

## Case Report

# Gastrointestinal Stromal Tumor with Autonomic Nerve Differentiation and Coexistent Mantle Cell Lymphoma Involving the Appendix

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Received 24 March 2009; Accepted in revision 28 March 2008; Available online 20 May 2008

**Abstract:** Gastrointestinal stromal tumor (GIST) and mantle cell lymphoma involving the appendix are rare as individual disease entities. Their coexistence has not been previously reported in the literature. We describe a 65-year old female who presented with extensive ileocecal mantle cell lymphoma, which extended to the appendix. The appendix was involved by mantle cell lymphoma and an incidental coexistent GIST was noted in the appendiceal wall. The GIST was CD117 positive but did not harbor mutations in the *c-kit* and *PDGFR* genes. In addition, it was unusual in showing S-100 immunoreactivity and ultrastructural evidence of autonomic nerve differentiation. This is the first description of the association of a GIST with autonomic nerve differentiation coexisting with mantle cell lymphoma in the appendix.

**Key Words:** Gastrointestinal stromal tumor, gastrointestinal autonomic nerve tumor, mantle cell lymphoma, appendix

## Introduction

Gastrointestinal autonomic nerve tumors (GANTs) were first called a "plexosarcoma" by Herrera *et al* in 1984 [1]. It is defined as a gastrointestinal mesenchymal stromal tumor, which ultrastructurally mimics an autonomic nerve tumor [2-7]. The stomach is by far the most common site of occurrence followed by jejunum, ileum and duodenum [8, 9]. It is believed that GANTs are a subgroup of gastrointestinal stromal tumors (GISTs) with autonomic nerve differentiation. This assumption is based on morphological, immunohistochemical and molecular similarity between GANTs and GISTs [8, 10]. The majority of these tumors exhibit *c-kit* expression and contain a specific *c-kit* mutation [8, 10]. In the absence of a specific discriminating antibody for GANT, GANTs can only be diagnosed by ultrastructural examination [8].

Gastrointestinal involvement by mantle cell lymphoma (MCL) is a relatively frequent

occurrence and is thought to occur in 20-30% of cases. Involvement is usually in the form of multiple lymphomatous polyposis [11-13]. Appendiceal involvement by MCL is not well documented. We present a case of an appendiceal GIST showing autonomic nerve differentiation combined with background MCL involving the appendix.

## Materials and Methods

### Case History

The patient was a 65-year old female with a past medical history of peritoneal MCL, diagnosed and treated in 1997. She had a symptom-free period of 5 years and then had recurrence. Abdominal and pelvic computerized tomographic scans revealed numerous intra-abdominal lymph nodes ranging from 1 to 1.5 cm in size, distributed throughout the mesentery and retroperitoneum. There were also two small intestinal masses identified. A right hemicolectomy was performed and she received post-operative

**Table 1** Immunohistochemical markers

Antibody	Source	Clone	Dilutions
GFAP	Dako Cytomation	Polyclonal	1/1000
CD117	Dako	Polyclonal	1:100
S100	Dako	Polyclonal	1:3000
Desmin	Dako	D33	1:100
Vimentin	Dako	3B4	1:100
Chromogranin	Ventana	LKH2H10	Predilute
Mart-1/Melan A	Ventana	A103	Predilute
CD20	Dako	L26	1:400
CD21	Ventana	2G9	Predilute
CD23	Ventana	1B12	Predilute
CD34	Ventana	QBEND/10	Predilute
CD56	Ventana	123C3.D5	Predilute
CD57	Ventana	NK1	Predilute
Ki-67	Ventana	30-9	Predilute
Bcl-1/Cyclin D1	Neomarker	SP4	1:50
PAX5	Novocastra	24	1:20
Bcl-2	Novocastra	100/DS	1:100
CD79a	Novocastra	11E3	Predilute
CD10	Novocastra	56C6	1:50
CD3	Ventana	2GV6	Predilute
CD5	Novocastra	4C7	1:100
Cytokeratin	Chemicon	AE1/AD3	1:1000

chemotherapy. Two years after the operation, the patient is alive and well.

#### *Histology and Immunohistochemistry*

The specimen was fixed, processed and cut according to standard protocols. For immunohistochemistry, tissue sections were cut using sterile disposable microtome blades on a rotary microtome. They were floated on a water bath (50°C) and picked up on poly-L-lysine coated glass slides. The sections were baked on a hot plate at 60°C for 20 minutes. Deparaffinization was carried out in 2 changes of xylene for 5 minutes each. This was followed by rehydration in 2 changes of absolute ethanol for 3 minutes each and 2 changes of 95% ethanol for 3 minutes each before a stay of 5 minutes under running tap water. For the antibodies requiring microwave heat retrieval, the sections were processed to unmask antigens by conventional microwave heating in 0.01 M sodium citrate retrieval buffer. Immunohistochemical antibodies used were listed in **Table 1**. The staining was performed using the standard streptavidin-biotin system on an automated immunostainer. Appropriate positive and negative controls for each antibody were run in parallel.

#### *Electron Microscopy*

The formalin fixed tissue (measuring less than

1 mm on a slide) were placed in buffered glutaraldehyde for 60 to 90 minutes at room temperature and then washed with phosphate buffer. The blocks were fixed in Osmium tetroxide (1%) for 30 minutes, rinsed rapidly in distilled water and dehydrated with acetone. The specimen was transferred to Epon embedding medium admixed with 100% acetone for 30 minutes, followed by replacing the medium with pure embedding medium twice for 10 minutes. The blocks were polymerized in fresh embedding medium in flat molds for 60 to 90 minutes at 99°C and sectioned.

#### *Molecular Analysis*

DNA was extracted from paraffin embedded tissue and analyzed for mutation in exons 9, 11, 13 and 17 of the *c-kit* gene, and exons 12, 14, and 18 of the *PDGFR* gene using denaturing high-performance liquid chromatography as per methodology described by Cohen et al [14]. The MCL had been previously worked up and showed the typical histopathology, immunophenotype and genotype.

## **Results**

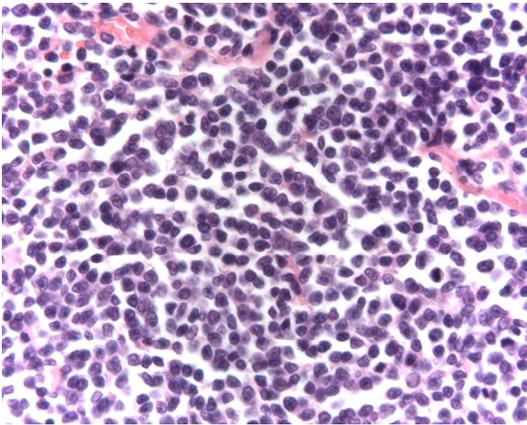
#### *Gross Findings*

The specimen consisted of terminal ileum,



**Figure 1** The gross specimen of the ileocecal region showing large, fleshy polypoid masses of mantle cell lymphoma constituting lymphomatous polyposis.

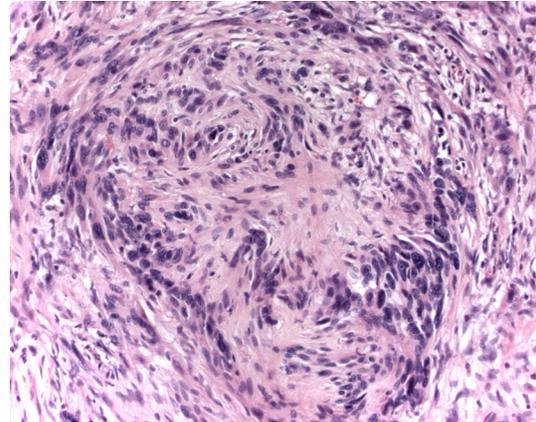
appendix and ascending colon. Ileocecal valve was replaced by a diffuse infiltrate. The mucosal surface showed multiple polypoid tumors (multiple lymphomatous polyposis) that were white-gray, glistening and had a fish-flesh appearance (**Figure 1**). The appendix measured 6.5 in length and up to 3.1 cm in diameter and on cut section showed a pale, solid intramural mass measuring 1.1 cm in largest dimension.



**Figure 2** The lymphoma was composed of small to intermediate cells with a high nuclear to cytoplasmic ratio, round to slightly irregular nuclear contours, delicate chromatin and indistinct nucleoli. Occasional larger cells with open chromatin and distinct to prominent nucleoli are admixed (H&E X 400).

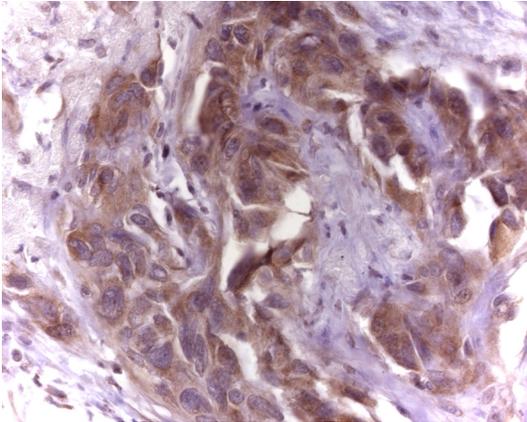
*Microscopic Findings*

The ileal and colonic sections revealed variable effacement of the intestinal wall



**Figure 3** The GIST was composed of plump epithelioid and spindle cells arranged in a whorled pattern. There is mild nuclear pleomorphism but no mitoses (H&E X 400).

architecture with sheets of small to intermediate-sized lymphoid cells, exhibiting round to slightly irregular nuclear contours, delicate to clumped chromatin and indistinct nucleoli, with a high nuclear to cytoplasmic ratio (**Figure 2**). Occasional larger cells with open chromatin and distinct to prominent nucleoli are admixed. Sections of the appendix showed a similar infiltrate with diffuse permeation of the wall. There was also a circumscribed tumor within the muscularis propria and extending into the mesoappendix. The tumor consisted of epithelioid and spindle cells arranged in short fascicles admixed with lymphocytes, eosinophils and occasional mast cells. The epithelioid cells were large, polygonal and pleomorphic with abundant eosinophilic and granular cytoplasm (**Figure 3**). The nuclei showed delicate, salt and pepper chromatin, inconspicuous nucleoli, rare intranuclear inclusions and less than 1 mitotic figure per 50 high power fields. The stroma contained occasional dilated thin walled vessels. This tumor was categorized as a GIST of very low malignant risk. Immunohistochemistry of the appendiceal tumor showed strong positivity for CD117 (c-kit) (**Figure 4**), S100, CD34 and vimentin; focal positivity for CD56 and CD57, and it was negative for CD20, chromogranin, synaptophysin, Melan A/Mart1 and AE1/AE3. The lymphoid infiltrate in the bowel and appendix was positive for CD20, CD5, PAX5, Bcl-1, Bcl-2 and negative for CD10 and CD23; features in keeping with recurrent MCL.



**Figure 4** The tumor cells of the GIST showed strong immunolabeling for c-kit (CD117) (anti-CD117 X 400).

#### Molecular Analysis

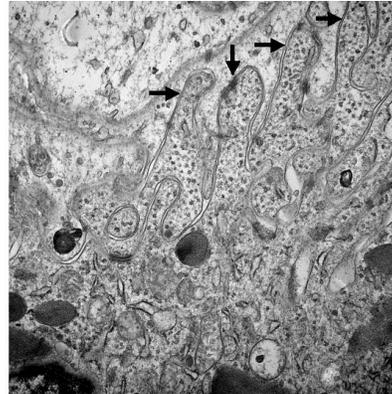
The appendiceal tumor did not reveal mutations in the *c-kit* and *PDGFR* genes.

#### Electron Microscopy

The tumor cells were densely packed together with interdigitating cell processes in a comb-like pattern (**Figure 5**). These interdigitating processes also joined together at the periphery of the cell by rudimentary cell junctions. The cytoplasm contained electron dense vesicles, mitochondria, rough endoplasmic reticulum and ribosomes. Occasionally, the cells showed bulbous synaptic terminals and synaptic-like vesicles. However, basal lamina and skeinoid fibers could not be demonstrated.

#### Discussion

MCL usually involves the gastrointestinal tract as either single, isolated or multiple polyps as so-called multiple lymphomatous polyposis [11-13]. These polyps are usually found throughout the gastrointestinal system but frequently there is a larger polyp in ileocecal valve region accompanied by mesenteric lymph node involvement. Appendiceal involvement is by contiguity from ileocecal disease [15]. With regards to the spindle and epithelioid cell tumor of the appendix, GIST is the first diagnosis to be considered since they are the commonest primary mesenchymal tumor of the gastrointestinal system. Appendiceal GIST is extremely uncommon, and to the best of our knowledge, only 7 cases



**Figure 5** An ultrastructural image of the GIST displaying numerous interdigitating cell processes (see arrows) (EM X 20,000).

have been reported thus far [16-18]. These cases were all c-kit immunopositive; however *c-kit* mutations were studied only in two cases [17]. All reported cases are c-kit and CD34 positive but uniformly negative for S100, desmin, smooth muscle actin [16-18]. The case described in this report showed a GIST pattern of immunohistochemistry with neuronal differentiation seen ultrastructurally.

In studies of GANT, 100% positivity for c-kit was noted, vimentin 92%, neuron specific enolase 90%, CD34 58%, S100 39% to 44%, synaptophysin 31%, chromogranin A 11%, neurofilament 16%,  $\alpha$  smooth muscle actin 10%, vasoactive intestinal peptide 20% [8, 9]. Cytokeratin, desmin and HHF35 are usually negative. Thus, the case described in this report fulfills the morphological and immunophenotypic features of a GIST showing neuronal differentiation. The absence of a *c-kit* mutation does not exclude this diagnosis as only 50% of such GISTs demonstrate a molecular aberration in the *c-kit* gene.

Amongst the several spindle cell lesions that are considered in the light microscopic differential diagnosis, a schwannoma is an important consideration in view of the S-100 positivity. It is a rare entity in the gastrointestinal tract, mainly involves the stomach but has also been reported in the colon, esophagus and rectum [19-21]. Although our case was GFAP and S100 positive, it was also c-kit positive and schwannomas are negative for c-kit. Also the common histological features of gastrointestinal schwannomas such as a

lymphoid cuff with the germinal centers, mainly spindle cell component with indistinct cytoplasm and wavy nuclei trapped between linear collagen were absent in our case [19-21]. Furthermore, absence of ultrastructural features of schwannoma such as basal membrane which tapered in both side and the tumor did not exhibit lymphocytic infiltration.

Synchronous presentation of GIST with another tumor has been reported and coexistence with hematological disorders has also been documented [22-24]. These hematological disorders include: chronic lymphocytic leukemia, mucosa-associated lymphoid tissue lymphoma (MALT-lymphoma), non-Hodgkin lymphoma, Burkitt lymphoma, anaplastic large cell lymphoma, high grade follicle center lymphoma (follicular lymphoma) and plasmacytoma [25-26]. However, synchronous presence of mantle cell lymphoma with either GIST or GANT has not been reported previously. GANT on the other hand, has been reported synchronously with adrenal ganglioneuroma, neurofibromatosis and Carney's triad. In this report, we described two unusual coexistent lesions of the appendix: a GIST showing autonomic nerve differentiation (GANT) in a background of mantle cell lymphoma.

#### Acknowledgments

The authors would like to thank Jocelyne Jacques for her able assistance with the immunohistochemistry.

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