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Original Article

Cytomorphologic diagnosis of adult granulosa cell tumors at the metastatic sites with an emphasis on the cytologic mimics

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Abstract: Introduction: Adult granulosa cell tumor (AGCT) is a rare ovarian sex-cord malignancy notorious for late recurrences and metastases. The cytologic features of AGCT at the metastatic sites have been documented sporadically. Hence, knowledge of the characteristic cytomorphologic features is essential for an accurate diagnosis and distinguishing it from its pathologic mimics, especially at the metastatic sites. Materials and methods: This was a retrospective study conducted over six years. The cytopathology electronic database was searched for the fine needle aspirates (FNA) reported as metastatic AGCT. A detailed cytomorphologic assessment was done for multiple cytologic features, including overall cellularity, cellular arrangement of the tumor cells, cell size, cell shape, nuclear pleomorphism, nuclear grooving, chromatin pattern, nucleolar prominence, mitotic figures, amount and character of cytoplasm, and the extracellular background. Results: There were 6 cases reported as metastatic AGCT on aspiration cytology. The smears in all the cases were cellular, with tumor cells arranged in loose aggregates, three-dimensional clusters, perivascular papillary fronds, and scattered singly. The most consistent cytologic features included microfollicular arrangement of monomorphic tumor cells with round-oval nuclei, fine chromatin, longitudinal nuclear grooving, and scant cytoplasm. Typical *Call-Exner bodies* and metachromatically stained extracellular hyaline material were noted sporadically. None of the smears showed anaplasia, prominent macronucleoli, atypical mitoses, or necrosis. Conclusions: The current study not only outlines the distinct cytologic attributes of AGCTs across various metastatic locations but also highlights its prevalent cytologic mimics. Additionally, it outlines key clinicopathologic traits that can aid in distinguishing and precisely diagnosing these tumors through cytological analysis.

Keywords: Granulosa cell tumor, adult granulosa cell tumor, metastatic AGCT, fine needle aspiration cytology, cytology, immunocytochemistry

Introduction

Granulosa cell tumors (GCT) of the ovary are rare sex-cord-stromal tumors constituting 2-3% of all ovarian tumors. These tumors exhibit a slow and gradual clinical progression, yet they are widely known for their tendency to recur at a later stage [1, 2]. Etiopathogenetically and morphologically, ovarian GCTs have been divided into two distinct types: adult granulosa cell tumor (AGCT) and juvenile granulosa cell tumor (JGCT). The AGCTs are more common than the juvenile ones and are hormonally active neoplasms wherein the neoplastic granulosa cells secrete excessive estrogens, inducing a hyperestrogenic state in the patient. This, in turn, can lead to the development of endometrial hyperplasia and/or endometrial adenocarcinoma (25-65% of patients) [3].

Clinically, most of these women are postmenopausal and present with adnexal mass, with or without endocrine manifestations. Women who develop endometrial hyperplasia or adenocarcinoma may present with abnormal uterine bleeding. Serum inhibin levels are often elevated in these patients; however, such elevations are considered suggestive and not conclusively diagnostic. Radiologically, AGCTs may show a spectrum of findings, and the most common finding is a solid-cystic mass. Depending upon the site of recurrence or metastasis, a large recurrent or metastatic tumor can mimic a primary tumor originating from the pelvis, liver,

spleen, small intestine, or a primary retroperitoneal tumor [4].

Treatment is primarily surgical; however, chemotherapy is recommended for recurrent and metastatic tumors [5]. Around 20-25% of AGCTs develop recurrences late after surgical resection, with a mean duration of 4-6 years and latent periods as long as 40 years after the initial treatment. Such recurrent cases have a poor prognosis. The most common sites for recurrence and metastases include the pelvis (70%), lung, liver, and lymph nodes [6].

Definitive diagnosis requires a microscopic examination of the tumor tissue. The histopathologic features of these tumors have been well-described in the literature; however, the cytologic features have been described sparsely. The limited literature and the reluctance to utilize fine-needle aspiration (FNA) for sampling adnexal masses play a significant role in the lack of awareness of cytomorphologic attributes of AGCTs among pathologists, which can frequently result in misdiagnoses. Furthermore, the cytomorphologic features of these tumors at the metastatic sites mimic a variety of other neoplasms, adding to the diagnostic challenge. In an attempt to address these lacunae, we present a detailed cytomorphologic analysis of six metastatic adult granulosa cell tumors and emphasize their cytologic mimics at different metastatic sites.

Materials and methods

Study design

This retrospective study was conducted over six years (2015-2021). The cytopathology electronic database was searched for the fine needle aspirates reported as metastatic AGCT during the study period.

Inclusion criteria

All the fine needle aspirates reported as metastatic adult granulosa cell tumors, for which the smears were available for review, were included in this study.

Exclusion criteria

All the aspirates reported as metastatic tumors other than AGCT.

All the available smears for the included cases were retrieved and reviewed by two cytopathologists. The available clinical and radiologic details were recorded in the FNA requisition forms. A detailed cytomorphologic assessment was done, and the cytologic features, including overall cellularity, cellular arrangement of the tumor cells, cell size, cell shape, nuclear pleomorphism, nuclear grooving, chromatin pattern, nucleolar prominence, mitotic figures, amount and character of cytoplasm, and the extracellular background, were recorded for each aspirate. The cell block sections and the immunocytochemical stains were also reviewed, wherever available.

Results

A total of six cases were reported as metastatic AGCT on fine needle aspiration cytology (FNAC) during the study period. The patient's age ranged from 42 to 59 years. Five of these patients presented with pelvic mass (n=5; 83.3%), one each with a hepatic space-occupying lesion (SOL), peritoneal, and mesenteric deposits in addition to pelvic mass (n=1 each; 16.7%) (**Figure 1**), and one each with splenic SOL (n=1; 16.7%), and intestinal and vaginal wall deposits (n=1; 16.7%). The size of these lesions ranged from 2 to 8 cm in maximum dimensions.

Three (50%) of these cases had an antecedent history of abdominal surgery. However, the original histopathologic diagnosis was available in only 1/3 (33.3%) cases. The duration between the primary surgery and the detection of metastasis by FNA ranged from 3 to 10 years, with an average of 6 years. All the available clinical and radiologic details of these cases are presented in **Table 1**.

The smears in all the cases were moderate to highly cellular, with tumor cells arranged in loose aggregates, three-dimensional clusters, perivascular papillary fronds, and scattered discretely. Microfollicle-like arrangement of the tumor cells was seen in 5/6 (83.3%) cases. The characteristic *Call-Exner bodies*, composed of a central round eosinophilic hyaline material surrounded by a peripheral rosette-like arrangement of tumor cells, were noted in 4 (66.7%) cases. In all the aspirates, the tumor cells were relatively monomorphic, small, round to oval, with round to oval nuclei, fine nuclear chromatin, indistinct to small nucleoli, and a scant to moderate amount of pale cytoplasm, with 2 (33.3%) aspirates showing fine cytoplasmic

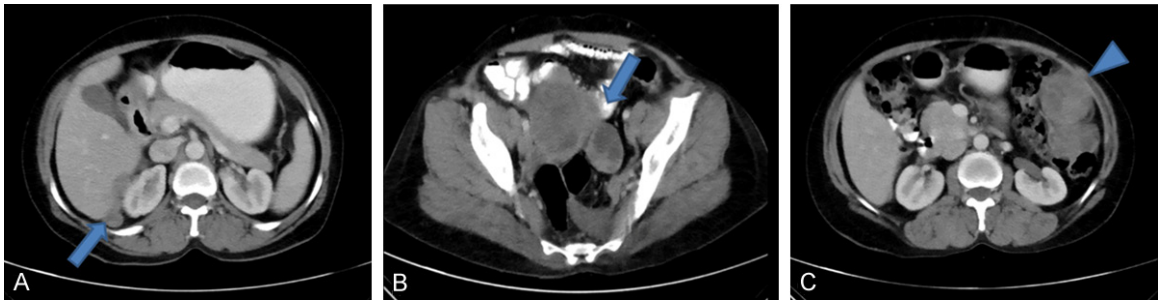

Figure 1. Radiologic features in a case of metastatic adult granulosa cell tumor with liver and pelvic space-occupying lesions: (A-C) Post-contrast axial multidetector computerized tomography images showing a solitary hypodense subcapsular deposit in segment 6 of the liver (arrow in A), two well-defined soft tissue deposits in the pelvis (arrow in B), and a large heterogeneously enhancing soft tissue deposit along the transverse mesocolon (arrowhead in C).

Table 1. Clinical and radiologic details of all the metastatic adult granulosa cell tumor cases (n=6)

Cases	Age	Clinical presentation	Radiologic features		Past surgical history
			Metastatic sites	Radiological findings	
Case 1	42 years	Abdominal swelling	Pelvic mass, mesenteric deposit, and liver SOL*	Predominantly solid-cystic left pelvic mass measuring 7×8 cm and solid liver SOL measuring 3 cm diameter	Surgery 10 years back; histopathologic records not available
Case 2	43 years	Abdominal pain	Peritoneal, intestinal, and vaginal wall deposits	Peritoneal deposit measuring 3×2 cm, bowel, and vaginal wall deposits measuring 2×1 cm	No surgical history available
Case 3	44 years	Abdominal pain and swelling	Abdominopelvic mass	Solid-cystic pelvic mass measuring 2.8×2 cm	No surgical history available
Case 4	47 years	Abdominal pain and swelling	Abdominopelvic mass	Solid-cystic pelvic mass anterior to urinary bladder measuring 4×3 cm	Surgery 3 years back. Histopathologic diagnosis: adult granulosa cell tumor
Case 5	52 years	Abdominal pain and swelling	Abdominopelvic mass	Solid-cystic pelvic mass measuring 4×5 cm	No surgical history available
Case 6	59 years	Abdominal pain and swelling	Splenic SOL	Solid-cystic lesion at the splenic hilum measuring 2×3 cm	Surgery 5 years back; histopathologic diagnosis not available

*SOL: space occupying lesion.

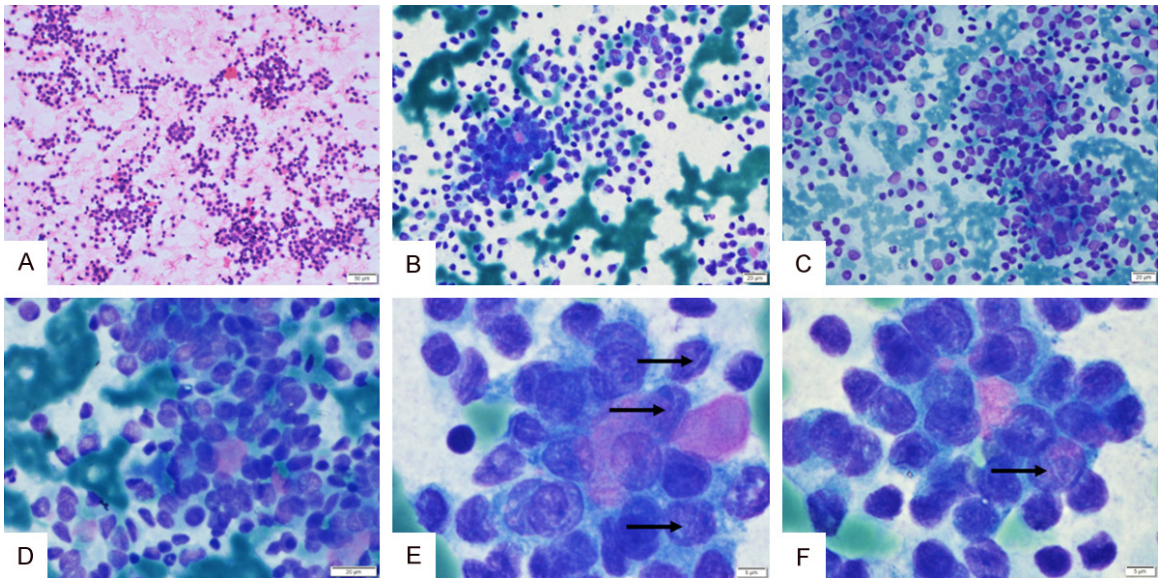

Figure 2. Cytomorphologic features in the aspirates from metastatic adult granulosa cell tumors: (A-F) Panel of photomicrographs from the hepatic lesion showing cellular smears with singly scattered as well as microfollicle-like arrangements of the tumor cells with minimal nuclear pleomorphism, round nuclei, fine chromatin, inconspicuous nucleoli, longitudinal nuclear grooves (arrows), and a scant amount of indistinct to amphophilic cytoplasm. Scattered Call-Exner bodies with central eosinophilic secretions can also be seen (Hematoxylin and Eosin; A: 10×) (May Grunwald Giemsa; B, C: 20×; D: 40×; E, F: 100×).

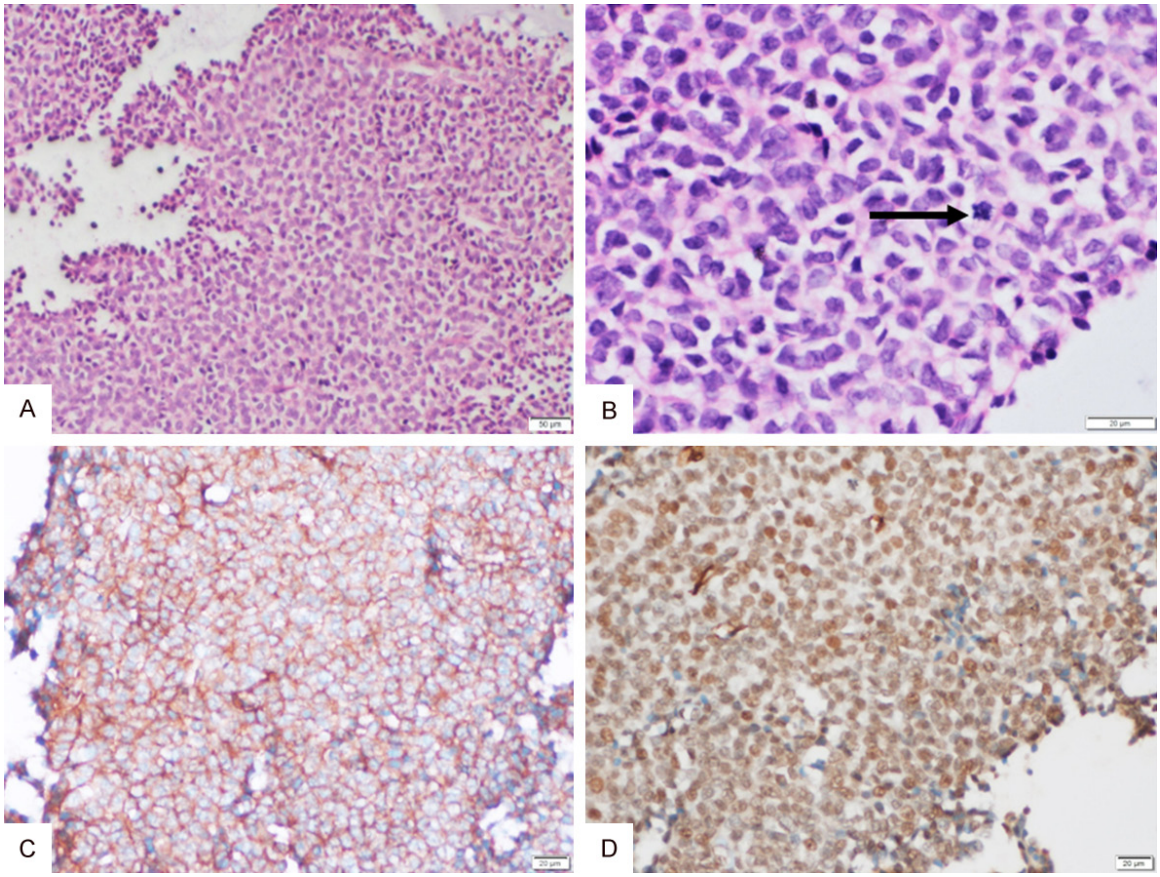

Figure 3. (A, B) Sections from the cell block showing sheets of mildly pleomorphic tumor cells with fine chromatin, inconspicuous nucleoli, longitudinal nuclear grooves, and scant cytoplasm. An occasional mitotic figure can also be noted in (B) (arrow) (Hematoxylin and Eosin; A: 4×; B: 40×); (C, D) Immunocytochemical (ICC) staining for inhibin (cytoplasmic positivity; C) and WT1 (nuclear positivity; D) showing diffuse positivity in the tumor cells (20×).

vacuolations. Longitudinal nuclear grooves were noted in all (n=6; 100%) of these cases. Randomly distributed metachromatically stained extracellular hyaline material was noted in 4/6 (66.7%) cases. Occasional mitotic figures were recorded in 1 (16.7%) case; however, atypical mitotic figures were not identified in any of these cases. None of the smears showed anaplasia, prominent macronucleoli, or necrosis. The most consistent and characteristic features included the presence of round to oval tumor cells in microfollicular arrangement with minimal nuclear pleomorphism, fine chromatin, longitudinal nuclear grooving, and scant cytoplasm (**Figure 2**). Cell blocks were available for 4 cases and showed sheets of similar tumor cells. Immunocytochemistry for inhibin, calretinin, and CD99 was performed on two cell block sections and confirmed the diagnosis (**Figure 3**). The cytomorphologic features of these aspirates are presented in detail in **Table 2**.

Discussion

Adult granulosa cell tumor (GCT) is a low-grade malignant neoplasm of the ovary with a long natural history and notoriety for late recurrences [7, 8]. It occurs mainly in postmenopausal women. Establishing an accurate clinical diagnosis is challenging as the presenting symptoms and radiologic features are often non-specific. A definitive diagnosis requires a pathologic examination of the tumor tissue. It is often difficult to diagnose AGCT on cytology, owing to the rarity of the tumor and the scarcity of the literature. This diagnostic dilemma is augmented while examining the smears from the metastatic sites, especially when the original histopathologic diagnosis is unknown. Adult granulosa cell tumors most commonly metastasize to the pelvis [5, 6]. Other reported metastatic sites include the lung, liver, lymph nodes, bone, and soft tissues [6, 7]. In addition, metastases to unusual sites, including the spleen, bowel wall, and vagina, have also been documented.

Table 2. Cytomorphologic features noted in the fine needle aspirates of metastatic adult granulosa cell tumors

Cases	Cellularity	Loose aggregates and cell dispersion	Microfollicle-like arrangement	Call-Exner bodies	Perivascular papillary fronds	Cell shape	Pleomorphism	Longitudinal nuclear Grooves	Chromatin pattern	Cytosol	Mitotic figures
Case 1	Moderately cellular	Present	Present	Present	Absent	Round to oval	Mild	Present	Fine granular	Scant	Absent
Case 2	Moderately cellular	Present	Present	Present	Present	Round to oval	Mild	Present	Fine granular	Scant	Occasional; no atypical mitoses
Case 3	Highly cellular	Present	Present	Absent	Absent	Round to oval	Mild	Present	Fine granular	Scant	Absent
Case 4	Cellular	Present	Absent	Absent	Absent	Round to oval	Mild	Present	Fine granular	Scant	Absent
Case 5	Highly cellular	Present	Present	Present	Absent	Round to oval	Mild	Present	Fine granular	Moderate with focal vacuolations	Absent
Case 6	Cellular	Present	Present	Present	Present	Round to oval	Mild	Present	Fine granular	Moderate with focal vacuolations	Absent

Table 3. Useful clinicopathologic, immunocytochemical, and molecular genetic features to differentiate metastatic adult granulosa cell tumor from its cytologic mimics [14-21]

Differential diagnosis	Most common age group	Clinical presentation	Cytologic features	Immunocytochemistry	Molecular genetic abnormalities
Metastatic adult granulosa cell tumor	Peri and post-menopausal women, 50-55 years	Abdominopelvic mass, pain (pelvic metastasis), metrorrhagia, and/or endocrine manifestations (due to hormone secretion)	Microfollicles and discretely scattered tumor cells with minimal to mild nuclear pleomorphism, round to oval nuclei with inconspicuous to tiny nucleoli, longitudinal nuclear grooves, and scant cytoplasm. Typical Call-Exner bodies composed of a microfollicular arrangement of tumor cells with central eosinophilic material may also be noted.	Positive: Inhibin, calretinin, FOXL2, SF-1, CD99 Negative: CD10, h-caldesmon, chromogranin, synaptophysin, TTF1, thyroglobulin, CD117, Mammaglobin, GCDFP-15	*FOXL2* 402C->G (C134W) point mutation is regarded as the genetic hallmark
Metastatic adenoid cystic carcinoma	40-60 years	Variable depending upon the metastatic site; mostly associated with pain due to perineural invasion	Cellular smears with well-demarcated hyaline globules and cylinders of metachromatically staining matrix surrounded by basaloid tumor cells with hyperchromatic angulated nuclei.	Positive: CD117 and p63 Negative: Inibin, calretinin, WT1, chromogranin	Around 60-90% of cases demonstrate *MYB-NFIB or MYBL1-NFIB fusion* genes
Metastatic follicular carcinoma thyroid	40-60 years	Variable depending upon the metastatic site	Microfollicles with nuclear enlargement, overlapping, and crowding.	Positive: Thyroglobulin, TTF1 Negative: Inhibin, calretinin, WT1, CD99	Most common are the *RAS* mutations and PAX8/PPAR gamma rearrangements
Small cell carcinoma of the ovary, hypercalcemic type	Young women; less than 40 years	Abdominopelvic mass, pain	Uniform round cells with hyperchromatic nuclei and scattered mitotic figures with scattered larger cells with rhabdoid morphology. Focal follicle-like spaces filled with eosinophilic secretions.	Positive: WT1, CK, EMA, vimentin, calretinin Negative: Inhibin, chromogranin, CD117	Inactivating mutations in *SMARCA4/BRG1*, or *SMARCA2/BRM* or *SMARCB1/INI1* genes

Low-grade endometrial stromal sarcoma	40-50 years	Abdominopelvic mass, pelvic pain, abnormal uterine bleeding	Moderate cellularity, discretely scattered and loose clusters of relatively monomorphic cells with small round to oval nuclei with fine chromatin and scant cytoplasm. Longitudinal nuclear grooves are not seen. Admixed vascular channels with a perivascular arrangement of cells are also noted commonly.	Positive: CD10, IFITM1, ER, PR, WT1 Negative: FOXL2, inhibin, calretinin (except in cases with sex-cord differentiation), and cyclin D1, BCOR, CD117, and DOG1	Most common is the JAZF1-SUZ12 fusion gene resulting from t(7;17)(p15;q21) (50% cases); Other fusion genes include: JAZF1-PHF1 from t(6;7)(p21;p15), EPC1-PHF1 from t(10;17)(q22;p13), MEAF6-PHF1 from t(1;6)(p34;p21) and MBTD1-Cxorf67 from t(X;17)(p11.2;q21.33)
Neuroendocrine tumor	40-50 years	Variable depending upon the site, clinical manifestations due to hormone secretion may be noted in functional tumors	Predominantly dispersed tumor cells with minimal to mild pleomorphism, round, often eccentrically placed nuclei with salt and pepper chromatin, inconspicuous nucleoli, and moderate cytoplasm with some showing eosinophilic granules. Mitotic activity varies with the grade of the tumor.	Positive: chromogranin, synaptophysin, CD56, patchy cytokeratin Negative: inhibin, calretinin, SF-1, and WT1	Variable depending upon the primary site
Metastatic lobular carcinoma	50-60 years	Variable depending upon the metastatic site. History of breast carcinoma, bilateral involvement is common in these patients	Round to oval, relatively monomorphic tumor cells arranged in small chains or dispersed individually with eccentrically placed nuclei, and some showing intracytoplasmic vacuolation.	Positive: GATA3, ER, PR, AR, EMA, GCDFP-15 Loss of E-cadherin Negative: inhibin, calretinin, SF-1, and WT1	*CDH1* and *PIK3CA* mutations are common
Metastatic Papillary thyroid carcinoma (PTC)	30-50 years	Variable depending upon the metastatic site. History of a thyroid tumor	Cellular smears with papillary fragments of tumor cells with nuclear enlargement, overlapping, nuclear grooving, intranuclear cytoplasmic inclusions, chromatin clearing, and inconspicuous nucleoli. Microfollicles with central thick colloid, resembling Call-Exner bodies may be seen in the follicular variant of papillary thyroid carcinoma.	Positive: TTF1, PAX8, BRAF, HBME1, GALECTIN 3 Negative: inhibin, calretinin, SF-1, and WT1	*BRAF* V600E mutation is seen in around 60% of cases
Metastatic adenocarcinoma	Elderly, usually more than 60 years	Variable depending upon the metastatic site	No nuclear grooving. AGCT has less nuclear atypia and mitosis as compared to this entity.	Adenocarcinomas are usually diffusely and strongly positive for EMA and CK7 and/or CK20 depending upon the primary site Negative: inhibin, calretinin, SF1, and FOXL2	Variable depending upon the primary site of adenocarcinoma

The previous case series documenting the cytologic diagnoses of AGCTs on FNAs and body fluids have noted variable cellular smears with monotonous tumor cells arranged as three-dimensional clusters and in micro follicles. Researchers have also identified typical *Call-Exner* bodies in cytologic smears from the AGCTs. Longitudinal nuclear grooving is one of the classic features of AGCT on histopathologic examination and has also been noted in cytologic smears [9-13]. In the present study, longitudinal nuclear grooving and a predominant micro follicle-like arrangement of the minimally pleomorphic, round to oval tumor cells with fine chromatin, indistinct nucleoli, and scant cytoplasm were noted to be the most consistent cytomorphologic features and were recorded in almost all the cases. The characteristic *Call-Exner* bodies with central eosinophilic secretions were also noted frequently.

The cytomorphologic features, although distinctive, may overlap with a variety of other neoplasms, especially at the metastatic sites. The differential diagnoses might vary with the location of the metastasis; however, the commonest ones include metastases from follicular carcinoma of the thyroid, follicular variant of papillary thyroid carcinoma, adenoid cystic carcinoma, neuroendocrine tumor, adenocarcinoma, small cell carcinoma, low-grade endometrial stromal sarcoma, juvenile granulosa cell tumor, and lobular carcinoma of the breast. Immunocytochemistry is helpful in challenging cases as AGCTs show positivity for FOXL2, inhibin A, steroidogenic factor-1 (SF-1), calretinin, and CD99. The key clinicopathologic, immunocytochemical, and molecular genetic features that can aid in differentiating metastatic AGCT from its cytologic mimics are presented in **Table 3** [14-21].

Isolated case reports and short series of metastatic granulosa cell tumors have been published, documenting metastases in the pelvis, liver, lungs, abdominal wall, perirectal tissue, lymph nodes, pleural, and peritoneal fluid [12, 13, 22-31]. In a study by Lal et al., the authors assessed cytological features of recurrent and metastatic granulosa cell tumors in fluids and FNAs and observed similar cytomorphologic features [30]. In another study by Harbhajanka et al., the authors studied the cytologic features and clinicopathologic correlation of 8 cases with recurrent and metastatic AGCTs on FNAs. They documented AGCT metastasis in the abdominal wall, pelvic mass, liver, and lung. They identified the presence of naked tumor nuclei (100%) as the most consistent cytologic feature, while nuclear grooves were noted in only 25% of their cases [31]. The current study found longitudinal nuclear grooves and microfollicular arrangement of the monomorphic tumor cells to be the most consistent cytologic features of metastatic AGCTs.

Conclusion

In addition to describing the cytomorphologic features of six metastatic AGCTs and documenting metastases at unusual sites, the current study highlights the common cytologic mimics and delineates the valuable clinicopathologic features to differentiate and accurately diagnose these tumors based on cytology.

Disclosure of conflict of interest

None.

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