16 is expressed in the endocervical epithelium (different from the stratified exocervical epithelium, in which several cytokeratins are expressed). The endocervical epithelium is in fact a pseudoglandular epithelium: it does not actually contain glands, but epithelial invaginations occur, on a depth of 5-8 mm, forming crypts.

The role of the endocervix is that of a boundary between the lower genital tract (vulva and vagina) and the upper genital tract, that is between an environment rich in microorganisms (vaginal microenvironment) and the sterile endometrium and endosalpinx. At this level there are various influences, from the fluctuations of the hormonal secretion of the different phases of the menstrual cycle and the presence of the commensal flora (pathogenic condition), to the aggression of the germs with sexual transmission, etc. Also, the vaginal and exocervix squamous epithelia may be aggressed by chemical agents or physical changes. The endocervix is the gateway for germs such as *Chlamydia trachomatis, Neisseria gonorrhoeae*, Mycoplasma etc., which have a tropism for the columnar epithelium.

The major barrier role belongs to the cervical mucus. The main constituents of cervical mucus are mucins (glycosylated proteins, so far 19 have been identified). Mucins can be membrane-forming or soluble (small molecule) gel-forming. Another important component is antimicrobial peptides, capable of destroying bacterial membranes. It is interesting to note that microorganisms selectively adhere to certain mucins, produce proteases that can degrade mucins as well as other mucus defense factors.

INFECTIOUS PATHOLOGY – ENDOCERVICITIS

The patient with endocervicitis may present with any of the following symptoms or signs: leukorrhea, vaginal bleeding (intermenstrual or postcoital), pelvic pain. Also, the following may be found: dysuria, pollakiuria (by association of urethritis, periurethritis, skenitis), dyspareunia or discomfort/ vulvovaginal irritation (1).

At the gynecological examination, a white-yellowish, yellowish or yellow-green leukorrhea can be observed. Endocervical mucus can be opaque or even purulent. When a swab is inserted, its yellowish coloration is observed. A suggestive sign may be bleeding when touching the cervix (during the Pap smear).

The etiological factors of endocervicitis are, in the order of frequency: *Chlamydia trachomatis, Neisseria gonorrhoeae,* Mycoplasma and Ureaplasma (1). The focus will be on *Mycoplasma genitalium*, whose role in genital pathology is increasingly better defined. It is a microorganism of the genus Mycoplasma, class Mollicute (without its own wall, it cannot be identified by Gram staining), being the smallest prokaryote that can multiply (2). It has a parasitic lifestyle, taking most of the nutrients from the host (which it cannot synthesize). It has its own motility, attaches, and adheres to cells and internalizes, similar to Chlamydia (different from other mycoplasmas). Upon entry into the cell, it evades the host's immune system (lymphocyte suppression and up-regulation of cytokine expression). It can destroy the host cell by secreting a toxin and/ or releasing hydrogen peroxide. It has a slow growth on culture media, detectable by nucleic acid amplification tests (NAATs). It is certainly different from *Mycoplasma hominis* and *Ureaplasma urealyticum*. There are still debates if it belongs to the germs that cause sexually transmitted diseases (STDs), although its transmission is demonstrated by identifying an identical genome in partners. It is located on the ciliated epithelia of the urinary and genital tract, and the environment rich in progesterone promotes colonization. It has been isolated in the respiratory tract and synovial fluid, but appears to be pathogenic only at the urogenital level. The resulting clinical picture is the consequence of the host organism's response to cell invasion by mycoplasmas. It seems to persist for years in the infected body, many specialists suggesting a possible involvement in the triggering of autoimmune diseases (3).

Risk factors for endocervical infection are: age under 20 years, non-white race, lower level of education, smoking, increased number of sexual partners (over 2 in the last 12 months), vaginosis, its prevalence being 1% in the sexually active adult population (between Gonococcus 0.4% and *Chlamydia trachomatis* 2.4%).

Mycoplasma endocervicitis is considered to have a similar incidence as Chlamydia infection in women considered "at risk" - early onset of sexual life, large number of sexual partners (6% Mycoplasmas, 10% Chlamydia); it is often asymptomatic or with symptoms that if carefully sought, suggest cervicitis, urethritis ("urethral syndrome" – dysuria, discomfort during and after urination, pollakiuria), endometritis, pelvic inflammatory disease, most often chronic, with reactivations.

The possible association with bacterial vaginosis should be mentioned (clue cells are observed on the smear collected from the vagina, but paradoxically also an inflammatory reaction). Long-term effects (male and female infertility) and probable obstetric complications (premature birth, less the risk of miscarriage) should not be forgotten. Finally, the hematogenous dissemination at the site of the primary infection should be noted, with manifestations at

imaging tests (transvaginal ultrasound of the cervix) (Figures 3, 4).

At the transvaginal ultrasound, it is recommended to apply additional pressure with the transducer. The presence of a hyper or hypoechoic mobile formation can be observed in the endocervical canal, which "slides" during the examination. The presence of a blade of fluid (mucus) in the endocervical canal makes the examination much easier. Doppler examination allows the identification of the vascular pedicle and especially its origin (thus differentiating a formation with cervical origin from an endometrial polyp).

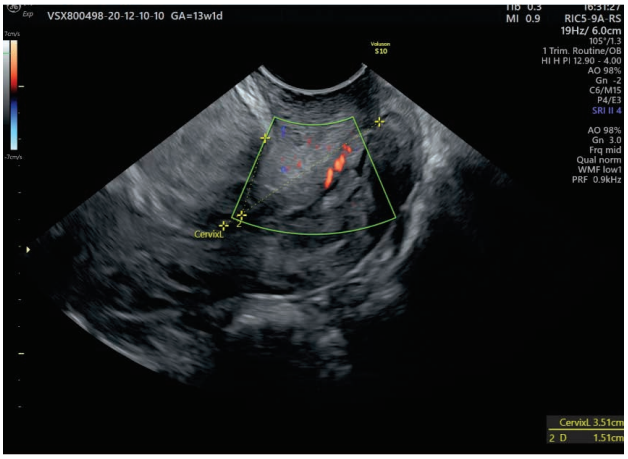

FIGURE 3. Transvaginal two-dimensional ultrasound image of the cervical canal, containing a hyperecogenic protrusive lesion. The color Doppler window looks for pedicle artery and its origin (personal collection of Roxana Bohîlțea)

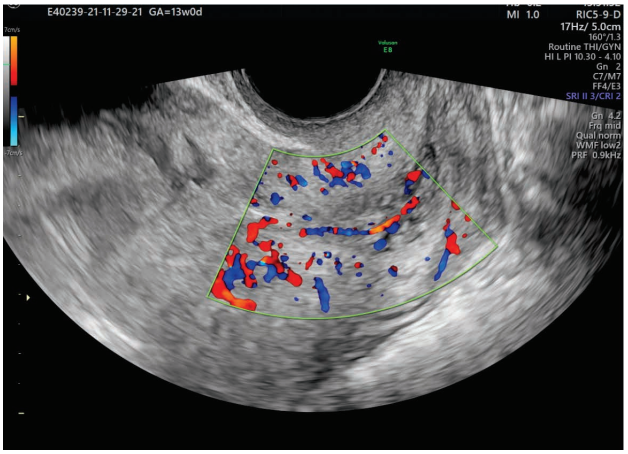

FIGURE 4. Transvaginal 2D ultrasound image of the cervix containing a mobile hyperecogenic lesion. The color Doppler reveal the implantation site that is at the level of internal os (personal collection of Roxana Bohîlțea)

NABOTH CYSTS

Naboth cysts are retention cysts present in the endocervical epithelium. They are formed by covering the holes of the "glands" (crypts) at this level, as a spontaneous defense response of the cervix (usually post-infectious, inflammatory or after interventions on the cervix) (7). This defense process consists in stimulating the squamous metaplasia, with the consequent obstruction of the glandular orifice and the progressive accumulation of mucus. They can be single or multiple and do not exceed an average size of one centimeter (8).

They can be observed both on the surface of the exocervix (by direct or colposcopic examination), but also at the cervical canal (by transvaginal ultrasound - single or multiple anechoic or hypoechoic images in the endocervical mucosa, some with expansion on stromal cells, with negative Doppler signal) (Figure 5). Very rarely, an agglomeration of Naboth cysts can be seen on ultrasound, which appears as a complex multicystic formation (9).

They are not considered pathological, because they do not present any annoying symptoms.

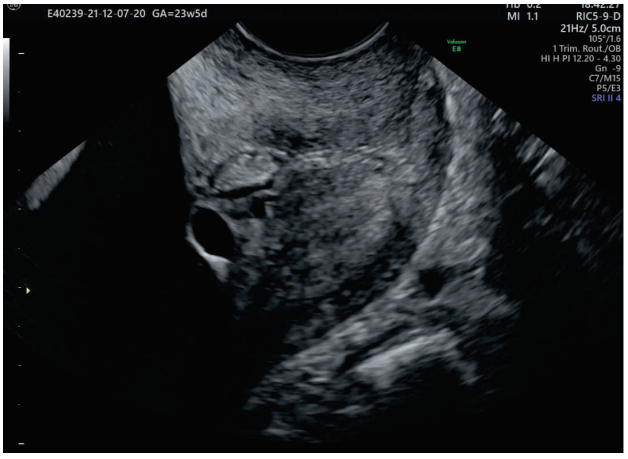

FIGURE 5. Transvaginal ultrasound show an irregular cervical canal; the arrow point to a Naboth cyst, above of it being noticed a probable old calcified one (personal collection of Roxana Bohîlțea)

CERVICAL FIBROIDS

Cervical fibroids are rare (less than 10% of all uterine fibroids). Their origin is probably the isthmic area, because the cervix is almost devoid of muscle fibers. Depending on the direction of growth of these smooth muscle formations, the neighboring symptoms appear (dysuria, pollakiuria, ureteral obstruction, dyspareunia, or obstruction of the endocervical canal). Sometimes, a submucosal uterine fibroid can protrude into the endocervix or even outside, into the vagina.

They are diagnosed during the examination with valves, at the digital vaginal examination or most frequently on imaging examination, by transvaginal ultrasound.

Transvaginal ultrasound shows a hypoechoic, regular, well-defined formation, located in the en-

docervical stroma. Depending on its structure (cystic, fatty degeneration, or calcification) the ultrasound appearance may be more complex (Figure 6). Doppler examination can show the uterine origin of fibroids by visualizing the vascular pedicle.

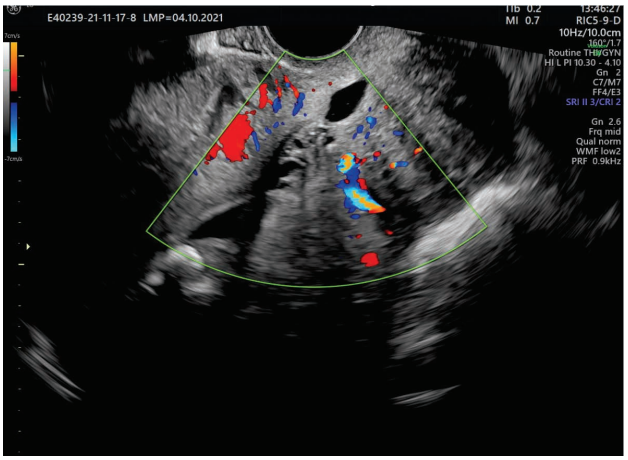

FIGURE 6. 2D transvaginal ultrasound reveals complex lesionsof the cervix; Naboth cyst (blue arrow), protrusive smooth surface lesion (orange arrow) and a cervical fibroid with its feeding pedicle (dashed line) (personal collection of Roxana Bohîlțea)

SECONDARY STENOSES OF THE UTERINE CERVIX

Secondary stenoses of the uterine cervix are obstructions in the internal os and may be consecutive to infections, surgery in the area, post-irradiation, and due to postmenopausal or neoplastic atrophy (10). The most common are iatrogenic, occurring after diathermy loop excisions, conization or electrocoagulation of the cervix (11,12).

The symptoms vary depending on the degree of stenosis (complete or incomplete) and the presence or absence of menstruation. Women of reproductive age may have symptoms such as pelvic pain, dysmenorrhea, abnormal bleeding, amenorrhea, or infertility (in the context of secondary endometriosis), while in postmenopause, patients are asymptomatic for a long period of time, then developing hematometra, hydrometra or even pyometra. The diagnosis is established based on clinical suspicion.

Ultrasound examination can detect collections in the uterine cavity. If the stenosis occurred at the level of the "new" external os postoperatively, accumulation of mucus and/ or blood may appear in the endocervical canal (inhomogeneous appearance on ultrasound examination, with negative Doppler signal). Stenosis is demonstrated by the inability to penetrate the cervical canal with a Hegar dilator 1 mm or 2 mm.

CERVICAL ENDOMETRIOSIS

Cervical endometriosis is one of the rarest locations of the disease (0.15-2.5% of cases of endometriosis). The location can be shallow, on the exocervix, or deeper.

Patients may be asymptomatic or present with pelvic pain, vaginal bleeding, or superficial dyspareunia. In cases of deep, infiltrative endometriosis with evolution towards the cervix, the symptoms can become important, even disabling (severe dysmenorrhea, pelvic pain, deep dyspareunia).

Superficial endometriosis of the uterine cervix is found on examination of the cervix or on colposcopy (bluish-violet lesion, much more obvious during menstruation). There are endometriotic lesions that develop in the depth of the stroma, but also deep, infiltrative lesions that extend from the pelvic cavity to the back of the cervix, pushing it backward or deforming it, which can be suspected at the gynecological examination.

Ultrasound examination describes a formation with a complex structure, quite difficult to differentiate from a polyp or myoma (hypoechoic images, worse delimited (ill-defined)). Association with other endometriotic lesions in the pelvis may aid in establishing the diagnosis (Figure 7).

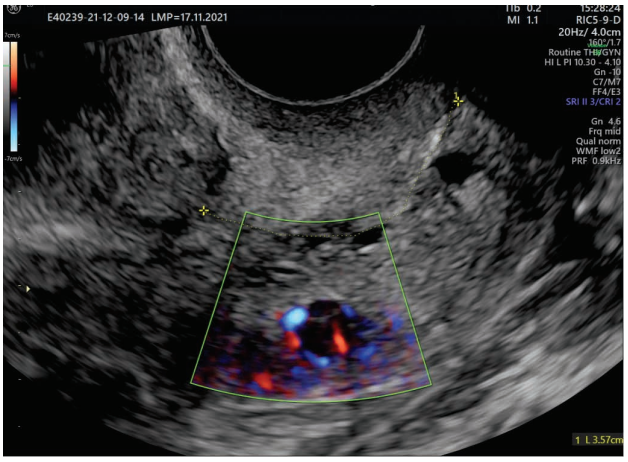

FIGURE 7. Cervical hypoecogen adenomiosis lesion with translezional flow on color Doppler (personal collection of Roxana Bohîlțea)

GLANDULAR PRENEOPLASTIC CERVICAL LESIONS

Diagnosing glandular intraepithelial preneoplastic lesions remains a challenge for any clinician. Severe glandular atypia can coexist in over 50% of cases with squamous lesions (so-called mixed lesions). It should also be emphasized that glandular lesions may not be detected on cytology tests, representing surprises of the pathological diagnosis on the excision sample. These diagnostic difficulties are determined on the one hand by the fact that the exfoliative cytology (Pap test) is superior in diagnosing squamous lesions compared to glandular ones, and on colposcopic examination the lesions may not be visible (located high in the endocervical canal) or, even if they are visible, they are difficult to recognize (13).

Risk factors for high-grade cervical lesions are: immunosuppression (concomitant HIV infection or immunosuppressive medication), smoking (nicotine degradation products present in the cervical mucus decrease local immunity), concomitant genital infectious diseases (Mycoplasma genitalium) and familial factors (familial aggregations are described, without the identification of a specific risk factor).

The main oncogenic factor is persistent high-risk strains HPV infection. Among the known strains, strains 16, 18, 45, 52, 35, 31, 33 are frequently found in endocervical glandular lesions. Although HPV infection underlies cervical oncogenesis, a viral infection is detected in less than 45% of patients with glandular lesions (14).

For the diagnosis of intraepithelial glandular lesions, the following can be used: cervical cytology by Pap smear, HPV testing, colposcopic examination, confirmation of lesions is mandatory by histopathological examination (the examined samples can be taken by endocervical biopsy curettage or cone biopsy).

It is recommended to complete the examination with a transvaginal ultrasound (thorough examination of the cervix and endometrium), and in patients over 35 years old it is mandatory to perform endometrial cavity curettage, with histopathological examination. Young patients, less than 35 years old, with atypical glandular cytological result and risk factors for chronic anovulation also benefit from examination of the endometrium (15).

INTERPRETATION OF PAP SMEAR RESULTS WITH GLANDULAR AFFECTION

Atypical glandular cells are found in a few Pap smear results (0.4-0.6%), being more common in women over 40 years old. In most cases, they reveal only intraepithelial lesions, but in about 20% of cases, a neoplastic lesion (endocervix, endometrium or endosalpinx) is confirmed, which must be sought and should not be missed under any circumstances.

According to current Bethesda terminology, abnormal cytology results in glandular cells can be the following: atypical glandular cells (AGC) (endocervical cells not otherwise specified (NOS); endometrial cells – NOS; glandular cells – NOS); atypical glandular cells – endocervical cells, in favor of neoplasia (cells have marked changes, but still insufficient to be interpreted as adenocarcinoma); glandular cells, in favor of neoplasia; endocervical adenocarcinoma in situ; adenocarcinoma: endocervical; endometrial; extrauterine; not otherwise specificed (NOS).

From the cytologist's point of view, AGC is a difficult cytological diagnosis. An endometrial cell/ reactive endocervical cell/ tubal metaplasia/ cervical endometriosis may mimic adenocarcinoma in situ (AIS). When squamous cell changes coexist, the main substrate is most likely a squamous lesion. If AGC is detected in a patient over 50 years old, endometrial cancer is most commonly diagnosed. If AGC is detected in women under 40 years old, a major squamous lesion (more than CIN 2) can be expected. In patients with repeated AGC NOS, the most likely substrate is endometrial cancer (16).

In addition to intraepithelial or neoplastic lesions, atypical glandular cells may be caused by glandular and squamous reactive and regenerative changes, Arias Stella reaction, cervical polyps, tubal or serous metaplasia, cervical endometriosis, microglandular hyperplasia, or changes associated with an intrauterine device (Figure 8) (17).

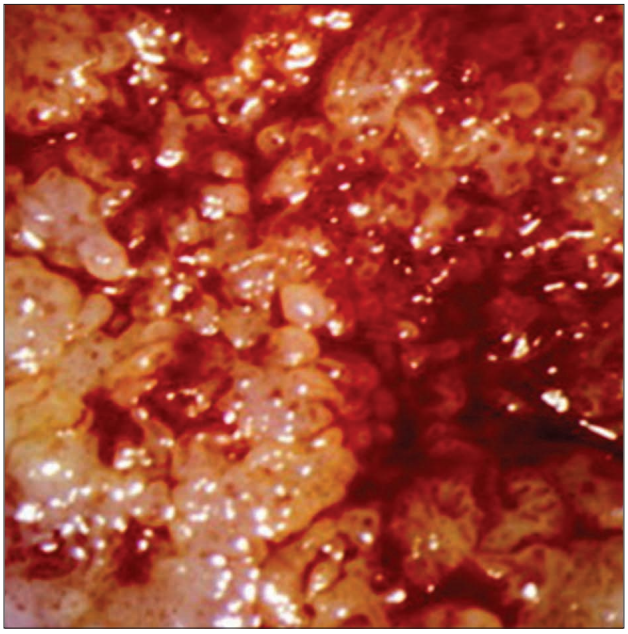

FIGURE 8. Colposcopic image of glandular lesion (personal collection of Corina Grigoriu)

MOLECULAR DIAGNOSIS OF HPV INFECTION

1. HPV DNA detection tests (detect the presence of oncogenic HPV DNA, without specifying the type).
2. HPV genotyping tests allow the identification of high-risk oncogenic viruses; the tests approved and currently used worldwide are the Hybrid Capture 2, Cervista or Cobas 4800 (PCR). The Cobas test identifies the types separately. HPV 16 and 18 and another group of 12 oncogenic types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68). This test is the first HPV test to be approved as the only screening test for cervical cancer.
3. HPV RNA isolation tests, which identify the expression of E6 and E7 genes (e.g., Aptima test).

molecular tests for the diagnosis of HPV infection, colposcopy, but also by thorough ultrasound examinations. Ultrasound examination of the cervix should be part of the routine examination, because its systematic evaluation can significantly contribute to refining the diagnosis.

Conflict of interest: none declared
Financial support: none declared

REFERENCES

1. Workowski KA, Bachmann LH, Chan PA, Johnson CM, Muzny CA, Park I, Zenilman JM, Bolan GA: Sexually Transmitted Infections Treatment Guidelines, 2021. *MMWR Recomm Rep.* 2021;70(4):1-187.
2. Gaydos C, Maldeis NE, Hardick A, Hardick J, Quinn TC: Mycoplasma genitalium as a contributor to the multiple etiologies of cervicitis in women attending sexually transmitted disease clinics. *Sex Transm Dis.* 2009;36(10):598-606.
3. Lis R, Rowhani-Rahbar A, Manhart LE: Mycoplasma genitalium infection and female reproductive tract disease: a meta-analysis. *Clin Infect Dis.* 2015;63):418-426.
4. Mitchell L, King M, Brillhart H, Goldstein A: Cervical Ectropion May Be a Cause of Desquamative Inflammatory Vaginitis. *Sex Med.* 2017; 5(3):e212-e214.
5. Gorgos LM, Sycuro LK, Srinivasan S, Fiedler TL, Morgan MT, Balkus JE, McClelland RS, Fredricks DN, Marazzo JM: Relationship of Specific Bacteria in the Cervical and Vaginal Microbiotas With Cervicitis. *Sex Transm Dis.* 2015;42(9):475-481.
6. Kletzel HH, Rotem R, Barg M, Micheli J, Reichman: Ureaplasma urealyticum: the Role as a Pathogen in Women's Health, a Systematic Review. *Curr Infect Dis Rep.* 2018;20(9):33.
7. Taylor SN, Lensing S, Schwebke J, Lillis R, Mena LA, Nelson AL, Rinaldi A, McNeil L, Lee JY: Prevalence and treatment outcome of cervicitis of unknown etiology. *Sex Transm Dis.* 2013;40(5):379-385.
8. Oliveto JM, Muinov L. Cystic Cervicitis: A Case Report and Literature Review of Cystic Cervical Lesions. *J Comput Assist Tomogr.* 2016; 40(4):564-546.
9. Mattson SK, Polk JP, Nyirjesy P. Chronic Cervicitis: Presenting Features and Response to Therapy. *J Low Genit Tract Dis.* 2016;20(3):e30-33.
10. Phadnis SV, Atilade A, Bowring J, Kyrgiou M, Young MPA, Evans H, Paraskevaidis E, Walker P: Regeneration of cervix after excisional treatment for cervical intraepithelial neoplasia: a study of collagen distribution. *BJOG.* 2011;118(3):1585-1591.
11. Kyrgiou M, Athanasiou A, Paraskevaidi M, Mitra A, KAlliala I, Martin-Hirsch P, Arbyn M, Bennet P, Paraskevaidis E: Adverse obstetric outcomes after local treatment for cervical preinvasive and early invasive disease according to cone depth: systematic review and meta-analysis. *BMJ.* 2016;354:i3633.
12. Niyibizi J, Zanré N, Mayrand MH, Trottier H: Association Between Maternal Human Papillomavirus Infection and Adverse Pregnancy Outcomes: Systematic Review and Meta-Analysis. *J Infect Dis.* 2020; 221(12):1925-1937.
13. Zhong P, Yin C, Jin Y, Chen T, Zhan Y, Tian C, Zhu L, Zheng X: More focus on atypical glandular cells in cervical screening: Risk of significant abnormalities and low histological follow-up rate. *Cytojournal.* 2020; 17:22.
14. Kawano K, Yamaguchi T, Nasu H, Nishio S, Ushijima K: Subcategorization of atypical glandular cells is useful to identify lesion site. *Diagn Cytopathol.* 2020; 48(12):1224-1229.
15. Aitken CA, Jansen EEL, Siebers AG, van Haaften-de Jong ALD, van Kemenade FJ, de Kok IMCM: Risk of Gynecologic Cancer after Atypical Glandular Cells Found on Cervical Cytology: A Population-Based Cohort Study. *Cancer Epidemiol Biomarkers Prev.* 2021;30(4):743-750.
16. Komatsu H, Oishi T, Osaku D, Sawada M, Kudoh A, Nonaka M, Chikumi J, Sato S, Harada T: Significance of High-Risk Human Papillomavirus Testing for Atypical Glandular Cells on Cervical Cytology. *Acta Cytol.* 2018;62(5-6):405-410.
17. Fachetti-Machado G, Figueiredo-Alves RR, Moreira MAR: Performance of Conventional Cytology and Colposcopy for the Diagnosis of Cervical Squamous and Glandular Neoplasias. *Rev Bras Ginecol Obstet.* 2018; 40(7):410-416.
18. Cleveland AA, Gargano JW, Park IU, Griffin MR, Niccolai LM, Powell M, Bennett MN, Saadeh K, Pemmaraju M and all: Cervical adenocarcinoma in situ: Human papillomavirus types and incidence trends in five states, 2008-2015. *Int J Cancer.* 2020;146(3):810-818.
19. Gadducci A, Guerrieri ME, Cosio S: Adenocarcinoma of the uterine cervix: Pathologic features, treatment options, clinical outcome and prognostic variables. *Crit Rev Oncol Hematol.* 2019;135:103-114.
20. Perkins RB, Guido RS, Castle PE, Chelmow D, Einstein MH, Garcia F, Huk WK, Kim JJ, Moscicki AB and all: 2019 ASCCP Risk-Based Management Consensus Guidelines for Abnormal Cervical Cancer Screening Tests and Cancer Precursors. *J Low Genit Tract Dis.* 2020;24(2):102-131.