

ORIGINAL STUDY

The accuracy of ascites cytology in diagnosis of advanced ovarian cancer in postmenopausal women prior to neoadjuvant chemotherapy

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Abstract

Objective: To evaluate the accuracy of ascites cytology in the diagnosis of epithelial ovarian cancer among postmenopausal women.

Methods: Ascites samples of women older than 51 years sent for cytology evaluation at our institution between 2010 and 2015 were retrospectively compared to final histology. The sensitivity, specificity, negative, and positive predictive values were calculated. Immunohistochemistry stain results were collected to determine diagnostic profiles.

Results: A total of 551 patients, 51 years and over had both cytology and diagnostic histology samples. Of those, 161 patients had pathology confirmed ovarian tumors, 155 of which were malignant. Of the 155 cases of ovarian cancer, 125 patients had malignant cells on cytology examination (true positive); in 30 cases, ascites was negative for malignancy (false negative). In six cases both ascites and final pathology were negative for malignancy (true negative). There were no cases of positive cytology and negative final pathology (ie, no false-positive cases). The sensitivity, specificity, positive, and negative predictive value for cytology diagnosis of ovarian cancer were 80.6%, 100%, 100%, and 16.7%, respectively. Immunohistochemistry was done on cell blocks in 79 cases of ovarian cancer, 75 (94.9%) had profiles diagnostic for ovarian origin.

Conclusions: Ascites cytology for postmenopausal women older than 51 years with immunohistochemistry is highly accurate in diagnosis of ovarian cancer. Neoadjuvant chemotherapy can be safely prescribed based on paracentesis evaluations.

Key Words: Ascites – Cytology – Epithelial ovarian cancer – Immunohistochemistry – Malignancy – Postmenopausal.

Video Summary: <http://links.lww.com/MENO/A570>.

Ascites is a common finding in both malignant and benign conditions.¹ Because many patients with advanced epithelial ovarian cancer (EOC) have ascites, cytology examination of this easily obtained fluid is an appealing method for establishing diagnosis. This is true especially for older patients, in whom other diagnostic procedures such as laparoscopy may be postponed due to comorbidities. Data on the sensitivity and specificity of cytological

evaluation are scarce and even more so among postmenopausal patients. Ascitic peritoneal fluid, which is rich in detached cancer cells, enables the transcoelomic spread of tumor cells to other pelvic and peritoneal organs.² Malignant ascites is frequently seen in patients with EOC but also with malignancies from other origins such as the gastrointestinal tract, pancreas, and breast cancer.³ Yet, the majority of the carcinomas found in malignant ascites of female patients are

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adenocarcinomas of gynecological origin, more than 80% of which are of tubo-ovarian origin. With the introduction of neoadjuvant chemotherapy (NACT), many patients with EOC are referred for chemotherapy, based on cytology/pathology diagnosis obtained before debulking surgery.⁴⁻⁶ The introduction of immunohistochemistry (IHC) staining of cell blocks obtained from ascitic fluid enables a more accurate diagnosis as to the origin of malignant cells identified.⁷ The expressions of cytokeratin 7 (CK7) and cytokeratin 20 (CK20) using IHC are a common and useful assay for the assessment of the primary sites of metastatic adenocarcinomas. The differential expression patterns of CK7 and CK20 enable one to discriminate between the two most common ascites-producing metastatic adenocarcinomas in females, that is, colorectal and ovarian adenocarcinomas, with the latter showing CK7 positivity and CK20 negativity in more than 90% of the cases, whereas adenocarcinomas arising in the intestinal tract show the opposite pattern, especially with CK20 positivity.⁸ Other CK7+/CK20- nongynecological carcinomas can cause peritoneal ascites, mainly gastric carcinomas, pancreatic ductal carcinomas, breast carcinomas, and lung adenocarcinomas. Immunostaining with carcinoembryonic antigen (CEA) can be used for the differentiation of ovarian adenocarcinoma from lung and breast carcinomas, being negative in ovarian adenocarcinomas (with the exception of mucinous adenocarcinomas) and positive in lung, breast, stomach, and pancreatic carcinomas.⁹ Malignant mesotheliomas, which also show CK7+/CK20- and negative CEA expression, can be distinguished from ovarian carcinomas by using calretinin expression staining.¹⁰

Zivadinovic et al¹¹ published a series of 170 cases in which cytology was compared to histology. Those authors found that cytology had a sensitivity of 98.92% and a specificity of 93.6%. Freedman et al¹² concluded that the diagnosis of EOC based on cytology and histology is superior to clinical factors (radiologic evaluation, CA 125 levels, and clinical symptoms) with diagnostic accuracies of 98%, 92%, and 87%, respectively. Allen et al¹³ reported that the examination of ascites fluid has high false-negative results: because only 67.1% of their cancer patients were found to have positive cytology in the ascites fluid, they recommended further investigation by biopsy via either a percutaneous or a laparoscopic route. The objective of this study was to evaluate the performance of ascites cytology in the diagnosis of malignancy of ovarian origin among patients 51 years and over (the average age of menopause in Israel), and to determine whether it can replace a diagnostic tissue biopsy that precedes the prescription of NACT.

MATERIALS AND METHODS

This study was approved by our institution review board (0071-16-TLV). This work did not receive funding of any kind. Patients older than 51 years, the average age of menopause in Israel, whose ascites samples were sent for cytology evaluation in our tertiary medical center between 2010 and 2015, were retrospectively evaluated and compared to

available histology reports of diagnostic tissue biopsies. All available records of cytology were examined to determine whether they were either positive or negative for malignancy. Ascites fluid cells were processed using the Thin-Prep System (Hologic Inc, Marlborough, MA) together with a formalin-fixed paraffin-embedded cell block. Tissue samples and aspirated cells were fixed in 4% (final dilution) buffered formaldehyde solution. Fixation time ranged between 4 and 10 hours for small tissue samples and aspirated cells. The tissue material was processed in an automated tissue processor (VIP6 Sakura, Torrance, CA) by exposure to gradually increasing percentages of ethanol (70%, 80%, 90%, 95%, and 100%), followed by exposure to xylene and finally to paraffin. The tissues were then embedded in paraffin, cut into 3 to 5 μm sections and stained with hematoxylin-eosin in an automated staining-covering device (Prisma, Sakura, Torrance, CA). IHC was used at the discretion of the cytopathologist for both cytological preparations and tissue samples and was performed on paraffin-embedded 3-5 μm tissue sections that underwent deparaffinization and heat-induced antigen retrieval at controlled temperatures, followed by incubation with the appropriate antibody according to the supplier's instructions for dilution and antigen retrieval. The antibody clones used included Clone OV-TL 12/30 Cell Marque, Ks20.8 Cell Marque, Clone 618F Bio Legend, Clone Col-1 and Clone (SP1) Cell Marque for CK7, CK20, CA125, CEA, and ER, respectively. All staining steps were performed at a temperature of 42°C controlled by an automated staining device (Benchmark XT, Ventana, Indianapolis, IN). The stained tissue developed a dark brown stain, which was further visualized by counterstaining with Gill's hematoxylin (Cat. No. 1.05174.2500, Merck, Darmstadt, Germany). Each section also included a good quality positive control tissue as specified by the manufacturer of the antibody.

All cytology samples were obtained from patients with advanced stage disease, with clinical ascites large enough for paracentesis. Cases with only washing cytology obtained during surgery (without clinical ascites) were not included in our study. Cytology results were compared to available tissue biopsy results. For ovarian cancer cases, in patients found eligible for primary debulking surgery, cytology was compared to a pathology specimen of tissue obtained from surgery. In cases in which primary debulking was not considered, negative cytology led to further investigation usually percutaneous biopsy from abdominal metastases (omental or peritoneal) or laparoscopy. After establishing the diagnosis, the patients were referred for NACT. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of ascites fluid in diagnosis of malignancy were calculated. Further analysis was done to evaluate the performance of IHC of cell blocks of ascites fluid in diagnosing malignancy of ovarian origin.

RESULTS

Between 2010 and 2015, 551 patients older than 51 years had both cytology and diagnostic histology samples taken

TABLE 1. Sensitivity, specificity, positive, and negative predictive value of ascites cytology in the diagnosis of malignancy of any origin and of epithelial ovarian cancer

	Sensitivity, %	Specificity, %	Positive predictive value, %	Negative predictive value, %	Test accuracy, %
Any malignancy (n = 551)	46.1	100	100	19.8	52.4
Epithelial ovarian cancer (n = 155)	80.6	100	100	16.7	81.4

either percutaneously or during surgery at our institution. Of those 551 patients, 224 had malignant cells on both the cytology and histology (ie, true positive cytology), whereas 65 patients had benign results from both cytology and histology (ie, true-negative cytology). There was disagreement between the cytological and histological evaluations in 262 cases, all these cases were negative for malignancy on cytology and positive for malignancy in the final pathology (ie, false-negative cytology). There were no cases of positive cytology and negative final histology (ie, no false-positive cytology results). The sensitivity of peritoneal cytology for diagnosing malignancy of any origin was 46.1%, specificity 100%, PPV 100%, and NPV 19.8%. Test accuracy was 52.4% (Table 1). The sites of malignancy of discordant cases, where cytology was negative and final pathology positive are detailed in Table 2.

Of the 551 patients with both cytology and histology available, 161 patients had pathology confirmed ovarian tumors, and in 155 cases these tumors were malignant. Stage and histology type are shown in Table 3. As expected, with few exceptions, most cases were advanced stage with high-grade histology. Of the 155 cases of ovarian cancer, 125 patients had malignant cells on cytology examination of ascites fluid collected (true-positive cytology), in the remaining 30 patients, ascites was negative for malignant cells (false-negative cytology). In six cases of ovarian tumors, both ascites and final pathology were negative for malignancy (true-negative cytology), whereas there were no cases of positive cytology and negative final pathology (ie, no false-positive cytology). The sensitivity, specificity, PPV, and NPV for cytology diagnosis of tubo-ovarian cancer were 80.6%, 100%, 100%, and 16.7%, respectively. Test accuracy was 81.4% (Table 1).

IHC, which is used at the discretion of the pathologist, was performed on 117 out of the 224 fluid samples of patients with positive malignant cells, and the findings were compared to the type of tumor that was determined on histology. During the study period, the most common antigens used for

establishing diagnosis of ovarian cancer were CK7 which was positive in 100% of cases, CK20 which was positive in only 1.4% of cases and CA-125 which was also positive in 100% of cases. CEA was negative in all cases of ovarian cancer. IHC confirmed the histologically determined origin in 75 of 79 cases of tubo-ovarian carcinoma (94.9%). IHC results of tubo-ovarian cancer and other malignancies are depicted in Table 4.

DISCUSSION

The last few years have witnessed the growing use of NACT for advanced ovarian cancer and the deferral of debulking surgery until after several courses of chemotherapy.¹⁴ Before initiation of NACT, tissue diagnosis is customarily obtained either by means of imaging-guided biopsies or by surgery (laparoscopy or laparotomy). Ascites fluid paracentesis is an appealing, less invasive technique for retrieving cells for cytology evaluation and diagnosis of malignancy. This technique may be particularly useful in older, postmenopausal patients, saving the need for surgical diagnostic procedures. Moreover, ascites aspiration could be initiated by general gynecologists, family practitioners, and other primary care physicians even before referral to oncologists/gynecologic oncologists, thereby saving valuable diagnostic time and patient discomfort. Our results confirm that cytology examination of ascites fluid among postmenopausal patients is both sensitive and highly specific for diagnosis of ovarian cancer with a PPV of a 100% (Table 1) and as such, is highly reliable for use in clinical practice. In cases in which ascites fluid is negative but clinical suspicion is high for ovarian malignancy, further biopsy is indicated. Our data are well in line with previously published literature.¹¹⁻¹³ Noteworthy, no other study, including our own, reported false-positive cytology results which could have led to unnecessary NACT treatment.

TABLE 3. Stage and histology subtypes of ovarian cancer cases

	Positive ascites (n = 125)	Negative ascites (n = 30)
Stage		
1	0	3
2	0	3
3	116	24
4	9	
Histology		
High-grade serous	115	21
Low-grade serous	0	0
Endometrioid	7	4
Mucinous	1	2
Undifferentiated	0	1
Nonepithelial	0	2
PPC	2	0

PPC, primary peritoneal carcinoma.

TABLE 2. Discordant cases between ascites cytology and final histology according to the origin of malignancy (cytology false-negative cases n = 262)

Origin of malignancy	Percent of cases (n)	Origin of malignancy	Percent of cases (n)
Colon	49.6% (130)	Liver	3.4% (9)
Ovary	11.4% (30)	Neuroendocrine	1.5% (3)
Pancreas	9.5% (25)	Renal cell	1.5% (3)
Endometrium	9.5% (25)	Lymphoma	1.5% (3)
Sarcoma	3.8% (10)	Urothelial carcinoma	1.5% (3)
Breast	3.8% (10)	Cervix	0.4% (1)
Cholangiocarcinoma	3.4% (9)	Lung	0.4% (1)

TABLE 4. Immunohistochemistry panel and reactivity in the assessment of ovarian origin

IHC stain Origin of adenocarcinoma	Malignant ascites cases (224)	Malignant ascites cases IHC performed (117)	CK7 (%)	CK20 (%)	CA125 (%)	ER (%)	CEA (%)
Ovary	125 (55.8%)	79 (67.5%)	76/76 (100%)	1/73 (1.4%)	75/75 (100%)	9/16 (56.3%)	0/32 (0%)
Colorectal	27 (12.1%)	16 (13.7%)	8/11 (72.7%)	7/13 (53.8%)	0/4 (0%)	NP	3/3 (100%)
Lung	8 (3.6%)	5 (4.3%)	4/4 (100%)	1/4 (25%)	0/2 (0%)	NP	0/2 (0%)
Breast	8 (3.6%)	3 (2.6%)	NP	NP	NP	3/3 (100%)	0/2 (0%)
Pancreas	5.4 (4.5%)	3 (2.6%)	2/2 (100%)	1/2 (50%)	1/1 (100%)	NP	2/3 (66.7%)
Cholangiocarcinoma	7 (3.1%)	6 (5.1%)	6/6 (100%)	2/6 (33.3%)	1/5 (20%)	NP	4/4 (100%)
Gastric	22 (9.8%)	4 (3.4%)	3/3 (100%)	3/3 (100%)	0/1 (0%)	NP	1/1 (100%)
Mesothelioma	1 (0.004%)	1 (0.85%)	0/1 (100%)	NP	NP	NP	NP

CA, cancer antigen; CEA, carcinoembryonic antigen; CK, cytokeratin; ER, estrogen receptor; IHC, immunohistochemistry; NP, not performed.

The most common ascites-producing ovarian tumors are the serous adenocarcinomas, which can show papillary configuration and psammoma bodies on cytology evaluation. These morphological features are usually absent from the other nonovarian ascites-producing carcinomas, and may be diagnostic for EOC. In cases in which diagnosis is uncertain, IHC staining of cellblocks can be added for the investigation of the site of origin. Because tumor cell content in cellblocks is, however, usually low, the number of the initial sections “sacrificed” for the assay should be selective and limited to the most efficient antibodies required for diagnosis.^{7,15-17} In our cohort CK7 was positive in 100% of ovarian cancer cases, whereas CK20 was positive in only one case (1.4%) (Table 3). CA125 was also positive in 100% of cases of ovarian cancer, whereas there were no cases with positive CEA, proving this pattern of positive CK7 and CA125, with negative CK20 and CEA highly discriminatory for ovarian cancer. Among colorectal cancers cases, malignant ascites was less common and IHC was less commonly used. Among these cases, CEA was positive in all (100%), CA125 was negative in all (100%), whereas CK7 and CK20 were positive in 72.7% and 53.8% of colorectal cancer cases, respectively (Table 3).

The major drawback of our work is the retrospective design of the study which did not enable us to control for IHC staining used by the cytopathologists, and these were used at their discretion. For example, only in recent years have we begun to routinely include PAX8 staining when a malignancy of a mullerian/gynecological origin is suspected. The rate of positivity of PAX8 in ovarian cancer is 92%, with no significant difference between the various histotypes.¹⁸ Our cohort included cases from 2010 through 2015, before PAX8 was routinely used and hence was not reported in our work. P53, another important marker for high-grade serous carcinoma was also not reported. This surrogate marker for the ubiquitous *TP53* gene mutations of high-grade serous carcinoma can aid in the discrimination between low-grade and high-grade serous carcinoma and may be important in the decision of which patients to refer for NACT before surgery, as those with low-grade malignancy are less likely to respond. In our cohort, as in others, the incidence of low-grade serous ovarian cancer is low, compared to high-grade histology (Table 4); hence, we could not make a meaningful remark regarding this histological subtype. The main strength of our study is the

large sample size of both malignant ascites cases in general and of EOC cases that had IHC staining that enabled drawing meaningful conclusions as to the performance of cytology in the diagnosis of EOC.

CONCLUSIONS

The present study demonstrated that cytology examination of ascites fluid in postmenopausal patients is a feasible and reliable way to diagnose metastatic adenocarcinoma with a high degree of sensitivity and specificity for ovarian cancer. In the era of NACT the role of the cytopathologist in the initial diagnosis of advanced EOC is crucial.

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