CASE REPORT

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A rare case of a double high-risk gastrointestinal stromal tumor of jejunum with KIT-negative/PDGFRA-positive immunophenotype

MĂDĂLINA BOŞOTEANU[1,2)], GABRIELA IZABELA BĂLŢĂTESCU[1,3)], MARIANA DEACU[1,2)], MARIANA AŞCHIE[1,2)], CĂTĂLIN ADRIAN BOŞOTEANU[1)]

[1)]*Clinical Service of Pathology, "Sf. Apostol Andrei" Emergency County Hospital, Constanţa, Romania*

[2)]*Department of Pathology, Faculty of Medicine, "Ovidius" University of Constanţa, Romania*

[3)]*Center for Research and Development of the Morphological and Genetic Studies of Malignant Pathology, "Ovidius" University of Constanţa, Romania*

Abstract

Gastrointestinal stromal tumors (GISTs) are mesenchymal tumors that represent the second most common type in the gastrointestinal system, but clinical outcomes vary due to complex molecular changes. The aim of our study is to highlight a unique case of a 5th decade male, presenting a double primary GIST of the jejunum, associated with a rare immunophenotype and with a high risk of malignancy. Ancillary studies were performed using several biomarkers [cluster of differentiation (CD) 117/c-kit, discovered on GIST1 (DOG1), desmin, S-100, vimentin, cytokeratin 7 (CK7), Ki67, actin, platelet-derived growth factor receptor alpha (PDGFRA) and CD34], in order to confirm the diagnosis and to evaluate prognostic and predictive factors. A KIT-negative/PDGFRA-positive immunophenotype was obtained in our case and it was associated with a poor prognosis. Its unfavorable clinical evolution was sustained by recurrence as malignant (GIST) with dedifferentiation and metastases developed in less than one year after the initial diagnosis. Clinico-morphological features of GISTs with an impact on survival must be identified and a tailored therapy should be applied for each case.

Keywords: gastrointestinal tumor, jejunum, immunohistochemistry, dedifferentiation.

Introduction

Gastrointestinal stromal tumors (GISTs) are mesenchymal tumors that represent the second most common type in the gastrointestinal (GI) system after adenocarcinomas [1]. It was proved that they originate from the interstitial cells of Cajal (ICC) and have a similar immunohistochemical profile: both express the cluster of differentiation (CD) 34 and *KIT* mutation [2]. GISTs can be identified in any portion of the GI tract and the small bowel location occupies the second place, with a 31.8% recorded frequency after gastric involvement, which is encountered in 55.6% of cases [1]. The vast majority of cases located in the small bowel are identified in ileum and jejunum, and only 5% in the duodenum [3]. Median age is 60 years old, but any age between 10 and 100 years old can be affected with an almost equal distribution between female and male gender [4].

Even if GISTs share similar morphological features, the clinical outcomes vary, depending on the either benign or malignant characters of the proliferation that diverge due to complex molecular changes. The oncogenic *KIT* mutations are the most frequent encountered in GIST, with 80–95% incidence recorded [5], mostly in exon 11 and rarely in exons 9 and 13, which can be usually highlighted by immunohistochemistry (IHC) [6]. Another mutation, which can be associated with GIST, is that one which involved platelet-derived growth factor receptor (*PDGFR*) gene, present in 6.5% of all cases [5, 7]. All these discoveries associated with tyrosine kinase inhibitor (TKI) therapy (Imatinib or Trastuzumab) had a great impact on survival of patients [8]. However, a small percentage of cases had a poor clinical course it was proved that these lack *KIT* or *PDGFR* mutations and there were designated as wild-type GIST (WT-GIST), with a poor prognosis [9].

The aim of our study is to highlight the unique case of a 5th decade male, presenting a double primary GIST of the jejunum. Notably, this tumor is associated with a rare immunophenotype and with a high risk of malignancy, proved by an unfavorable clinical evolution, fact sustained by the recurrence and metastases developed in less than one year after the initial diagnosis.

Case presentation

We report a rare case of a double small bowel GIST in a 42-year-old obese and chronic smoking male patient. His past medical history revealed a left pulmonary tumor in May 2016. The tumor was large, measuring 15×10 cm, and it was located in the superior lobe, with extension to the inferior lobe. The left lung along with regional mediastinal lymph node resection have been performed in other medical center and the pathological report of the surgical specimen proved to be a pleomorphic carcinoma with metastasis in one mediastinal lymph node. Adjuvant chemotherapy was applied after surgery and follow-up in the next months noted a good clinical evolution. In June 2017, he was referred to the Department of Emergency, "Sf. Apostol Andrei" Emergency County Hospital, Constanţa, Romania, accusing abdominal pain and vomiting. Abdominal

distension and tenderness were noticed at the physical examination. The ultrasound evaluation showed dilated, fluid-filled loops of the small bowel and an area of narrowed lumen, suggestive for intestinal occlusion. Laboratory tests demonstrated a severe iron-deficiency anemia. An enterectomy was performed and the surgical specimen was sent to the Department of Pathology. Macroscopically, two lesions were identified in the 32 cm of jejunum, with free resection margins: the first, slightly elevated, measuring 2.2 cm and the second one, a pedunculated mass of 4.5 cm in greatest dimension (Figure 1).

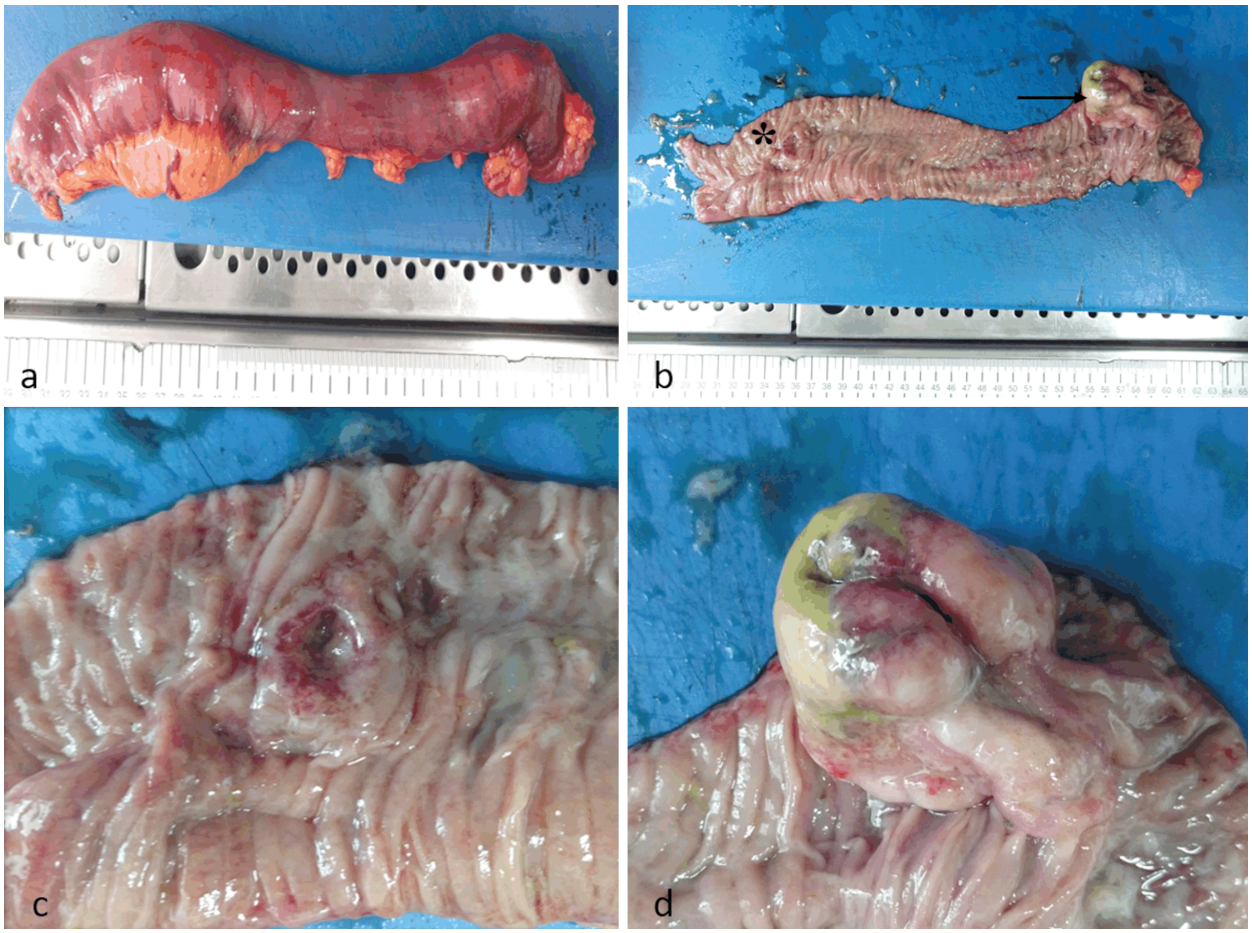

Figure 1 – ***Macroscopic aspect of enterectomy specimen: (a) Serosa has no macroscopically modification; (b) Cut section of the surgical specimen revealed a double tumor; (c) The first lesion is slightly elevated and ulcerated with 2.2 cm in greatest diameter (*); (d) The second tumor is a pedunculated lesion with 4.5 cm in greatest dimension and covered with necrotic deposits on the surface.***

The morphological features of these double tumors were similar and strongly suggestive for GIST, with a high risk of malignancy, being hypercellular neoplasms, predominantly composed of spindle cells with focal perinuclear vacuolization, arranged in short fascicles that randomly intersect. An epithelioid component represented by polygonal cells with eosinophilic or clear cytoplasm, arranged in nests or sheets, was accompanying the previously described population. The nuclear pleomorphism was moderate, but with more than 5 mitoses/50 high-power fields (HPFs). The neoplastic cells infiltrated the lamina propria of the mucosa, submucosa, muscular layer and subserosa (Figure 2). Tumoral necrosis, focal hemorrhage, angiotropism were morphological features also present in both lesions.

Immunohistochemical tests were performed on 4 µm-thick sections of formalin-fixed, paraffin-embedded tissue blocks of the tumor. After the epitope retrieval, tissue sections were incubated with the following antibodies from Biocare Medical (ready-to-use): CD117/c-kit (EP100 clone), discovered on GIST1 (DOG1) (DOG1.1 clone), desmin (D-33 clone), S-100 (15E2E2+4C4.3 clone), vimentin (SP20 clone), cytokeratin 7 (CK7) (BC-1 clone) and Ki67 (SP6 clone). We used 3,3'-diaminobenzidine (DAB) as chromogen, with brown staining. The sections were finally counterstained with Mayer's Hematoxylin. A strong nuclear and diffuse immunostaining was obtained for Ki67, with an 80% proliferative index (Figure 3). The IHC reaction for vimentin was also diffusely and strongly positive in the cytoplasm of tumor cells (Figure 3). A negative reaction was noticed in the rest of the antibodies (Figure 4). The absence of a positive immunostaining for CD117 or DOG1 raised the suspicion of a WT-GIST phenotype and further immunohistochemical tests were required. Moreover, actin, PDGFR alpha (PDGFRA) and CD34 immunostainings were subsequently evaluated in another medical unit. A negative reaction of tumor cells for actin and a positive one for the last two confirmed the initial diagnosis.

A year later, in July 2018, the tumor relapsed and an abdominal computed tomography (CT) scan identified both a tumoral lesion in the GI tract and a liver metastasis. Therefore, a second enterectomy was performed and

revealed a tumoral block due to an infiltrating and ulcerated 9 cm lesion, adherent to adjacent loops (Figure 5). The morphological evaluation emphasized a highly cellular tumor with predominantly epithelioid malignant cells and only focal spindle-shape cells, with more than 5 mitotic figures/50 HPFs and focal multinucleated giant tumor cells (Figure 5). The microscopic features were consistent with a recurrent malignant GIST with dedifferentiation. No metastasis was identified in the 45 regional lymph nodes. Unfortunately, the prognosis was poor and the patient died shortly after surgery. All these data were collected with the written approval of the patient.

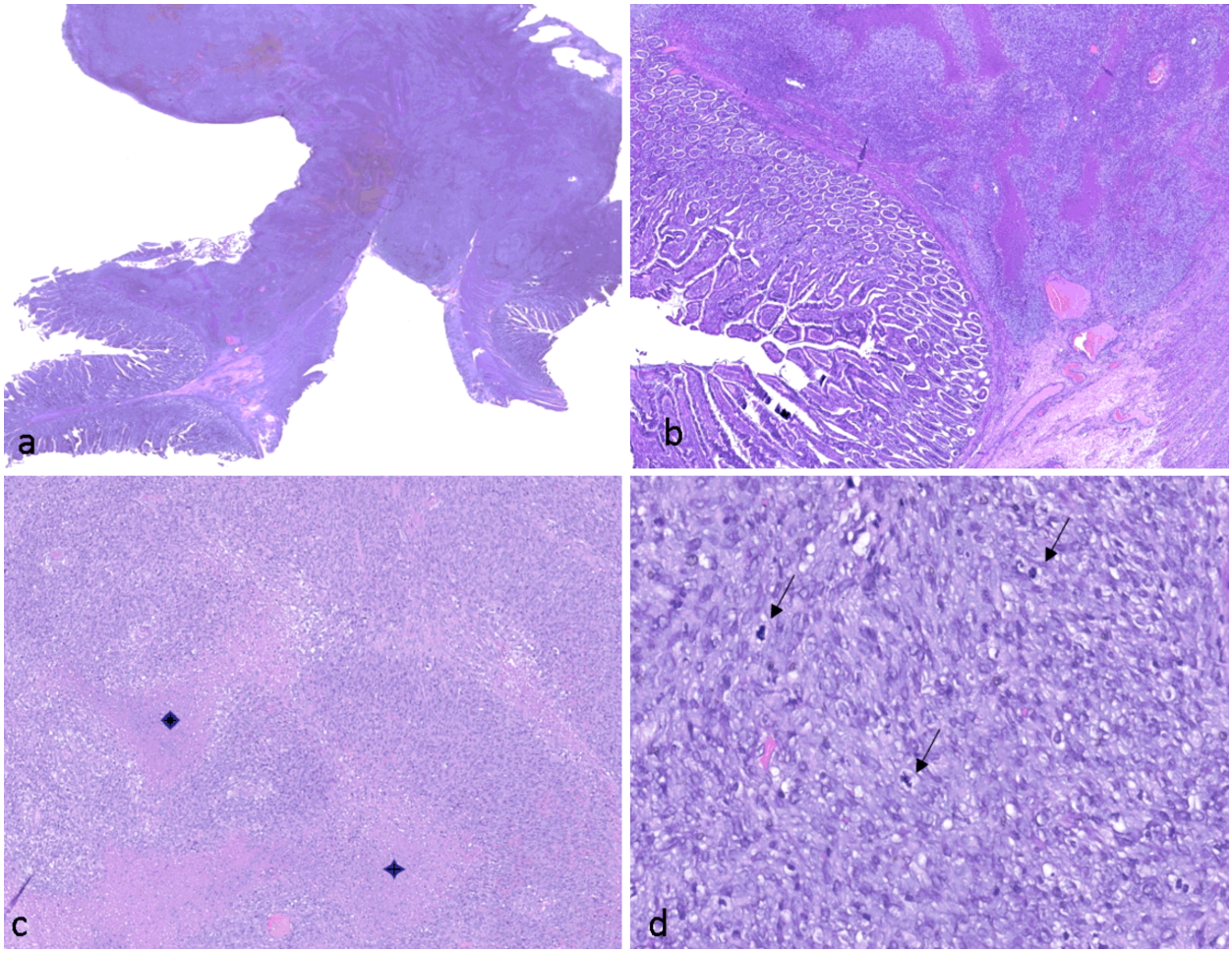

Figure 2 – ***Morphological features of the tumor: (a) A malignant proliferation of spindle-type cells arranged in fascicles; (b) The tumoral cells infiltrate submucosa and lamina propria of the mucosa; (c) Areas of necrosis are identified inside the tumor mass (★); (d) On higher objective, numerous mitoses were counted. Hematoxylin–Eosin (HE) staining: (a) Scanned image; (b and c) ×40; (d) ×100.***

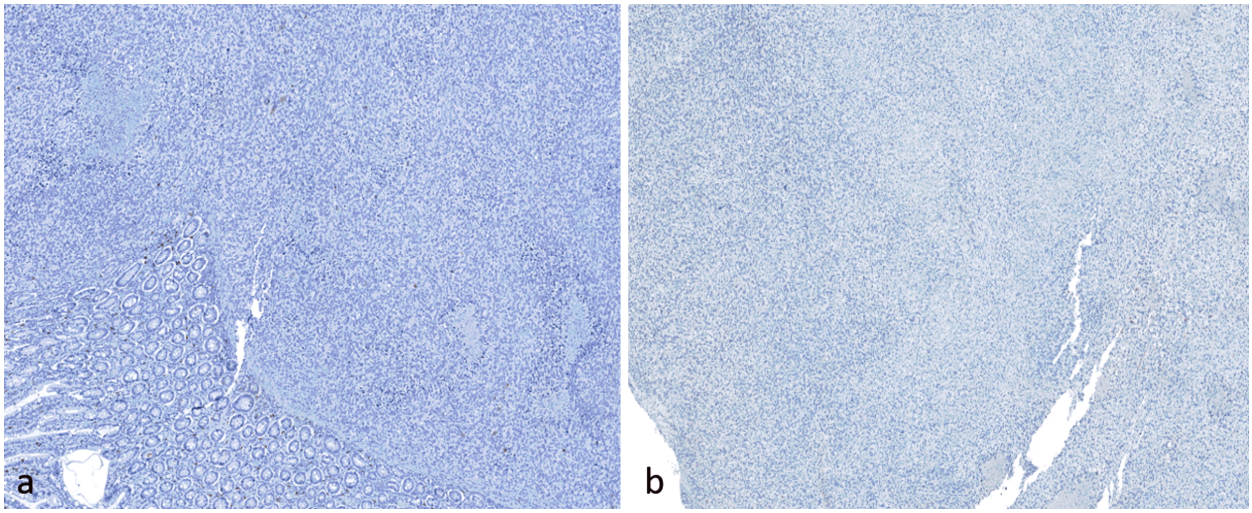

Figure 3 – ***Immunohistochemical examination: (a) Negative immunostaining for CD117 biomarker; (b) Negative reaction for DOG1 biomarker. Immunomarking for: (a) Anti-CD117 antibody, ×40; (b) Anti-DOG1 antibody, ×40. CD117: Cluster of differentiation 117; DOG1: Discovered on GIST1; GIST: Gastrointestinal stromal tumor.***

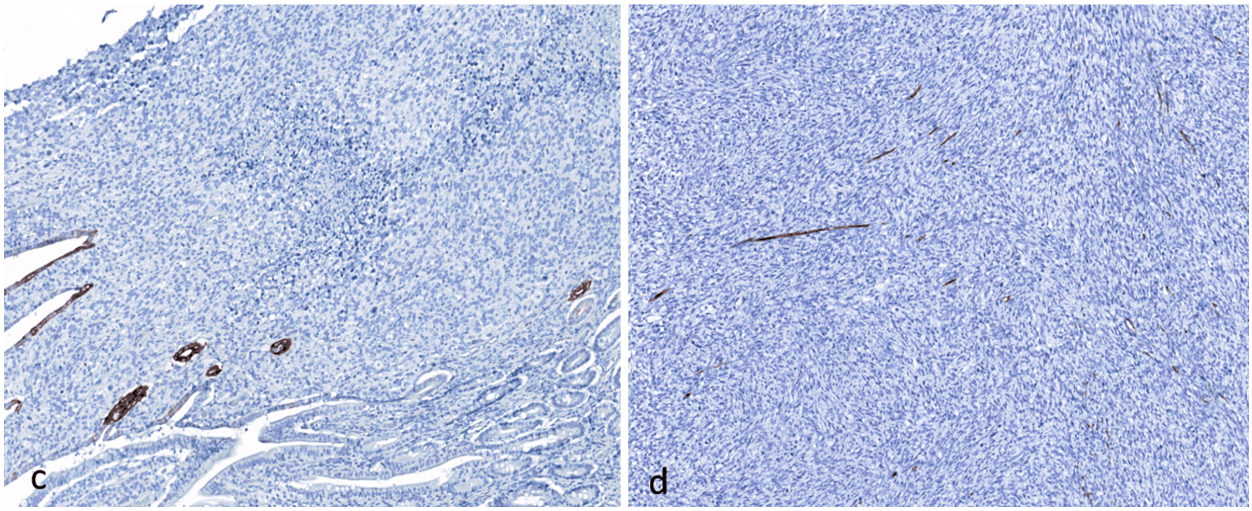

Figure 3 (continued) – ***Immunohistochemical examination: (c) No positive reaction for CK7 biomarker; (d) Negative reaction for desmin biomarker. Immunomarking for: (c) Anti-CK7 antibody, ×40; (d) Anti-desmin antibody, ×40. CK7: Cytokeratin 7.***

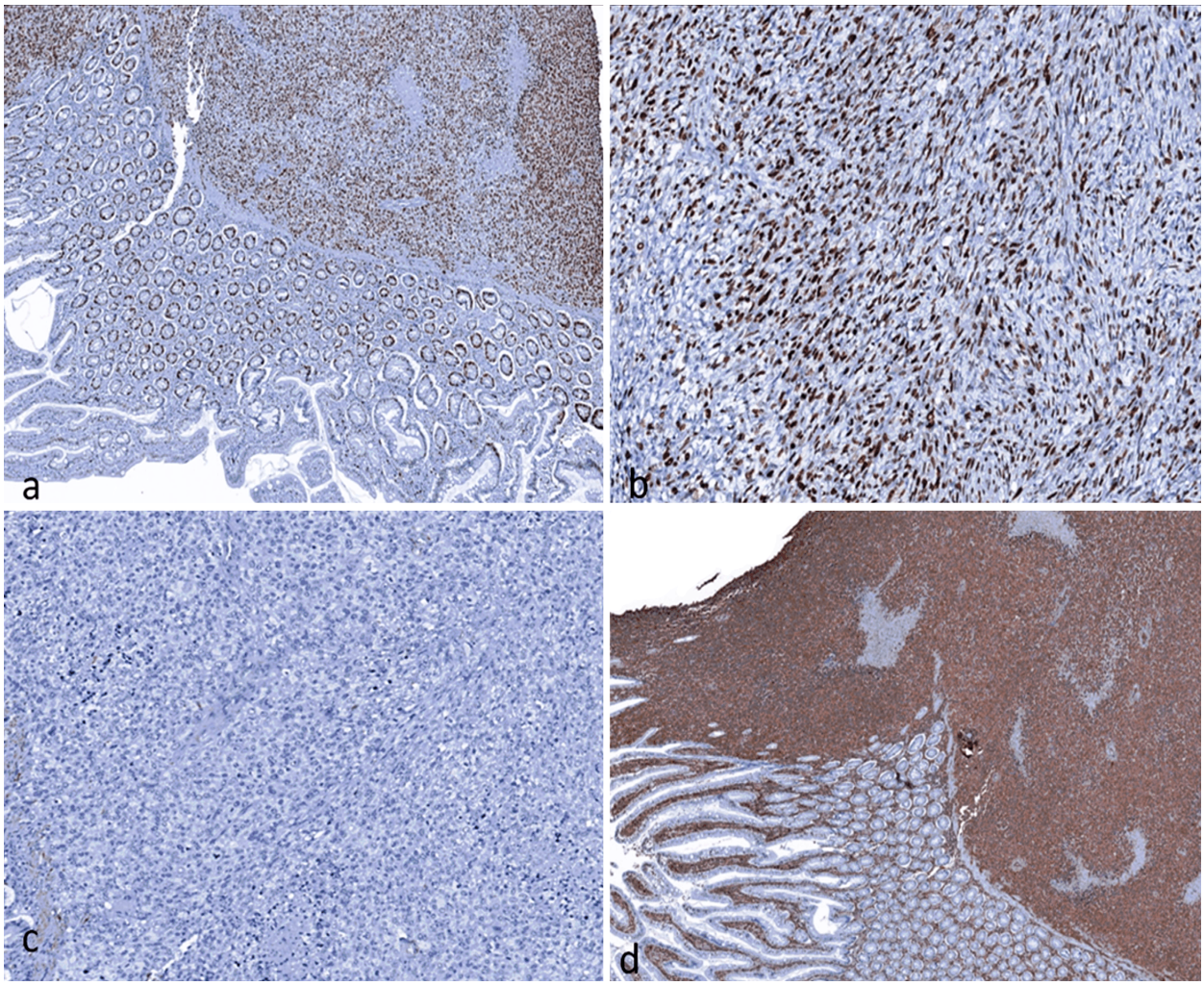

Figure 4 – ***(a) Ki67 immunostaining with a diffuse and intense nuclear positive reaction in the epithelioid cell component of tumor cells; (b) Similar immunohistochemical reaction for Ki67 biomarker in the spindle cell component of tumor cells; (c) Negative immunostaining for S-100 biomarker; (d) Intense and diffuse response of malignant cells to vimentin biomarker. Immunomarking for: (a and b) Anti-Ki67 antibody, ×40 and ×100, respectively; (c) Anti-S-100 antibody, ×40; (d) Anti-vimentin antibody, ×40.***

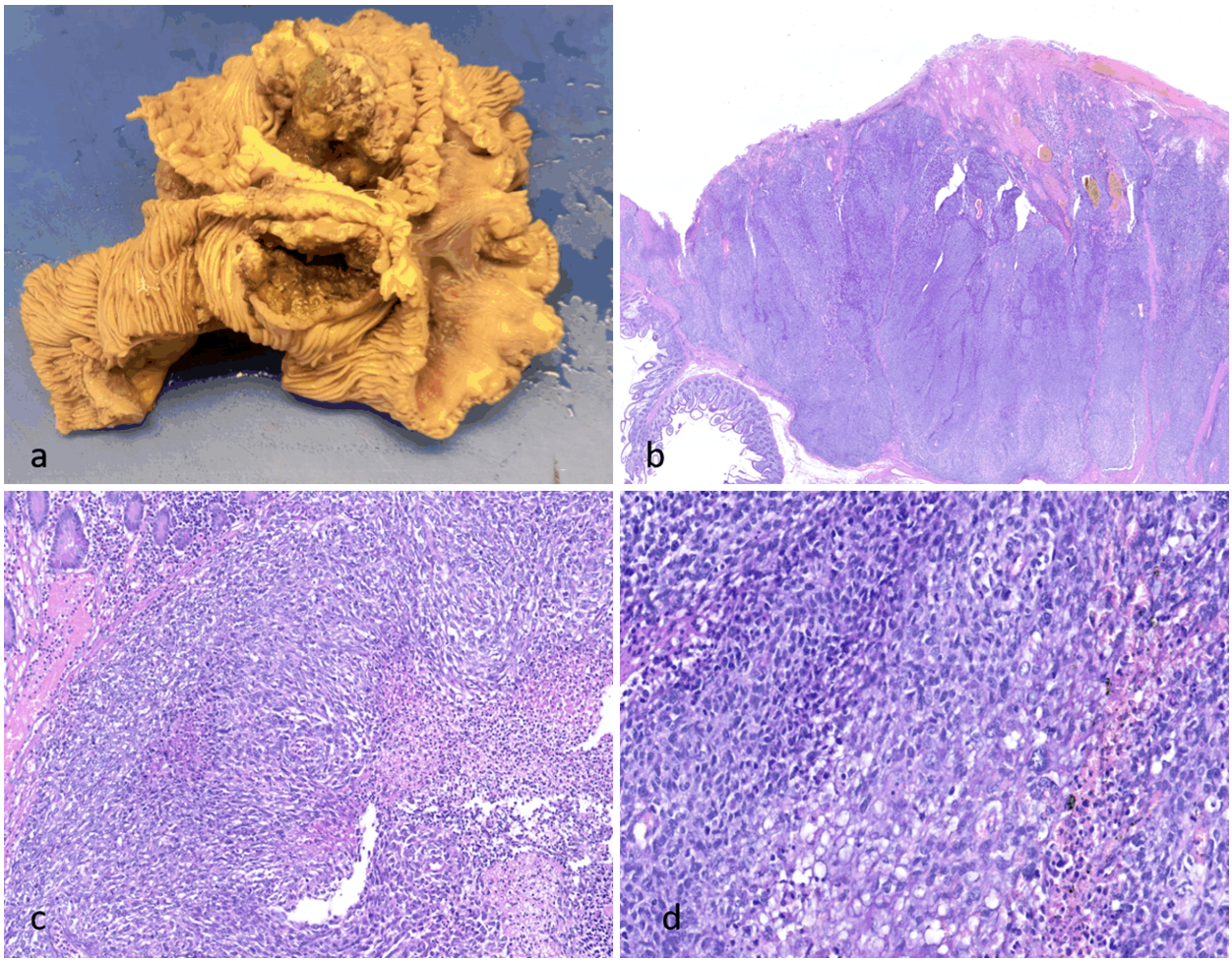

Figure 5 – ***(a) Macroscopic aspect of recurrence tumor with an adherently tumoral bloc of the enterectomy specimen, due to an ulcerative and infiltrative tumor; (b) Morphological features of recurrent malignant GIST with invasion of all intestinal layer; (c) There are an extensive epithelioid component and numerous areas of tumoral necrosis; (d) The content of tumor proliferation stands frequent atypical mitosis and nuclear pleomorphism. HE staining: (b) Scanned image; (c) ×40; (d) ×100. GIST: Gastrointestinal stromal tumor; HE: Hematoxylin–Eosin.***

Discussions

GISTs are uncommon tumors and they are characterized by a wide spectrum of clinical behavior, from benign to malignant, which are caused by sequential genetic mutations. They can affect any part of the GI tract, the primary gastric location being the most frequent [1]. Small bowel involvement is rare, correspondingly the jejunum being affected in 0.1–3% of all GISTs [10]. Not only it is a rare location, but it is also associated with a poor prognosis compared to the gastric localization of a GIST, even if the morphological features are similar [4, 11]. From its first recognition, it is compulsory that factors with a predictive and prognostic value are identified and used to stratify the risk of malignancy. Several classifications have been proposed during the years, the first one being that of the *National Institutes of Health* (NIH) based on the tumor size and mitotic count in order to evaluate the risk for malignancy [12]. Based on these criteria, there are identified four grades for malignant risk, the most frequent were proved to be cases with a high risk (33%), followed by those with low risk (30%) [12]. In the present case, we established a high potential risk, based on the recent criterion introduced by the *Armed Forces Institute of Pathology* (AFIP) in the classification, which supplementary assesses the tumor location (gastric *versus* other) as an additional prognostic factor [13]. In the last edition of the *International Union Against Cancer* (UICC) utilizing the Tumor, Node, Metastasis (TNM) Classification, the grade of a GIST was quantified, based on mitotic count, as low mitotic rate (5 or fewer mitoses/50 HPFs) and high mitotic rate (over 5 mitoses/50 HPFs), highlighting the importance of using the 40× objective (total area 5 mm^2 in 50 HPFs) [14]. Recent studies have shown that other morphological features may also play an important role in stratifying the prognosis. Tumoral necrosis, high cellularity, invasion of the serosa or of the adjacent structures and rich vascularity are linked to a higher risk of malignancy [15]. It was also noticed that some factors are associated with a higher risk of recurrence, like incomplete resection margin, tumor rupture and spillage during surgery [15]. In our study, we report a unique case of two primary GIST tumors located in the jejunum, penetrating the mucosa, but without reaching to the serosa and lacking perforation. Its worrisome morphological features led to frame these tumors in high-risk category for malignancy.

A tumor with morphological features suggestive for GIST must be further supplementary investigated by IHC.

Since ICCs were proved to be the original cell for GIST, the first antibody used in IHC was CD117/c-kit and its positivity is considered to be the gold standard of a precise diagnosis [13]. The *KIT* gene mutations, discovered by Hirota *et al.*, are the most frequent and some of the first mutations which lead to the progression of GIST [16]. The activation of its proto-oncogene – *KIT*, known as *CD117* (*c-KIT*), a receptor tyrosine kinase –, determines activation of pro-growth signals within the cells and it can be identified in up to 80–95% of GISTs [5]. Thus, it opens the way for a successful targeted therapy with Imatinib, a TKI [17]. More than that, this large group of GIST is not homogeneous in terms of prognosis and predictive factors, because an effective TKI therapy depends on the specific mutation [8]. The mutation on exon 11 (juxta-membrane domain) represents the most frequent type of *KIT* mutation with up to 70% of cases, the second one being those on exon 9 (extracellular dimerization) with 10–15% rates [15].

There are cases with CD117 negative and, as an alternative biomarker, DOG1/GIST1 was added, which is a transmembrane protein encoded by transmembrane member 16A (*TMEM16A*) gene located on chromosome 11q13 and its main role is to control the cholinergic activity of the GI smooth muscle [18, 19]. DOG1 is involved in GIST progression, independently of the *KIT* and *PDGFRA* activation and it is positive in up to 85% of GISTs, especially in those cases with a KIT/PDGFRA-negative immunophenotype [18, 20]. Even if its specificity is not high, there are still strong recommendations that DOG1 is included in the first panel of antibodies together with others markers necessary for differential diagnosis [18]. In the present case, a negative immunostaining for both biomarkers have raised the possibility for a WT-GIST phenotype of this tumor, but it was proved that there is a positive immunohistochemical expression for PDGFRA. *PDGFRA* is another gene with an important role in the pathogenesis of GISTs, present in 6.5% of all cases [5, 7]. One of the most important mutations of this gene is that on the exon 18, which is associated not only with resistance to Imatinib, but also to Sunitinib, a second-line TKI [3, 7]. Several studies proved that between cases with *KIT* mutant GIST and those with *PDGFRA* mutation, there are differences not only on molecular level, but also concerning the morphological traits. GISTs cases associated with *PDGFRA* mutation present more frequently an epithelioid morphology [21]. Moreover, it was demonstrated that the vast majority of *PDGFRA* mutated GIST cases are associated with the gastric, omentum or mesentery location, with a 95% rate and are less observed in the small bowel location, which harbors more frequent mutations on *KIT* gene [9, 21]. A few cases of GISTs do not have *KIT* or *PDGFRA* mutations and were previously considered as WT-GIST. These tumors have a higher prevalence in younger patients and have a different clinical behavior compared to those with *KIT* or *PDGFRA* mutations [22]. Some authors recommended avoiding the WT-GIST terminology, because there are other rare gene mutations involved, like the B-Raf proto-oncogene, serine/threonine kinase (*BRAF*) gene mutations localized on exon 15 V600E [23] or those on the succinate dehydrogenase (*SDH*) gene, which encodes the protein succinate dehydrogenase and is present in 90% of pediatric GISTs, compared with 12% to 15% in adult GISTs [15, 24]. It was also recommended to mention the genes which are not involved beside the WT terminology, like *KIT*/*PDGFRA* WT-GIST or *KIT*/*PDGFRA*/*BRAF* WT-GIST.

Numerous evidences regarding the prognostic value of Ki67 index have been collected in the last years. The research conducted by Pyo *et al.* included 1967 GIST cases from 24 other scientific reports, demonstrating a strong correlation between a high Ki67 and a poor prognosis, with low disease-free and overall survival rates [25]. The meta-analysis study conducted by Zhou *et al.* revealed that NIH-intermediate and NIH-high GIST categories are associated with higher rates of overexpression of Ki67 than the low and very low groups [26]. Zhao *et al.* demonstrated that the Ki67 index can be used as a predictor factor, as it was observed that a Ki67 index higher than 8% is associated with a poor response to adjuvant Imatinib therapy [27]. It was also demonstrated that the recurrence of a GIST can be predicted by a high Ki67 index [25]. In our case, a value of 80% of the Ki67 index revealed the aggressiveness of this double GIST tumor with a rapid recurrence in the dedifferentiated form.

Differential diagnoses of GIST take in consideration the morphology of the tumoral cells. Other tumors with spindle cell morphology must be differentiated by those GISTs with a predominant spindle cell proliferation. In this category, there must be included desmoid tumors, leiomyoma/leiomyosarcoma, Schwannoma, perivascular epithelioid cell tumors (PEComas) – spindle cell type. IHC may help distinguish between these entities by incorporating the following antibodies in the first panel: S-100, desmin, alpha-smooth muscle actin (α-SMA), β-catenin antibodies. In our report, a negative reaction for S-100 and for desmin excludes other types of tumors. When an epithelioid morphology is present in a GIST, the differential category must include tumors with a similar morphology, like adenocarcinoma, PEComa with epithelioid cell or neuro-endocrine tumors. In our case, taking into consideration the past history of the patient, the main differential diagnosis was with a metastasis from a lung carcinoma, but a complete negative reaction for CK7 excluded this possibility.

The gold standard treatment for GISTs without metastases is complete surgical resection (Ro), which is associated with a very good five-year survival rate of 48–70% [28]. The latest recommendations of *European Society for Medical Oncology* (ESMO) regarding the adjuvant therapy include Imatinib and other TKI [29]. Adjuvant therapy is extremely useful for GIST cases with *KIT* exon 9 mutation, but is not effective in *PDGFRA* D842V-mutated GISTs and *SDH* expression-negative GISTs [29]. Patients who have localized tumors, but are associated with a high risk of recurrence or those with unresectable tumors or with metastases must be also treated with Imatinib. In case of no response, others drugs are recommended as second-line or as third-line treatment (Regorafenib, Sunitinib) [29].

The dedifferentiation process is rare in GISTs, even if it is common in other types of sarcoma. It was first described in 2005 as a shift from a typical morphology

of a CD117-positive GIST to unrecognizable features with CD117-negative tumor recurrence after TKI treatment [30]. An anaplastic or epithelioid morphology is frequently reported in these cases, compared to the rarer angiosarcomatous aspects [31]. In our report, knowing the past history of the patient prevented us from establishing an incorrect diagnosis, due to the unusual morphological aspect. Antonescu *et al.* proved that dedifferentiation can occur in GISTs either *de novo*, or after chronic treatment with Imatinib and it is mostly secondary to genetic instability, rather than to new additional mutations [31].

Conclusions

Small bowel GISTs are rare tumors with a wide range of clinical outcomes. The assessment of the prognosis does not represent an easy task and it requires corroboration of clinical, morphological and ancillary tests, performed by a multidisciplinary team. A double small bowel GIST with a rare phenotype associated with recurrence and dedifferentiation process represent a challenge regarding therapy. Tailor therapies emerged from the last decade discoveries improved the prognosis of the patients but those with rare molecular mutations associated with worrisome morphological features usually are associated with a poor outcome. The aggressiveness of a tumor is usually associated with a lack of treatment response and death of the patient. As a further matter, the peculiar morphological traits of a recurrent GIST in the dedifferentiated form must be appraised for a veracious diagnosis.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgments

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Corresponding author
Gabriela Izabela Bălţătescu, MD, PhD, Clinical Service of Pathology, "Sf. Apostol Andrei" Emergency County Hospital, Constanţa; Center for Research and Development of the Morphological and Genetic Studies of Malignant Pathology, "Ovidius" University of Constanţa, 1 Universităţii Lane, 900470 Constanţa, Romania; Phone +40241–605 002, e-mail: gabrielabaltatescu@yahoo.com

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