CLINICAL PRACTICE

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Diagnostic difficulties in c-kit negative gastrointestinal stromal tumors: report of four cases

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Abstract: *Introduction: The gastrointestinal stromal tumors (GIST) are dominated by KIT and PDGFRA mutation. The immunohistochemical detection of CD117, a protein express by KIT gene, is essential for the diagnosis and those that are negative always represented a diagnostic challenge*

Case reports: In this article we present a series of 4 cases of CD117 negative GIST tumors, diagnosed and surgically resected in Fundeni Clinical Institute and an overview of the histogenesis, diagnostic problems and management of c-kit negative GIST. All patients were males and the tumors were located in the stomach and small bowel.

Conclusion: It is important for the pathologists to beware of the fact that a CD117 negative in the context of a typical morphological appearance does not exclude a GIST tumor and also the oncologist must be aware not to exclude the therapy with imatinib based on the negativity of CD117.

Keywords: *GIST, gastrointestinal stromal tumors, c-kit negative, CD117, DOG1, immune-histochemistry*

INTRODUCTION

GISTs are rare tumors accounting for less than 1% of all gastrointestinal tumors but they are the most common mesenchymal tumors of the GI tract.[1] The incidence in Europe and USA is 7-10 cases/1,000,000 [2] and in Korea is 16-22/1,000,000 [3]. Large population based studies from Iceland, Netherlands and Sweden found the incidence to be 11, 12.7 and 14.5 cases/1,000,000 [4,5,6]. However there are studies that explain this increased incidence by a better diagnosis using modern diagnosis criteria rather than a real increase [7].

The most common age of the diagnosis is between 50 to 60 years old (a mean age of 65) and a peak incidence between 70 and 79, before 20 years old being quite rare ≈ 1% [7,8]

GIST affects both sexes equally [6] though some authors found them to be more common in men [7,9]. They arise most often in the stomach (50-60%) and small intestine (30-35%) but it can occur in any segment of the gastrointestinal tract only with a lower frequency.[10]

Gain of function mutation in KIT or PDGFRA oncogenes lead to ligand-independent kinase activation [11] which gives rise to the majority of GIST. Immunohistochemistry is useful for diagnosis and

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typically detects c-kit protein expression, but if the tumor is negative for c-kit, a final diagnosis can be challenging.

In this article we present a series of 4 cases of c-kit negative GIST tumors, diagnosed and surgically resected in Fundeni Clinical Institute and an overview of the histogenesis, diagnostic problems and management of c-kit negative GIST.

CASE REPORTS

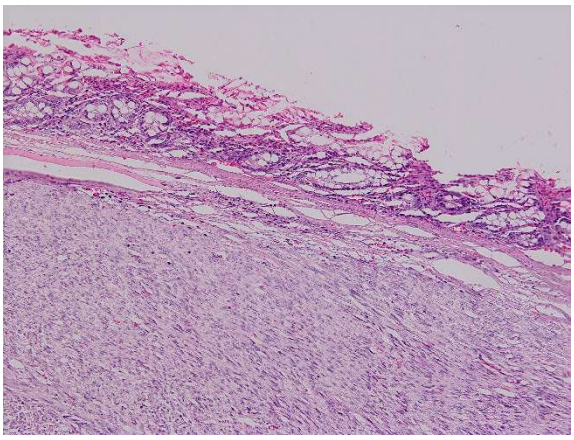
In the pathology archives of Fundeni Clinical Institute, between 2004 and 2017 we have traced four cases of c-kit negative GIST. We have examined available clinical data from the hospital database and also HE slides. Immunohistochemically investigations were performed with biotin-streptavidin method [12] in order to assess the phenotype and provide a definitive diagnosis.

Antibody suppliers were Novocastra, and Labvision/Thermo Fisher Scientific as previously reported [13] and the dilutions respected the manufacturer recommendation. The stratification risk according to Miettinen&Lasota (2006) was used [2]. For the mitotic rate we counted the number of mitosis on 50 high power fields (HPF). Tumor cellularity was divided in three categories: spindle, epithelioid and mixed.

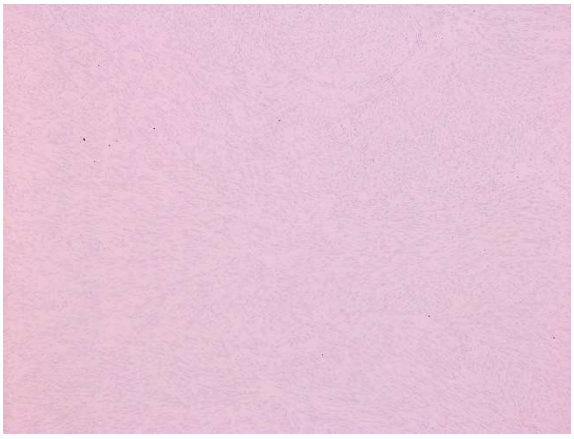
Case no. 1

A 47 years old male who presented with lower gastrointestinal bleeding, was investigated with capsule-endoscopy procedure and found with a tumor on the ileum, close to the ileocecal valve. A segmental enterectomy was performed. The surgical specimen was 12 cm long and presented centrally a well circumscribed white nodule of 4/3/4 cm, expanded in the submucosa and muscularis propria with mucosal ulceration. Histologic examination revealed a tumor consisting of spindle cells with a mitotic rate of 1-2/50 hpf. Immunohistochemical stains showed negativity for CD34, CD117, PDGFRA, Desmin, a weak positivity for SMA and a diffuse cytoplasmic positivity for DOG1. The prognostic group was 2, and 7 years later the patient is alive with no recurrent disease.

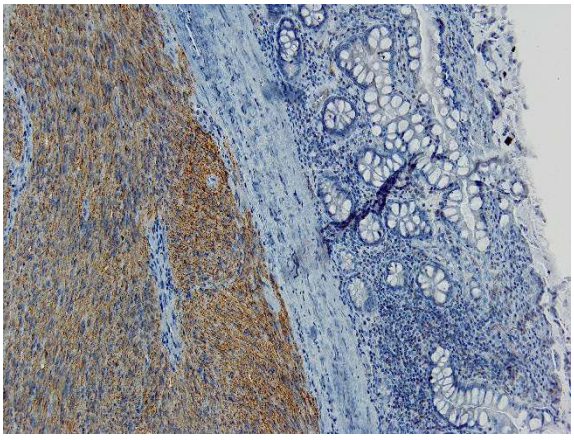
Figures case 1



HEx100



CD117x100



DOG1x100

Case no. 2

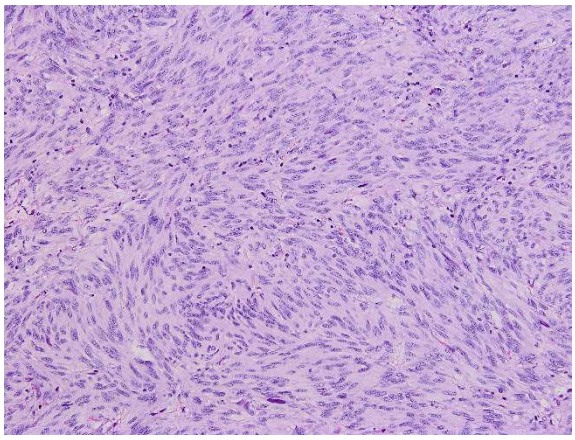
A 69 years old male presented with abdominal pain, nausea and vomiting for 12 hours before admission. The abdominal CT scan revealed a pelvic tumoral mass on the left paramedian side, developed behind the

abdominal wall that involved a small intestinal loop. A segmental enterectomy was performed. The surgical specimen was 30 cm long and presented a solid white tumor with irregular contour 8.5/3.5/2cm with predominantly intramural and subserosal development and focal mucosal ulceration.

Microscopic examination reveal a mixt morphology with spindle and epithelioid cells, with mild pleomorphism, intratumoral hemorrhagic foci and a mitotic rate of 2/50 HPF.

Immunohistochemical stains were negative for SMA, Desmin and CD117 and positive for DOG1. CD34 and PDGFRA were not done.

Figures case 2



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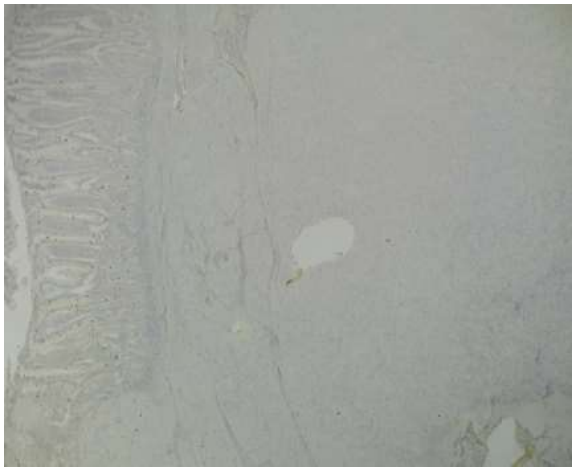
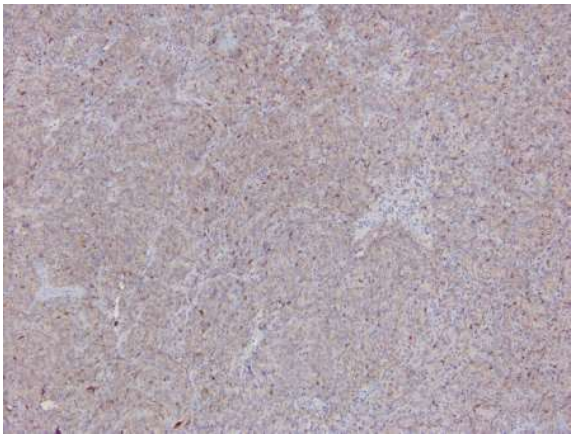


CD117x100



DOG1x100

On the bases of these results the tumor was diagnosed as a c-kit negative GIST with moderate risk (group 3a) and survival from the time of the diagnosis until death was 33 months.

Case no. 3

A 67 years old male was admitted to the hospital with melena, fatigue and weight loss. An esophagogastroduodenoscopy was performed, revealing a 1.5 cm lesion in the gastric fornix.

The tumor biopsy showed a stromal gastric hemorrhagic proliferation associated with a chronic gastritis with Helicobacter Pylori infection.

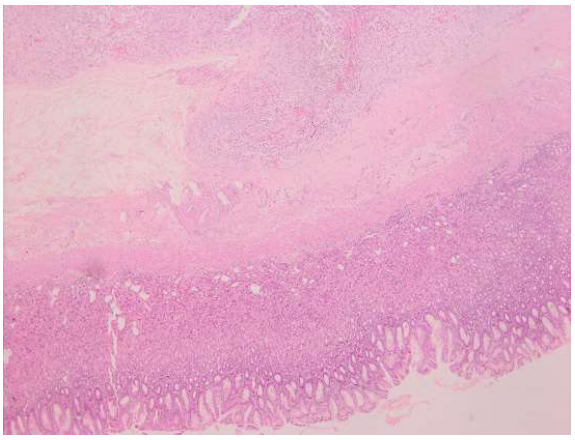
A local excision was carried out and on the surgical specimen was found a firm rubbery white intramural nodule with a diameter of 3/3/3 cm.

Microscopy revealed a tumor proliferation with spindle cell morphology, without mucosal involvement, with 1-2 mitotic figure/50HPF.

Immunohistochemistry showed negativity for CD117, Actin, Desmin and positivity for DOG1. CD34 and PDGFRA were not done.

The diagnosis of c-kit negative GIST with low risk (group 2) was established and the patient is alive with no recurrence.

Figures case 3



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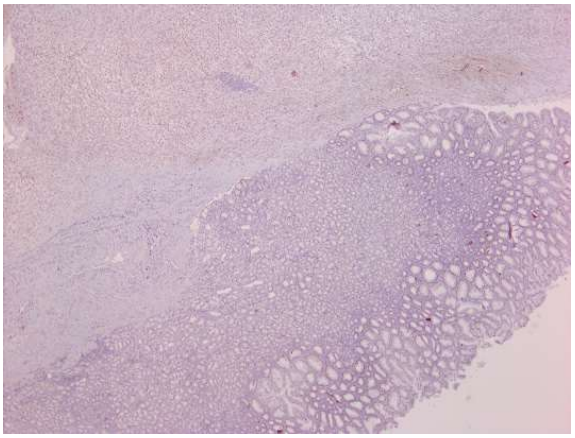
CD117x100



DOG1x100

Case no. 4

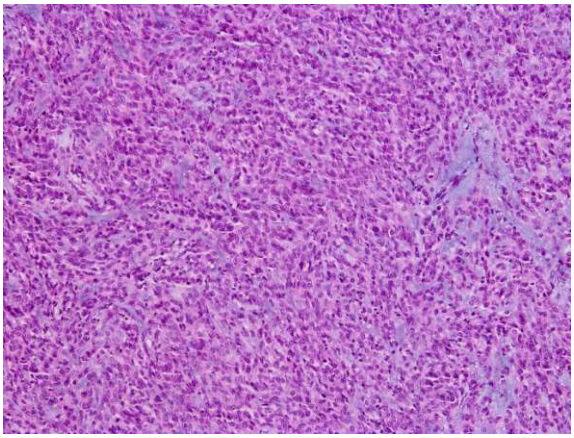
59 years old male with loss of appetite, abdominal pain, weight loss, is admitted to the hospital for investigations.

Esophagogastroduodenoscopy revealed an ulcerated tumoral proliferation on the body of the stomach and an ultrasound discovered multiple localized proliferations in left and right hepatic lobes.

A total gastrectomy was performed and the surgical specimen showed a12 cm white grey tumor with intramural development and cystic degeneration. On the microscopic examination it showed an epithelioid and spindle cell morphology with fascicles that intersect and intertwine at various angles, with more than 10 mitotic figures/50 hpf Immunohistochemistry showed negativity for CD117 and Desmin, and positivity for SMA and DOG1.

A diagnosis of GIST with high risk of recurrence (group 6b) was established and the patient survived time from the initial diagnosis was for 9 months.

Figures case 4



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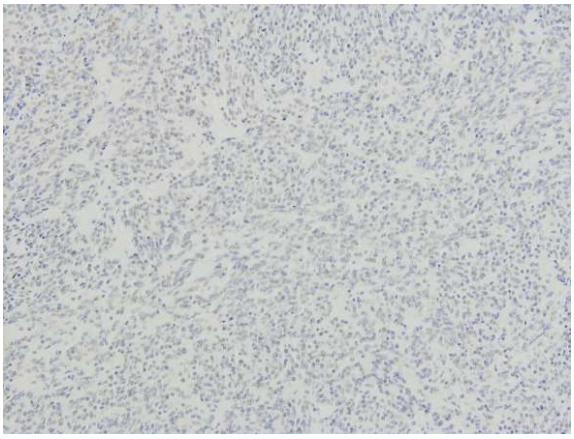
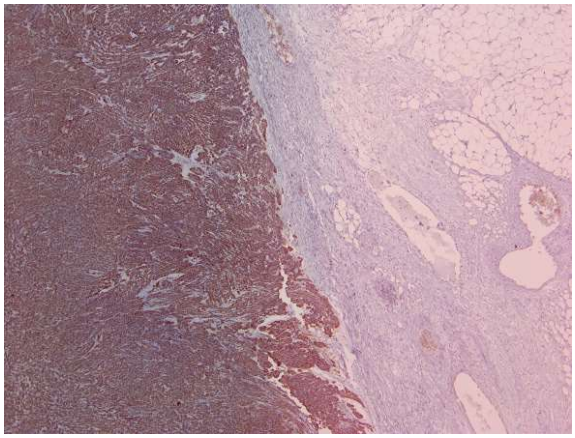


CD117x100



DOG1x100

DISSCUSSION

Arising in the interstitial cell of Cajal, GIST tumors are an entity dominated by mutations in KIT receptor tyrosine kinase which accounts for almost 80% of all cases. The histopathological diagnosis of such tumors is built upon the microscopic morphology and immunohistochemically stains.

The detection of CD117, a protein express by KIT gene, is essential for the diagnosis and we can find it in approximately 95% of GIST [8, 14].

Table 1: Clinical-pathological characteristics of the four cases presented

Case no.	Age	Location	Metastasis	Tumor diameter	Mitosis/ HPF	Cell type	Group	Death	Survival months	Resection
1	47	SI	no	4.0	<5	fusiform	2	no	84	R0
2	69	SI	peritoneum lung	8.5	<5	mixt	3a	yes	33	R1
3	67	S	no	3.0	<5	fusiform	2	no	135	R0
4	60	S	liver peritoneum	12.0	>5	mixt	6b	yes	9	R1

Table 2

	CD117	DOG1	Desmine	SMA
Clone Dilution	T595 1:40	K9 1:100	DE-R-11 1:100	αsm-1 1:50
Case no.1	negative	positive	negative	positive
Case no.2	Negative	positive	negative	negative
Case no.3	Negative	positive	negative	positive
Case no.4	negative	positive	negative	positive

However, there are 4-5% of GISTs that are negative for CD117 and in these cases immunomarkers like DOG1 (ANO1) are very useful for the confirmation of the diagnosis [9,15,16].

DOG1 shows higher prevalence of positivity in gastric epithelioid GISTs, which are often KIT negative.[17]

Depending of the antibody used, the positivity of DOG1 in KIT-negative GISTs varies: with clone DOG1.1, the positivity is 36% [18] and with clone K9, the positivity is 50–76%. [17,19]. We also used K9 clone in our cases, which we found very helpful.

Other immunohistochemical markers used, that can guide the diagnosis are CD34 (with a much higher positivity in the stomach-80% then in the small intestine-35%) together with SMA Desmin, Vimentin. CD34 was done only in one of our cases, a small intestine GIST and it was negative so the only reliable marker was DOG1.

Most of the GISTs are reported in the stomach (50-60%), jejunum and ileum (30-35%), duodenum (5%), colorectal (4%), and rarely in the esophagus and appendix (<1%) [10, 20]. Primary tumors outside the GI tract have been reported in small numbers in omentum [21,22], mesenteries and retroperitoneum [23,24]. The location of c-kit negative GIST is more

frequently reported in the stomach [25, 26, 27] and less in the small intestine [28,29]. Two of our cases were located in the stomach but the other two located in the small intestine gave rise to diagnostic suspicion so we had to pay more attention at the differential diagnosis due to the rarity of the tumor site.

Location and size of the tumor influence the apparition of symptoms. Small tumors are usually silent and are being discovered incidentally during investigations or surgical procedures for other disease However if a GIST becomes symptomatic, it usually cause non-specific symptoms like weight loss, fatigue, nausea, bowel obstruction or overt or occult gastrointestinal bleeding due to mucosal ulceration or tumor rupture[30,9]. Two of our patients presented with nonspecific symptoms like abdominal pain, nausea, fatigue, weight loss, and the other two with bleeding signs. Non-specific symptoms delays the diagnosis and we can see from Table 1 that those two cases have also the largest diameter (8.5 cm in the small intestine and 12 cm in the stomach).

The prognosis and predictive factors for survival of patients with C-kit negative GISTs are still unclear and difficult to assess because of insufficient data. The tumor diameter, metastatic disease, incomplete resection is associated in our cases with a shorter survival rate (Table1).

DOG1 especially clone K9 is an invaluable marker of the diagnosis but mutational status comes in hand in the treatment management. Complete resection and inhibitors of tyrosine kinase are considered for the treatment as in c-kit positive GIST [31].

However when we encounter a c-kit negative GIST we need to take into consideration:
- The antibody that we use, the clone and the laboratory technique
- The fixation methods of the surgical specimen may influence the reaction
- The CD117 negativity does not exclude a GIST diagnosis and other markers like DOG1 are necessary to complete the immunohistochemical diagnostic panel; we also must think at GIST with other mutations like PDGFRA
- For a possible treatment with inhibitors of tyrosine kinase (imatinib) we must have in mind mutation analysis as well
- GIST wild type is a constantly changing concept, at first considered a KIT negative GIST, now is described as GIST with no identified gain of function mutation. Besides the KIT gene, GISTs may present mutations in another receptor tyrosine kinase - the PDGFRA which accounts for less than 10% of all GISTS [32].

According to most studies, activating mutations in KIT or PDGFRA are present in 85 - 90% and are mutually exclusive [33]. Other driver mutations studied, like BRAF, RAS, PIC3K, SDHA, NF1 need to have other genetic event in order to develop and progress [34,33, 35,36]

CONCLUSIONS

Kit negative GIST is a problematic entity which makes the diagnostic difficult.

In conclusion, we report four cases of CD117 negative, DOG1 positive GIST located in the stomach and small intestine, all being male patients.

It is important for the pathologists to beware of the fact that a CD117 negative in the context of a typical morphological appearance does not exclude a GIST tumor and also the oncologist must be aware not to exclude the therapy with imatinib based on the negativity of CD117.

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Conflict of interests
The authors declare no conflict of interests.

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