REVIEW Libri Oncol., Vol. 43 (2015), No 1-3, 33 – 39

CYTOLOGY IN DIAGNOSIS OF OVARIAN CANCER

VESNA RAMLJAK, MERDITA AGAI MUHAXHIRI, IVA BOBUŠ KELČEC

Department for Cytology, University Hospital for Tumors,
University Hospital Center Sestre milosrdnice, Zagreb, Croatia

Summary

Cytology has so far been underutilized as a modality for the primary diagnosis of ovarian cancer. Lately the outlook on fine needle aspiration cytology (FNAC) has greatly shifted. With the availability of modern techniques, USG and CT guided FNAC is becoming an optimum modality for the diagnosis of primary and metastatic ovarian neoplasms and evaluation of recurrent malignant tumors, which consequently has a great impact on patient management. The most common indication for peritoneal washing cytology is staging or upstaging of ovarian carcinomas (First - look). Second - look procedures are performed in patients previously treated by surgery, radiotherapy and/or chemotherapy to determine the presence of residual or recurrent ovarian cancer. In both procedures cytology has proven itself as a useful diagnostic method.

KEY WORDS: *ovary, aspiration cytology, peritoneal cytology, ovarian tumors, diagnosis*

CITOLOGIJA U DIJAGNOZI RAKA JAJNIKA

Sažetak

Kao dijagnostička metoda, citologija je do nedavno bila nedovoljno uključena u primarnu dijagnostiku raka jajnika. No, u novije se vrijeme stav prema aspiracijskoj citologiji tankom iglom jasno mijenja. Uz dostupne suvremene tehnologije, ultrazvukom i CT-om vođena citološka punkcija postaje optimalna metoda za dijagnozu primarnih i metastatskih novotvorina jajnika, te za rano otkrivanje relapsa maligne bolesti. Time se citologija izravno upliće i u management liječenja pacijenta. Citološka pretraga ispirka trbušne šupljine najčešće je indicirana u određivanju stadija karcinoma jajnika (tzv. „prvi pogled“). Zahvati tzv. „drugog pogleda“ izvode se na bolesnicima koji su prethodno tretirani kirurški, zračenjem i/ili kemoterapijom da bi se odredila ostatna bolest ili recidiv karcinoma jajnika. U oba navedena slučaja („prvi i drugi pogled“) citologija se dokazala kao korisna dijagnostička metoda.

KLJUČNE RIJEČI: *jajnik, aspiracijska citologija, peritonealna citologija, tumori jajnika, dijagnoza*

INTRODUCTION

The very high mortality from ovarian cancer reflects the dissemination of the tumor at the time of the diagnosis because of absence of symptoms in the early stage of the disease. The disease occurs mainly in women past the age of 40 with median age of 58 at the time of diagnosis. The overall 5 year survival rate is only 40% and is stage depended.

Cytology has so far been underutilized as a modality for the primary diagnosis of ovarian cancer. This can be ascribed to (i) accuracy of imaging techniques like ultrasound and CT scan in detecting malignancy and omental or peritoneal deposits, (ii) large variety of tumours are difficult to subtype by cytology alone, (iii) fear of dispersing tumor cells into the peritoneal cavity, (iv) inexperience of cytopathologists in interpreting ovarian lesions and (v) the view among a substantial num-

ber of clinicians that preoperative fine needle aspiration cytology (FNAC) might delay an already indicated surgical treatment. There have been limited studies on FNAC in diagnoses of ovarian cancers (1).

However there are certain situations where cytology both FNA as well as exfoliative have an important role to play:

1) In distinguishing non neoplastic cyst from true neoplasms of the ovary
2) In detecting suspected pelvic recurrences in known cases of ovarian cancers
3) In arriving at a primary diagnosis of ovarian cancer in patients who are poor surgical risk
4) Patients already having a disseminated disease at presentation
5) In detected metastatic tumour deposits at unusual sites like cutaneous location in known cases of ovarian cancers
6) Ascitic or pleural fluid cytology for detection of malignant cells
7) Peritoneal washings at the time of surgery to detect peritoneal deposits (1).

There is clear association between the stages and prognosis of ovarian malignant tumors. Since two-thirds of epithelial ovarian cancer cases present at advanced stages and have a low 5-year survival rate, early evaluation of ovarian lesions is very important (2,3). Those women diagnosed with disease confined to ovary often require less aggressive surgical intervention, may not require chemotherapy and have an overall 5-year survival rate of approximating 90% (2).

OBTAINING THE SPECIMEN

Taking cytological matherial should always be performed under radiologic guidance, preferably ultrasound. Transvaginal route is a favoured approach. Transabdominal, transrectal, laparoscopic and aspiration at the time of laparotomy are some of the other approaches possible. Transabdominal approach is suited for large masses with omental deposits. The method used depends on the size of the lesion, its location, and the resources available to the person performing the aspiration (4). Complications are uncommon. Although common wisdom holds that puncture of a malignant tumor can cause seeding of the peritoneal cavity, documented cases are actually rare (5). If any ultrasound features are worrisome, the physician will usually forego aspiration and recommend surgery. Aspiratin of suspicious ovaran masses should be avoided, except to confirm malignancy in patient with inoperable or metastatic disease.

PREPARING THE SPECIMEN AND REPORTING RESULTS

Specimens are usually cyst fluids. Standard method are used in preparation. A portion of fresh fluid can be used to measure the level of E2 or tumor-associated antigens cancer antigen 125 (CA-125), carcinoembryonic antigen (CEA), and alphafetoprotein (AFP). Elevated levels are typical of some ovarian lesions and can be useful adjunct to cytologic examination (6,7). FNA of the ovary is generally reserved for small, incidental masses that appear benign on sonographic or laparoscopicexaminarion (1-3). Incidental ovarian cysts are often discovered in women with infertility or during pregnancy (4). Aspiration cytology, in combination with a benign ultrasound appearance, is used to reassure the patient that an oophorectomy is not necessary (5). In some laboratories, estradiol (E2) levels in the fluid are also measured because these are usually elevated in folicle-derived cysts but not in epithelial lesions (6,7). Thus, ultrasound, cytology and E2 levels can form an effective „triple test" for distinguishing benign from malignant ovarian cyst.

Specimens that are virtually acellular are best termed *non-diagnostic* (8,9). The percentage of FNAs of the ovary that are non-diagnostic ranges widely, from a low of 13% to a high of 72% (4,10). This variation is likely related to the type of lesion selected for aspiration and the definition of a non-diagnostic specimen. The cytology report should state that malignant cells are either absent(no malignant cells identified) or present (positive for malignant cells). If the finding is equivocal, the report should state either that atypical or suspicious cells are present. Benign ovarian FNA results fall into two broad categories: 1. Follicular or lutein cysts, and 2.epithelial cyst. This has significant clinical relevance because surgery is unnecessary for follicle-derived cysts, which usually regress over time. Surgery may be indicated, how-

ever, for a benign-appearing epithelial cyst, particulary if the sonographic findings are worrisome (11).

BENIGN TUMOR-LIKE LESIONS OF THE OVARY

Non-neoplastic cysts

Benign ovarian cysts are common; the majority are discovered incidentally by USG laparoscopy, or laparotomy. The most common are the so-called functional cysts and the second major categorie are nonfunctional cysts derived from ovarian surface epithelium or endometriosis. Precise classification by cytologic examination is not always possible, particulary when only cyst contents (fluid and macrophages) are obtained (12). One of the most common, cystic lesions of the ovary, they arise from an ovarian follicle and are not neoplastic but rather physiologic. Follicle cysts can be solitary or multiple and range in size up to 8cm or more in diametar. Because follicle cyst appear benign sonographically and laparoscopically, they are managed conservatively, with an eye on preserving the ovary. FNA is commonly used as a minimally invasive method for sampling the lesion and confirming it is in fact benign. When ciliated or mucinous epithelium is identified, the cyst is of surface epithelial origin and not a follicle cyst. E2 levels in cyst fluid as measured by radioimmunoassay are helpful; elevated E2 concentracion correlates strongly with cysts of folicular origin (10,13). Eighty-one percent to 90% of follicle cysts have an e2 content greater than 20nmol/L, and in 97% to 99% of nonfollicular cysts the E2 content is less than 20 nmol/L. The other non-neoplastic cysts are corpus luteum cyst, endometriotic cyst, simple ovarian, paraovarian and paratubal cyst (7,14).

Hydrosalpinx, a complication of salpingitis, presents as large cystic adnexal mass. The distended fallopian tube is filled with clear fluid and is lined by ciliated epithelium. These findings are identical to those seen with serous ovarian and parovarian cysts (15).

Tubo-ovarian abscess is an advanced complication of acute salpingitis, known clinically as pelvic inflamatory disease. Most cases resultfrom an ascending infection of the lower genital tract by sexually transmited pathogens, of which the most common are Neisseria gonorrhoeae, Chlamydia trachomatis, and Mycoplasma genitalium (15).

SCOPE OF CYTOMORPHOLOGY IN DIAGNOSIS OF COMMON MALIGNANT OVARIAN NEOPLASMS

Borderline tumours of ovary

Borderline serous & mucinous neoplasms are difficult to differentiate from malignant counterpart as invasion cannot be documented on cytology. They usually present as highly cellular smears with nuclear features ranging from bland to highly atypical. A definitive diagnosis of a borderline ovarian surface epithelial tumour is not possible on cytologic material (1).

Malignant tumours of ovary

The cytologic diagnostic accuracy for ovarian carcinoma is in the range of 90-95% tumours. A negative diagnosis in a suspected case does not rule out malignancy and should warrant a repeat procedure. Subtyping is relatively easier in the low and intermediate grade tumours, but is not possible in the high grade varieties (1). The principal groups of ovarian carcinomas are: Serous carcinomas, Mucin-producing carcinomas and Endometrioid carcinomas. Direct aspiration from ovarian carcinomas are, as a rule, richer in cells than are aspirates from benign epithelial tumors. The cytologic recognition of a malignant tumor is usually not difficult. Even in well-differentiated types of carcinoma, the smeared aspirate contains approximately spherical (papilary)groups of cancer cells, often with characteristic nuclear features, such as enlargement andhyperchromasia, large nucleoli, and thickening of the nuclear membrane. In serous adenocarcinoma, the cells may form a monolayer, but such a finding is insufficient for a reliable distinction between a serous and endometrioid carcinoma. Psammoma bodies are rarely seen in direct aspirates. Mucinous ovarian carcinoma may be recognized by the presence of mucus-producing columnar cells embedded in masses of mucin. Clear cell adenocarcinomas may resemble clear cell adenocarcinoma of the kidney. The germ cell tumours (GCT) constitute the majority of ovarian masses in the pediatric age group. Most GCTs can be reliably diagnosed and sub-

typed on FNAC, in conjunction with tumour marker levels and immunocytochemistry. Endometrioid tumours display features like those of the endometrial counterpart, but may be difficult to distinguish from serous cytadenocarcinomas. Malignant mixed mullerain tumours can only be suspected when both epithelial and sarcomatous component are aspirated, otherwise it may be classified as an adenocarcinoma. Granulosa cell tumour can be diagnosed on FNA in the presence of acinar like structures with central reddish-violet bodies called as Callexner bodies and cells with nuclear grooves. Aspirates from stromal tumours of ovary may yield very sparse material and may be difficult to diagnose on FNAC.

The accuracy of aspiration biopsy cytologic diagnosis in patient with ovarian enlargement or cancer was reported by Kjellgren and Angstrom (1971, 1979), Geier et all (1975), Nadji et all (1979) and Geier and Strecker (1981). In these reports, ovarian cancer was accurately identified in approximately 85% to 9o% of cases. The proportion of false-positive cytologic reports in histologically benign lesions varied from 0% to about 5%. None of these authors reported on spread of tumor cells after the procedure (16).

In about 20% to 30% of patients with advanced ovarian carcinoma, regardless of histologic type, malignant cells may be observed incervicovaginal preparations and occasionally in endocervical and endometrial aspirates. (Jobo et all) Conversely, the presence of ovarian cancer cells in cervicovaginal smears usually, but not always, indicates advanced disease. The cancer cells may be derived from a primary tumor, via the fallopian tubes and the endometrial cavity, but may also reflect metastatic foci either within the endometrial cavity or in the vagina. When seen in cervicovaginal smears or in endometrial aspirates, the tumor cells of nearly all ovarian cancers form clusters, often of papillary configuration, made up of large malignant cells with prominent, large nuclei, containing multiple, often large, irregular nucleoli (16, 15).

Psammoma bodies are commonly found in serous carcinoma of the ovary, less commonly in the bordeline serous tumors, very rarely in endometrioid carcinomas,and practically never in mucous tumors. Endosalpingiosis is the most important entity in the differential diagnosis of serous ovarian carcinomas with which it shares the presence of numerous psammoma bodies in cervicovaginal smears and other cytologic preparation. The presence of psammoma bodies in a cervicovaginal preparation or in endocervical or endometrial aspiration, particulary in the absence of an intrauterine contraceptive device, calls for a thorough investigation of the female genital tract to rule out of malignant tumor, most likely of ovarian origin (16).

Role of immunocytochemistry

Most of the surface epithelial malignancies can be diagnosed without resort to immunocytochemistry. Immunocytochemistry for inhibin in

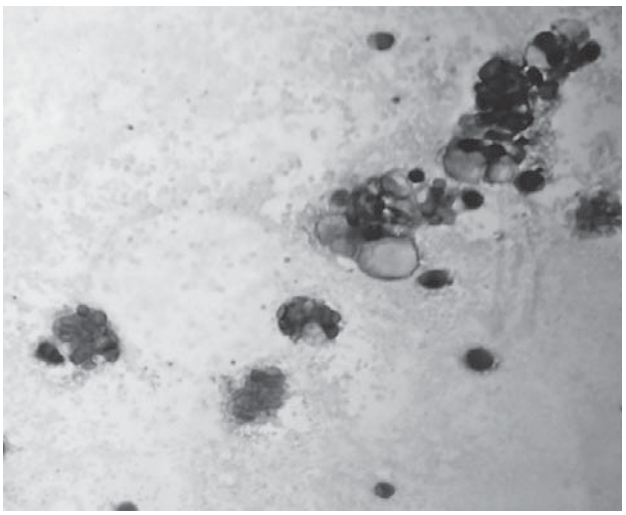

Figure 1. Immunocytochemistry, CK 7

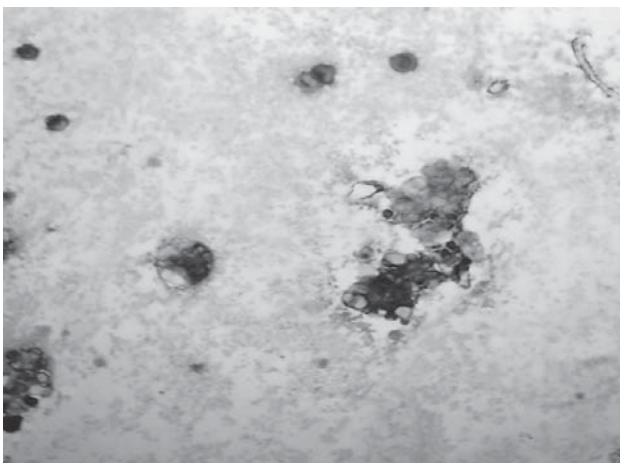

Figure 2. Immunocytochemistry, Ca125

conjunction with Ber EP4, CK 7 and CA-125 may help in differentiating follicular cells in nonneoplastic cyst aspirates from epithelial proliferations in neoplastic masses. Metastatic tumours may also be distinguished from primary using animmunocytochemistry panel. Antibodiesagainst PLAP, AFP and beta HCG may help in classifying germ cell tumours (1).

INTRAOPERATIVE FNA FROM IN SITU MASS LESIONS DURING OPERATION

As a diagnostic tool, intraoperative cytology offers simplicity, speed, accuracy, excellent cellular detail and thus several adventages over conventional frozen section techniques. It provides accurate results in minutes while the patient is under anesthesia. The surgeon often needs microscopic confirmation of the clinical diagnosis before undertaking major resection. Microscopic diagnosis is required for planning subsequent menagement of irresectable lesions. Intraoperative cytology permits microscopic evaluation of lesions that are not suitable for surgical biopsy and can be used as an alternative to surgical biopsy in selected case. Also, FNAC permits more accurate sampling from deeply seated lesions. Unfortunately, it is not being used to its fullest extend. Percutaneous fine needle aspiration is associated with advanced disease and is highly specific in predicting unresectability of some adenocarcinomas, resulting in decreased survival.

METASTATIC TUMORS

The most common tumors that metastasize to the ovaries arise in the urogenital tract, colon, stomach and breast. Approximately 15% to 20% of bilateral ovarian malignancies are metastatic. Tumors that occur as multiple nodules on the ovarian surface are likely to be metastatic. Krukenberg tumors are characterized by mucin-filled signet ring-shaped cells metastatic to the ovary (17). The majority of these tumors arise in the stomach, but tumors of the colon, appendix, andbreast also cause this pattern of spread.

In many cases, distinguishing between a primary ovarian carcinoma and a metastasis is impossible by cytomorphology alone. Measuring the concentration of tumor-associated antigens in cyst fluid is useful;unlike ovarian carcinomas, metastatic colon cancers have a high CEA level combined with a low CA-125 level (7).

PERITONEAL CYTOLOGY

Peritoneal dissemination of malignant cells is a significant prognostic variable in gastric and gynecologic malignancies which means that peritoneal cytology is clinically useful in both of them (Figure 3,4,5). FIGO incorporated the results of peritoneal washing cytology into the staging classification of ovarian cancer in 1975. Cytologic

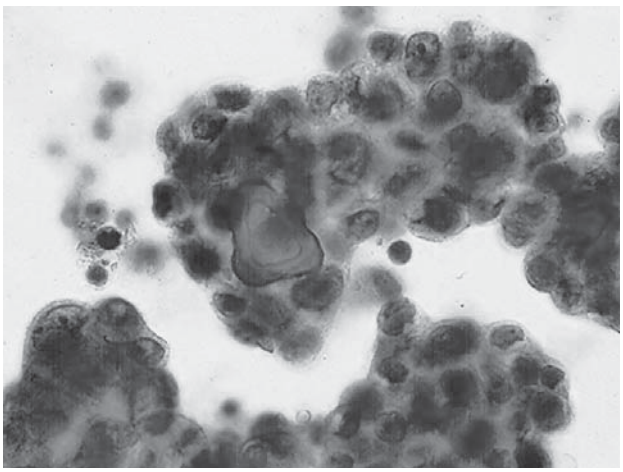

Figure 3. Ca serosum, Peritoneal washing, Psamoma body (MGGx400)

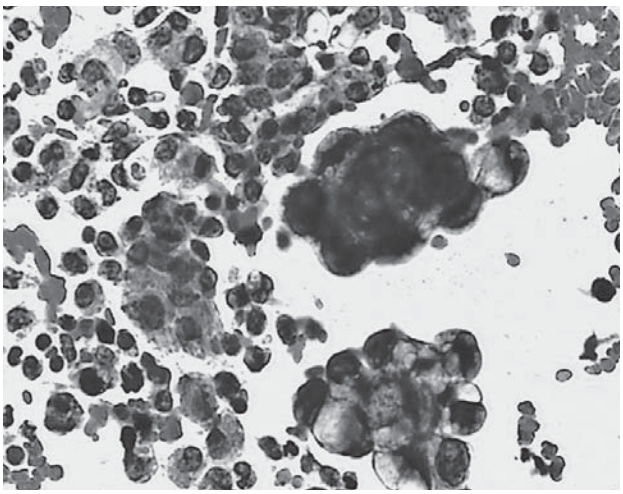

Figure 4 Ca serosum, peritoneal washing (MGGx200)

samples of peritoneal fluid are routinely obtained during staging procedures for ovarian cancer. These findings are important in substaging early (FIGO I and II) ovarian cancer; malignant cells in peritoneal washing or ascites warrant assignment of tumors to stage IC or IIC. Cytology is more sensitive in detecting ovarian carcinomain ascites than in peritoneal washes, as well as in patients with peritoneal metastases measuring greater than 0,5 cm (Fig 1.). The most important pitfall in the examination of peritoneal cytology specimens in women involves benign epithelial proliferation. Women with or without cancer can have endometriosis or endosalpingiosis involving peritoneal surfaces. These lesions often shed epithelial fragments into peritoneal washings or ascites if present (Figure 1,2). Benign fallopian tubal epithelium, if salpingitis is present, and benign eutopic endometrial tissue may also be shed into the fluid.

STAGING OF PRIMARY CARCINOMA OF THE OVARY (FIGO)

stage I.		Growth limited to the ovaries
	I. a	One ovary, no ascites and negative peritoneal washes
	I.b	Both ovaries, no ascites, negative peritoneal washes
	I. c	One or both ovaries, positive ascites or peritoneal washes
stage II.		Growth involving one or both ovaries with pelvic extension
	II.a	Extension and/or metastases to the uterus and/or tubes
	II.b	Extension to other pelvic tissues
	II.c	Tumor eighter stage Iia or Iib with positive ascites or peritoneal washing
stage III.		Tumor involving one or both ovaries with peritoneal implants including small bowel and omentum i mezenterij, pozitive retroperitoneal or inguinal lymph nodes
stage IV.		Tumor involving one or both ovaries with distant metastases

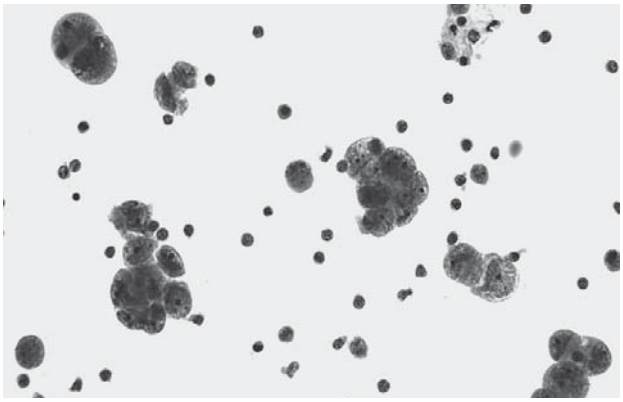

Figure 5. Ca serosum, Ascites (Papanicolaou x 200)

CONCLUSION

Cytology has so far been underutilized as a modality for the primary diagnosis of ovarian cancer. This can be ascribed to (i) accuracy of imaging techniques like ultrasound and CT scan in detecting malignancy and omental or peritoneal deposits, (ii) large variety of tumours are difficult to subtype by cytology alone, (iii) fear of dispersing tumour cells into the peritoneal cavity, (iv) inexperience of cytopathologists in interpreting ovarian lesions and (v) the view among a substantial number of clinicians that preoperative fine needle aspiration cytology (FNAC) might delay an already indicated surgical treatment.

But lately the outlook on FNAC has greatly shifted. With the availability of modern techniques, USG and CT guided FNAC is becoming an optimum modality for the diagnosis of primary and metastatic ovarian neoplasms and evaluation of reccurent malignant tumors, which consequently has a great impact on patient management. The most common indication for peritoneal washing cytology is staging or upstaging of ovarian carcinomas (First - look). Second - look procedures are performed in patients previously treated by surgery, radiotherapy and/or chemotherapy to determine the presence of residual or recurrent ovarian cancer. In both procedures cytology has proven itself as a useful diagnostic method.

REFERENCES

1. Mathur SR, Ovarian Cancer. Role of Cytology. Indian Journal of Medical & Paediatric oncology 2007;27(1): 5-6.

2. Fishman DA, Bozorgi K. The scientific basis of early detection of epithelial ovarian cancer: The National Ovarian Cancer Early Detection Programme (NOCEDP) Cancer Treat Res. 2002;107:3–28.
3. Young JG, Perez CA, Hoskins WJ. Cancer of the ovary. In: Devita VT, Hellman S, Rosenberg SA, editors. Cancer: Principles and practice of oncology. Philadelphia: Lippincott; 1993.str. 1226–3.
4. Mulvany NJ.Aspiration cytology of ovarian cysts and cystic neoplasms: A study of 235 aspirates. Acta Cytol. 1996;40:911-920.
5. Trimbos JB, Hacker NF. The case against aspirating ovarian cysts. Cancer 1993;72:823-831.
6. Geier GR, Strecker JR: Aspiration cytology and E2 cintent in ovarian tumors. Acta Cytol. 1981;25:400-406.
7. Pinto MM, Bernstein LH, Brogan DA et al. Measurement of Ca125, carcinoembryonic antigen, and alphafetoprotein in ovarian cyst fluid. Diagnostic adjunct to cytology. Diagn Cytopathol. 1990;6:160-163.
8. Greenebaum E, Mayer JR, Stangel JJ, Hughes P. Aspiration cytology of ovarian cysts in in vitro fertilization patients. Acta Cytol. 1992;36:11-18.
9. Stanley MW, Horwitz CA, Frable WJ: Cellular follicular cyst of the ovary: Fluid cytology mimicking malignancy. Diagn Cytopathol. 1991;7:48-52.
10. Mulvany Nj: Aspiration cytology of ovarian cysts and cystic neoplasms: A study of 235 aspirates. Acta Cytol. 1996;40:911-920.
11. Volmar KE, Herman CM, Creager AJ: Fine-needle aspiration cytology of endometrioid adenofibroma of the ovary. Diagn Cytopathol. 1990;31(1):38-42
12. Ramzy I, Delaney M, Rose P: Fine needle aspiration of aovarian masses: II. Correlative cytologic and histologic study of nonneoplastic cysts and noncelomic epithelial neoplasms. Acta Cytol. 1979;23:185-193.
13. Allias F, Chanoz J, Blache G et all. Value of ultrasound-guided fine-needle aspiration in the management of ovarian and paraovarian cysts. Diagn Cytopathol. 2000;22:70-80.
14. Pinto MM, Greenebaum E, Simsir A et all.Ca-125 and carcinoembryonic antigen assay versus cytodiagnostic experience in the classification of benign ovarian cysts. Acta Cytol. 1997;41:1456-1462.
15. Cibas ES. Ovary. In: Cibas ES, Ducatman BS,editors. Cytology Siagnostic Principles and Clinical Correlates. Philadelphia: Saunders, Elsevier; 2009. str. 433-451.
16. Koss LG. Koss'Diagnostic Cytology and its Histpathologic Bases. Philadelphia, Baltimore: Lippincott Williams & Wilkins; 2006. str. 503-507.
17. Uguz A, Ersoz C, Bolat F. Et all. Fine needle aspiration cytology of ovarian lesions. Acta Cytol. 2005;49(2): 144-148.

Author's Address: Vesna Ramljak, Department for Cytology, University Hospital for Tumors, University Hospital Center Sestre milosrdnice, Ilica 197, 10000 Zagreb, Croatia. e-mail: vesna.ramljak@kbcsm.hr