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A 68 years old woman underwent radio-chemotherapy for a squamous cell carcinoma of the cervix. Follow up was uneventful until, eight years after radio-chemotherapy, imaging exams detected a diffuse enlargement of the uterine body. Radical hysterectomy revealed a multiphasic lesion with both sarcomatous and mixed carcinomatous components. The carcinomatous, component presented neuroendocrine histologic and ultrastuctural features and an intense expression of neuroendocrine immunohistochemistry markers. No residual cervical carcinoma was documented (pR0). The patient died of disease after 9 months.

Reported cases further demonstrate how the irradiation of the uterus for cervical cancer carries a not negligible risk of developing a second endometrial cancer. The second cancer may develop years after initial therapy and may have aggressive histologic and clinical features. This case underlines the importance for a long follow-up in women having received radio-chemotherapy alone.

Key words
MMMT • Neuroendocrine tumor • Radio-chemotherapy • Ultrastructure • Immunohistochemistry

Summary
Chemo-radiation represents an effective therapy for carcinoma of the uterine cervix. The endometrium may however receive a consistent dose of mutagenic radiations and patients may have an increased risk of secondary malignancies. Endometrial mixed malignant mullerian tumor (MMMT) is a rare, highly aggressive disease, and neuroendocrine features are even rarer.

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Introduction
Endometrial mixed malignant mullerian tumor (MMMT) or carcinosarcoma is a rare, highly aggressive disease, accounting for approximately 3% of all uterine neoplasms typically occurring in elderly postmenopausal women with a median age of 65 years.

By definition, these are neoplasms composed of an admixture of malignant epithelial and mesenchymal components. Both the components may show a wide pattern of differentiation: the carcinomatous component may have endometrioid, serous, undifferentiated or clear cell morphology while the sarcomatous component may show diverse histotypes resembling both typical uterine sarcomas (homologous) and other soft tissues tumors (heterologous).

Neuroendocrine features are infrequently observed in the lower female genital tract and predominantly in the cervix. Radiation therapy is the standard treatment for most patients with stage IIb–IVa cervical cancer. Recent randomized trials demonstrating improved survival when concurrent chemotherapy is added to radiation have led to the adoption of chemo-radiotherapy as the standard treatment for advanced cervical cancer with or without subsequent surgery. While chemo-radiotherapy is often curative, the radiation used can damage normal tissues, including the uterine corpus, and, if surgery is not performed, patients may survive long enough to be at risk of developing second uterine malignancies.

Neuroendocrine features are considered an extremely rare event in MMMT. In particular, no reports exist up till now of MMMT with neuroendocrine features in a

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MIXED MALIGNANT MULLERIAN TUMOR WITH NEUROENDOCRINE FEATURES IN AN IRRADIATED UTERUS FOR CERVICAL CARCINOMA. A UNIQUE ASSOCIATION?

Case report

A 68 year old post-menopausal woman, without significant medical history, presented with meno-metrorrhagia and intermittent pelvic pain. Subsequent colposcopy revealed a mass deforming the uterine cervix. Bioptic samples revealed a moderately differentiated (G2), invasive, squamous cell carcinoma. Staging magnetic resonance imaging (MRI) demonstrated a 6 cm mass located in the uterine cervix infiltrating the right paracervical connective tissue. The tumour was thus assigned a IIB FIGO stage and the patient was treated with chemotherapy (cistplatin and taxol) and radio (brachi)therapy, according to validated international protocols of treatment. Although chemio-radiotherapy was complicated by significant morbidity (actinic colitis and endometritis), the gynecological follow up, performed regularly, showed no evidence of residual disease.

Eight years later follow up computed tomography and MRI detected a diffuse enlargement of the uterine body within which 43 x 32 x 49 mm mass was seen. Clinical and radiological suspicion of a new, different primitive neoplasm of the uterine corpus was raised and the patient underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy.

Pathologic findings

At macroscopy, the neoplasm measured 40 x 30 x 50 mm and was solid and polypoid in nature, friable and necrotic. It occupied a large part of the uterine cavity with ulceration of the endometrial mucosa and deep infiltration throughout the myometrium with focal involvement of the serosa. Ovaries and salpinges were unremarkable. Microscopically, a carcinosarcoma (Mixed Müllerian Malignant Tumor) composed of different intermingled cancer cell populations was diagnosed.
The carcinomatous component was mixed, consisting of three different histotypes: moderately differentiated endometrioid carcinoma (70%) (Fig. 1A) neuroendocrine carcinoma (20%) (Fig. 1B); serous carcinoma (10%) (Fig. 1C).

The sarcomatous component consisted of spindle cells with marked cytological atypia and high mitotic count (Fig. 1D), immunopositive for vimentin (Fig. 2B) and negative for cytokeratins (Ck AE/AE3, CAM 5.2, Ck MNF-116) (Fig. 2A), smooth muscle actin (SMA), desmin and caldesmon, CD68 and CD10.

The neuroendocrine carcinomatous component was characterized by typical architectural and cytological features such as solid and insulo-trabecular growth and presence of wide areas of confluent necrosis. Cells showed irregular nuclei with vesicular chromatin and with inconspicuous nucleoli and amphophilic granular cytoplasms. The mitotic count was high (more than 20 mitosis/10HPF) with an high ki67 staining index (90%). Immunohistochemistry displayed strong and intense expression of neuroendocrine markers Chromogranin, Synaptophysin and CD56 only in the carcinomatous component (Fig. 2C-E).

Complete evaluation of the surgical specimen showed total regression of the cervical cancer and evidence of actinic cervicitis along with other radio-induced alterations such as vascular sclerosis and stromal fibrosis. No residual tumor was documented (pR0)\textsuperscript{12}.

Electronic microscopy confirmed the multiphasic nature of the neoplasm. Epithelial looking polygonal cells arranged in chords and nests, rarely forming glandular lumens (Fig. 3A), with tight junctions and cytoplasmic small electron dense neurosecretive were seen. Furthermore sarcomatoid spindle cells with irregular nuclear outlines (Fig. 3B) were also documented. The neoplasm infiltrated the myometrium and metastatic deposits were documented on the uterine serosa; no other metastases were evident at surgery (FIGO stage IIIA).

The patient received three courses of platinum-based chemotherapy but died of metastatic disease after 9 months.

**Discussion**

MMMT are relatively rare diseases and show an extremely aggressive clinical course. In the past, four different pathogenetic theories have been proposed for this disease: the collision theory suggests that the carcinoma and sarcoma are two independent neoplasms; the combination theory suggests that both components are derived from a single stem cell which undergoes divergent differentiation early in the evolution of the tumour; the conversion theory suggests that the sarcomatous element derives from the carcinoma during the evolution of the tumour; the composition theory suggests that the spindle cell component is a pseudosarcomatous stromal reaction to the presence of the carcinoma\textsuperscript{13}. 
Recent studies have shown that uterine carcinosarcoma should be regarded as a metaplastic carcinoma: indeed, the carcinomatous component is considered the “driving force” of the disease, being the most frequently found element in tumor-involved lymph-vascular spaces, metastatic lesions, and representing, above all, the major determinant of clinical outcome. The emergence of sarcomatous elements would therefore represent the evolution of subclones arising within an aggressive, poorly differentiated endometrial carcinoma with endometrioid, serous or clear cell histology. Even in metastases, endometrial carcinoma can progress to carcinosarcoma. All these findings seem to support the conversion theory which considers MMMT a particular subtype of endometrial carcinoma rather than a sarcoma. MMMT should therefore be included in the Type II subgroup having a p53-mediated pathogenesis, an aggressive behavior and adverse prognosis.

Aside from anecdotal reports, neuroendocrine differentiation in mixed mesodermal (Müllerian) tumors of the female genital tract have accounted for up to 17% in a relatively large series of 47 cases. This percentage, based on a rather old study, seems to be high. It may be possible that the neuroendocrine pattern is underestimated in routine pathology practice, and interpreted merely as undifferentiated carcinoma. Though some published data suggest an aggressive course and poorer prognosis for carcinosarcomas with neuroendocrine or neuroectodermal differentiation, it remains to be clarified whether the neuroendocrine pattern bears clinical significance, both for diagnosis and therapeutic approaches. The present case confirmed an aggressive behavior and resistance to therapy leading to patient death despite prompt surgery and adjuvant chemotherapy.

Cervical cancer remains the second most common female malignancy worldwide. Women treated with radiation for cervical carcinoma are usually young and often survive for many years. The addition of concurrent chemotherapy may further improve survival rates for those with loco-regional advanced disease.

Previous studies have been carried out on radiation-induced second cancers. There are some epidemiologic surveys for second cancers following radiation treatment for cervical cancer. A study from Czesnin et al. found the risk of uterine sarcomas, including carcinosarcoma, to be 5.4 times that of the control population. Fehr et al. observed 2294 patients who were irradiated for cervical carcinomas, and found 12 patients with proven endometrial cancer. This was more than double the expected annual spontaneous incidence of endometrial cancer. More recently, Pothuti et al. reporting the experience of two large US Cancer Centres, described 23 post irradiation endometrial cancers, 7 of which with combined carcinomatous and sarcomatous features. Radiation-associated endometrial cancers carry a poor prognosis because they are more likely to be non-endometrioid, poorly differentiated and advanced stage cancers. The longer latency, in general more than a decade, of radiation-associated endometrial cancers may suggest a possible delay in clinical presentation and diagnosis.

In the presented case the patient had scrupulously adhered to follow-up, however she came to surgery in an advanced stage confirming the aggressive behavior of the disease.

The mechanism for tumorigenesis of post radiation carcinoma is still unclear. Parkash et al. have pointed out that it is possible that the obliteration of the cervical os by previous radiation therapy favors the development of an inflammatory process in the uterine cavity, which might lead to necrosis and cancer. It should be underlined that the endometrial cavity is very close to the cervix and during radiotherapy, it receives a considerable dose of irradiation. The relationship between radiation and carcinogenesis is complex. Radiation-induced DNA damage is most commonly found in the form of double strand breaks, which may result in mutation when the normal mechanisms for DNA repair or apoptosis fail.
Endometrial tissue can persist after radiation therapy for cervical cancer and undergo neoplastic transformation. Furthermore, young women treated with radiation for cervical cancer receiving hormone replacement therapy with estrogen may have an increased risk of endometrial cancer.

In our experience on surgically removed uteri after neoadjuvant radio-chemotherapy for uterine cervix carcinoma, the endometrial lining is often modified by treatment and, in general, shows atrophic features sometimes associated with nuclear enlargement, mild hyperchromasia with irregular nuclear outlines and rarely, nuclear p53 accumulation can be observed with immunohistochemistry. These latter “atypical” cases, in an intriguing hypothesis to be verified by further studies, may represent a very early radiation induced pre-neoplastic lesion may result in a second endometrial cancer in time. In a recent, interesting in vitro study, Tsukamoto et al. experimentally demonstrated a radiation induced epithelial-mesenchymal transition on cultured endometrial cells, explaining the unexpectedly high rates of MMMT in patients with irradiated uteri. In conclusion all these observations demonstrate how the irradiation of the uterus for cervical cancer carries a not negligible risk of developing a second endometrial cancer, in those patients were the uterus not surgically removed after completion of radio-chemotherapy. The second cancer may develop many years after therapy and can have aggressive histologic features such as sarcomatoid and neuroendocrine morphology, rapidly carrying these patients to exitus.

Patients where the uterus is left in its anatomic site after the completion of the curative-intention radio-chemotherapy, probably require a longer and more thorough follow-up for the risk of a second malignancy. This latter observation should be taken into account when curative-intention radio-chemotherapy protocols are comparatively evaluated with neo-adjuvant treatments followed by radical surgery.

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