EXPRESSION OF c-KIT ONCOPROTEIN IN GASTROINTESTINAL STROMAL TUMORS IN ADULTS AND CHILDREN: GUIDELINE FOR DIAGNOSIS AND TREATMENT

Jankulovski N.,1 Popov Z.,2 Bojadzieva S.,3 Validire P.,4 Gogusev J.5

1Department of Digestive Surgery, Clinical Centre, Medical Faculty, Ss. Cyril and Methodius University, Skopje, R. Macedonia
2Department of Urology, Clinical Centre, Medical Faculty, Ss. Cyril and Methodius University, Skopje, R. Macedonia
3Pediatric Clinic, Clinical Centre, Medical Faculty, Ss. Cyril and Methodius University, Skopje, R. Macedonia
4Departement d’Anatomie Pathologique, Institut Mutualiste Montsouris, Paris, France,
5INSERM U507, Hôpital Necker Paris, France.

A b s t r a c t: Gastrointestinal stromal tumors (GISTs) represent a distinct oncogenetic entity that is now center stage in clinical trials of kinase-targeted therapies. These neoplasms express the c-KIT oncoprotein and occur predominantly in adults, more rarely in children. Two selected cases of GIST expressing c-KIT, including one adult patient and a 9-year-old boy are presented. The adult patient was admitted for palpable abdominal mass without other clinical symptoms. On biopsies obtained by scanner-guided procedure, diagnosis of ganglioneurinoma was proposed with the remark that GIST tumor could not be categorically excluded. At surgery, voluminous encapsulated tumor located at the jejunal wall was found and totally excised. The second patient presented with acute upper gastrointestinal hemorrhage associated with several months history of general fatigue and loss of appetite. Computed tomography (CT) and magnetic resonance imaging (MRI) showed a tumoral mass arising from the lesser curvature of the stomach compatible with GIST. Two small metastatic lesions in the liver were also detected. Combined treatment by surgery and systemic therapy by the tyrosine kinase inhibitor imatinib mesylate was applied.

K e y w o r d s: Gastrointestinal stromal tumor GIST, c-KIT, Fluorescence in Situ Hybridization (FISH), imatinib mesylate (Glivec)
Introduction

Gastrointestinal stromal tumors (GISTs) are mesenchymal tumors of the digestive tract and they typically express KIT oncoprotein and have activating KIT or PDGFRA mutations [1, 2, 3, 4]. GISTs arise predominantly in the stomach (60%) [5] and small intestine (25%) [1] but also occur in the rectum (5%), esophagus (2%), and a variety of other locations (5%) including appendix, gallbladder, pancreas, mesentery, omentum, and retroperitoneum [6, 7, 8, 9, 10, 11, 12, 13].

GISTs were consistently underrepresented in the older literature, and they have been frequently classified as tumors of either smooth muscle (leiomyomas or leiomyosarcomas) or neural (schwanoma, malignant peripheral nerve sheath tumor) origin [14, 15, 16, 17]. More recently, it has been shown that the cells in GISTs have characteristics similar to those of the interstitial cells of Cajal [15]. These cells are present in the myenteric plexus and play a role in the intestinal movements [4, 18, 19]. In view of the fact that the immunohistochemical features of the GIST cells are shared with the interstitial cells of Cajal, it was proposed that perhaps these, or a common progenitor are the cells of origin of this neoplasia [4].

At present, consensus guidelines for prognosis emphasize tumor size and mitotic index for risk stratification of primary GIST tumors [16, 20]. Mitotic index assessed by direct counting or immunohistochemistry has been linked to prognosis and should be included in the evaluation of any primary tumor [14, 21, 22]. Presence of aneuploidy is a negative prognostic factor in GISTs [23]. Another prognostic feature is tumor location; tumors arising from small intestine, colon mesentery and omentum have a less favorable outcome compared with those arising from the stomach [3, 14, 16].

In this report, we evaluated the clinicopathological features of two cases with GIST, including one adult and a 9 year-old boy. The treatment approaches based on surgery and systemic therapy for each patient are described. We review the literature on GIST as a model for understanding the crucial role of oncogenic kinase mutations in clinical guidance of such patients.

Case reports

Case N°1

Fifty six years old patient was admitted in GI surgery department for a clinically palpable intra abdominal tumoral mass without specific symptoms. Guided biopsy under computer tomography was performed on the lesion that was diagnosed as ganglioneurinoma, but a mesenchymal tumor (Tm) of the GI tract was not categorically excluded (Figure 1). At laparotomy, encapsulated submucous voluminous tumor (10 × 11 cm), located at the jejunal wall at 15 cm
from the ligament of Treitz and invading part of the duodenum was discovered. The tumor was totally excised as well as part of the duodenum and omentum. No metastatic invasion of the liver or lymph nodes was observed. Tumor markers such as α–foetoprotein, CEA and CA 19-9 were all in the normal range.

Figure 1 – Computer tomography image showing an isolated large abdominal mass (Tm) originating from the jejunum (case 1). No infiltration of the surrounding organs was detectable.

Pathology. The histopathological examination of the scanner-guided biopsy was in favor of either ganglioneurinoma or neurofibroma, but the possibility of mesenchymal type tumor was also considered. The gross anatomy of the resected lesion showed an encapsulated tumoral mass, 11 cm in diameter, slightly lobulated and soft in consistency, the capsule being formed by the intestinal muscularis mucosae layer. On histological examination, the lesion was mostly composed of mesenchymal spindle-like elongated cells infiltrating the intestinal myenteric plexus (Figure 2A). Immunohistochemically, the tumor stained consistently positive for c-KIT (CD117), and CD34 (Figure 2 B,C), moderately for S-100 (Figure 2D) and smooth muscle actin (not shown) whereas desmin, vimentin, and neuron specific enolase were not expressed (not shown). The mitotic count showed less than 5 mitoses per 50 high power field (HPF) (not shown). Given the histological structure and the immunohistochemical algorithm, the pathologic findings were interpreted as a GIST of uncertain malignant potential. In accordance with the classification proposed by Miettinen et al. [24], the lesion was graded as 3a. Post operatively, adjuvant imatinib
mesylate was not applied. On clinical follow-up, twelve months after surgery, the patient remains well without clinical or radiological evidence of recurrence.

Figure 2 – Histopathologic and immuno-histochemical examination of sections from the GIST tumor in the adult patient (case 1). Hematoxylin and Eosin stained section showing mixture of epithelioid and spindle cells, (A) Area showing cells with moderate expression of c-KIT (CD117), (B) consistent expression of hSMA (human Smooth Muscle Actin), (C) and S-100 protein, (D) Original magnification ×120.

Case N° 2

The second patient was a 9-year old boy who presented a recurrent history of iron deficiency anemia associated with several month history of general fatigue and loss of appetite. An abdominal ultrasound and CT scan identified a well-defined tumoral mass arising from the lesser curvature of the stomach. Two small metastatic lesions in the liver were also found. On esophagogastro-duodenoscopy, an unusual prominent sub mucous tumor was detected at gastric wall. The patient underwent a partial gastrectomy and a 5 cm × 3 cm × 3 cm tumor was resected. There was no infiltration in to surrounding organs. On histological examination, there was no evidence of tumor in the surgical margin. Adjuvant imatinib mesylate was used because of the considerable risk of recurrence (liver metastases). On follow-up CT scans including one at the 12-th month post-opera-
tively, the initial liver metastases were not detectable. Postoperative annual surveillance endoscopies have all been normal and 3 years after surgery the patient remains well with no evidence of tumor recurrence.

Pathology. The resected specimen consisted of a portion of stomach measuring 5 cm × 4 cm × 3 cm. There was an ulcerated, polypoid lesion into the gastric lumen with a diameter of approximately 4 cm. The cut-surface showed a multilobulated, firm and fleshy tumor within the gastric wall. Histology showed a mixed spindle cell, neoplasm with high cellularity but no necrosis (Figure 3A). While a diffuse growth pattern predominated, there were areas with trabecular pattern and foci of nuclear palissading. In some areas, the lesion contained a scarce stroma appearing pale and liquefactive. Focal cellular pleomorphism and paranuclear vacuoles were not identified. Immunohistochemical stains confirmed that the tumor cells were strongly positive for CD117 (c-KIT), CD34 (Figure 3 B, C ) and vimentin (not shown). The mitotic rate evaluated by MIB-1 antigen staining, varied from 2/50 to 4/50 HPF (Figure 3 D). The tumoral cells were

Figure 3 – Histopathologic and immunohistochemical analysis of sections from the GIST described in case 2. Aspect of sarcomatous epithelioid GIST with a moderate mitotic activity on Hematoxylin and Eosin staining, (A) Consistent expression of KIT-oncoprotein (CD117), (B) Expression of CD34 antigen, (C) and scattered MIB-1 positive cells, (D) Original magnification ×100

Slika 3 – Хистопатолошка и имунохистохемска анализа на зреани GIST неоазма кaj случајот 2. Прикажана е GIST неоазма од саркоматоидно епителоден тип со среден степен на делебена активност (боеле со хематоксилин и еозин), (A) Присуство на засилена експресија на c-KIT-онкопротеинот (CD117), (B) Експресија на антигенот CD34, (C) и присуството на неколку MIB-1 позитивни клетки, (D) Помеѓу зголемување × 100

negative for the cytokeratins, muscle markers desmin, smooth muscle actin as well as for the neuroendocrine markers including S-100 and chromogranin (not shown). These histopathological and immunohistochemical findings were interpreted as GIST tumor with malignant potential. To correlate c-KIT, and c-MYC genes structure (amplification) with the level of the corresponding oncoprotein expression, Fluorescence in Situ Hybridization (FISH) analysis was carried out on imprints from the tumor of case 2. The cells on slides were prepared and hybridized with double color probes specific for c-KIT and C-Myc genes according to the procedure recommended by the manufacturer (MP Biomedicals, Illkirch, France). No alteration of c-KIT gene was observed by FISH (4 A), while an elevated number of specific red signals corresponding to c-MYC gene amplification was found in approximately 24% of the GIST cells (Figure 4B).

![Figure 4](image_url)

**Figure 4** – Gene structure analysis of c-KIT (A) and c-MYC (B) oncogenes by Fluorescence In Situ Hybridization (FISH) on prints from the GIST tumor (case 2). Two large aneuploid cells are observed without c-KIT amplification, the green signals being from the gene probe, the red ones from the centromeric probe, (A). In (B), detection of c-MYC gene amplification showing at least two-fold increase in red signals in comparison to the euploid number of centromeres (green signals) (B).

**Discussion**
In this report we evaluated the clinicopathologic features of two distinct GIST tumors in an adult and a young boy, the first located at the small jejunum, the second arising from the stomach. Both tumors were histologically consistent with GISTs and were KIT and CD34 positive on immunohistochemistry. At genomic level, C-KIT gene amplification was not present in any case, while a moderate increase in the c-MYC copy number was detected by FISH analysis in case 2 (Figure 4). Although an increased c-MYC gene copy number was present in case 2, the low level of c-MYC oncoprotein expression found (not shown) suggests a transcriptional regulation of this gene in GISTs. Unusual features of the described GISTs were the jejunal location, the presence of moderate expression of smooth muscle actin and absence of neural markers in the adult patient, as well as the young age of the second patient at first occurrence. Another particular feature in the child GIST was the presence of two small liver metastases, while less than 5 mitoses per 50 HPF were observed on MIB-1 staining. In this respect, it is believed that tumors with high mitotic rate are more likely to metastasize, although metastatic behavior of GIST with no detectable mitotic activity was also reported [25]. Concerning the age of GIST tumors occurrence, while several series in adults have been published [17, 26], these neoplasms are rare in children and are not clearly understood. Therefore, awareness of their existence in children and their clinical characteristics are important for early diagnosis and treatment [27].

In adults, approximately 47% of patients have metastatic disease at the time of primary presentation [17]. Of these, 65% are liver metastases, and others are peritoneal, bony and lung metastases [17]. In children, only several cases had metastatic disease at the time of primary presentations, all being liver metastases [27]. In both adults and children, tumors of large size (> 5 cm) usually have high frequency of recurrence and liver metastasis [14, 28].

The recommended primary intervention for GISTs is complete surgical resection. In this context, the extensive lymphadenectomy has not been shown to improve survival [29], because the neoplasm does no metastasize to lymph nodes. Considering the systemic therapy, the response to conventional chemotherapy and radiotherapy is poor [30], the GISTs being highly resistant to chemotherapeutic agents, including the newer approved (e.g., temozolomide) [31] and investigational (e.g., ecteinascidin-743) drugs [32, 33]. In several clinical trials, single-agent and combination chemotherapy have failed to yield partial response rates greater than 5% [30]. It was proposed that these disappointing results are possibly due to high-level expression of BCL-2 and multidrug resistance proteins in many GISTs [34]. However, the historically grim prospects for patients with locally advanced or metastatic GIST are much brighter today with the advent of imatinib therapy. The new drug, imatinib mesylate is a potent and specific inhibitor of the KIT protein-tyrosine kinase, which is constitutively activated in more than 90% of GISTs as result of gain-
of-function mutations in the KIT proto-oncogene [35, 36, 37]. The c-KIT (CD117) oncoprotein that establishes the diagnosis of a gastrointestinal tract neoplasm with characteristic histological features is easily detected through reactivity with the anti CD117 antibody on immunohistochemical staining [16]. Another target of imatinib is the platelet-derived growth factor receptor alpha gene (PDGFRA) encoded tyrosine kinase. Mutations in the PDGFRA gene have also been identified in approximately one third of a subset of GISTs that appear to lack KIT mutations [38, 39]. The success rate of imatinib to induce complete remission is 4–5%, partial remission (decrease of at least 50% in tumor burden) is 47–67%, and stable disease is 18–32% in patients with advanced CD117-positive GISTs [40, 41, 42].

Clinically, the tyrosine kinase inhibitor imatinib, registered as Glivec or Gleevec by Novartis Pharma (Basel, Switzerland) is approved for the treatment of KIT (CD117)-positive unresectable and/or metastatic malignant GISTs, as well as the first–line treatment of chronic myeloid leukemia in all phases [40]. The drug is administered orally in tablet form and the standard dosage for the treatment for GIST is 400 or 600 mg once daily. In a recent clinical trial, the authors suggest that in patients with widespread metastatic disease, 400 mg twice a day might be more convenient dosage [42]. New-evidence based treatment guidelines were established recently, that recommend imatinib as first-line therapy in cases of marginally resectable and pathology-confirmed GISTs, followed by surgery and postoperative imatinib administration [43]. However, before considering imatinib administration, it should be kept in mind that GISTs are highly vascularized tumors that are prone to bleeding [44]. Hemorrhage is frequent presenting symptom as well complication of the disease, and was observed in a small subset of patients in a phase II trial of imatinib in GIST, probably as a consequence of tumor degeneration in response to therapy [41, 44]. Other questions to be asked remain, some of which may be answered as the current clinical trials mature and data become available. For example, we still do not know the optimum dose of imatinib to be used in the treatment of GIST. We need to learn how best to use the drug in the preoperative setting, will it be helpful, and could it lead to easier, less hazardous surgery. Yet it should be determined whether neo-adjuvant or adjuvant treatment result in prolonged survival, and how to circumvent resistance in some patients to imatinib.

In conclusion, contemporary management of GISTs appears to be centered on combined use of systemic tyrosine kinase inhibitor therapy and surgical resection to optimize treatment outcomes. As illustrated by the presented cases, a multidisciplinary team approach that integrates the expertise of surgeons, medical oncologists, pathologists, diagnostic radiologists, and gastroenterologists is evolving as a new paradigm for GIST diagnosis and treatment.

REFERENCES


Резиме

ЕКСПРЕСИЈА НА ОНКОПРОТЕИНОТ c-KIT
ВО ГАСТРОИНЕСТИНАЛНИТЕ ТУМОРИ ОД СТРОМАЛНО ПОТЕКЛО КАЈ ВОЗРАСНИ И ДЕЦА: КЛЮЧЕН ФАКТОР ЗА ПОСТУПУВАЊЕ НА ДИЈАГНОЗАТА И ЛЕКУВАЊЕТО

Jankulovski N.,1 Popov @.,2 Bojaxieva S.,3 Validire P.,4 Gogu{ev J.5
Гастроинтестиналните тумори од стромално потекло (ГИСТ) претставуваат посебен онкогенетски енитет каде што терапијата со инхибитори на ензимот тирозин киназа е необичан ефикасен. Молекулярната патогенетска основа опфака мутации на онкогените ц-КИТ и ПДГФРА како и нивна засилена клеточна експресија. ГИСТ туморите посебно се поjavуваат кај врзрасти, а поретко кај деца. Опишани се два случаи на ГИСТ кај кои беше откривена значајна експресија на ц-КИТ онкопротеинот, вклучувајќи еден 56-годишен пациент и една 9-годишно дете. Каж врзрастиот пациент беше откривен волумноносен интраабдоминален тумор врз кој беше направена предоперативна биопсија под контрола на компјутерска томографија (КТ). Histоморфолошкото наод беше во прилог на ганглионеврон, но диференцијално дијагностици, гастроинтестинален тумор од стромално потекло – ГИСТ не можеше да се исключи. Лапаротомијата покажа дека се работи за инкапсулиран тумор сместен во видот на проксималното јејуум, кој беше целосно изваден. Вториот пациент беше примени под клиничка слика на хематемеза, како и со анамнеза за неколку месечени замор и губење на аппетит. Испитувањата со КТ и со магнетна резонанца (МРИ) покажаа присуство на неоплазма поставена на малата кривина на стомакот што на означителни одвеше во прилог на ГИСТ. Две мали метастатски лезии беа откривени во црнобробниот паренхим. Каж пациентот, пост операативно се примени дополнителна системска терапија со инхибиторот на ензимот тирозин-киназа познат како иматиниб месилат.

Ключни зборови: гастроинтестинален тумор од стромално потекло (ГИСТ), ц-КИТ онкопротеин, ФИСХ (флуоресцентна ин сио хибридизација), иматиниб месилат.

Corresponding Author:

Jankulovski Nikola
Clinic for Surgical Diseases
Department of Digestive Surgery

Vodnjanska 17, 1000 Skopje, Macedonia

E-mail: nikolajankulovski@yahoo.com