Case Report

Primary adenosquamous carcinoma of the endometrium with glassy cell features. A diagnostic pitfall as a very rare tumour type in the endometrium

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Summary
Primary adenosquamous carcinoma of the endometrium with glassy cell features (ASC-GCF) is an extremely rare entity and to date, 16 cases of this entity have been reported in the literature. ASC-GCF is an aggressive histological subtype of cervical carcinoma with rapid growth and early metastases; however, very little is known about those originating from the endometrium as they are limited to only a few case reports. Herein, we report a case of primary adenosquamous carcinoma of the endometrium with extensive glassy cell features which posed a major diagnostic challenge by mimicking many entities with its histological diversity.

Key words: adenosquamous, carcinoma, endometrium, glassy cell, pathology

Introduction
Adenosquamous carcinoma with glassy cell features (ASC-GCF) was first described as a poorly differentiated variant of cervical adenosquamous carcinoma in 1956 by Glucksmann and Cherry and termed as “Glassy cell carcinoma (GCC)” 1. GCC is a rarely reported cancer which most commonly originates in the uterine cervix and accounts for only about 1-5% of all cervical cancers 2,3. Cases occurring in the endometrium, fallopian tube, vagina and colon have also been reported 4,5. GCC arising in the endometrium is much rarer and to the best of our knowledge, the number of GCC cases originating from the endometrium is only 16 in the literature to date. Herein, we present a case of endometrial adenosquamous carcinoma with extensive glassy cell features which posed a major challenge in the differential diagnosis.

Case report
A 82-year-old patient was admitted to the Department of Obstetrics and Gynecology of Ankara University Medical School with the complaint of abdominal distension. Abdominal CT imaging of the patient revealed a cystic and solid mass lesion, 86x69 mm in size, in the right pelvis (Fig. 1). The patient underwent total abdominal hysterectomy and bilateral salpingooopherectomy (TAH-BSO), bilateral pelvic and paraaortic lymph node dissection, omentum resection.
Pathology findings

Gross examination of TAH-BSO material revealed an exophytic mass in uterine corpus, 8.5 cm in long diameter, completely filling the endometrial cavity. It was a cream-white colored solid nodular lesion and infiltrated to the uterine serosa by traversing the uterine wall (Fig. 2). The tumor did not extend to the cervical wall. Microscopically, the tumour cells were arranged in solid sheets, cords and the background stroma was heavily infiltrated by lymphocytes, plasma cells and eosinophils. Polygonal shaped tumour cells had pale granular eosinophilic, clear or ground glass type cytoplasm with large nuclei, distinct nucleoli and sharp cell borders. Nuclear pleomorphism was prominent and high mitotic activity was observed. Bizarre cells with multinucleation were seen. Extensive emperiploesis of tumour cells was present throughout the neoplasm. Histologic evidence of glandular or squamous differentiation were very focal and required extensive sampling. There was clear cell differentiation focally and spindling of the tumour cells in some areas imitated a sarcomatoid component (Figs. 3, 4). Tumor cells infiltrated the full thickness of the uterine corpus wall and perforated the serosal surface. The tumor was completely localized in the uterine corpus and there was no extension to the cervix. Cervix wall was free of tumour. This finding ruled out the cervix origin of the tumour. Lymphovascular space invasion was detected. Histochemically mucicarmen was negative in the tumour cells. Periodic acid-schiff (PAS) staining revealed distinct cell membranes of tumour cells more clearly. Glassy cells were negative with PAS and PAS diastase.

A large panel of immunohistochemistry was performed for the differential diagnosis. Spindled tumour cells showed strong and diffuse cytokeratin expression. P40 and p63 were positive in the squamoid areas. Napsin A and AMACR were focally positive in the tumour cells with clear cytoplasm. Multifocal positivity was observed with MUC1. Tumour cells especially in squamoid areas showed membranous, web-like positivity with CD44. CEA (monoclonal) staining in tumour cells was sparse. Loss of E-cadherin expression was detected. p53 showed wild-type staining pattern. P16 overexpression was not seen. ER and PR were negative. Conserved expression of MSH2, MSH6, PMS2 and MLH1 suggested that the tumour is most likely microsatellite stable. High risk HPV (hrHPV) positivity of rare tumour cells were detected by chromogenic in situ hybridisation (CISH) method (Fig. 5). Our diagnosis was poorly differentiated endometrial ASC-GCF.
Figure 3. (a) Solid sheets of squamous cells, admixed with inflammatory cells (H&E slide, x10). (b) Higher magnification reveals clear evidence of squamous differentiation. Extensive emperipolesis of tumour cells can be seen (H&E slide, x20). (c) Histologic evidence of glandular differentiation (H&E slide, x10). (d) Glandular structures composed of round or polygonal tumour cells with prominent nucleoli, eosinophilic cytoplasm (H&E slide, x20).

Figure 4. (a) Epithelioid polygonal shaped tumour cells, individually or in small groups, in a background stroma heavily infiltrated by inflammatory cells (H&E slide, x20). (b) Tumour cells with eccentric irregular nucleus, ground glass type cytoplasm and sharp cell borders, some with rhabdoid features. Prominent nuclear pleomorphism (H&E slide, x20). (c) Spindling of the tumour cells imitating a sarcomatoid component in a background stroma heavily infiltrated by inflammatory cells (H&E slide, x12,1). (d) Tumour cells with clear cytoplasm mimicking clear cell carcinoma (H&E slide, x20).
Of the 41 pelvic and para-aortic lymph nodes dissected, 7 left pelvic and 4 para-aortic lymph nodes were metastatic, and the largest metastasis was 4.2 cm in long diameter. There was extracapsular spread of lymph node metastases. Omentum was free of tumour. The stage of the patient was determined as FIGO IIIC2. The patient received adjuvant paclitaxel and carboplatin chemotherapy.

**Review of the literature and discussion**

ASC-GCF is a very rare tumour type among gynaecological malignancies and is more likely to be seen in the cervix. It causes a diagnostic pitfall when it is seen in endometrium due to its intratumoural histological diversity and the fact that it is not an expected tumor type among endometrial tumours. To the best of our knowledge, there are only 16 reported cases of endometrial ASC-GCF in the English literature. Our case is the 17th one with a different clinical presentation. Our patient presented with abdominal distension, while previous cases presented with postmenopausal bleeding. Cases of endometrial ASCGCF reported to date in the literature are listed in Table I. Cases of ASC-GCF seen in cervix occur mainly in younger patients (mean 41 years) and some studies have noted an association with pregnancy or long term use of oral contraceptives 6. Whereas cases arised in the endometrium occur mainly in postmenopausal period. Dawson et al. published the oldest case, a 96-year-old endometrial GCC case 7 and our patient is the second oldest one after that.

Glassy cell features in an adenosquamous carcinoma are charactesized by the presence of tumour cells with ground-glass or granular cytoplasm, large eosinophilic nuclei, prominent nucleoli, and prominent cell membranes 2. High mitotic activity, atypical mitoses and intense inflammatory cell infiltration are seen. These features can be seen at varying rates in an adeno-
squamous carcinoma and tumours which are composed entirely of glassy cell features may have no histological evidence of glandular or squamous differentiation. There is confusion in the literature regarding the definition of GCC. While adenosquamous carcinomas containing 30% to 100% glassy cell features were designated as GCC previously, the latest World Health Organization (WHO) classification of female genital tumours no longer recommends the use of the terminology of GCC. The present case caused a diagnostic challenge in our routine practice and we started with a wide list of various tumour types for differential diagnosis. The areas of spindled tumour cells gave the impression of a sarcomatoid component and led to a preliminary misdiagnosis of uterine carcinosarcoma consisting of squamous cell carcinoma as an epithelial component and rhabdomyosarcoma as a heterologous sarcomatous component. Diffuse and strong cytokeratin positivity of the tumour cells indicated that the spindled tumour cells were also of epithelial nature. Desmin and myogenin negativity of the glassy cells with rhabdoid features ruled out the morphological impression of rhabdomyoblastic differentiation. These findings excluded the preliminary diagnosis of uterine carcinosarcoma. Poorly differentiated endometrioid carcinoma with areas of squamous metaplasia was another possibility in the differential diagnosis since the tumour was localised in the uterine corpus and consisted of squamous and glandular components. However, ER and PR negativity of the tumour cells and the absence of endometrioid-like appearance in the glandular areas ruled out this possibility. Tumour cells with clear cytoplasm showed focal Napsin A and AMACR positivity mimicking clear cell carcinoma, but the documentation of squamous differentiation by p63 and p40 immunostaining in tumour cells were contrary to the diagnosis of clear cell carcinoma. Malignant melanoma was also included in the differential diagnosis list due to the presence of large eosinophilic nuclei in tumour cells, however diffuse and strong cytokeratin expression precluded this possibility. The tumour also showed areas mimicking lymphoepithelioma-like carcinoma, with sheets of poorly differentiated epithelioid cells surrounded by prominent inflammatory cell infiltration, but the obvious glandular and squamous differentiation of the tumour were inconsistent features with this entity. In conclusion, all the histological and immunohistochemical features led us to the diagnosis of “Poorly differentiated ASC-GCF”. Since adenosquamous carcinoma is very rare in the uterine corpus in comparison with the cervix, origin of the cervix had to be ruled out first. Localization of the tumour mass entirely in the uterine corpus and absence of any tumour infiltration to the cervical wall excluded the cervix origin, which is the primary site of origin for uterine adenosquamous carcinomas.

Table I. Reported cases of endometrial ASC-GCF in the literature.

| Author                       | Age (years) | Symptoms     | FIGO Stage | Treatment          | Follow-up          |
|------------------------------|-------------|--------------|------------|--------------------|--------------------|
| Cristopherson, et al.        | 56          | Vaginal bleeding | I          | RT, H              | DOD at 5 months    |
|                              | 70          | Vaginal bleeding | I          | RT, H              | DOD at 32 months   |
|                              | 71          | Vaginal bleeding | I          | RT, H              | Death of pneumonia after 6 years, NED |
|                              | 78          | Vaginal bleeding | I          | RT                 | Suicide after 7 months |
|                              | 78          | Vaginal bleeding | III        | RT                 | DOD after 5 months  |
| Arends, et al.               | 59          | Vaginal bleeding | IIIA       | TAH, BSO, RT, Progestins | NED after 24 months |
| Dawson, et al.               | 96          | Vaginal bleeding | IVB        | Megestrol          | NED after 16 months |
| De Rosa, et al.              | 58          | Metrorrhagia    | IB         | TAH, BSO           | DOD after 12 months |
|                              | 52          | Metrorrhagia    | IB         | TAH, BSO, PND      | DOD after 16 months |
|                              | 58          | Metrorrhagia    | IB         | TAH, BSO, PND      | ED at 36 months    |
| Hachisuga, et al.            | 62          | Vaginal bleeding | IIIC       | TAH, BSO, PND, RT  | NED after 66 months |
| Mhawech, et al.              | 60          | Vaginal bleeding | IB         | TAH, BSO, PND, RT  | NED after 60 months |
| Ferrandina, et al.           | 63          | Vaginal bleeding | IB         | TAH, BSO, PND      | NED after 69 months |
| Nagy and Sipos, et al.       | 68          | Abnormal cytology | IA        | TAH, BSO, RT      | NED after 24 months |
| Callegari, et al.            | 64          | Abnormal cytology | IA        | TAH, BSO, PND      | NED after 14 months |
| Fox, et al.                  | 58          | Vaginal bleeding | IA         | TAH, BSO, PND      | NED after 18 months |
| Sevim, et al. (Current case) | 81          | Abdominal distension | IIIC2    | TAH, BSO, PND, PaND, O, CT | NED after 15 months |

Abbreviations: BSO = Bilateral salpingo-oophorectomy, CT = Chemotherapy, DOD = Death of disease, ED = Alive with evidence of disease, NED = No evidence of disease, O = Omentectomy, PaND = Para-aortic lymph node dissection, PND = Pelvic lymph node dissection, RT = Radiotherapy, TAH = Total abdominal hysterectomy
Cervical ASC-GCF has been believed to originate from subcolumnar reserve cells and strong association has been found with hrHPV infection, especially HPV type 18. Histogenesis of endometrial ASC-GCF is unknown. Arends et al. suggested that the ASC-GCF seen in the endometrium might originate from endocervical or isthmic mucosal cells, based on the finding of lysozyme immunoreactivity of tumour cells, which is expected to be expressed in endocervical and isthmic mucosal cells. However, reported cases of stage I endometrial ASC-GCF limited to the uterine corpus without any association with cervix, like our present case, contradict to this hypothesis. Our case showed hrHPV by CISH, but not accompanied by p16 overexpression. The prevalence of HPV in endometrial cancers is low, even in tumours with squamous differentiation or in pure endometrial squamous tumors. HPV infection does not seem to have any role in the initiation of endometrial carcinoma. It may only be a mere “passenger” in the endometrium since endometrium is a suitable host for HPV replication. HPV-associated tumours show a strong overexpression of p16. Despite of the hrHPV positivity, p16 was negative in our case. Negative p16 immunostaining has been described in a small percentage of cases of hrHPV-positive carcinomas of the vagina, vulva and head and neck. The absence of p16 immunostaining in a hrHPV-positive tumour may be explained by the inactivation of the tumour suppressor gene p16 due to genetic or epigenetic alterations. Interestingly, p16 inactivation due to either mutation or methylation is a relatively frequent event in different cancers and has been associated with tumour progression and dissemination and with a more aggressive behaviour.

As adenosquamous cell carcinoma is a rare tumour type in the cervix and much rarer in the endometrium, very limited genomic profiling has been performed. In a case of endometrial glassy cell carcinoma reported in 2019, next-generation sequencing showed PTEN (phosphatase and tensin), KRAS (v-Ki-ras2 Kirsten rat sarcoma viral oncogene), and TP53 (tumor protein) gene mutations. In an in vitro study performed on cell culture obtained from endometrial adenosquamous carcinoma, it was determined that endometrial adenosquamous carcinoma cell lines have a PTEN termination mutant, are MSI-Low, are copy number high, and do not have a POLE mutation. Since endometrial adenosquamous carcinoma is a very rare tumour type and was not included in the genomic characterisation analysis of endometrial carcinomas within the Cancer Genome Atlas (TCGA) project, the genomic profile of this endometrial tumour type and in which group of the endometrial carcinoma molecular subgroups they should be included in the recent molecular classification are unknown. When we consider the molecular profile of cervical adenosquamous carcinomas according to the findings of the TCGA project, only 3 of the 178 cervical cancers included in were adenosquamous carcinomas. Among the novel genomic and proteomic characteristics that subclassify cervical cancers, cervical adenosquamous carcinoma cases were involved in the “Keratin-low Squamous” group and “Adenocarcinoma-rich” subgroups. They were characterised by overexpression of hormone receptor genes ESR1, PGR and PIK3CA amplification, and PTEN gene deletion.

An important problem with this tumour is the lack of standardised treatment protocols, due to their rarity. ASC-GCF of the cervix is an aggressive tumour with a tendency to metastasise early and be relatively resistant to radiation therapy. However, more recent reports suggest that multimodal treatment strategies with postsurgical radiochemotherapy based on paclitaxel-carboplatin has been shown to be effective in cases originating from cervix. Paclitaxel-carboplatin has also been shown to be effective in recurrent cases or in neoadjuvant treatment of tumours. ASC-GCF arised in the endometrium was also reported as a highly aggressive malignancy with a poor prognosis. Our patient received adjuvant chemotherapy and no local recurrence or distant metastasis have been detected for 15 months.

**Conclusion**

We presented this unusual case as a significant diagnostic pitfall in endometrial pathology. ASC-GCF causes diagnostic problems in the endometrium, because it does not come to mind among the endometrial malignancies as it is very rare in the endometrium (limited to a few case reports reported in the literature) and it mimics several endometrial carcinomas due to its intratumoural histological diversity.

**Conflict of interest**
The authors declare no conflicts of interest.

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**Ethical consideration**
None.

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