CASE REPORT

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Muscle metastases from cervical carcinoma – case report

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Abstract

Muscular metastases are rarely found in medical practice, and the reported cases in literature are not numerous. The diagnosis of these lesions involves an interdisciplinary collaboration. We present a case of secondary determination in the psoas muscle, with a starting point of cervical squamous carcinoma. In establishing the diagnosis, there contributed the clinical, imagistic and magnetic resonance evaluation and computed tomography (CT), the histopathological diagnosis being determined after the CT-guided biopsy puncture.

Keywords: muscular metastases, cervical carcinoma, muscular biopsy, immunohistochemistry.

Introduction

World widely, cervical cancer represents the third cause of death in women and the second as mortality frequency, with approximate 300 000 deaths every year. The incidence in developed countries is high, over 80% [1].

Cervical neoplasms metastasize more frequently through direct invasion and through the local lymphatics, the hematogenous metastasation being extremely rare. The muscular metastases of cervical cancer are reported in a percentage less than 1% in specialized literature, even though the muscular mass represents about half of the body weight [2, 3]. Most frequent muscle metastases came from lung carcinomas (the most frequent ones), pancreatic, renal, colorectal, ovary and melanoma carcinomas. There are quoted cases of muscular metastases in carcinomas, lymphomas and leukemia [3, 4].

Regarding the most frequent localization, the psoas, iliopsoas and paraspinal muscles are the most affected ones; also, there are observed metastases in the gluteal, paraspinal, abdominal and limb proximal muscles [3, 4].

Regarding the muscular metastases in the cervical cancer, there were reported cases of invasion in the biceps, psoas, masseter, deltoid and intercostal muscles [3, 4].

Every metastasis preserves the immunohistochemical characteristics of the primary tumor. When the muscular biopsy shows squamous neoplastic cells in men, the primary tumor is located most often in the lungs (81%), while in women the primary tumor is in the cervical (64%) [5].

Our study has the aim of presenting a rare case of muscular metastasis, with a starting point in the cervical tumor, the final diagnosis presenting successive imagistic investigations [computed tomography (CT) and magnetic resonance imaging (MRI)] and histopathological and immunohistochemical ones, leading to the accurate identification and specification of the muscular tumor origin.

Case presentation

We present the case of a patient with muscular metastasis in the psoas muscle, originating from a sqamous cervical carcinoma.

The patient P.E., aged 64 years, from the rural area, presented to the Clinic of Gynecology of Tîrgu Mureş (Romania), with climax metrorrhagia, in 2012; based on the gynecological investigation and on the histopathological investigation after local cervical biopsy, there was established the diagnosis of invasive adenosquamous carcinoma stage IB2. A hysterectomy with adnexectomy was performed, with pelvic lymphadenectomy within the limits of the oncological safety, and in the end there was established the tumor stage: T1b2 N0M0.

The histopathological investigation of the surgical exeresis tissue highlighted well-differentiated squamous tumor cells in the endocervical, with no keratinization tendency. These cells arranged in islands were associated with the proliferative tumor glandular component, the glands being lined by an atypical epithelium. Also, in the cervical channel, there were identified numerous areas of cells with the same morphological aspect (Figures 1 and 2).

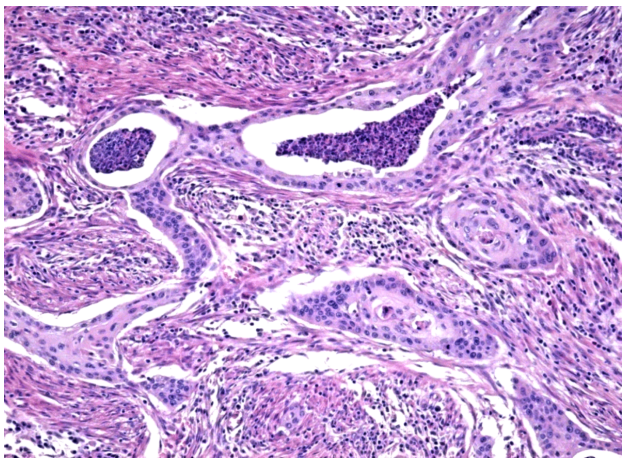

Figure 1 – ***Islands of tumor cells associated with glands, with the presence of an abundant acute inflammatory infiltrate with abscess areas. HE staining, ×10.***

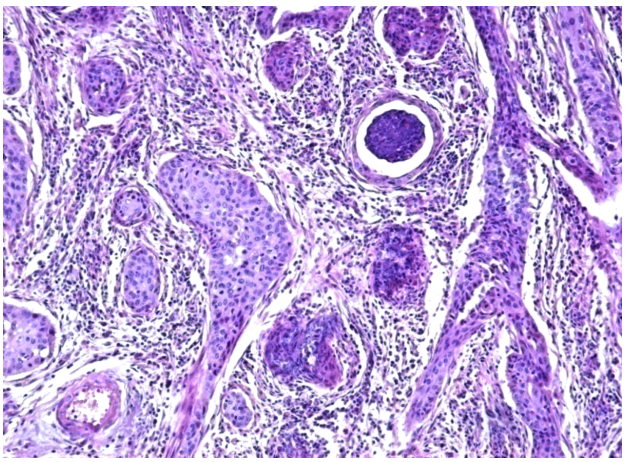

Figure 2 – ***Islands of tumor cells associated with glands. HE staining, ×10.***

For highlighting some particular characteristics of the tumor, there were performed various immunohistochemical determinations, based on serial sections following the ones utilized for the histopathological study. The immunohistochemical protocol started with the deparaffinization of the slides, antigen retrieval by boiling in citrate buffer (pH 6), in the microwave oven, at 650 W, for 21 minutes, blocking of endogenous peroxidase with 1% water peroxide for 30 minutes, blocking the non-specific antigen sites with 2% skimmed milk for 30 minutes, and overnight incubation at 4^0C in solutions of primary antibodies, in the dilutions recommended by the manufacturer (see below). The next day, the sections were washed in phosphate-buffered saline (PBS), then the signal was amplified with a polymeric detection system conjugated with horseradish peroxidase (HRP) (Nichirei-Histofine, Tokyo, Japan) for 60 minutes, and, in the end, the detection was performed with 3,3'-diaminobenzidine (DAB, Dako). Ultimately, the sections were contrasted with Hematoxylin, dehydrated, clarified and coversliped in a xylene-based mounting medium (DPX, Merck).

For the immunohistochemical study, there were used the following primary antibodies:

- anti-estrogen receptor (ER) (monoclonal mouse anti-human estrogen receptor alpha, clone 1D5, 1:50 dilution, Dako);
- anti-progesterone receptor (PR) (monoclonal mouse anti-human progesterone receptor, clone PgR 636, 1:50 dilution, Dako);
- pan-cytokeratin AE1/AE3 (monoclonal mouse anti-human pan-cytokeratin AE1/AE3, 1:50 dilution, Dako);
- high molecular weight cytokeratin (monoclonal mouse anti-human, clone 34βE12, 1:500 dilution, Dako);
- anti-Ki67 (monoclonal mouse anti-human, clone MIB-1, 1:50 dilution, Dako).

The microscopic study showed immunopositivity in the tumor cells, both in the undifferentiated cell islands, and in the tumor glandular epithelium for cytokeratins AE1/AE3 and 34βE12, the reaction intensity being higher for pan-cytokeratin AE1/AE3. The tumor cells were negative for ER and only rarely positive at nuclear level for PR. Ki67 was positively diffuse in the tumor cell islands, the positivity percentage ranging between 40–60% (Figures 3–5).

The patient was recorded in the Department of Oncology, where she underwent a cytostatic treatment with Cisplatin 45 mg/m^2/week and adjuvant radiotherapy, the box technique, T target volume and pelvis ganglionary areas, total radiation dose (TD) 50 Gy, with good tolerance.

After 34 months since establishing the diagnosis of cervical cancer, the patient presented pain in the thorax–lumbar spine, with insidious progress, which did not improve after the classical treatment with analgesics (Acetaminophen 2 g/day) and non-steroidal anti-inflammatory drugs, reason for which there was performed a abdominal–pelvis MRI investigation. MRI highlighted an area with an edema aspect in the left sacrum. There was subsequently performed a bone scintigraphy, invalidating the bone metastases, the aspect pleading for bone structure changes after external radiotherapy. The patient's state progressively deteriorated, with increasing pain, being required stronger analgesics like Tramadol 100 mg/dose, three doses per day. There was performed a new MRI investigation (Siemens Magnetom Essenza 1.5T) that highlighted an area with high signal of T2 and T2-STIR (short *tau* inversion recovery)-weighted, a signal similar to the muscles of T1, with contrast, relatively homogenous presentation (Figure 6, a and b). There were not identified any other abdominal or pelvic lesions.

For completing the imagistic investigations, there was performed a whole body CT investigation (Siemens Biograph mCT) that did not highlight the presence of lung metastases. The lesion described in the MRI investigation in the psoas muscle presented a peripheral contrast capture, extension in the left foramen L1–L2, with no bone structure changes (Figure 7, a and b).

The imagistic aspects suggested the presence of a muscular metastasis in the psoas muscle. In order to ascertain the diagnosis, there was performed a CT-guided biopsy puncture (Figure 8). The histopathological investigation on the biopsy puncture material highlighted a muscular tumor in the left psoas. The histological investigation of muscular biopsy showed fibrocollagenous tissue including islands of large carcinomatous cells, intense chronic inflammatory infiltrate, adjacent to the striated muscular tissue. The tumor cells were arranged as heterogeneous cordons and whorls, which infiltrated the muscular fibers and the surrounding conjunctive tissue.

The tumor cells did not present any polarization, had an intense basophilic cytoplasm and relatively heterogeneous nuclei, without the agglutination of chromatin (Figures 9 and 10). As a result, it was concluded that the entity in the psoas muscle presented the characteristics of a muscular metastasis that could have originated most probably within the cervical neoplasm.

The patient continued the treatment with a new line of chemotherapy with Cisplatin, 45 mg/m^2/week, the prognosis of the patient remaining low. There was proposed a new surgical intervention for removing the muscular tumor, but the patient refused.

The general state of the patient progressively aggravated, with accentuated neurological signs. A new MRI investigation after nine months since the histo-pathological confirmation of the muscular metastasis, showed the extension of the tumoral process in the entire left psoas muscle, iliac muscle and lumbar square muscle, on the left side; also, there was observed the presence of a left hydronephrosis, secondary to the left ureter inclusion in the tumoral process (Figure 11, a–c). Also, a CT investigation highlighted the presence of some osteolysis processes in the transverse processes L1 and L2 of the left side (Figure 12, a and b).

The patient's death occurred two months after the last imagistic investigation.

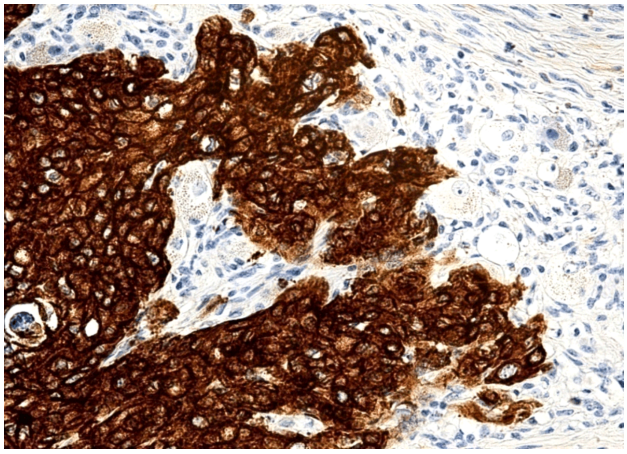

Figure 3 – ***Extremely intense marking of the glandular and tumor epithelium with the anti-AE1/AE3 antibody, ×200.***

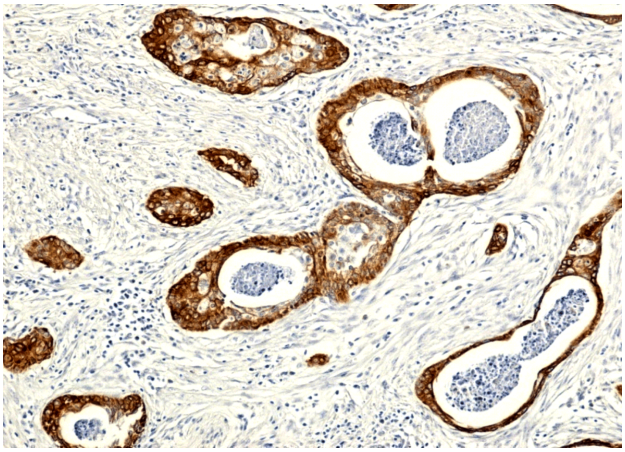

Figure 4 – ***Intense marking of the tumor glandular epithelium with the anti-34βE12 antibody, ×100.***

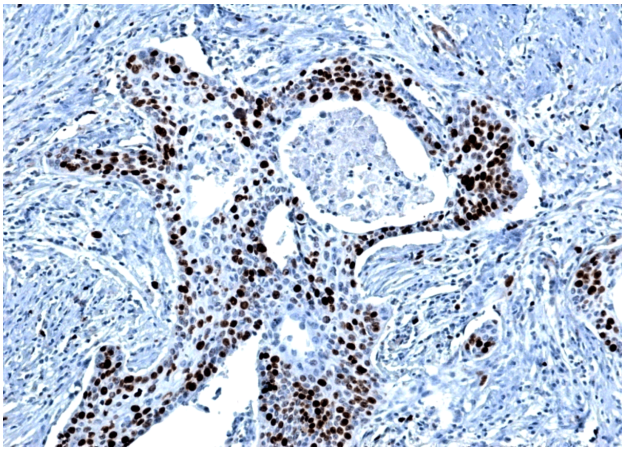

Figure 5 – ***Ki67 intense and frequent nuclear marking in the nuclei of the tumor epithelium, ×100.***

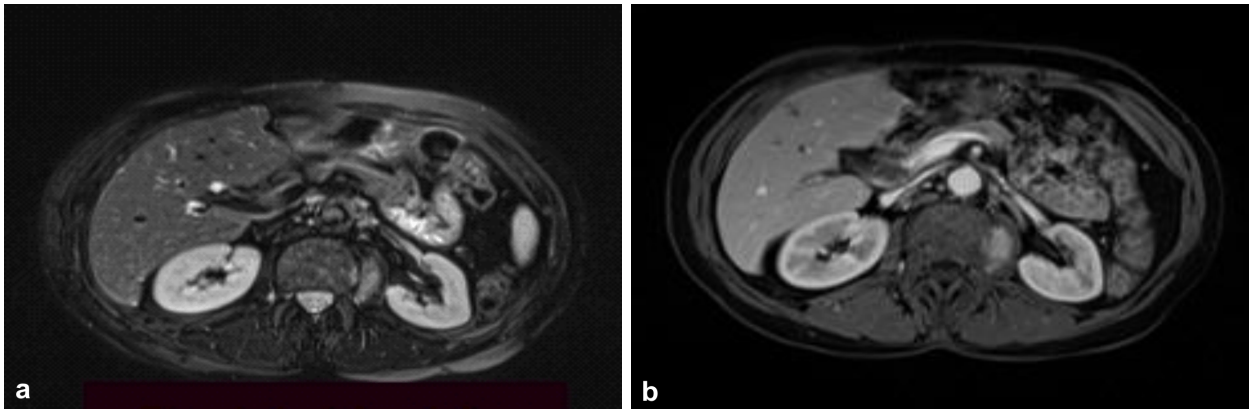

Figure 6 – ***(a and b) MRI axial sections: contrast enhancement of the left psoas muscle.***

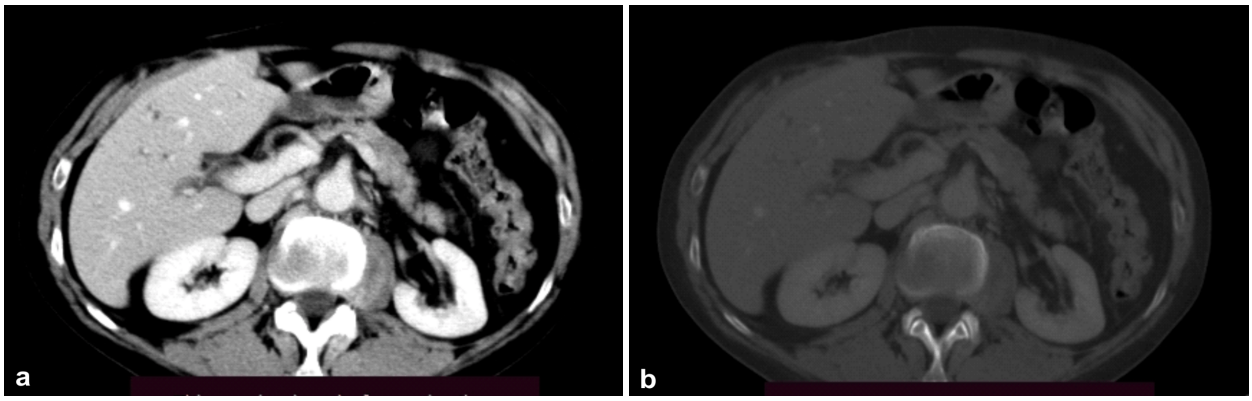

Figure 7 – *(a and b) CT axial sections (parenchymatous and bone window): contrast enhancement of the psoas muscle, without bone invasion.*

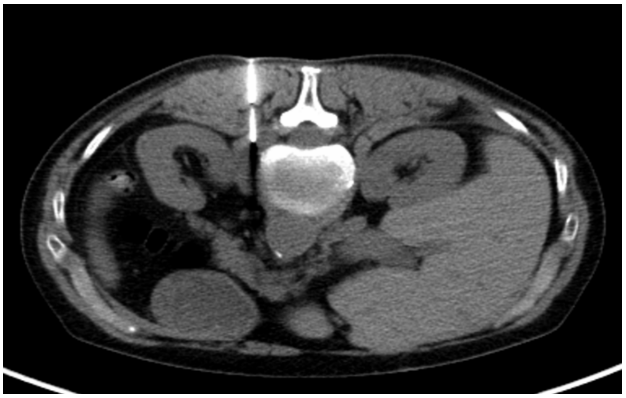

Figure 8 – *Muscular CT-guide biopsy puncture.*

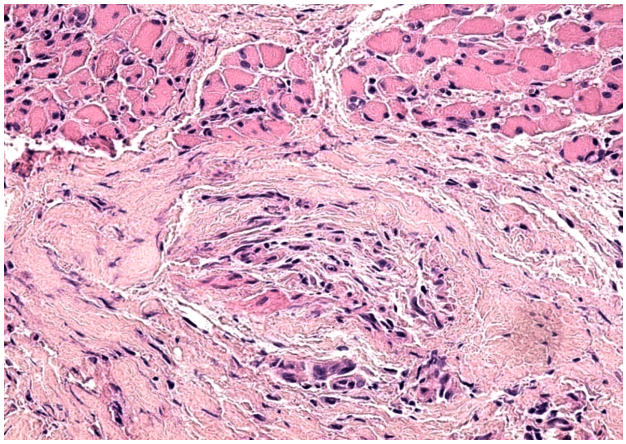

Figure 9 – *Tumor cellular islands in the stroma around some skeletal muscular fibers. HE staining, ×200.*

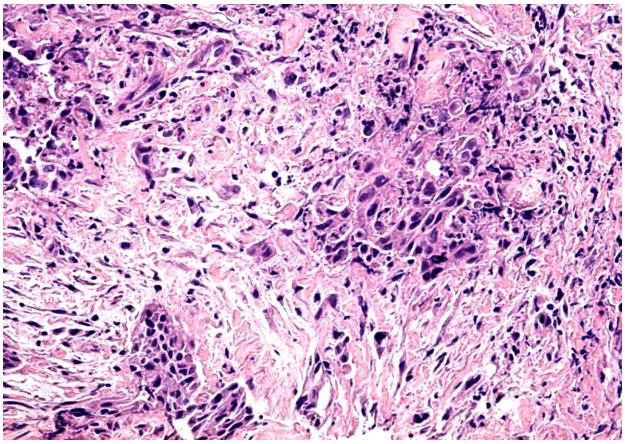

Figure 10 – *Irregular tumor cellular islands and detached cells with an infiltrative characteristic. HE staining, ×200.*

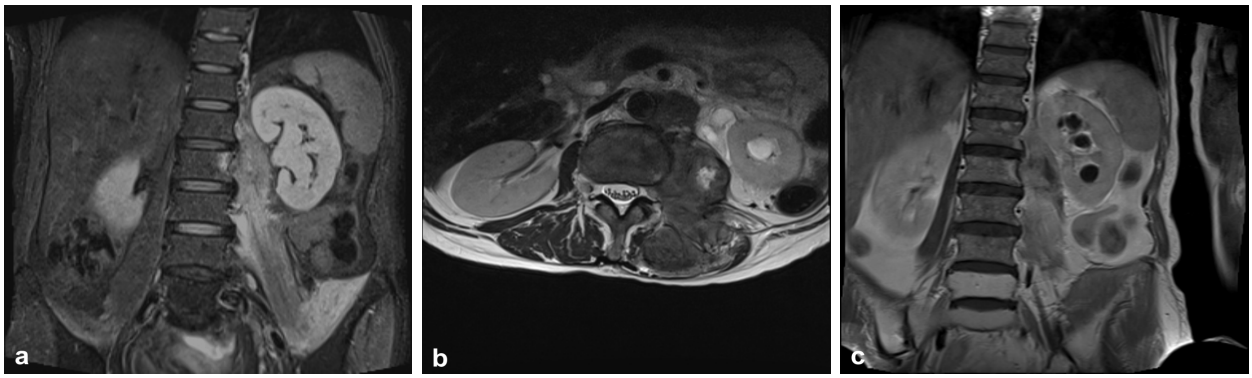

Figure 11 – *MRI examination: (a) Coronal plane section T2-STIR-weighted – high signal of the left iliac and psoas muscle, left hydronephrosis; (b) Axial plane section T2 weighted – extension of the tumor process in the lumbar square muscle, the longissimus dorsi muscle; (c) Coronal plane section, T1 weighted with contrast: heterogeneous enhancement capture of the left psoas muscle.*

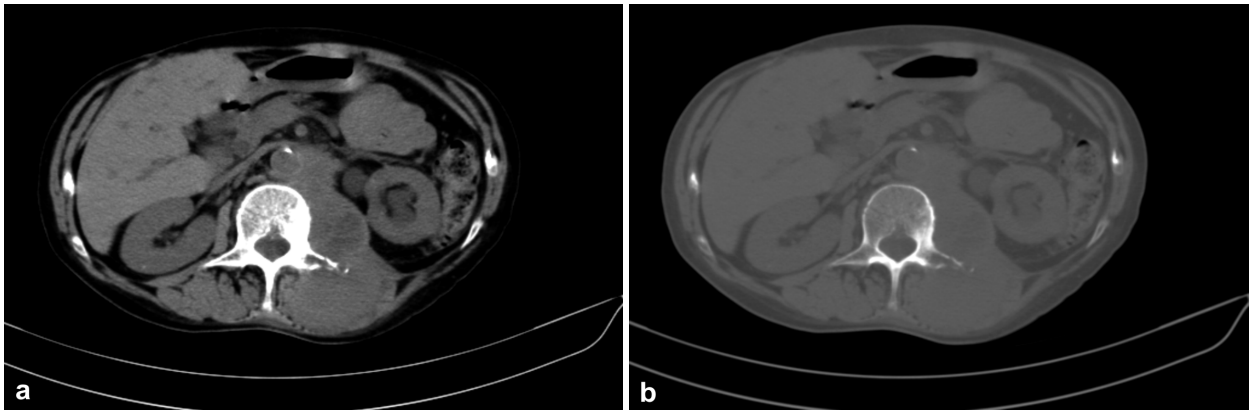

Figure 12 – ***(a and b) CT axial sections, parenchymatous and bone window: osteolysis of the transverse process left L1.***

Discussion

The tumoral secondary determinations in the skeletal muscles are rare, representing less than 1% of the total metastases identified by clinically and imagistically evaluation procedures [6–8]. However, the number of muscular metastases reported by the pathological anatomy departments in autopsy is a lot higher, reaching 17.5% of the total of metastases [9]. These differences are due to the fact that the identification and diagnosis of metastases in the striated skeletal muscles is difficult without any specific clinical symptoms. Lately, MRI investigations allowed the increase of the percentage of diagnosed muscular metastases, as MRI provides a better resolution of the soft tissues than CT or other imagistic investigations [10–12]. Also, the introduction of the imagistic method positron emission tomography–computed tomography (PET–CT) considerably enhanced the precision of the diagnosis for detecting skeletal metastases [13].

The lung, renal, thyroid and melanoma neoplasms are the ones that most frequently metastasize in the psoas, iliopsoas, paraspinal muscles and in the limb muscles. The muscular metastases from a cervical neoplasm are quite rare, the reported incidence being less than 1%, most of them human immunodeficiency virus (HIV)-positive and in advanced stages of the disease.

The exact pathway of neoplastic cell dissemination from a primary cervical tumor is not well known yet. The low frequency of muscular metastases is the consequence of possible various factors responsible for the high resistance of muscles to cancer metastasation [2, 4]:

- Reduced stasis caused by constant movements of the skeletal muscles;
- Presence of lactic acid resulted from the muscular contraction represents an unfavorable environment for the development of metastasis cells;
- Presence of natural killer (NK) lymphocytes in the muscles that present an anti-tumor activity;
- Presence of protease inhibitors localized in the basal membranes that inhibit cellular invasion;
- Presence of some muscular peptide factors may negatively influence the dissemination of tumor cells.

The pH changes, accumulations of metabolites, a variable blood flow determined by the adrenergic receptors and by the pressure variations in the muscles affect the implantation of tumor cells in the skeletal muscles [14].

As an experiment, Weiss proved that the tumor cells survive in the enervated muscle, which does not contract, but they develop in electrically stimulated muscles [4].

In our study, the presence of tumor metastases was suggested by the pain in the thorax–lumbar area that required imagistic investigations. The MRI examination was the one highlighting the changes of the left psoas muscle, and the biopsy puncture associated to the histopathological examination confirmed the suspicion of muscle metastasis.

The imagistic semiological characteristics of muscle metastases include the intermediary signal T1 weighted, the T2 weighted high signal and the present contrast enhancement. Starting from these aspects, the lesions present a differential diagnosis with the abscess of the soft part, the hematoma and the primary muscle tumors [4]. That is why, from the images presented by us, there could also be observed that the imagistic aspects, including CT with the bone window, were not pathognomonic, being able to direct the diagnosis to a muscular metastasis, taking into consideration the patient's anamnesis data and clinical evolution.

Due to the fact that the clinical aspects of the muscular metastases may be similar to a primitive muscular sarcoma, the differentiation between the two entities is extremely important for the treatment strategy [14].

In the same line, in our case, the certainty diagnosis was the histopathological and the immunohistochemical one. These microscopic studies confirmed the origin of the muscular lesion in the primary cervical tumor, the tumor lesion being certainly with a secondary determination.

According to the cases reported in the specialty literature, next to the psoas, the metastases were identified in the biceps, deltoid, iliopsoas, masseter and intercostals muscles [3, 15–20]. In the present case, we reported the presence of a metastasis in the psoas muscle, with cervical cancer as starting point. Actually, the psoas muscle is mentioned as the most frequent localization of muscular metastases in cervical cancer [13].

The case presented by us is included in the characteristics of the patients with cervical cancer and muscular metastases, according to review [3], the aspects being similar ones: the squamous primary tumor, the chemotherapeutical treatment was performed according to the existing protocols, and the interval of metastasis onset was 24 up to 60 months since the initial diagnosis of the

cervical neoplasm [13, 15–21], but there is also reported a case of muscular metastasis onset even after 76 months, its localization being in the intercostals muscles [3].

Also, there is a reference to the HIV status, the previously quoted articles presented negative HIV cases, but there are also works mentioning the higher frequency of muscular metastases in the immunocompromised, HIV-positive patients, as the HIV virus causes the decrease of the body resistance [21, 22]. In some situations, there may also exist multiple metastases secondary to the squamous cervical carcinoma, detected by PET–CT [23].

Various studies showed that muscular metastases may be single, but there may also exist multiple muscular metastases even in the cervical squamous carcinoma [23].

In our case, the muscular metastasis was at first single in the left psoas muscle and there was not detected the presence of HIV virus. The metastatic process continued with an extension in the iliac muscle and the lumbar square muscle and osteolysis in the transverse processes of vertebras L1 and L2 of the left side.

Regarding the disease stage, in the cases reported by other authors, there are observed different stages, from IB up to IIIB [13, 15–21]; in our study, the initial stage of the disease was IIB, with the onset of muscular metastasis after 34 months, after the surgical intervention, radio-therapy and chemotherapy. Thus, there is no correlation between the initial stage of the disease and the onset of a muscular metastasis.

The studies analyzed by us did not present any conclusive data regarding the time period from the onset of muscular metastasis and the death of patients; also, we cannot report to the rate of survival of the patients with such pathology, in the situation of performing or not a surgical intervention of muscular tumor excision. In our case, the patient's denial for the surgical intervention led to a rapid extension of the tumor within the whole psoas muscle, with the invasion of the iliac muscle and a consequent inclusion of the left ureter, lesions that most probably led to the rapid death of the patient, after 45 months since the first diagnosis of primary tumor, namely after 11 months since the first imagistic diagnosis of muscular metastasis.

The certainty diagnosis was the histopathological and immunohistochemical one. The microscopic studies suggested most probably the origin of the muscular lesion in the primary cervical tumor, the biopsy puncture from the secondary determination of the psoas muscle high-lighting the epithelial-like cells that infiltrated the muscular fibers. There are numerous presentations of cervical cancer patients that presented metastases, most frequently in the psoas muscles, and also in the deltoid, masseter, intercostals and biceps muscles. In these cases, the muscular biopsy made the difference between metastasis, sarcoma and other muscular conditions, like cysts or abscesses, as it highlighted the presence of squamous tumor cells similar to those in the origin tumor that infiltrated the muscular fibers [24–27].

All the presented arguments allowed us to consider the muscular formation as being a metastasis, from the cervical neoplasm and the presented case may be included in the short list of the muscular metastases of cervical neoplasms.

Conclusions

Even though the muscular metastases are quite rare, these lesions may also be suspected in the patients with a cervical cancer in their medical record, who present with muscular pain with an insidious progress, with no improvement after classical pain killer treatment. The imagistic evaluation is required in these cases, guiding the diagnosis and taking into consideration the elements of differential diagnosis, and the histopathological and immunohistochemical examination are essential for the diagnosis confirmation.

Conflict of interests

The authors declare that they have no conflict of interests.

References

[1] Abudukadeer A, Azam S, Mutailipu AZ, Qun L, Guilin G, Mijiti S. Knowledge and attitude of Uyghur women in Xinjiang province of China related to the prevention and early detection of cervical cancer. World J Surg Oncol, 2015, 13:110.
[2] Sudo A, Oghihara Y, Shiokawa Y, Fujinami S, Sekiguchi S. Intramuscular metastasis of carcinoma. Clin Orthop Relat Res, 1993, (296):213–217.
[3] Ferrandina G, Salutari V, Testa A, Zannoni GF, Petrillo M, Scambia G. Recurrence in skeletal muscle from squamous cell carcinoma from the uterine cervix: a case report and review of the literature. BMC Cancer, 2006, 6:169.
[4] Williams JB, Youngberg RA, Bui-Mansfield LT, Pitcher JD. MR imaging of skeletal muscle metastases. AJR Am J Roentgenol, 1997, 168(2):555–557.
[5] Mathis S, Fromont-Hankard G, du Boisguéheneuc F, Godenèche G, Mahieu F, Balaboi I, Nocon C, Marsac E, Neau JP. [Muscular metastasis]. Rev Neurol (Paris), 2010, 166(3):295–304.
[6] Ménard O, Parache RM. [Muscle metastases of cancers]. Ann Med Interne (Paris), 1991, 142(6):423–428.
[7] Kozyreva ON, Mezentsev DA, King DR, Gomez-Fernandez CR, Ardalan B, Livingstone AS. Asymptomatic muscle metastases from esophageal adenocarcinoma. J Clin Oncol, 2007, 25(24): 3780–3783.
[8] Li Q, Wang L, Pan S, Shu H, Ma Y, Lu Z, Fu X, Jiang B, Guo Q. Skeletal muscle metastases on magnetic resonance imaging: analysis of 31 cases. Contemp Oncol (Pozn), 2016, 20(3):242–250.
[9] Surov A, Hainz M, Holzhausen HJ, Arnold D, Katzer M, Schmidt J, Spielmann RP, Behrmann C. Skeletal muscle metastases: primary tumours, prevalence, and radiological features. Eur Radiol, 2010, 20(3):649–658.
[10] Glockner J, Sundaram M, White L. Incidence of solitary soft-tissue metastases revealed by MR imaging. AJR Am J Roentgenol, 2002, 179(6):1644.
[11] Jiang H, Wang ZC, Xian JF, Li J, Zhao PF. [Age-related changes of normal adult inferior rectus muscle: analysis with dynamic contrast-enhanced magnetic resonance imaging]. Zhonghua Yi Xue Za Zhi, 2011, 91(27):1899–1903.
[12] Schmidt GP, Reiser MF, Baur-Melnyk A. Whole-body imaging of the musculoskeletal system: the value of MR imaging. Skeletal Radiol, 2007, 36(12):1109–1119.
[13] Basu S, Mahajan A. Psoas muscle metastasis from cervical carcinoma: correlation and comparison of diagnostic features on FDG-PET/CT and diffusion-weighted MRI. World J Radiol, 2014, 6(4):125–129.
[14] Plaza JA, Perez-Montiel D, Mayerson J, Morrison C, Suster S. Metastases to soft tissue: a review of 118 cases over a 30-year period. Cancer, 2008, 112(1):193–203.
[15] Mariya Y, Watanabe S, Yokoyama Y, Tarusawa N, Takekawa S, Kattou K, Kaimori M, Ise N. [Metastasis of uterine cervical cancer to the biceps muscle of right upper arm; report of a case]. Rinsho Hoshasen, 1990, 35(11):1447–1450.
[16] Pathy S, Jayalakshmi S, Chander S, Thulkar S, Sharma MC. Carcinoma cervix with metastasis to deltoid muscle. Clin Oncol (R Coll Radiol), 2002, 14(6):447–448.

[17] Wong BJ, Passy V, DiSaia P. Metastatic small cell carcinoma to the masseter muscle originating from the uterine cervix. Ear Nose Throat J, 1995, 74(2):118–121.
[18] Bar-Dayan Y, Fishman A, Levi Z, Rachmani R. Squamous cell carcinoma of the cervix with psoas abscess-like metastasis in an HIV-negative patient. Isr J Med Sci, 1997, 33(10):674–676.
[19] Devendra K, Tay SK. Metastatic carcinoma of the cervix presenting as a psoas abscess in an HIV-negative woman. Singapore Med J, 2003, 44(6):302–303.
[20] Saâdi I, Hadadi K, Amaoui B, Errihani H, Mansouri A, Benjaafar N, El Gueddari BK. [Muscle metastasis of squamous cell carcinoma of the uterine cervix]. Cancer Radiother, 2003, 7(3):187–189.
[21] Schwartz LB, Carcangiu ML, Bradham L, Schwartz PE. Rapidly progressive squamous cell carcinoma of the cervix coexisting with human immunodeficiency virus infection: clinical opinion. Gynecol Oncol, 1991, 41(3):255–258.
[22] Singh GS, Aikins JK, Deger R, King S, Mikuta JJ. Metastatic cervical cancer and pelvic inflammatory disease in an AIDS patient. Gynecol Oncol, 1994, 54(3):372–376.
[23] Vishnoi MG, Jain A, John AR, Paliwal D. A rare case of extensive skeletal muscle metastases in adenocarcinoma cervix identified by ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography scan. Indian J Nucl Med, 2016, 31(3):215–218.
[24] Trăistaru R, Rogoveanu O, Popescu R, Enăchescu V, Ghiluşi M. Periarticular diffuse neurofibroma of the upper limb. Rom J Morphol Embryol, 2011, 52(4):1377–1383.
[25] Kalra N, Aiyappan S, Nijhawan R, Sharma SC, Khandelwal N. Metastatic carcinoma of cervix mimicking psoas abscess on imaging: a case report. J Gynecol Oncol, 2009, 20(2):129–131.
[26] Karunanithi G, Sethi P, Reddy KS, Rani PR. Skeletal muscle metastasis from carcinoma cervix: a case report. J Gynecol Oncol, 2010, 21(3):196–198.
[27] Skenderi F, Chikha A, Ibisevic N, Tatarevic-Suko A, Kantardzic N, Vranic S. Skeletal muscle metastases from squamous cell carcinoma of the cervix: report of two cases with literature preview. Int J Gynecol Pathol, 2017, 36(1):95–100.

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