binding with *Lens culinaris* agglutinin. AFP-L3 is predominantly found in the serum of mothers of children with DS — its transplacental passage is probably promoted relative to the other 2 isoforms. It was later proven that the use of AFP-L3 instead of AFP increases the sensitivity of the triple test [13, 14], while the use of AFP-L2 and AFP-L3 improves the detectability of open neural tube defects and abdominal wall defects [15]. AFP-L3 is the predominant isoform of AFP found in hepatocellular carcinoma based on hepatic cirrhosis and hepatitis [3].

Congenital deficiency of AFP

It is known that a gene mutation causes complete inhibition of AFP production [7, 16], which occurs at a frequency of 1 out of 105 000 neonates. This mutation occurs in exon 5 on chromosome 4 (c543 G>A) and causes the stop codon to be inserted prematurely, leading to the completion of AFP transcription. As shown by previous research, AFP is not necessary for the normal development of a fetus, because in the absence of this protein its functions are taken over by albumins and alpha-albumins. This was also proven in an animal model, in which the synthesis of AFP and albumin mRNA takes place at the same time from the early stages of pregnancy.

Hereditary persistence of AFP (HPAFP)

The last several decades of research on AFP have shown that autosomal dominant inherited gene mutation is responsible for the persistently elevated levels of AFP in adults [hereditary persistence of AFP (HPAFP)] [5, 17–19]. In such a case, constantly elevated levels of AFP, in the range of 0.009–3.564 μg/mL, are observed in the serum. From 1983 to 2010, HPAFP was recorded in 19 families. Two-point mutations on chromosome 4 were found (a-55 C>A and a-119 G>A) in the site of binding of HNF-1 (hepatocyte nuclear factor-1) to the AFP gene promoter. HNF-1 (responsible for stimulation) and NF-1 (mainly responsible for suppression) are 2 important transcription factors of the AFP gene. Genetic mutations increase the affinity of HNF-1 to the AFP promoter and cause elevated AFP transcription [5]. In addition, elevated binding of HNF-1 to the promoter results in decreased binding of NF-1 (due to the partial overlap of HNF-1- and NF-1-binding sites), which further stimulates transcription [17].

FETO-MATERNAL CIRCULATION OF AFP

AFP in fetal serum

In the first trimester of pregnancy, the production of AFP commences in the yolk sac, and concomitantly from 4 weeks in the fetal liver [4, 20], which is the predominant source of this protein. The AFP synthesis increases till 20 weeks of pregnancy, then remains constant until 32 weeks, and gradually decreases until birth [1]. Trace amounts of AFP are produced in the gastrointestinal tract and kidneys of the fetus. The protein appears

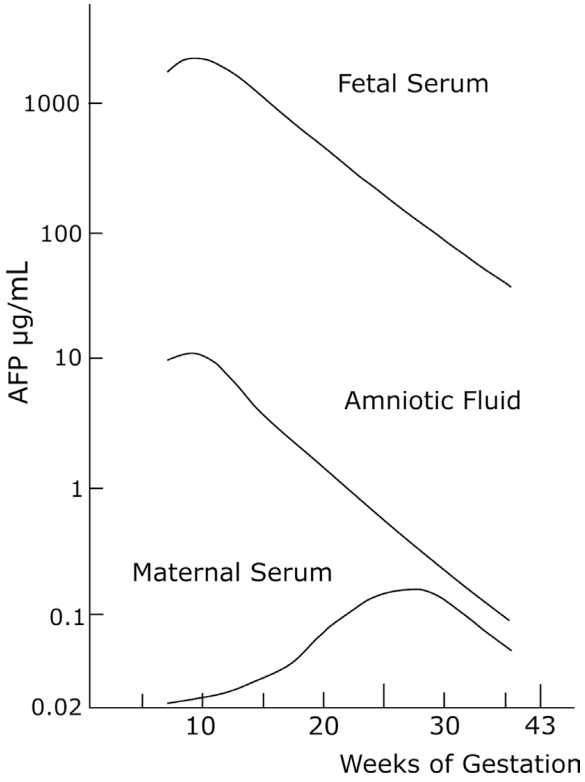

Figure 1. Alpha-fetoprotein concentration in fetal serum, maternal serum and amniotic fluid in relation to gestational age (modified from [1])

in the fetal serum 29 days postconceptional [1, 19]. It reaches the maximum concentration (approximately 3000–5000 µg/mL) at 10–13 weeks of pregnancy [21] and then gradually decreases with each week of pregnancy due to the dilution caused by the increased weight of the fetus until birth, reaching a concentration of approximately 200–300 µg/mL at 32–35 weeks and approximately 20–120 µg/mL on the day of birth [22] (Fig. 1). During the postpartum period, the gene expression undergoes changes and AFP is gradually replaced by albumins in the neonate vasculature [4, 23].

AFP in cerebrospinal fluid

AFP appears in the cerebrospinal fluid (CSF) of the fetus via filtration of the interstitial fluid from the neurons of the central nervous system and in the plasma via filtration through the blood–CSF barrier (choroid plexuses), as well as via back transport from motoneurons [24]. There are no published reference intervals for concentrations of AFP in the cerebrospinal fluid of normal fetuses. There are some short reports from investigations on AFP concentration in the CSF in aborted fetuses between 16 and 25 weeks of pregnancy. Levels of AFP are highly variable and were found to decline from 160–1220 µg/mL in 16–17 weeks of pregnancy to 60 µg/mL in 23–25 weeks of pregnancy [24, 25]. Concentrations of AFP in the CSF rapidly decrease postnatally, with a half-life of about 11 days. By the age of 6 weeks, the concentrations are close to those found in adult plasma and should be in the region of 0.014 µg/mL or less. In the cerebrospinal fluid, AFP is undetectable already after 2 months postpartum [26]. In the cases of acrania and open myelomeningocele (85% [21]), AFP is leaked from the CSF to the amniotic fluid, and from there the protein is absorbed into the maternal bloodstream [2, 28]. Such leakage does not occur in the case of closed neural tube defects, because the CSF is not washed into the amniotic fluid. Elevated AFP in the amniotic fluid may also be related to causes such as abdominal wall defects (omphalocele, gastroschisis) (Fig. 2). Thus, to confirm the relationship with neural tube defects, the concentration of acetylcholinesterase (ACHE) is determined in the amniotic fluid (typically ACHE is not present in the amniotic fluid, but only in fetal OUN) [28]. A significant finding is that elevated AFP level is not observed in the first trimester of pregnancy but only during the second trimester in the case of open myelomeningocele [31].

AFP in amniotic fluid

The maximum concentration of AFP in amniotic fluid (approximately 80 µg/mL) is reached [27] at 10 weeks of pregnancy, and the concentration gradually decreases to 0.2–3 µg/mL on the day of delivery. The AFP level in amniotic fluid decreases by about 10% per week between 14 and 20 weeks of pregnancy [21] and reaches 1% in the fetal serum at the second trimester of pregnancy [20]. In early pregnancy, AFP is introduced from the organism of the fetus to amniotic fluid through the skin, and later excreted by the kidneys with urine [20]. High fetal proteinuria indicates kidney immaturity; hence, during pregnancy, proteinuria, as well as the AFP level in the amniotic fluid, decreases [18]. AFP along with the amniotic fluid is swallowed by the fetus, and thus the protein is recirculated and then degraded in the liver. AFP penetrates the mother's bloodstream from the amniotic fluid via the amniotic sac and the decidua via a highly efficient hydrostatic gradient-based transport and, to a lesser extent, via the extracellular transport [1]. Congenital nephrotic syndrome of the Finnish type, *inherited* in an autosomal recessive manner, is an extremely rare cause of increased AFP in the amniotic fluid. This disease is caused by mutation in locus 19q13.1 in the *NPHS1* gene, responsible for coding the nephrin protein. Nephrin is an important transmembrane protein of the filtration slit in renal glomerulus, necessary for the proper functioning of the renal filtration barrier. The deficiency of nephrin results in severe proteinuria, presented in utero, typically from the second trimester of pregnancy [34].

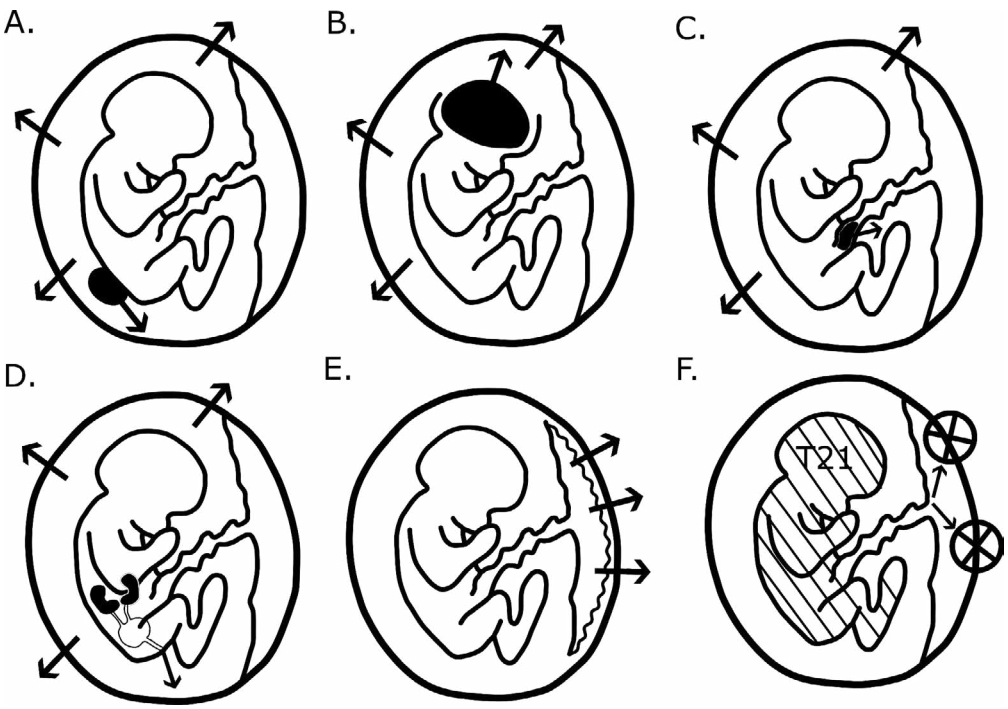

Figure 2. Fetal causes of changes in maternal serum alpha-fetoprotein (AFP) concentration; **A.** Open spina bifida: AFP increase in amniotic fluid, AFP increase in maternal serum; **B.** Acrania: the same as in A; **C.** Omphalocele: the same as in A and B; **D.** Congenital nephrotic syndrome of the Finnish type: the same as in A, B and C; **E.** Placental damage: AFP normal in amniotic fluid, AFP increase in maternal serum; **F.** Trisomy 21: AFP normal in amniotic fluid, AFP increase in placenta, AFP decrease in maternal serum

AFP in maternal serum

In the mother's serum, AFP is detected from six weeks of pregnancy [20], and its level gradually increases, reaching 0.05 µg/mL in the second trimester. The maximum concentration (approximately 1 µg/mL) is observed at 32 weeks of pregnancy, and later the concentration decreases till the day of delivery, reaching approximately 0.05–0.1 µg/mL [1] [20]. This scheme is different from the one observed in fetal serum and amniotic fluid. AFP is transported to the mother's bloodstream mainly via placental vessels with the contribution of AFP-R in the placenta and to a considerably lower extent via the amniotic sac. It has been confirmed that the placenta itself does not produce AFP [20]. The expression of receptors binding AFP placental villi is observed only in the second and third trimester, and not in the first [23], and until that time, AFP is transported through the amniotic fluid and amniotic sac. Transplacental transport is challenged by 4 barriers: syncytiotrophoblast immersed in the maternal blood in the intervillous space, trophoblast basement membrane, endothelial basement membrane, and fetal vascular endothelium. The transplacental transport is predominantly unidirectional — from fetus to mother — and takes place in 2 ways: in the first route, the protein leaves the fetal vessel lumen through the core of the placental villi and is transitioned through discontinuities/cavities within the syncytiotrophoblast; in the second route, the protein is transported through the recovery vessels passing through the decidua. The transport is facilitated by the hydrostatic gradient between the high pressure in fetal vessels and the low pressure in the intervillous space [8]. In the case of a genetically and anatomically healthy fetus, increased fetus–mother transport of AFP occurs even if the placental barrier is damaged at the level of placental villi [29]. On the other hand, defective placental transport of AFP is observed in the case of DS, and thus the low level of AFP in the maternal serum, despite normal fetal production. This theory is further confirmed by the histopathological determination of high AFP levels in the placenta [20, 30] (Tab. 1).

REFERENCE VALUES AND LABORATORY DETERMINATION METHODS

Based on the long-term screening programs used to detect neural tube defects and fetuses with DS, it was determined that the AFP norms in maternal serum range from 0.5 to 2.5 MoM in the first and second trimester [32, 33]. Until the 1970s, AFP detection was widely carried out by immunoelectrophoresis, which is a method focusing on qualitative determination and does not produce accurate results. In the 1970s, RIA methods were introduced, which utilized anti-AFP antibodies labeled with I125 and enabled AFP detection in the range of 0.002–0.005 µg/mL. This led to the inclusion of AFP analysis on a wide scale in screening

Table 1. Normal ranges of AFP in corelation to compartment, gestational age and period of life (wks = weeks of gestation)

AFP (compartment)		Physiological ranges
Fetal serum	10–13 wks	3000–5000 µg/mL [21]
	32–35 wks	200–300 µg/mL [22]
	40 wks	20–120 µg/mL [22]
Cerebrospinal fluid	16–17 wks	160–1220 µg/mL [24, 25]
	23–25 wks	60 µg/mL [24, 25]
Amniotic fluid	10 wks	80 µg/mL [27]
	40 wks	0.2–3 µg/mL [27]
Maternal serum	32 wks	ca 1 µg/mL [20]
	40 wks	0.05–0.1 µg/mL [20]
HPAFP mutation		0.009–3.564 µg/mL [5, 17–19]

AFP — alpha-fetoprotein; HPAFP — hereditary persistence of AFP

tests used for the diagnostics of neural tube defects [2]. Subsequently, ELISA tests were introduced, which are based on the use of anti-AFP antibodies labeled with the enzyme responsible for the transformation of a substrate into a colored product. The 1990s were marked by the development of immunoenzymatic techniques. Then, chemiluminescence immunoassay was introduced, involving the use of anti-AFP antibodies with a chemiluminescent marker. In this technique, following the bonding between 2 antibodies and the addition of a substrate, a light reaction is produced, which is measured with a chemiluminometer. The latest method of AFP determination is time-resolved fluorescence immunoassay, which is based on the use of antibodies labeled with lanthanide chelates—typically europium isotopes, because they are characterized by long radioactive decay times, considerably longer than typical fluorescing compounds. In this technique, following the binding of antibodies with AFP and addition of a booster solution, lanthanide is released from the bond with the antibody and chelate is bound with the fluorescing compound, responsible for transferring the triggering wave onto the lanthanide; subsequently, the triggering wave is emitted and measured (Tab. 2).

CONCLUSIONS

- In numerous gestational complications, pathologically high levels of AFP are observed in the serum of a pregnant woman, which is not related to elevated fetal production, but the increased fetal–maternal leak due to placental injury.
- AFP determination can be useful for the diagnosis of neural tube defects only from the second trimester and only in the case of "open" defects, because in such case elevated release of AFP with CSF to the amniotic fluid occurs.

Table 2. Analitycal methods and their accuracy (detection limits) in AFP measurements [35–38]

Method	Accuracy
RIA	From 5×10^{-4} µg/mL
ELISA	From 6×10^{-7} µg/mL
CLIA	From 6×10^{-5} µg/mL
TRFIA	From 1.21×10^{-4} µg/mL

CLIA — chemiluminescence immunoassay; ELISA — enzyme-linked immunosorbent assay; RIA — radioimmunoassay; TRFIA — time-resolved fluorescence immunoassay

- In the case of "closed" neural tube defects, increased AFP level is not observed in the mother's serum, because such defects do not feature AFP leakage with CSF to the amniotic fluid.
- Defective placental transport of AFP is observed in DS; thus, the level of AFP is low in the maternal serum, despite normal fetal production.
- Congenital nephrotic syndrome of the Finnish type is a rare cause of increased AFP in the amniotic fluid and maternal serum, characterized by severe fetal proteinuria, starting from the second trimester of pregnancy.
- Rare genetic mutations cause complete inhibition of AFP production by the fetus. This leads to erroneously negative results in screening tests during pregnancy, and constantly high levels of AFP in adults, which are not linked to oncogenesis, thus resulting in erroneously positive tumor diagnosis.

Conflict of interest

All authors declare no conflict of interest.

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VIA MEDICA

RECCOMENDATIONS

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Recommendations of procedures to follow in the case of ovarian lesions in girls

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ABSTRACT

This study presents current recommendations of the Polish Association of Pediatric Surgeons (PTChD) regarding diagnostics and treatment of ovarian lesions in girls. They are based on many years of the authors' clinical experience as well as a review of international literature and include practical clinical guidelines. The recommendations were formulated in cooperation with the Polish Association of Pediatric Oncology and Hematology (PTOHD), Polish Pediatric and Adolescent Gynecology Section of the Polish Society of Gynecologists and Obstetricians (PTG) and Polish Pediatric Section of the Polish Society of Radiology (PLTR). Only better understanding of prepubertal ovarian biology and natural history of its pathology may help to introduce efficient and safe diagnostic and therapeutic strategies for girls. The prepared document has been supplemented with treatment algorithms.

Key words: ovarian neoplasms; ovarian masses; child; management; algorithms

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INTRODUCTION

This study presents current recommendations of the Polish Association of Pediatric Surgeons (PTChD) regarding diagnostics and treatment of ovarian lesions in girls. They are based on many years of the authors' clinical experience as well as a review of international literature and include practical clinical guidelines. The recommendations were formulated in cooperation with the Polish Association of Pediatric Oncology and Hematology (PTOHD), Polish Pediatric and Adolescent Gynecology Section of the Polish Society of Gynecologists and Obstetricians (PTG) and Polish Pediatric Section of the Polish Society of Radiology (PLTR). The guidelines can only be useful in clinical practice and cannot be regarded as ultimate therapeutic recommendations. Followed by new clinical experience, they will be subject to modifications (validation every 3–5 years) aimed at attaining the most efficient therapeutic effect possible while maintaining the highest level of safety and keeping side effects and complications to a minimum. The prepared document has been supplemented with treatment algorithms. We expect these guidelines to be helpful in detecting malignancy, in preserving more ovarian tissue during surgery of ovarian lesions and torsions and in reducing the rate of surgical management of noncomplicated ovarian cysts.

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EPIDEMIOLOGY OF ADNEXAL MASSES

Functional ovarian lesions [1–4]

Types:

a) Follicular cyst
b) Hemorrhagic follicular cyst
c) Corpus luteum
d) Corpus luteum cyst
e) Hemorrhagic corpus luteum cyst

Adnexal functional lesions are the most frequent reason for ovarian "tumors" in children. According to relevant publications, they constitute from 17% to 44% of all ovarian pathologies in children. Functional lesions may be discovered accidentally or in an emergency room due to experienced pain. Imaging tests usually reveal a simple cyst, which is small in size (1–3 cm). The cysts can also grow larger (> 5 cm) and cause acute pain because of rupture or adnexal torsion. Data concerning the incidence of functional lesions in children are limited. Nevertheless, it is known that they occur more often and reach larger sizes in the peripubertal period due to an increase in gonadotropic stimulation. In infants, they occur as a result of hormonal stimulation by the mother's organism in the fetal period. According to several available publications, the incidence of these lesions oscillates between 2% and 5% in the period between 0 and 9 years of age and between 4% and 32% in the period between 10–18 years of age.

It should be noted that follicular cysts may also produce estrogens and cause symptoms of precocious puberty (peripheral precocious puberty). Such lesions are usually self-limiting and do not require treatment. In order to differentiate them from other reasons for precocious puberty (hormonally active tumors, McCune-Albright syndrome, central cause), a series of tests should be performed taking into consideration physical examination, bone age measurement, estradiol level measurement, GnRH (gonadotropin-releasing hormone) stimulation test (gold standard) as well as imaging tests (ultrasound, possibly also MRI — magnetic resonance imaging). After eliminating other causes of precocious puberty, surgical treatment is only advisable in the event of cyst torsion or development of secondary sex characteristics, but also when the cyst does not disappear within three months.

Ovarian endometrial cysts ("chocolate cyst") is a benign estrogen-dependent cyst occurring in women in the reproductive period. It affects 17–44% of women suffering from endometriosis. The incidence of the lesions in girls before and after menarche is extremely rare (single published descriptions of cases). A non-surgical treatment is possible [3, 4]

Ovarian tumors [1, 3–11]

Ovarian tumors can derive from three cell lines: epithelial tissue cells, ovarian stroma cells and pluripotent germ cells. In children, most ovarian tumors (> 70%) originate from germ cells: these are mostly benign mature teratomas. In patients over 18 years of age, a vast majority of tumors come from the epithelial tissue: benign adenomas and cancers constitute > 60% of all ovarian tumors and 80–90% of malignant neoplasms. The classification presented below is based on WHO's (World Health Organization) classification and includes lesions occurring in children.

Benign tumors

Germ cell tumors

a) Teratoma maturum (mature teratoma) is the most common neoplastic ovarian tumor in children. These neoplasms are composed of cells that are derivatives of all the three germ layers. A special type of a mature teratoma is a dermoid cyst, which is made of one germ layer (monodermal). Most publications state that the peak incidence of these lesions is in the early teenage years. The exact incidence remains unknown. Mature teratomas constitute approximately 50% of all ovarian tumors in children and often occur bilaterally (10–15%). Clinically, mature teratomas are most often slow-growing cystic structures that demonstrate characteristic features in an ultrasound examination.
b) Teratoma immaturum (immature teratoma) may macroscopically resemble a mature teratoma and is difficult to classify histologically. The key difference between the two types of teratoma is the presence of immature (germinal) cells, mainly of neurogenic origin, in the immature one. Measurements of tissue quantity allow to classify their maturity level. They occur much less frequently than mature teratomas. It is difficult to specify their exact incidence since these lesions are often misclassified. It was believed that these tumors have a tendency to demonstrate features of "local malignancy". However, the newest publications assume that, in their pure form, these lesions are benign tumors. The essence is to understand the natural course of development of an immature teratoma. Removed completely and well-assessed histologically, as a histopathology specimen, it is a benign tumor. Frequent coincidence of small malignant tumor foci (most often as a yolk sac tumor that can be misdiagnosed as Heifetz lesions, which frequently occur in teratomas) may be responsible for its "malignant" clinical course with distant metastases. These cases are described in the literature as metastatic immature teratomas. Serum AFP (alpha-fetoprotein) cut-off level of 1000 ng/mL is used as one of the indicators of the presence of malignant elements. Higher values usually indicate the presence of a malignant tumor. According to the present state of knowledge, immature teratomas in their pure form require surgical treatment only. Nevertheless, a reliable histopathological test including grading is key to post-

operative management. Consultation with an oncologist is indicated.

Stromal tumors

a) Thecoma — produces estrogen, very rarely develops in children
b) Fibroma — very rarely diagnosed in children

Epithelial tumors

Epithelial tumors — typical in adults, rarely develop in children, however, the exact epidemiological data are not known.

a) Cystadenoma — serous or mucinous, usually cystic or complex masses in imaging tests. They can reach enormous sizes, occupy the entire abdominal cavity, exert pressure on organs in the chest and deform costal arches.

Malignant tumors

The exact epidemiological data for Poland are not known. In one American study of 2008, which involved 1037 children diagnosed with a malignant ovarian tumor, the incidence of this tumor was calculated. The age-adjusted incidence of malignant ovarian tumors in children amounted to 0.102 for children before the age of nine and 1.072 for children between 10 and 19 years of age per 100.000 per year. The authors of the majority of available publications agree that the incidence of malignant lesions is low. However, the characteristics of the patients' age is a disputable issue. Nevertheless, numerous publications emphasize the rarity of incidence of these lesions in very young children: girls before the age of four constituted a mere 1.5% of all the patients involved in the above study.

Germinal tumors

Germinal tumors — most frequent malignant tumors in children. According to the literature, they constitute between 60 and 80% of all malignant ovarian tumors. All malignant germ cell tumors in advanced stages require chemotherapy. However, the management in stage I/II depends on each individual case. In lower stages, the management is chosen by an oncological team based on the analysis of recurrence risk factors and the current treatment protocols. Recently created Malignant Germ Cell Tumor International Collaborative in its publications gathering data from four clinical trials recommends surgery alone for patients with stage I/II, grade 1/2 tumors and suggests conducting of a prospective trial of observation after surgery for patients with grade 2/3, stage II–IV tumors. This approach might be encouraged by the results of Park et al., as they revealed that most stage I malignant ovarian germ cell tumor recurrences can be successfully salvaged by surgery and BEP chemotherapy without compromising the overall survival.

a) Dysgerminoma – histologically, it is the equivalent of a germ cell tumor of the testicle (seminoma). It is most frequently diagnosed in teenage patients. Bilateral occurrence of this type of tumor is found in 5 to 20% of patients. The presence of multinuclear syncytiotrophoblastic giant cells is frequently associated with elevated LDH (lactate dehydrogenase) levels and rarely with elevated βhCG (β-subunit of human chorionic gonadotropin) levels.
b) Tumor sinus endodermalis/yolk sac tumor — produces the AFP marker.
c) Carcinoma embryonale (embryonal carcinoma) — usually occurs as part of a mixed tumor (hence the wrong assumption in the past that it produced βhCG and AFP or was endocrinologically active and caused precocious puberty by producing estrogens).
d) Choriocarcinoma — produces βhCG.
e) Gonadoblastoma — neoplastic tumor that forms most frequently in dysgenetic gonads in people with disorders of sex development.
f) Tumor germinalis mixtus malignus (malignant mixed germ cell tumor) — produces AFP (due to yolk sac tumor component).

Stromal tumors

Stromal tumors — constitute 6–17%. They originate from primary undifferentiated mesenchymal cells that possess potential abilities to differentiate in the direction of granulosa cells tumor and theca cells within the female gonad and in the direction of Leydig and Sertoli cells within a testicle. They are, therefore, hormonally active and may lead to precocious puberty or virilization.

a) Tumor granulocellularis typus juvenilis (juvenile granulosa cell tumor) — the most frequent in the group of malignant stromal tumors. Its level of aggressiveness differs depending on the details of the histological structure. Advanced forms require adjuvant chemotherapy.
b) Androblastoma (Sertoli-Leydig cell tumor) — produces estrogen or testosterone, advanced forms require adjuvant chemotherapy.
c) Gynandroblastoma — very rare.

Epithelial neoplasms

Epithelial neoplasms — constitute up to 2% of all malignant ovarian lesions. More frequently develop in older girls (their incidence increases after 14 year of age). Most of these tumors require adjuvant chemotherapy.

Cystadenocarcinoma (ovarian cancer) — serous or mucinous.

Other malignant neoplasms:

a) Epithelial tumor — borderline malignancy
b) Polyembryoma

c) Metastases and infiltrations of another neoplasm (e.g., lymphoma)

DIAGNOSTIC METHODS

Imaging tests and symptoms — see: TREATMENT ALGORITHMS (Fig. 1–6) [4, 6, 8, 12–19]

The goal of diagnostic procedures for ovarian tumors in children is to establish whether there is a risk that the lesion is of malignant nature, assess the possibility of fertility-sparing therapy if the lesion proves to be benign and verify the necessity of surgery in the case of a non-cancerous lesion. During the process, we recommend following algorithms that take presented symptoms (acute or mild), imaging results (basic ultrasound test) and evaluation of serum tumor marker concentration (depending on indications) into consideration. Based on the recent knowledge we have prepared separate algorithms for an ultrasound test result that includes Ueland Index (MI, morphology index — an ultrasound-based scoring system that include the tumor volume along with its structural appearance)

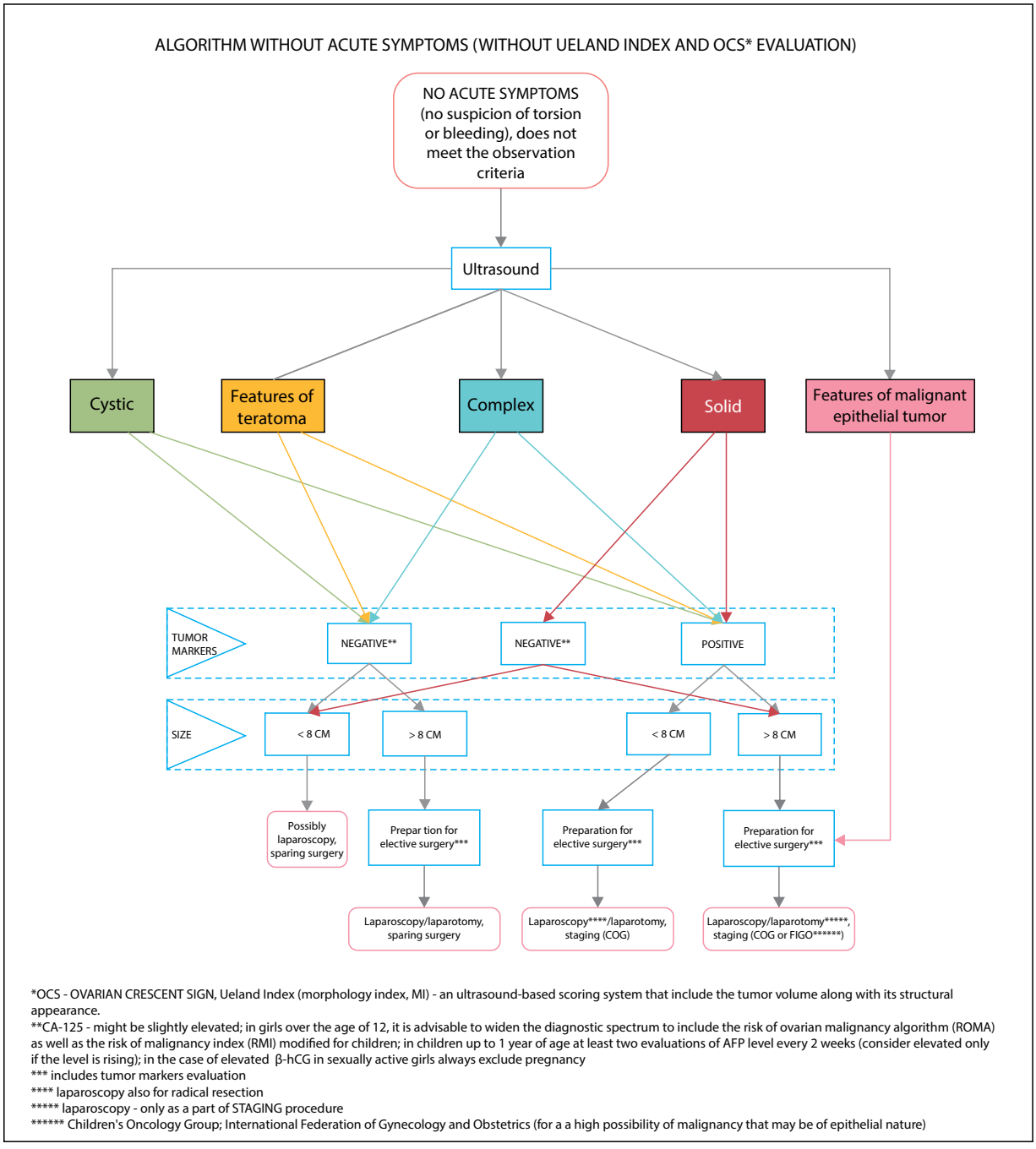

Figure 1. Algorithm — without acute symptoms (without Ueland index and OCS evaluation)

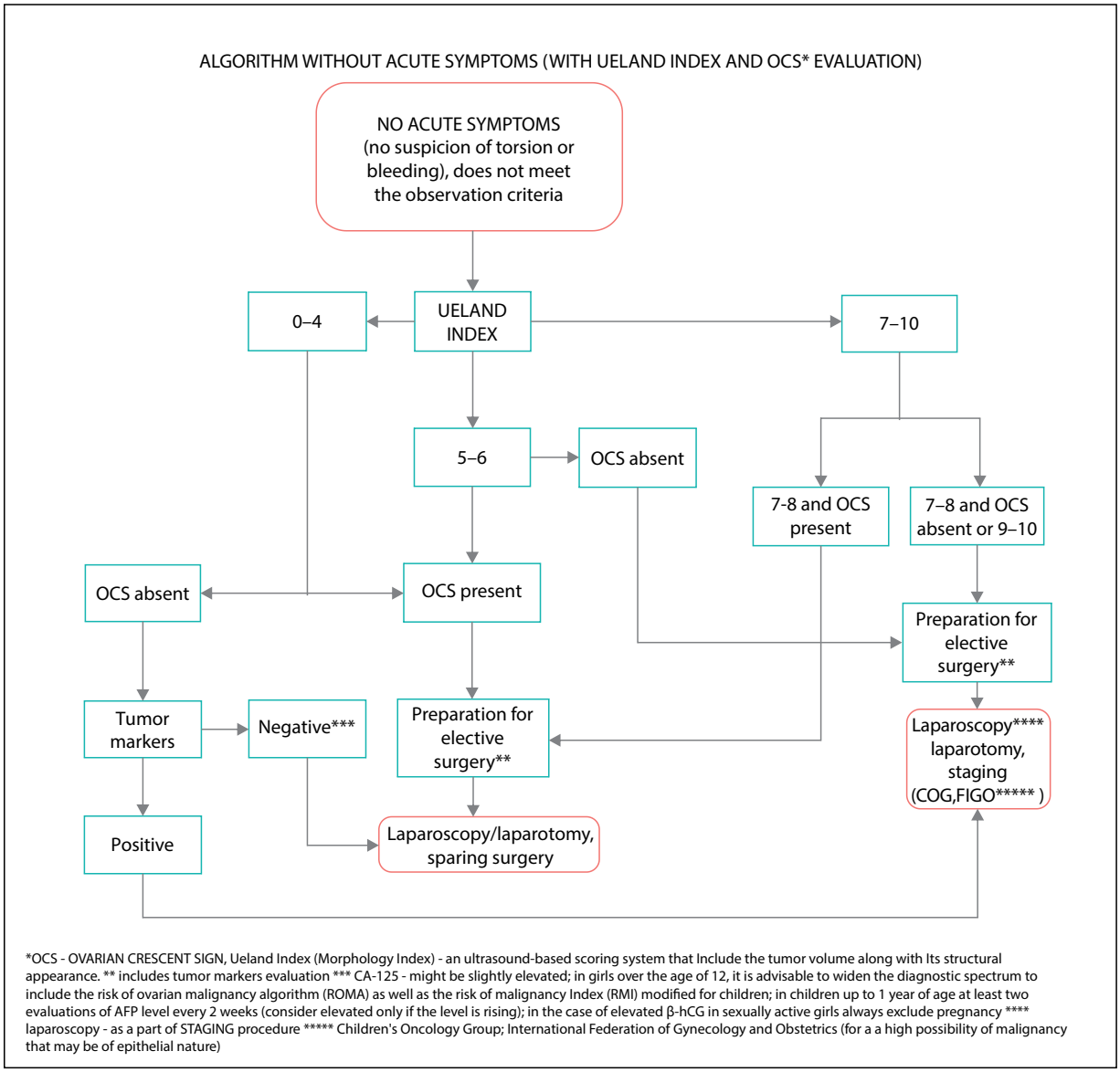

Figure 2. Algorithm — without acute symptoms (with Ueland index and OCS evaluation)

and ovarian crescent sign evaluation. A separate situation is also an accidental discovery of an ovarian lesion during a procedure performed for other indications. An additional file shows a detailed description of the imaging tests useful in the diagnosis of ovarian lesion (see Supplement 1).

Tumor markers [4, 6, 8, 12, 15–16, 20–23]

Due to the lack of randomized studies analyzing children, it is advisable to measure the below-mentioned serum markers when a lesion is first noticed in an imaging test in patients with a solid lesion of any size, a mixed (solid and cystic) lesion with a diameter over 5 cm or a cystic lesion larger than 8 cm. In other cases, the evaluation of markers may be performed during the next ultrasound test.

Markers: AFP (alpha-fetoprotein), βhCG (β-subunit of human chorionic gonadotrophin), LDH (lactate dehydrogenase), CA125 (cancer antigen CA-125) and, should it be possible in a given surgical center, estradiol, testosterone and inhibin A.

In newborns and infants, it is recommended to perform the βhCG and AFP (at least two evaluations tests every two weeks. A gradual decrease in marker concentration indicates a non-cancerous character of a lesion.

In girls over the age of 12, it is advisable to widen the diagnostic spectrum to include the risk of ovarian malignancy algorithm (ROMA) as well as the risk of malignancy index (RMI) modified for children. CA-125 might be slightly elevated even in non-malignant lesions.

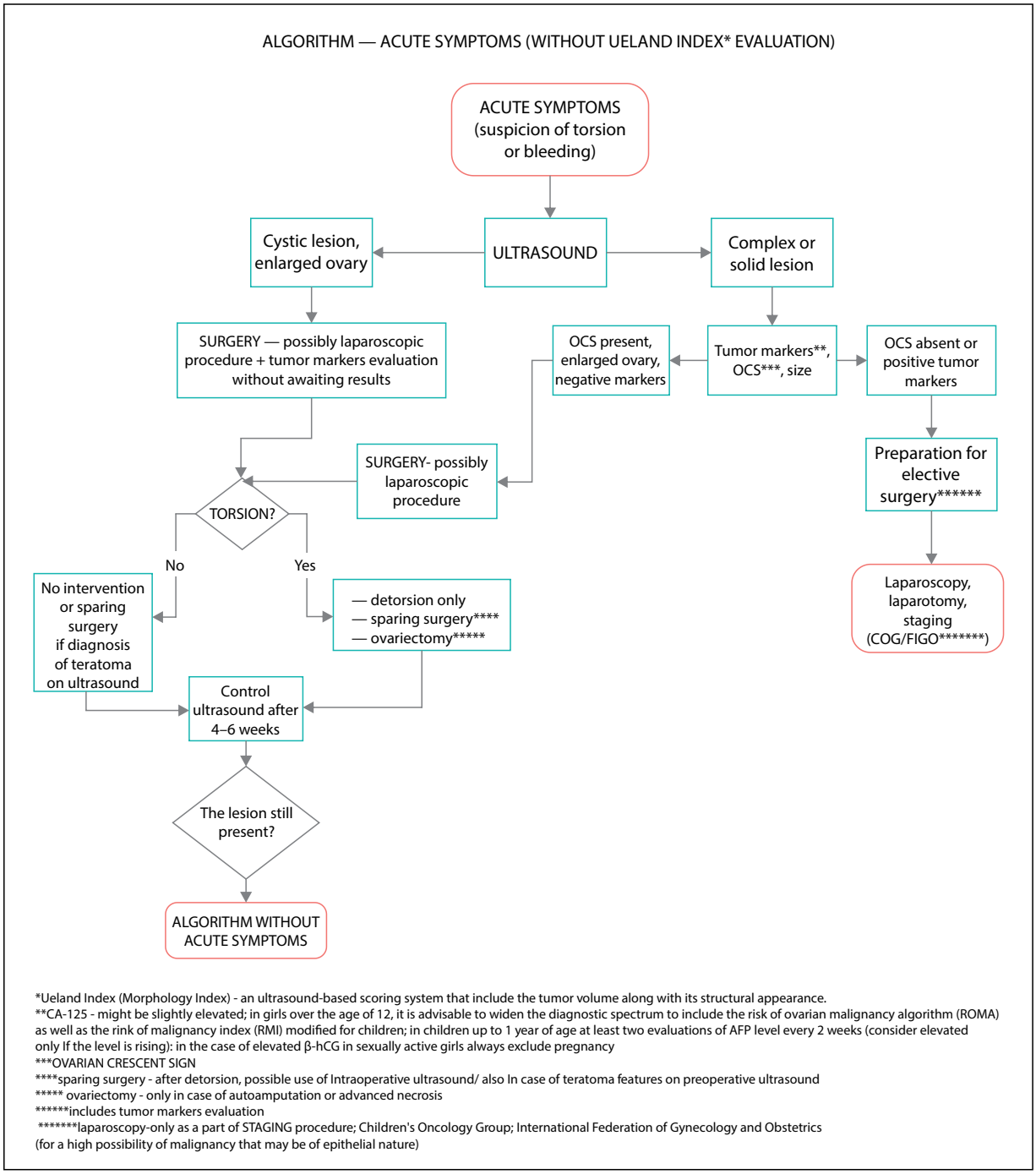

Figure 3. Algorithm — acute symptoms (without Ueland index evaluation)

In the case of elevated βhCG in sexually active girls, pregnancy should be always excluded. In the case of elevated LDH level a lymphoma should be excluded.

TREATMENT METHODS [1, 4, 6, 8, 12, 24–26]

Observation (see also: TREATMENT ALGORITHMS — Figure 6)

Possible in the case of functional lesions. The treatment plan should result from a risk-benefit ratio analysis:

Observation choice criteria

In everyone: certainty that the lesion is of ovarian origin

Newborn:

a) Any asymptomatic lesion regardless of an ultrasound test

Infant:

a) Lesions observed since the neonatal period (no progression of the lesion, decrease in marker level)
b) Asymptomatic lesions (in practice < 5–6 cm)
c) Simple and complex cysts

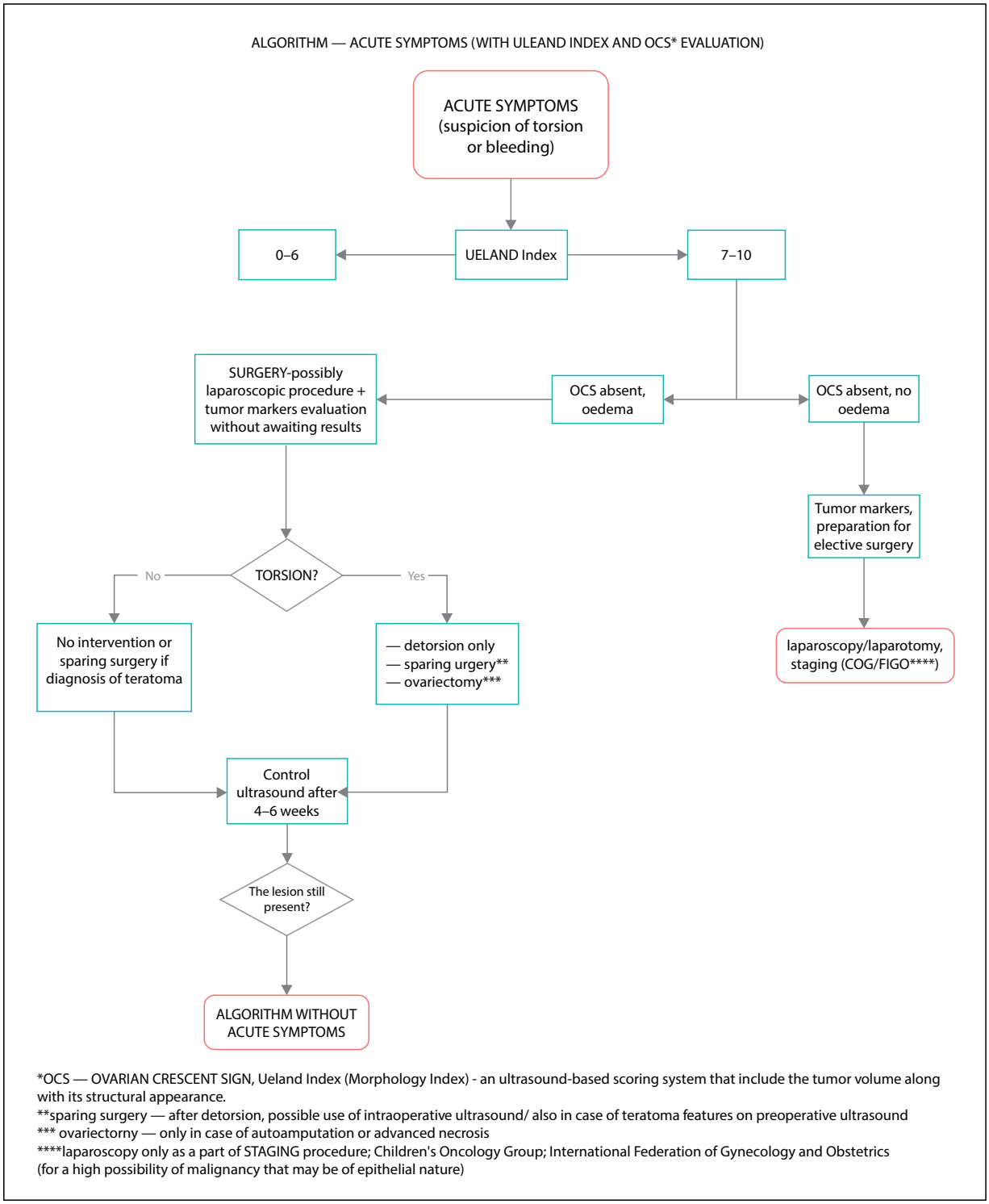

Figure 4. Algorithm — acute symptoms (with Ueland index and OCS evaluation)

Older child:

d) No acute abdominal symptoms, no additional symptoms (no precocious puberty, virilization, general symptoms, mass effect)
e) Size up to 8 cm
f) Simple and complex cysts

Procedures during observation

Newborns and infants:

a) Ultrasound test after 4 weeks
b) Monitoring marker concentration: βhCG, AFP, estradiol (minimum 2 markers)

Older children:

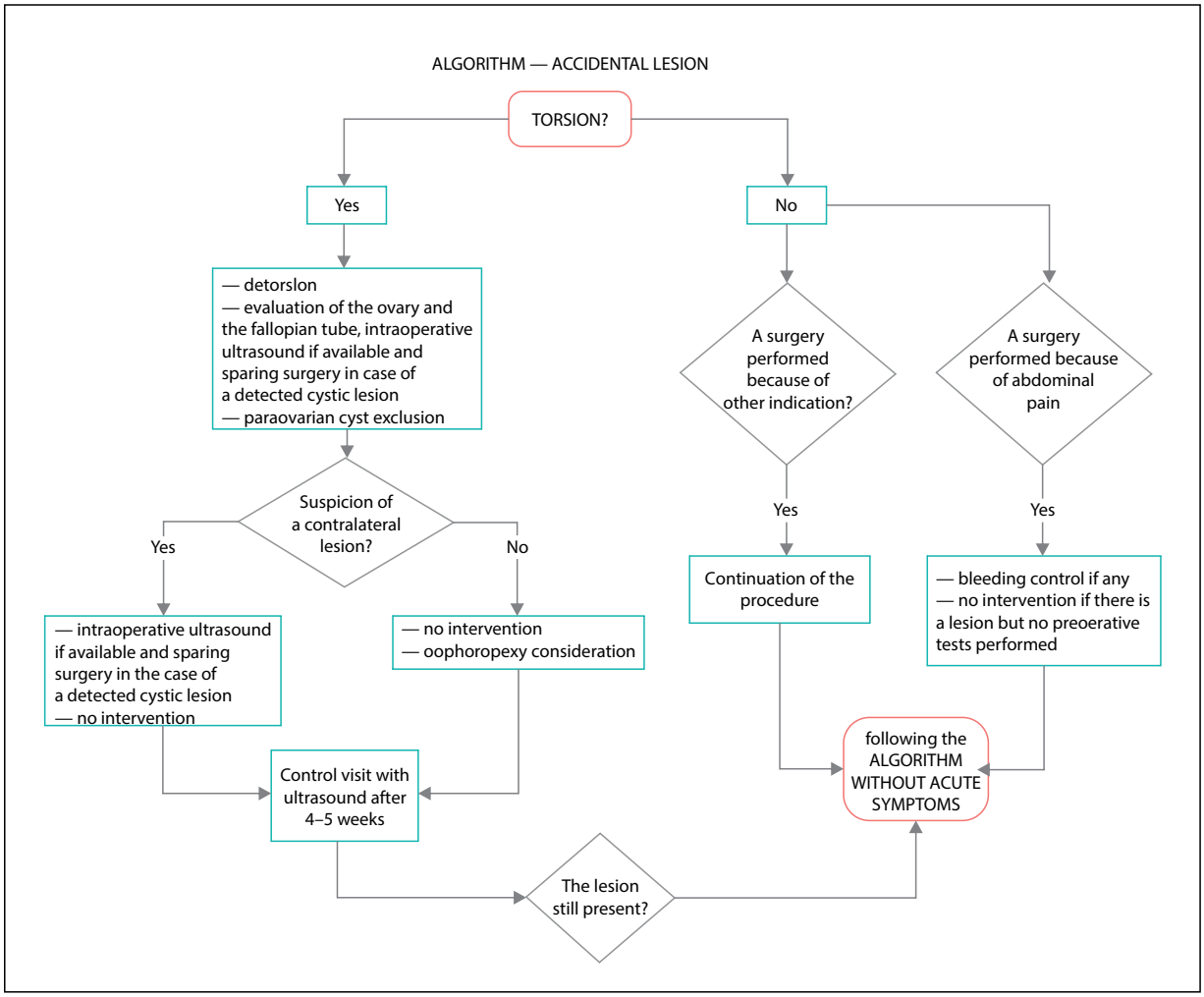

Figure 5. Algorithm — accidental lesion

a) Information about a possibility of torsion
b) Ultrasound test after menstruation for 3–6 consecutive cycles or after four weeks in prepubertal patients (assessment of changes in size and character)
c) Marker concentration evaluation (AFP, βhCG, CA-125, ROMA > 12 years of age) — in the case of a solid lesion, complex lesion with a diameter over 5 cm or a cystic lesion larger than 8 cm, a blood test is performed after the lesion is identified in an imaging test. In other cases, a blood test may be performed at the control ultrasound test
d) Considering hormone therapy: utilizing progestogens, contraceptive therapy for at least three months and another follow-up. Sometimes, indication to include GnRH/Tamoxifen analogues (in the case of symptoms of precocious puberty).

Should the lesion remain: qualification for surgical treatment (preparation for elective surgery):

a) Wait (3–6 months of observation)
b) Take age, imaging tests (the lesion's size and echogenicity, visible ovarian tissue), previous surgeries (tendency to frequently develop functional lesions in the remaining ovary after ovariectomy) into consideration
c) Always repeat an ultrasound of the abdomen/pelvis and tumor markers evaluation before surgery
d) In case of diagnostic uncertainty, consider CT (computed tomography) or MRI
e) Before surgery perform an anti-Müllerian hormone (AMH) test and a follicle-stimulating hormone (FSH) test or secure a blood serum sample (freeze) in order to perform the tests at a later time.

Laparoscopy

Laparoscopy is currently the method of choice in the case of lesions without malignant features in preoperative tests (see: TREATMENT ALGORITHMS — Figures 1–6). Endoscopy allows to assess the size of a lesion and its safe enuclea-

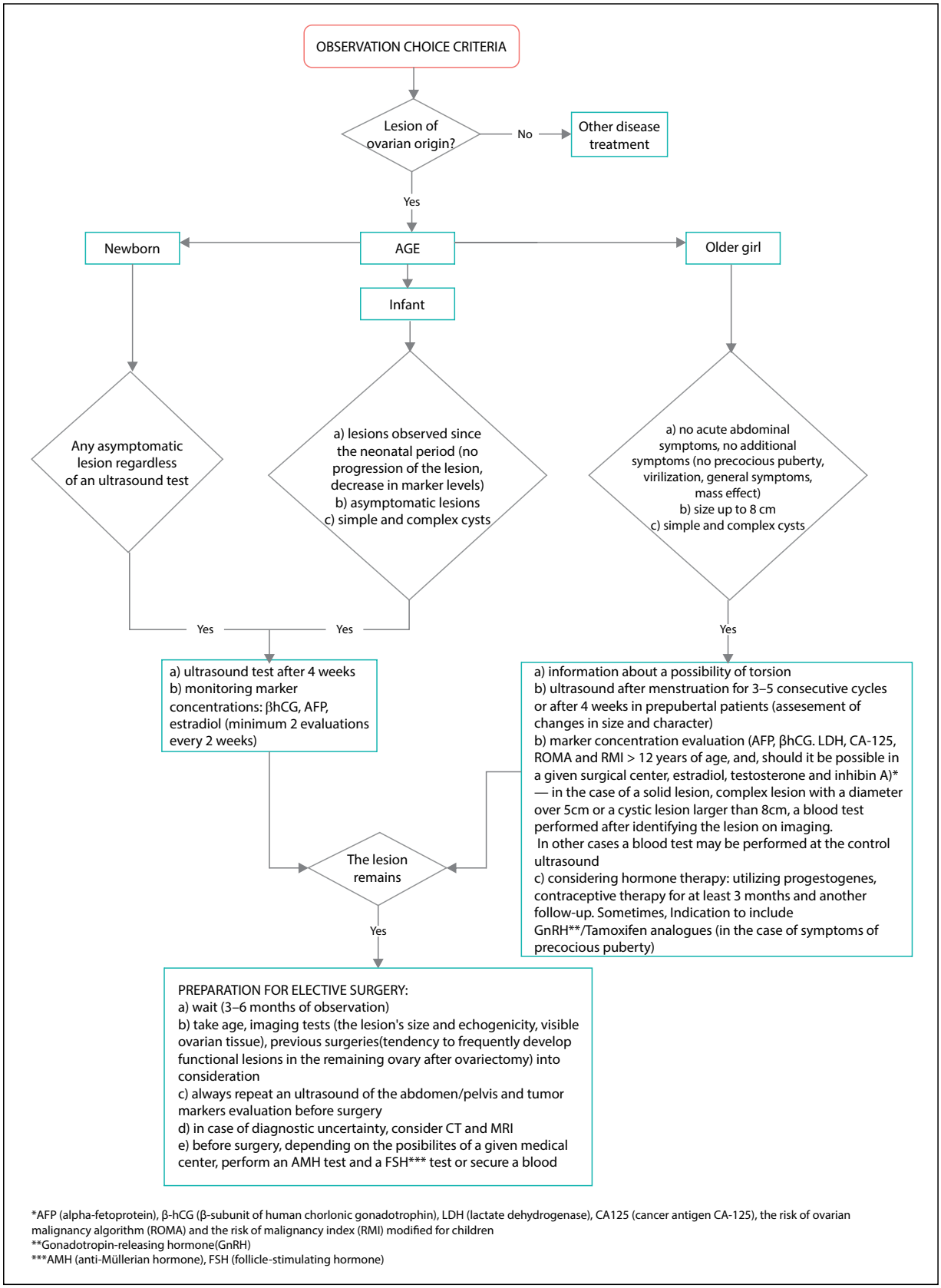

Figure 6. Algorithm — observation

tion or marsupialization. It is also an excellent diagnostic tool (including exclusion of ovarian torsion and making the final deciding concerning the surgical technique) and proves useful in the removal of benign lesions, including the most common malignant tumor, i.e., mature teratoma. However, in the case of lesions suspected of malignancy, laparoscopic resection has not been advised so far. Therefore, the assessment of the risk of malignant lesion incidence is the most difficult decisive element in the process of selecting the right therapeutic procedure. No randomized study that would allow to objectively choose the proper malignancy indicators of ovarian lesions has been conducted so far. Still, one of the goals of this study is to improve the efficiency of malignant lesion diagnoses based on the available knowledge. A large size of a lesion is one of the malignancy risk indicators. It poses the most significant obstacle in the application of the laparoscopic technique in the case of solid lesions. However, it has been proven that in the case of tumors smaller that 7 cm in diameter there is no increased risk of damaging their capsule during laparoscopy. That is why, it may soon be possible to demonstrate the safety of the application of endoscopy in the treatment of malignant ovarian tumors. As is the case with children, there have been no randomized studies on the use of laparoscopy in stage I malignant ovarian tumors conducted among adult patients either. Nevertheless, other studies suggest that the technique is efficient and does not pose danger to oncological principles. Moreover, ovarian cancer in adults is a tumor of higher malignancy than the majority of malignant neoplasms in children. The lack of randomized studies seems to be the only obstacle for laparoscopy to be applied in the resection of malignant ovarian lesions of smaller sizes, even though it can complement the treatment as its initialization performed in order to inspect the peritoneal cavity with consecutive conversion to minilaparotomy and a resection of the lesion performed in the open way, should it prove large. Properly performed staging guarantees the correct assessment of the disease's progression stage and the right choice of treatment to complement a surgical procedure and offers a possibility to make a decision not to perform the surgery at all. It is important to remember that the aim of the treatment is not only to save the patient's life but also to secure her future fertility in each possible case. The efficiency of the proposed algorithms and a possible reasonability to perform laparoscopy in the resection of malignant ovarian tumors need to be verified in multi-center prospective studies. In the case of a large benign lesion, should there be uncertainty as to the possibility of saving the ovarian tissue in laparoscopy, it is advisable to perform laparotomy by Pfannenstiel incision and remove the tumor from the abdominal cavity after decompression puncture in order to perform sparing surgery.

Laparotomy

This method is recommended when the presence of a malignant lesion is suspected. Should such a suspicion occur, it is necessary to perform staging. In the case of large benign lesions, it is advisable to perform laparotomy by Pfannenstiel incision and remove the tumor from the abdominal cavity after decompression puncture.

Staging (assessment of tumor's progression stage)

The current guidelines concerning the assessment of the disease's stage in the case of epithelial neoplasms in adults have been developed by the International Federation of Gynecology and Obstetrics (FIGO). The removal of the tumor together with an oviduct is accompanied by an assessment of the amount of fluid in the peritoneal cavity, its cytology and biopsies taken from the pelvic walls, the pouch of Douglas and the paracolic sulci area. The surface of the diaphragm should be evaluated by means of cytology or biopsy. Serous membrane of the intestine and the mesentery should also be evaluated and the greater omentum removed. Moreover, pelvic and aortic lymphadenectomy should be performed. This protocol allows to preserve the uterus and the contralateral ovary in young women in a lower stage who wish to maintain their reproductive potential.

The current procedures regarding the assessment of the disease's progression stage in the case of germ cell tumors of the ovary in children have been developed by the CHILDREN'S ONCOLOGY GROUP (COG). These guidelines include:

a) cytology of peritoneal cavity fluid or washing, assessment of the peritoneum with biopsy or removal of possible lesions;
b) palpatory examination of retroperitoneal lymph nodes with biopsy of the solid and enlarged ones;
c) inspection and palpation of the omentum and resection of abnormal parts;
d) inspection and palpation of the contralateral ovary in case of abnormalities;
e) resection of the affected ovary with possible preservation of the oviduct.

Due to positive results achieved in the treatment of patients diagnosed with stage I malignant germ cell tumor of the ovary even in the case of recurrence (and the application of chemotherapy in such case), proceeding in accordance with COG's guidelines seems highly justified. The treatment procedure in the case of malignant epithelial neoplasm or borderline malignancy is connected with more doubts. The few studies in the area of these neoplasms in children do not offer enough certainty to regard the COG's procedures as safe in their case. Nevertheless, they may be sufficient when lesions in their initial stages are concerned. An incom-

plete assessment of the stage of progression in these cases requires close observation for recurrence.

Conclusions

Intraoperative identification of a malignant lesion may be very difficult. The first step is a preoperative malignancy risk evaluation of the lesion (see: TREATMENT ALGORITHMS — Figure 1–6). In the case of a high possibility of malignancy of the lesion, which is limited to a gonad, in girls, it is advisable to follow the procedures developed by COG. In the case of lesions with a high possibility of malignancy that may be of epithelial nature [imaging test result (see: Additional file 1), markers characteristic of epithelial lesions, older girls (> 12 years of age)], an expansion of progression stage evaluation advised by FIGO should be taken into consideration (as in the case of patients who wish to retain their reproductive potential).

Scope of treatment

In each possible case, one should aim at fertility-sparing treatment. According to the few studies of treatment results in pediatric surgery centers, the percentage of fertility-sparing surgeries in the case of benign lesions oscillates between 24% and 83% worldwide, which is a highly uneven distribution. Efforts should be made to make these results more balanced and increase the percentage of the saved gonads. It is also important to understand what exactly fertility-sparing treatment consists in (see below).

Fertility-sparing treatment of ovarian tumors in children

This procedure is based on sparing the healthy part of an ovary after resection of an intraovarian lesion (sparing the contralateral ovary after surgical removal of the whole ovary due to a tumor is not considered a sparing procedure!). One of its types is cystectomy, which is resection of an ovarian cyst, and tumorectomy, which is a surgical removal of a neoplasm that is not a cyst. A so-called bivalving can be used in order to enucleate small benign lesions of the ovary, whereas large lesions can be resected with a margin and enucleated or a stripping technique can be used instead.

Important issues

Before dissecting, the type of the lesion should be identified (paraovarian or intraovarian);

a) One should always try to enucleate cystadenomas;
b) Intraoperative perforation and puncture are not adverse outcome risk factors (in the case of cystic lesions and negative markers);
c) Size of the lesion is not a limitation;
d) Torsion is not a limitation;
e) Long-time observation will prove whether these patients are more prone to cancer recurrence/development.

Cyst fenestration is the least invasive procedure. It can be performed when the follicular cyst has been identified as the reason for the symptoms (the lesion should not be dissected when it has been accidentally discovered during surgery performed due to other indications). Bilateral and recurring lesions occur in 8–20% of patients. Insufficient caution in determining indications for operative treatment may result in multiple interventions, loss of the ovary and, in extreme cases, castration. One should also remember about the option of puncture in the case of large or even giant lesions with a low-level risk of malignancy, which offers a possibility of an easy and minimally invasive removal of even very large lesions. A lesion can also be placed in an endobag and sucked out of the peritoneal cavity after exteriorizing the endobag through the umbilical incision. In case of any difficulties with the laparoscopic enucleation it is also possible to finish the procedure after exteriorizing the punctured benign lesion directly through the umbilical incision.

The available publications deem ovary-sparing treatment of the affected gonad plausible only in the case of non-cancerous lesions, benign neoplasms and possibly borderline lesions (it increases the risk of recurrence in the case of these neoplasms; however, the recurrences can be efficiently treated without affecting overall survival). Malignant lesions and immature teratomas currently constitute a contraindication to ovary-sparing treatment. In the case of immature teratoma found on the pathology report after sparing surgery it is acceptable to continue the follow up without the absolute necessity for oophorectomy if the tumor markers are negative.

Ovarian biopsy

This procedure is based on taking a sample of ovarian tissue in order to conduct a histopathological test. The procedure is currently used in the case of suspected pathologies in an ovary during surgical removal of a lesion in the other ovary. There are no studies recommending biopsy when the ovary has a correct macroscopic appearance, and no anomaly is found within the ovary during preoperative imaging tests. Some studies showed a very low percentage of pathological lesion detectability by means of biopsy of the other gonad. There are no studies evaluating the influence of biopsy on the healthy ovary either (only animal studies). That is why biopsy is currently advised only in the case of abnormal appearance of the ovary discovered intraoperatively. However, in the case of suspected dysgerminoma found in a preoperative examination, a biopsy should always be performed. A possibility to perform intraoperative ultrasound

facilitates the decision-making process concerning a biopsy of the contralateral ovary or even enucleation of a visualized ovary.

Intraoperative histopathological examination

The most recent guidelines of the American Pediatric Surgical Association recommend intraoperative examination of a frozen sample based on promising results in adults. However, the same data for children are limited. In one study conducted on a small group of pediatric patients, such an examination did not change the initial diagnosis in patients with a large lesion. It was also found that in the case of borderline, large, cystic and mucinous tumors the above-mentioned examination is not reliable.

To summarize, the Polish Association of Pediatric Surgeons does not currently recommend intraoperative histopathological examinations. When a lesion can be enucleated or is limited to an ovary, it should be removed in order to be histopathologically examined. When a lesion proves to be unresectable, a biopsy should be performed, and the surgical procedure should be terminated.

Ethical approval

The study was approved by the Ethical Committee of the Medical University of Wroclaw - available on request.

Conflict of interests

The authors declare that they have no competing interests.

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Parasitic fibroid of greater omentum in 31-years old woman

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ABSTRACT

Uterine leiomyomas are the most common benign tumors of the uterine smooth muscle. The latest FIGO classification, considering both their location and the degree of ingrowth into the uterine muscle, distinguishes eight classes of fibroids [1]. The location of leiomyomas in connection with their size may determine the characteristic symptoms: abdominal pain, pressure symptoms, difficulties in getting pregnant, recurrent miscarriages. Among the case reports there are also descriptions of the so-called parasitic leiomyomas [2]. The paper presents a case report of a parasitic leiomyoma in a young woman who has not been operated on in the abdominal or pelvic organs so far.

Key words: parasitic leiomyoma; parasitic myoma; parasitic fibroid

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CASE REPORT

The 31-year-old patient was admitted to the Gynecology Department of the 1st Department and Clinic of Obstetrics and Gynecology at the Medical University of Warsaw in June 2021. The patient, previously treated at the Infertility Clinic, was referred for a diagnostic laparoscopy with examination of the patency of the fallopian tubes.

Before admission to the Department, in a gynecological examination, the uterine body was slightly enlarged, mobile, and there were no changes in the reproductive organ. The ultrasound examination showed a normal uterine body of dimensions 38 × 41 mm, homogeneous endometrium 10 mm, appendages normal on both sides, a solid lesion of approximately 50 mm, without vascular flows, the ultrasound image most likely corresponding to uterine myoma.

The patient qualified for surgical treatment. The laparoscopy revealed a normal, smooth and mobile body of the uterus, ovaries and fallopian tubes on both sides macroscopically normal. Slightly above the bottom of the uterus, there was a tumor with the appearance of a myoma, 5 × 6 cm in size, fixed to the greater omentum. In the peduncle, a feeding vessel emerging from the greater omentum was visualized, the lesion had no contact with the uterine muscle (Fig. 1 A, B). The tumor was dissected from the omentum, placed in an end bag and after morcellation was taken outside. The lesion had a firm consistency, and in a cross-section with a whitish color, macroscopically clearly indicated the diagnosis of myoma. Moreover, a chromoscopy confirmed the patency of both fallopian tubes.

The patient was discharged home without any complaints the next day after the surgery. The histopathological examination confirmed the diagnosis of uterine leiomyoma.

DISCUSSION

Parasitic leiomyomas are extremely rare. So far, the literature has described the occurrence of primary tumors that have acquired new vascularization from organs other than the uterine muscle, and iatrogenic tumors resulting from previously performed laparoscopic procedures [3, 4]. In times of widespread ultrasonography, the diagnosis of uterine leiomyomas is usually not difficult and their suspicion in ultrasound is most often confirmed by the histopathological result. In 2016. Pradip described the case of a 31-year-old female patient with a 30 cm tumor filling the abdominal cavity, which turned out to be a parasitic myoma pedunculated to the greater omentum [5]. Although

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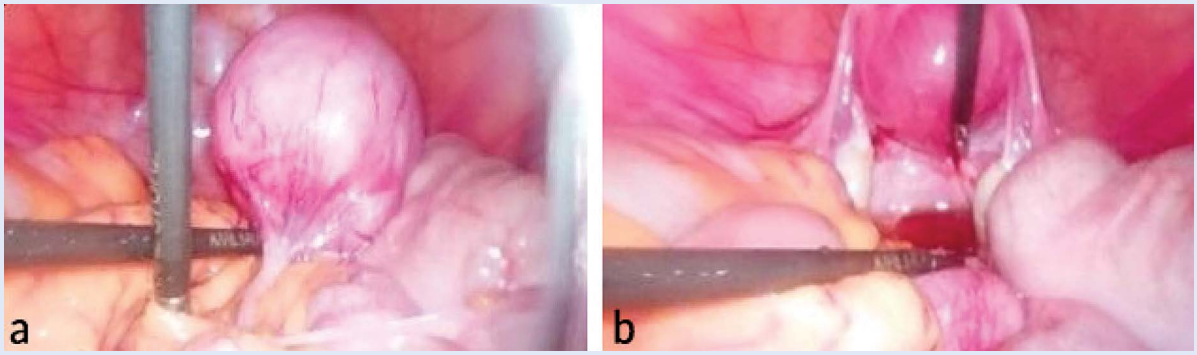

Figure 1. A. — laparoscopy photo: parasitic myoma fixed to the greater omentum, the peduncle is visible; **B. —** laparoscopy photo: uterus, fallopian tubes and ovaries; some fluid visible in rectouterine pouch

they have been described so far as casuistic cases, it is worth noting the possibility of their occurrence, especially in the era of the current development of laparoscopic techniques.

Conflict of interest

All authors declare no conflict of interest.

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