

Case Report

# Unusual Clinical Presentation of Gastrointestinal Clear Cell Sarcoma

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## Key Words

ATF1 · Cancer of unknown primary site · Ewing's sarcoma · EWSR1 · Gastrointestinal clear cell sarcoma · Melanoma · Polymerase chain reaction · Review · Translocation

## Abstract

**Background:** Use of molecular assays is gradually becoming a mandatory part of the clinical management of soft tissue tumors, however the choice and the interpretation of these tests may present a challenge. **Summary:** This report demonstrates an unusual presentation of sarcoma, which was initially diagnosed as a tumor of unknown primary site. Given the presence of vimentin, Fli-1, CD99 and S100 markers, lack of immunostaining for melan A, HMB45, MITF, synaptophysin, CD56, myf4, CKAE1/3 and WT-1, as well as the presence of EWSR1 translocation determined by a break-apart FISH assay, Ewing's sarcoma (ES) diagnosis seemed to be well justified. However, polymerase chain reaction testing for ES-specific rearrangements (EWSR1/FLI1, EWSR1/ERG, EWSR1/ETV1, EWSR1/ETV4, EWS/FEV) failed to confirm the ES origin of the neoplastic tissue. We further considered clinical, morphological, immunohistochemical and molecular diagnostic features of other types of EWSR1-rearranged sarcomas and performed molecular testing for gastrointestinal clear cell sarcoma. The polymerase chain reaction assay revealed EWSR1ex7/ATF1ex5 fusion, thus confirming the latter diagnosis. Subsequent high-precision computed tomography of the abdominal cavity revealed a 5-cm tumor of the small bowel, which was subjected to surgical resection. **Key Message:** This report exemplifies that the use of anonymous cytogenetic assays, such as break-apart FISH EWSR1 testing, may not be sufficient even in case of a perfect match with relevant morphological and immunohistochemical tumor features. **Practical Implications:** Explicit identification of the translocation gene partners is indeed important for proper sarcoma diagnosis management.

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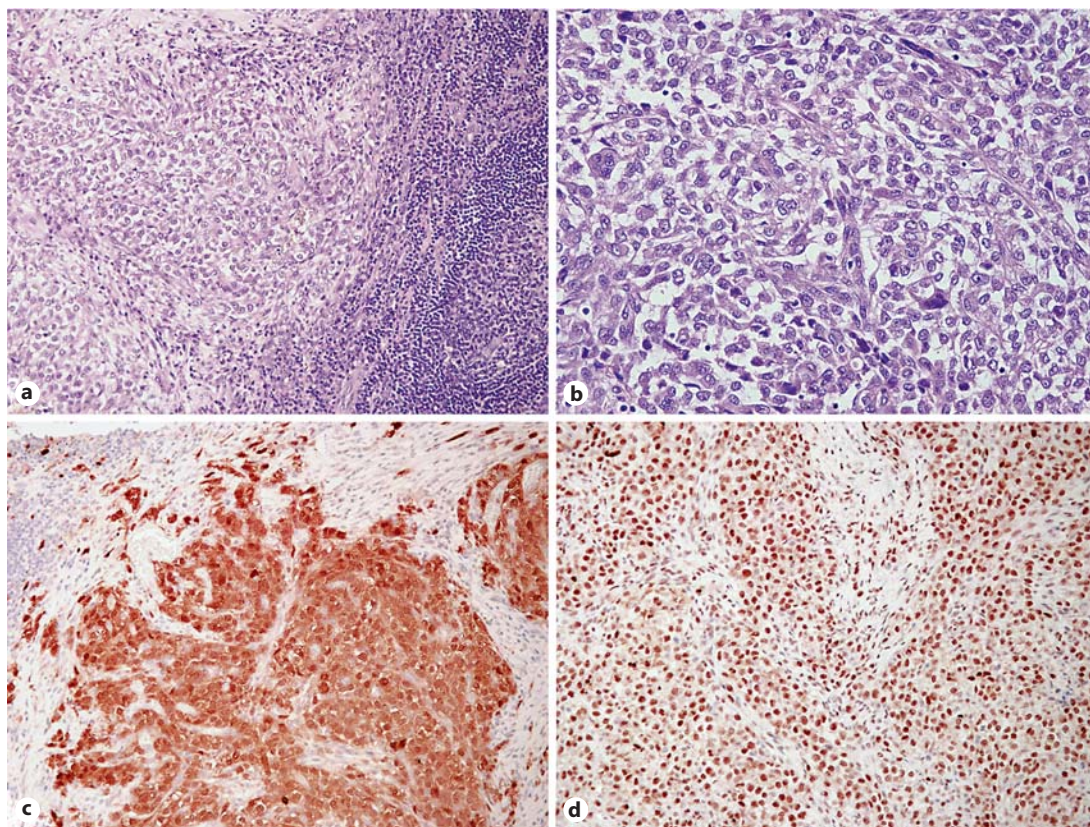
## Introduction

Gastrointestinal clear cell sarcoma (CCS) is an exceptionally rare tumor, with only a few dozen cases described in the literature [1, 2]. The diagnosis of CCS is complicated as it requires a combination of morphological, immunohistochemical and molecular techniques [3]. Here we describe an unusual presentation of CCS of the small intestine which manifested with non-specific symptoms and was initially considered as metastatic sarcoma of unknown primary site.

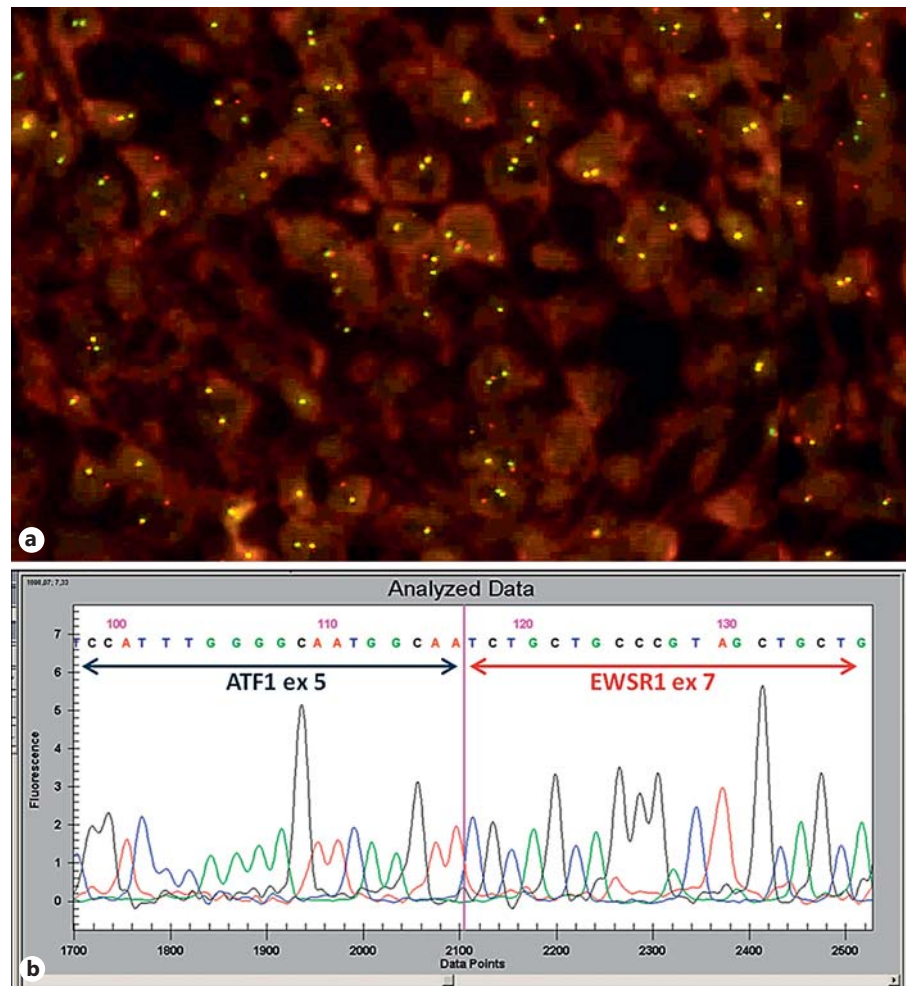
## Case Report

The 21-year-old patient was initially forwarded to a cardiology unit due to dyspnea, tachycardia, fatigue and night fever. These symptoms had been triggered by recent tonsillitis, so the patient was suspected to suffer from septic endocarditis and therefore received intensive antibacterial therapy. Further examination by spiral computed tomography revealed abdominal lymphadenopathy, while no abnormalities were detected by esophagogastroscopy and colonoscopy. Diagnostic laparoscopic biopsy of an affected lymph node was subsequently performed.

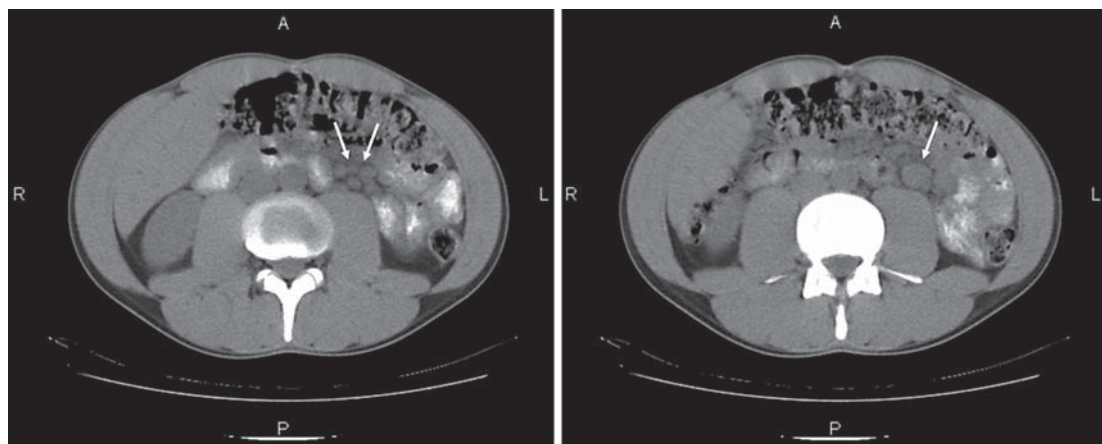
Morphological examination of the obtained specimen revealed features of sarcoma (fig. 1). Given immunopositivity with antibodies for vimentin, S100 (fig. 1c), CD99 and Fli-1 (fig. 1d), lack of reactivity for melan A, HMB45, MITF, synaptophysin, CD56, myf4, CKAE1/3 and WT-1, as well as presence of EWSR1 translocation determined by a break-apart FISH assay (fig. 2a), Ewing's sarcoma (ES) diagnosis was assigned to this



**Fig. 1.** Lymph node metastasis of CCS. **a** H&E staining.  $\times 200$ . **b** H&E staining revealed presence of multinucleated cells.  $\times 400$ . **c** Immunohistochemical staining with S100 polyclonal antibody (dilution 1:2,500, Dako).  $\times 200$ . **d** Immunohistochemical staining with Fli-1 antibody (clone MRQ-1, dilution 1:50, Cell Marque).  $\times 200$ .



**Fig. 2.** **a** EWSR1 translocation revealed by the Vysis LSI dual color break-apart probe.  $\times 1,000$ . **b** Nucleotide sequence of the chimeric EWSR1ex7/ATF1ex5 transcript.

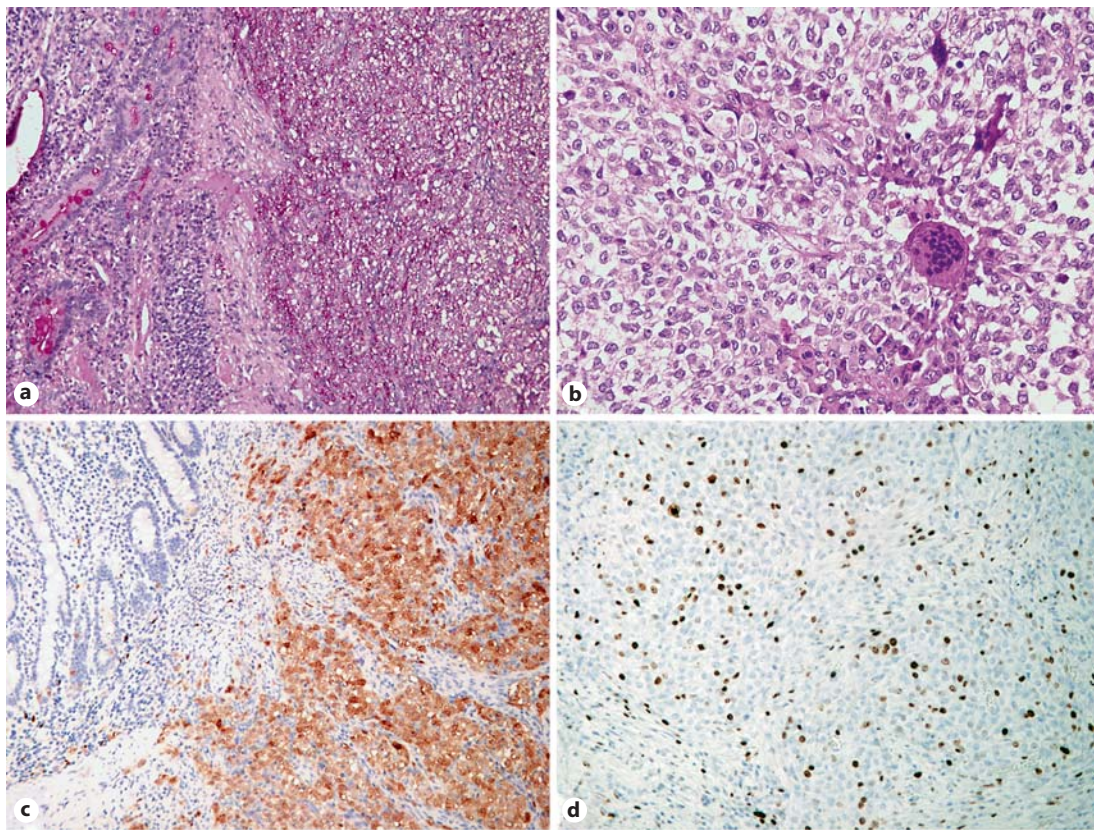


**Fig. 3.** Abdominal CT scans revealed tumor nodules (marked by arrows), which formed a conglomerate approximately 5 cm in diameter. The results of image analysis are in agreement with the macroscopic appearance of the tumor upon surgical resection (data not shown).

**Table 1.** Differential diagnosis of tumors bearing EWSR1 translocation [3, 5, 14]

Characteristics	ES	CCS, common type	CCS, gastro-intestinal type	Myoepithelial carcinoma	Desmoplastic small round cell tumor	Extraskeletal myxoid chondrosarcoma	Present observation
Anatomic location	bones (80%) or virtually any anatomic site (20%)	extremities, often associated with tendons or aponeuroses	small bowel, stomach, colon	limbs (75%), trunk, head and neck; rarely in bones or visceral organs	abdominal cavity, retro-peritoneum	proximal extremities, limb; rarely in retroperitoneum, pleura, bones	abdominal cavity (small bowel)
Median age at presentation	20 years	20–40 years	20–40 years	40 years	20–30 years	50 years	21 years
Clear cell appearance	often present	present	present	often present	absent	may be present	present
Osteoclast-like cells	absent	often present	often present	usually absent	usually absent	usually absent	present
PAS stain	positive	positive	positive	negative	negative	positive	positive
S100 expression	may be positive	positive	positive	positive	usually negative	positive in 20% of cases	positive
Melanocytic markers (melan A, HMB45, MITF)	negative	positive	often negative	negative	negative	negative	negative
Fli-1 expression	positive	usually negative	usually negative	usually negative	usually negative	usually negative	positive
CD99 expression	positive	usually negative	usually negative	usually negative	positive	usually negative	positive
Cytokeratin expression	may be positive	negative	negative	positive	positive	usually negative	negative
EWSR1 gene rearrangement (FISH break-apart assay)	positive	positive	positive	positive	positive	positive	positive
EWSR1 fusion partners	FLI1 (85%), ERG, ETV1, ETV4, FEV	ATF1, CREB1	ATF1, CREB1	POU5F1, PBX1, ZNF444	WT1	NR4A3	ATF1

case. However, comprehensive polymerase chain reaction testing for ES-specific rearrangements (EWSR1/FLI1, EWSR1/ERG, EWSR1/ETV1, EWSR1/ETV4, EWS/FEV) [4] failed to confirm the ES origin of the neoplastic tissue. EWSR1 translocations are known to occur in other types of tumors, including CCS, myoepithelial carcinomas, desmoplastic small round cell tumors, extraskeletal myxoid chondrosarcomas, myxoid liposarcomas, angiomatoid fibrous histiocytomas, B-lymphoblastic leukemia, etc. [5]. Based on the combination of clinical, morphological and immunohistochemical features (table 1), we further considered the diagnosis of gastrointestinal CCS and performed polymerase chain reaction analysis for EWSR1/ATF1 and EWSR1/CREB1 fusions [6]. This led to the identification of the EWSR1ex7/ATF1ex5 translocation, as confirmed by DNA sequencing (fig. 2b). Subsequently, the patient's abdominal cavity was again subjected to a high-precision computed tomography. This allowed to reveal the tumor conglomerate in the small intestine (approximately 5 cm in diameter), which was excised by surgical intervention (fig. 3). The microscopic and immunohistochemical appearance of this tumor was identical to that observed for lymph node metastasis (fig. 4).



**Fig. 4.** CCS of the small bowel. **a** Presence of glycogen in tumor cells, as detected by PAS staining.  $\times 200$ . **b** H&E visualization of multinucleated cells.  $\times 400$ . **c** Immunohistochemical staining with S100 polyclonal antibody (dilution 1:2,500, Dako).  $\times 200$ . **d** Immunohistochemical staining with Ki-67 (clone MIB-1, dilution 1:250, Dako).  $\times 200$ .

## Discussion

Several circumstances make this case unusual and therefore deserving presentation. First, though this patient had undergone a decent clinical examination, the primary site of metastatic sarcoma remained unknown until the definite morphological diagnosis was established. Second, while CCS often needs to be discriminated from melanoma, its ES-like appearance is uncommon. This tumor did not express melanocytic markers (melan A, HMB45, MITF) but presented with typical features of ES, including the presence of EWSR1 translocation and Fli-1 expression (fig. 1d, 2a). However, it is important to keep in mind that these characteristics may also occasionally be observed in other tumor types [7–9]; furthermore, the presence of multinucleated cells (fig. 1b, 4b) is somewhat more compatible with CCS than ES diagnosis [3]. Finally, this case exemplifies that the use of anonymous assays, such as break-apart FISH EWSR1 testing, may lead to erroneous results, so the identification of the translocation gene partners is indeed important for proper sarcoma management [10–12]. A series of similar tumors, which were characterized by gastrointestinal location, sarcoma-like histology, absence of melanocytic markers, distinct ultrastructural features and presence of EWSR1 translocations (including EWSR1-ATF1 and EWSR1-CREB1 fusions), has recently been described by Stockman et al. [13]; they suggest to designate this tumor entity as malignant gastrointestinal neuroectodermal tumors.

In conclusion, this report exemplifies the power of combined imaging-based, morphological, immunohistochemical, cytogenetic and molecular testing in diagnostic oncology.

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### Disclosure Statement

The authors disclose no potential conflicts of interest.

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