Cytomorphologic Features of Metastatic Urothelial Carcinoma in Serous Effusions

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Metastatic urothelial carcinoma (UC) to serous effusion (SE) is extremely rare and its cytomorphological features have only been described in case reports. In this study, we searched the pathology database at University of Michigan for SEs due to metastatic UC in the last 20 years. A total of 25 cases from 20 patients with clinically and pathologically confirmed metastatic UC in SEs were retrieved. The specimens consisted of 15 pleural, 8 peritoneal, and 2 pericardial effusions. Smears were reviewed and evaluated for the following features: cellularity, single cells, cell clusters or short cords, cell wrapping, "windows" between the cells, two-tone cytoplasm, cytoplasmic vacuoles, signet ring cells, nuclear to cytoplasmic (N/C) ratio, nuclear hyperchromasia, irregular nuclear membrane, nuclear centricity, double or multiple nuclei, nucleoli, anaplastic cells and mitosis. Our results showed that UC manifested in SEs predominantly as a single cell population with or without clusters or short cords, and frequently exhibited the "cell wrapping" of two or more cells. Individual UC cell in SEs exhibited nuclear enlargement with increased N/C ratio, irregular nuclear membranes, hyperchromatic coarse chromatin and frequently prominent nucleoli. Double or multinucleated cells, cells with vacuolated cytoplasm or signet ring appearance were also frequently present. Our results demonstrated that while certain features could suggest the diagnosis of UC, the cytomorphological features are not specific and often overlap with those of reactive mesothelium, mesothelioma, metastatic adenocarcinoma, or squamous cell carcinoma in SEs. Accurate diagnosis of UC rests on the combination of clinical history, cytomorphologic features and appropriate immunohistochemical panel. Diagn. Cytopathol. 2013;41:569–574. © 2012 Wiley Periodicals, Inc.

Key Words: urothelial carcinoma; serous effusions; cytopathology; differential diagnosis

Urothelial carcinoma (UC) of the bladder is the fifth most common carcinoma in the United States.[1] Approximately 5% of patients with bladder cancer present with de novo metastatic disease at the time of the diagnosis, and an additional 50% who are originally diagnosed with muscle-invasive disease will develop a recurrence.[2] UC usually disseminates through direct extension. The common sites of the metastasis of UC of the bladder include lymph node, bone, lung, liver, and adrenal gland.[3] Metastasis of the bladder UC to peritoneum and pleura accounts for 16 and 11% of the total bladder UC metastasis, respectively.[4] The upper tract UC accounts for 5% of the total UC[5] and contributes 19 and 15% of metastasis to peritoneum and pleura, respectively.[6] Involvement of the peritoneum and pleura by UC are commonly associated with widely spread metastasis to other organs. However, SE as the initial solitary site of metastasis of UC has also been reported.[7]

Cytological features of UC in pleural and peritoneal fluid were to date described only in case reports.[8–10] In this study, we report the cytomorphological features of UC in 25 SEs and discuss the relevant differential diagnoses.

Materials and Methods

A total of 25 specimens consisting of 15 pleural, 8 peritoneal, 2 pericardial fluids from 20 patients were retrieved from the pathology database at University of Michigan during the period 1990 through 2011. All relevant clinical information was obtained from the patients' medical records. For each specimen, two air-dried and Diff-Quik stained smears, two fixed and Papanicolaou-stained smears and a cell block were prepared. In bloody or low

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Table I. Clinical and Pathological Data

Patient	*Age/sex*	*Fluid source*	*Primary*	*Interval to metastasis*	*Metastasis to other sites*
1	71/F	pleural	bladder	1	pancreas
2	49/F	pleural	bladder	9	ND
3	72/M	peritoneal	bladder	7	Lung
4	77/M	peritoneal	kidney	26	Bone
5	57/F	pleural	bladder	11	Lung, liver
6	29/F	pericardial	kidney	2	ND
7	79/M	pleural	bladder	91	Bone
8	61/M	Pleural	bladder	13	Bone, lung, liver
9	84/M	pleural	bladder	43	Bone
10	77/M	pleural	bladder	9	ND
11	74/M	pleural	bladder	10	Lung
12	56/M	pleural	bladder	9	ND
13	69/M	pleural	bladder	228	Lung, liver
14	49/M	peritoneal	bladder	7	ND
15	43/M	peritoneal	bladder	12	Liver
16	67/F	pleural	bladder	13	Brain
17	70/M	peritoneal	bladder	30	Liver
18	61/M	peritoneal	bladder	8	Bone, liver
19	62/M	peritoneal	bladder	NA	NA
20	81/M	pleural	bladder	NA	NA

Month(s) is used for "interval to metastasis." ND, not detected; NA, not available.

Table II. Cytological Features of Urothelial Carcinoma in Serous Fluid

	Number of cases (%)[a]			
	3+	*2+*	*1+*	*0*
Smear patterns				
Cellularity	9 (36)	5 (20)	11 (44)	0 (0)
Single cell	13 (52)	5 (20)	7 (28)	0 (0)
Cell clusters	3 (12)	4 (16)	3 (12)	15 (60)
Short cords	0 (0)	1 (4)	7 (28)	17 (68)
Cell wrapping	2 (8)	4 (16)	6 (24)	13 (52)
Cell windows	0 (0)	2 (8)	0 (0)	23 (92)
Cytological features				
Two-tone cytoplasm	0 (0)	0 (0)	6 (24)	19 (76)
Cytoplasm vacuoles	4 (16)	3 (12)	5 (20)	13 (52)
N/C ratio	8 (32)	16 (64)	1 (4)	0 (0)
Nuclear hyperchromasia	5 (20)	9 (36)	5 (20)	6 (24)
Irregular nuclear membrane	1 (4)	10 (40)	10 (40)	4 (16)
Centric nuclei	6 (24)	7 (28)	11 (44)	1 (4)
Eccentric nuclei	1 (4)	11 (44)	7 (28)	6 (24)
Nucleoli	5 (20)	8 (32)	12 (48)	0 (0)
Double nuclei	2 (8)	2 (8)	11 (44)	10 (40)
Multiple nuclei	3 (12)	1 (4)	3 (12)	18 (72)
Signet ring cells	1 (4)	2 (8)	7 (28)	15 (60)
Anaplastic cells	0 (0)	2 (8)	10 (40)	13 (52)
Mitosis	0 (0)	2 (8)	2 (8)	21 (84)

[a]Total case number is 25.

cellular samples, a ThinPrep was also prepared. The smears, Hematoxylin and Eosin-stained cell blocks sections and relevant immunohistochemical stains (available in 18 cases from 13 patients) were reviewed. The smears were evaluated for the following features: cellularity, single cells, cell clusters or short cords, cell wrapping, "windows" between the cells, two-tone cytoplasm, cytoplasmic vacuoles, signet ring cells, N/C ratio, nuclear hyperchromasia, irregular nuclear membrane, nuclear centricity, double or multiple nuclei, nucleoli, anaplastic cells and mitosis. A semiquantitative method was used to quantitate each of the cytomorphological features. In general, a feature is considered as high (3+) when easily detected at 10× magnification, moderate (2+) when detected at 20× or low (1+) when only detected at 40× magnification. All the cases were separately reviewed by the three authors. Discrepant cases were reviewed by two of the authors including the senior pathologist (CWM) to achieve consensus.

Results

The 25 cases of SEs with metastatic UC were retrieved from 20 patients (15 males and 5 females) with age ranging from 29 to 84 (mean 63.5) years old at time of presentation with the malignant SEs (Table I). The malignant effusions consisted of 15 pleural fluids (60%), 8 peritoneal fluids (32%), and 2 pericardial fluids (8%). UC originated from the urinary bladder and renal pelvis constituted 90 and 10% of metastatic UC in the fluids, respectively. The interval between the initial diagnoses of UC to the presentation with malignant effusion ranged between 1 and 228 months. More than half of the patients (11 cases, 55%) presented with the effusion within 1 year after the initial diagnosis of the primary cancer. Bone, lung, and liver were the most common metastatic sites other than the pleural and peritoneal cavities. UC metastasis to brain or pancreas occurred less frequently.

The cytomorphological features of the UC in SE are summarized in Table II. The cellularity of the effusions varied widely among all three serous cavities. The smears presented with a predominantly singly dispersed cell pattern in all 25 cases. A pattern of mixed single cells and cell clusters was seen in 10 cases (40%). The cell clusters were easily recognizable even in low magnification and consisted of 10–20 cells each (Fig. 1). Occasional cytoplasmic vacuoles were noted within the cell clusters. Short cords, defined as three to six cells forming a row, were identified in 8 cases (32%) (Fig. 2). The short cords were commonly appreciated at high-power magnification. The cell clusters and the short cords usually coexisted in the same specimen. "Cell wrapping," defined in this study as a tumor cell partially or completely wrapped by one or more tumor cells with the nuclei molded in a crescent form around the cytoplasm of the central cell, was identified in 12 cases (48%) (Fig. 3). When present, cell wrapping was frequent and easily identified at low magnification. The "windows" between the UC cells (Fig. 4) were only seen in two cases (8%).

The UC cells (Fig. 4) exhibited high *N/C* ratio with, central or eccentric nuclei. The nuclei were usually round, oval or convoluted. Hyperchromasia, chromatin clumping, irregular nuclear membranes, and enlarged nucleoli were identified in the majority of cases. Binucleated or multinucleated malignant cells were identified in 60 and 28% of the specimen, respectively (Fig. 5). Cytoplasmic

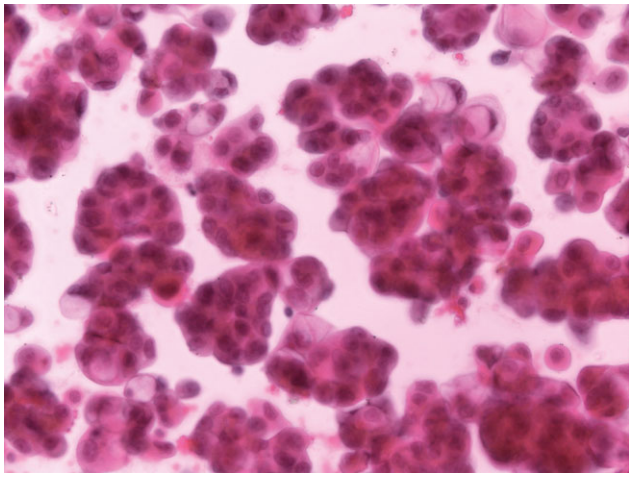

Fig. 1. Cell clusters consisting of 10–20 tumor cells each are easily recognizable in low-power magnification. Occasional cytoplasmic vacuoles and signet ring cells are noted. Papanicolaou stains ×400. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

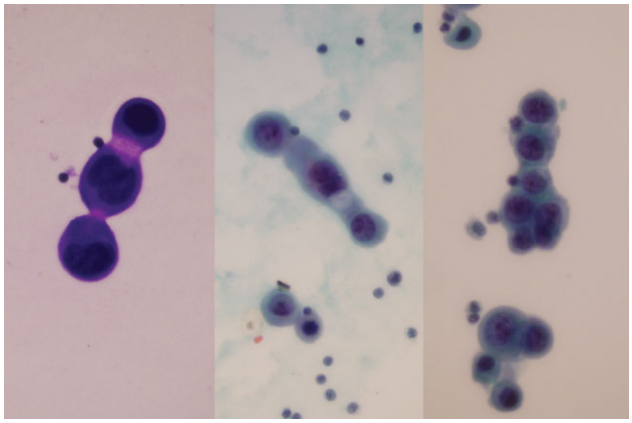

Fig. 2. The short cords consist of three to six cells forming a row with window-like spaces. Diff-Quik (left panel) and Papanicolaou (middle and right panels) stains ×600. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

vacuoles, both fine and large in size, were identified in 48% of the specimens (Fig. 6). Signet ring cells were identified in 40% of the specimens (Fig. 6). Small number of cells exhibiting two-tone cytoplasm was seen in 24% of the specimens (Fig. 3, insert). Anaplastic tumor cells (Fig. 5, insert) and mitotic figures (Fig. 3) were identified in 12 cases (48%) and 4 cases (16%), respectively. Multiple specimens retrieved from the same patient were morphologically similar.

Various panels of immunohistochemical staining were performed on the sections of cell blocks from 18 specimens (13 patients). The tumor cells stained positively for CK7, CK20 or high molecular weight keratin (HMWK) in 11/12, 10/12, or 4/4 of the patients, respectively. The tumor cells were negative for WT-1, calretinin or CD68 in all the cases stained (data not shown).

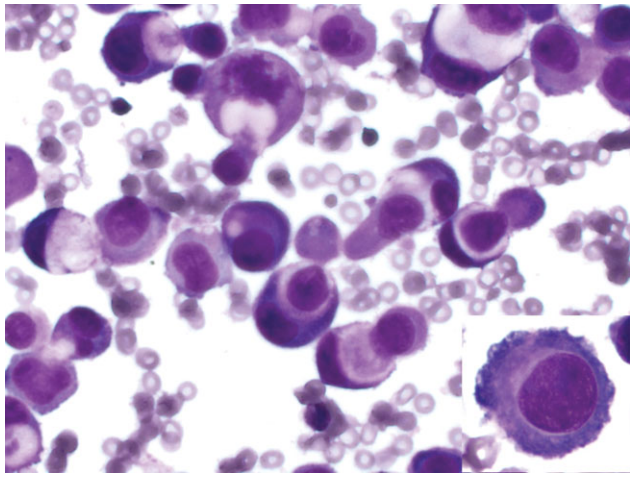

Fig. 3. UC cells show "cell wrapping" and mitotic activity. Diff-Quik stain ×600. Some cells exhibit two-tone cytoplasm (insert, Diff-Quik stain ×1,000). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

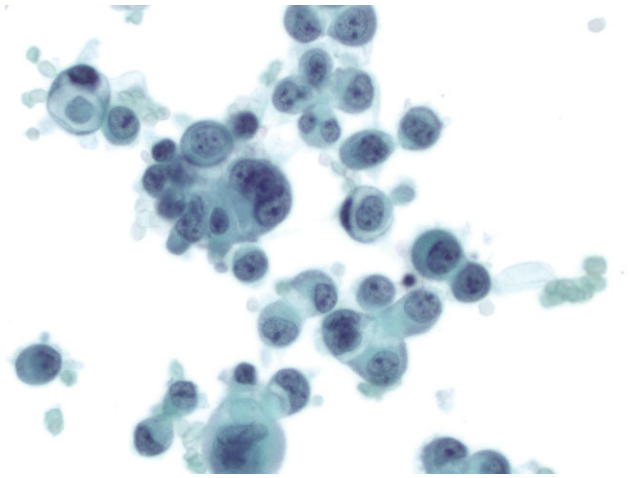

Fig. 4. UC cells occasionally exhibit "window-like" arrangement between the cells, increased *N*/*C* ratio, enlarged nuclei, irregular nuclear membrane, hyperchromatic clumped nuclear chromatin, and one to three nucleoli. Papanicolaou stain ×600. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Discussion

Malignant SE is usually a manifestation of disseminated and advanced stages of malignancy. Neoplasms of lung, breast, ovary, and lymphoma constitute >75% of malignant pleural effusions.[11] The direct and/or indirect malignant seeding of breast, ovarian, endometrial, gastric, colonic, and pancreatic carcinomas contribute up to 80% of malignant peritoneal effusions.[4] SE usually occurs as early as one to several months after the diagnosis of the primary cancer. Occasionally, it can be the first manifestation of the disease before the diagnosis of the primary malignancy. Metastasis of UC to the pleural or peritoneal cavities is not common. In our institution, where 120 cys-

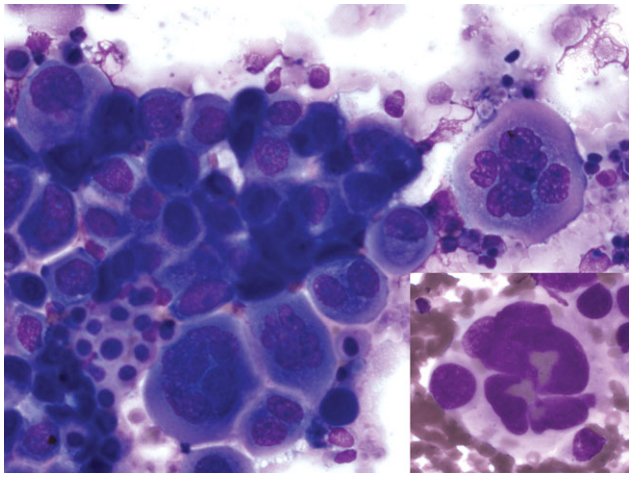

Fig. 5. Double or multiple nucleated cells are frequent. Diff-Quik ×400. Anaplastic cells were not uncommon (insert, Diff-Quik ×600). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

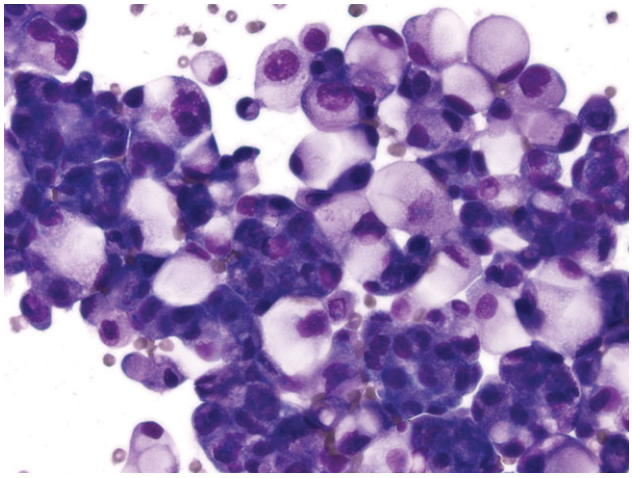

Fig. 6. Large or fine cytoplasmic vacuolation and signet ring cells are common in some cases. Diff-Quik ×400. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

tectomies for UC are performed annually, a search of our files during the last 20 years resulted in 25 fluids only from 20 patients. In this study, a urinary bladder carcinoma contributed 90% of the malignant effusions and the UC of the renal pelvis contributed the remaining 10%. More than half of the malignant effusions (11/18 cases) in this study occurred within 1 year after the primary diagnosis, which supports the aggressive nature of those UCs. The bone, lung and liver were the most common sites of UC metastasis. The pattern of metastasis to other organs in our study is consistent with those previously reported.[3,4]

Single tumor cells, with or without clusters or short cords, along with the "cell wrapping" were the main cytological patterns observed in our study. On the individual cellular level, the characteristic features were nuclear enlargement with increased *N/C* ratio, irregular nuclear membrane, and hyperchromasia with coarse chromatin and frequent prominent nucleoli. Double or multiple nuclei were also frequently identified. Tumor cells with vacuolated cytoplasm or signet ring cells were very common. Cytoplasmic inclusions or cercaria-like cells, features frequently reported in smear preparations from fine needle aspiration of metastatic UC,[8,12,13] were not identified in this study (data not shown). The presence of high number of mitotic figures and anaplastic tumor cells in this study supports the high grade status of these metastatic UCs.

The main differential diagnosis of UC in SEs includes reactive mesothelial cells (RM), malignant mesothelioma (MM), metastatic adenocarcinomas (ADC), and metastatic poorly differentiated squamous cell carcinoma (PDSCC). The Key features used in the differential diagnosis are summarized in Table III.[14] RM can present with moderate to high cellularity in the benign effusion. They mainly present as numerous single cells with variable number of small clusters. RM can exhibit significant nuclear atypia with nuclear enlargement, coarse chromatin, and irregular nuclear contour or prominent nucleoli. However, the presence of dense two tone cytoplasm with a clear outer rim, brush border, frequent "windows" between the mesothelial cells and occasionally few submembranous glycogen vacuoles are important cytological features in confirming the mesothelial origin of these cells.[11,15] RM cells typically form smaller, two-dimensional cellular clusters with scalloped borders rather than three-dimensional cell clusters. While the short cords in UC may mimic those of mesothelial cells, they lack the classic mesothelial features such as intercellular windows and cellular clasping with cytoplasmic pinching.

MM commonly present with a pattern of single, noncohesive tumor cells admixed with large cell clusters or morule-like spheres in serous fluids. At low magnification, the smear reveals a striking number of larger clusters of mesothelioma cells with scalloped berry-like borders or three dimensional (3D) spheres. The tumor cells are markedly enlarged, and the morules or spheres are relatively larger than those of UC. The pattern of "cell-in-cell"[16] rather than that of "cell wrapping" favors the diagnosis of mesothelioma. At the cellular level, in addition to the characteristic features of mesothelium such as two tone cytoplasm, glycogen vacuoles and intercellular windows, the single mesothelioma cells tend to be highly variable in size ranging from size of a normal mesothelial cell to a gigantic size approaching that of the small morules in the background. In contrast, UC cells tend to be relatively uniform in size and shape except for the background scattered anaplastic cells. MM tend to have subtle cytological atypia with low *N/C* ratio, relatively smooth

Table III. Predominant Cytologic Features in UCC and its Differential Diagnosis[14]

Features	*UC*	*RM*	*MM*	*ADC*	*PDSCC*
Cellularity	+/++	++	+++	+/++	+++
Cell variation (size and shape)	−/+	−/+	+++	−/+	−/+
Clusters					
Number	−/+	+/++	++/+++	++/+++	++/+++
Architecture	3D	2D	Morules	3D	3D
Border	Scalloped	Scalloped	Scalloped	Smooth	Smooth
Cell pleomorphism	++	−/+	+/++	+++	+++
Cell–cell arrangement	Rare window cell wrapping	Windows clasping cell-in-cell	Windows clasping cell-in-cell	cell-in-cell	No windows cell wrapping pearl
Cytoplasm					
N/C ratio	high	low	low	high	high
Two-tone cytoplasm	+	+++	+++	−	++
Cytoplasmic vacuoles	++	−/+	−/+	++/+++	−/+
Signet ring cells	+	−	−	++	−
Submembranous vacuoles	−	+/++	+++	−	−
Membrane brush border	−	++	+++	−	−
Nuclei					
Enlarged in size	++	+/++	+++	++/+++	+++
Nuclear centricity	Variable	Central	Central	Variable	Central
Chromatin	Coarse	Vesicular	Variable	coarse	coarse
Membrane irregularity	++	+	+/++	+++	+++
Enlarged nucleoli	++	+	+++	++/+++	+++
Double nuclei	++	+	+++	+	+
Multiple nuclei	+	+	+++	+	+
Anaplastic cells	+++	−/+	+	++	++
Mitotic figures	+	−/+	+/++	++/+++	++/+++

Abbreviations: UC, urothelial carcinoma; RM, reactive mesothelium; MM, malignant mesothelioma; ADC, adenocarcinoma; PDSCC, poorly differentiated squamous cell; Semi-quantitation: −, none; +, low or few; ++, moderate or medium; +++, high or marked.

nuclear contour and less nuclear hyperchromasia. Despite the subtle atypia, nuclei of MM usually contain a prominent nucleoli and frequently macronucleoli. In contrast, UC nuclei exhibit at least moderate atypia. The nucleoli are not as prominent and macronucleoli are rare. In contrast to the characteristic submembranous glycogen vacuoles of MM, UC cells exhibited large and diffuse cytoplasmic vacuoles mimicking adenocarcinoma in about 50% of the cases. The identification of double or multiple nucleated cells does not help in the differential diagnosis of UC, RM, and MM since these cells are frequently noted in all three entities.

Tumors of glandular origin usually produce cellular clusters with central lumina or pseudoacini in serous fluid.[17] The cytomorphology of cell aggregates, balloon-like vacuolation and signet ring cells can be appreciated in the fluids of both metastatic UC and ADC. Predominant single tumor cells and cell wrapping should raise the possibility of UC.

Metastatic SCC is an exceedingly rare cause of malignant SE. The common cytological features included dense cytoplasm, cytoplasmic keratinization with occasionally "pearl" formation, well-defined cell borders and intercellular bridging, and bizarre cell shapes such as tadpole cells, spider cells etc.[18] However, PDSCC is more difficult to differentiate from UC since it manifests as large cell balls mimicking mesothelioma and adenocarcinomas. Characteristically, small groups with attempt to form pearls, dense refractile cytoplasm with well-defined borders, and high degree of cellular pleomorphism are seen. The cellular clusters when present in cell blocks appear as large sheets with whorl pattern and well defined cell borders. Intercellular junctions can be appreciated in these fragments as well.

The immunohistochemical (IHC) staining plays an important role in the differential diagnosis of UC in the fluids. As demonstrated in this study, UC is commonly positive for CK7, CK20, and HMWK. In addition to reaction to mesothelial markers such as calretinin, WT-1 and D2-40, RM, and MM also show strong cytoplasmic staining with CK7. However, mesothelial cells are rarely reactive to CK20. Calretinin is strongly positive in most of MM, demonstrating a nuclear and cytoplasm staining pattern. In contrast, small numbers of adenocarcinomas show predominantly cytoplasm staining pattern.[19] While positive staining to EMA can separate UC for RM that is nonreactive, it is important to remember that MM acquires a strong membranous EMA staining. Therefore EMA should only be used in the context of an IHC panel.[20,21] Similarly, MOC-31 and CEA stains should be used with caution and only as part of an IHC panel since they positively react with UC, ADC, and SCC. MOC-31 and CEA are positive in 100 and 83% of ADC and in 47 and 22% of SCC in SEs, respectively.[22,23] Morphologically, the SCC is the greatest mimics of UC in the fluids. While HMWK, CK5/6, and p63 have shown positive staining in 100, 89, and 80% of SCCs, respectively,[22,23] these markers commonly cross react to both SCC and UC. The newer markers S100P and

GATA3 stain positively in 78 and 67% of UCs, respectively. When S100P and p63 were combined, 95% of urothelial carcinomas were stained by one or both markers.[12] More importantly, GATA3 does not stain SCC[24]; and therefore have a more promising role in differential of UC and SCC in the fluids.

In conclusion, metastatic UC is rare in SE. The cytomorphological features of UC often overlap with those in RM, MM, metastatic ADC, and metastatic PDSCC in serous fluid. Familiarity with the cytomorphologic features as well as the clinical history and utilization of immunostains are very important to reach a definitive diagnosis of UC in the fluid.

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