

## POINT OF VIEW

# Multiple non-metastatic gastrointestinal stromal tumors. Differential features

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## ABSTRACT

**Introduction:** gastrointestinal stromal tumors (GISTs) are specific, generally KIT (CD117)-positive, mesenchymal tumors of the digestive tract displaying *KIT* or *PDGFRA* gene mutations. Clinically, they tend to present as solitary tumors of the intestinal wall; more rarely, multiple tumors may occur in one or more organs.

**Objective:** to review the morphological, immunohistochemical and molecular features of multiple, non-metastatic forms of GIST.

**Sources:** review of the literature on Medline, and authors' own experience.

**Conclusions:** multiples GISTs may occur in three different contexts: as spontaneous lesions (in both adults and children); due to familial GIST syndrome (autosomal dominant inheritance); or in association with specific syndromes (e.g. Carney's triad, Carney-Stratakis syndrome, type I neurofibromatosis). Outside these contexts, the existence of multiple GISTs is deemed to be the result of tumor metastasis, and therefore indicative of advanced-stage disease. Clinicians need to be aware of these variants, whose prognosis and treatment differ.

**Key words:** Gastrointestinal stromal tumors. GIST. Carney's triad. Carney-Stratakis syndrome. Type I neurofibromatosis. Familial GIST syndrome. Pediatric GIST.

## RESUMEN

**Introducción:** los tumores del estroma gastrointestinal (GIST) son neoplasias mesenquimales del tubo digestivo que generalmente expresan el receptor KIT (CD117) y muestran mutaciones en los genes *KIT* o *PDGFRA*. Aunque la forma de presentación clínica habitual es como una neoplasia mural solitaria, excepcionalmente pueden presentarse formas múltiples en el mismo o diferente órgano.

**Objetivo:** revisar las características morfológicas, inmunohistoquímicas y moleculares de las formas de GIST múltiples no metastásicas.

**Fuentes:** revisión de la literatura en Medline y la propia experiencia.

**Conclusiones:** los GIST múltiples pueden presentarse en tres contextos diferentes: lesiones espontáneas (del adulto o de la edad infantil); síndrome familiar propio (transmitido con herencia autosómica dominante); y lesiones asociadas a síndromes específicos (triada de Carney, síndrome de Carney-Stratakis, y neurofibromatosis tipo I). Fuera de estos ámbitos, se interpreta que todo GIST múltiple es el resultado de siembras tumorales metastásicas y, por tanto, corresponde a enfermedad avanzada. Estas variantes deben ser conocidas por el clínico dado las connotaciones pronósticas y terapéuticas que ello conlleva.

**Palabras clave:** Tumores del estroma gastrointestinal. GIST. Triada de Carney. Síndrome de Carney-Stratakis. Neurofibromatosis tipo I. Síndrome de GIST familiar. GIST pediátrico.

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## GENERAL FEATURES OF GASTROINTESTINAL STROMAL TUMORS

Gastrointestinal stromal tumors (GISTs) are mesenchymal tumors of the gastrointestinal tract that differentiate towards interstitial cells of Cajal or their precursors; they are generally KIT (CD117)-positive, and display *KIT* or *PDGFRA* gene mutations (1,2). These

tumors have recently prompted particular interest due to their good response to new targeted therapies (imatinib mesylate, Glivec®, Novartis) (3). They generally arise as solitary tumors of the stomach (50%), small intestine (25%), large intestine (10%) or esophagus (5%) (Fig. 1); in 10% of cases they occur outside the gastrointestinal tract (EGIST: extra-gastrointestinal stromal tumors), in the mesenterium, epiploon and –more rarely– appendix, gall bladder or pancreas (1,4). Epidemiological studies report an annual incidence of 6.8-19.6 per million inhabitants, depending on the countries surveyed (5), affecting men and women roughly equally, over a wide age range; however, 75% of cases occur in adults aged over 50 (6). The following clinical signs and symptoms may be observed: fatigue, abdominal pain, dysphagia, feeling of fullness, hematemesis or melena (1,4,5,7,8). Smaller GISTs are often incidental findings during surgery, radiological studies or endoscopy (1). At histological examination, tumors tend to display spindle cell, epithelioid or mixed cell morphology (Fig. 2). Immunohistochemical features include positivity for

CD117 (95%), CD34 (60-70%), smooth muscle actin (30-40%), S-100 protein (5%), and more rarely desmin (1-2%) (1,4) (Fig. 3); it should be stressed that 5% of GISTs do not show positivity for CD117. Two new tissue markers (DOG-1, PKC $\theta$ ) have been identified, providing specificity and sensitivity equal to or greater than those of CD117 (9-15). Both proteins are selectively expressed on interstitial cells of Cajal (ICC), but their relationship with the *KIT* receptor is unknown. Molecular studies have revealed mutually exclusive mutations in *KIT* (60-80%) and *PGFRA* (5-10%) genes (1,4,5,16). Recently, *BRAF* mutations were detected in 13% of cases bearing no *KIT/PPDGFR* (wt *KIT/PDGFR*) mutations (16). Approximately 20-25% of gastric and 40-50% of small intestinal GISTs are clinically malignant. Local recurrences and metastases (early and late) commonly develop in the abdominal cavity and liver; more rarely, in bone, soft tissue and skin (1). Lymph-node metastases are extremely rare, and primarily affect children (1). Tumor size, mitotic activity and location are the major prognostic factors (17-19).

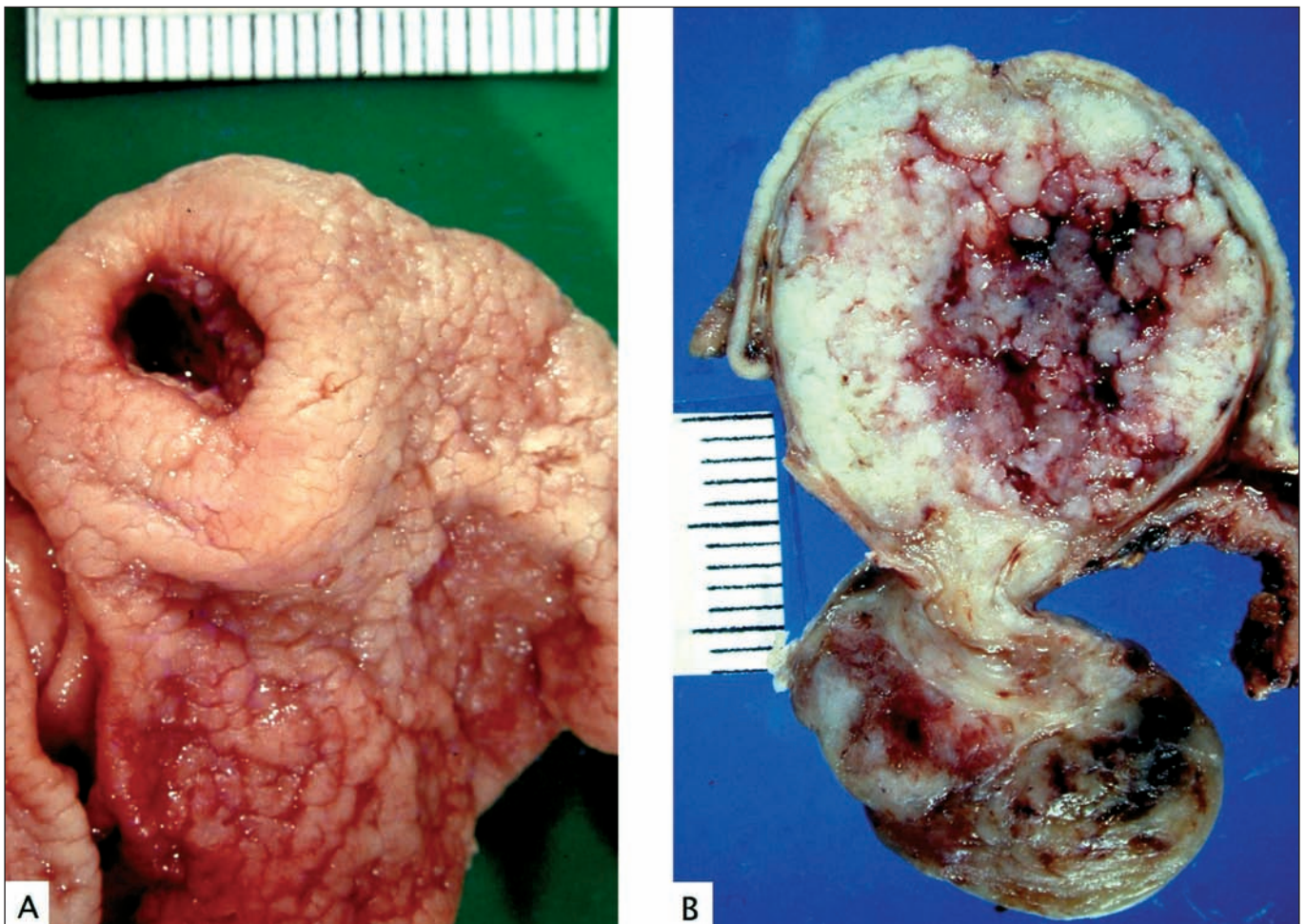


Fig. 1. Gastric gastrointestinal stromal tumor. A. Protruding lesion in the mucosa with central ulceration. B. Section showing hourglass growth pattern, focal necrosis and bleeding.

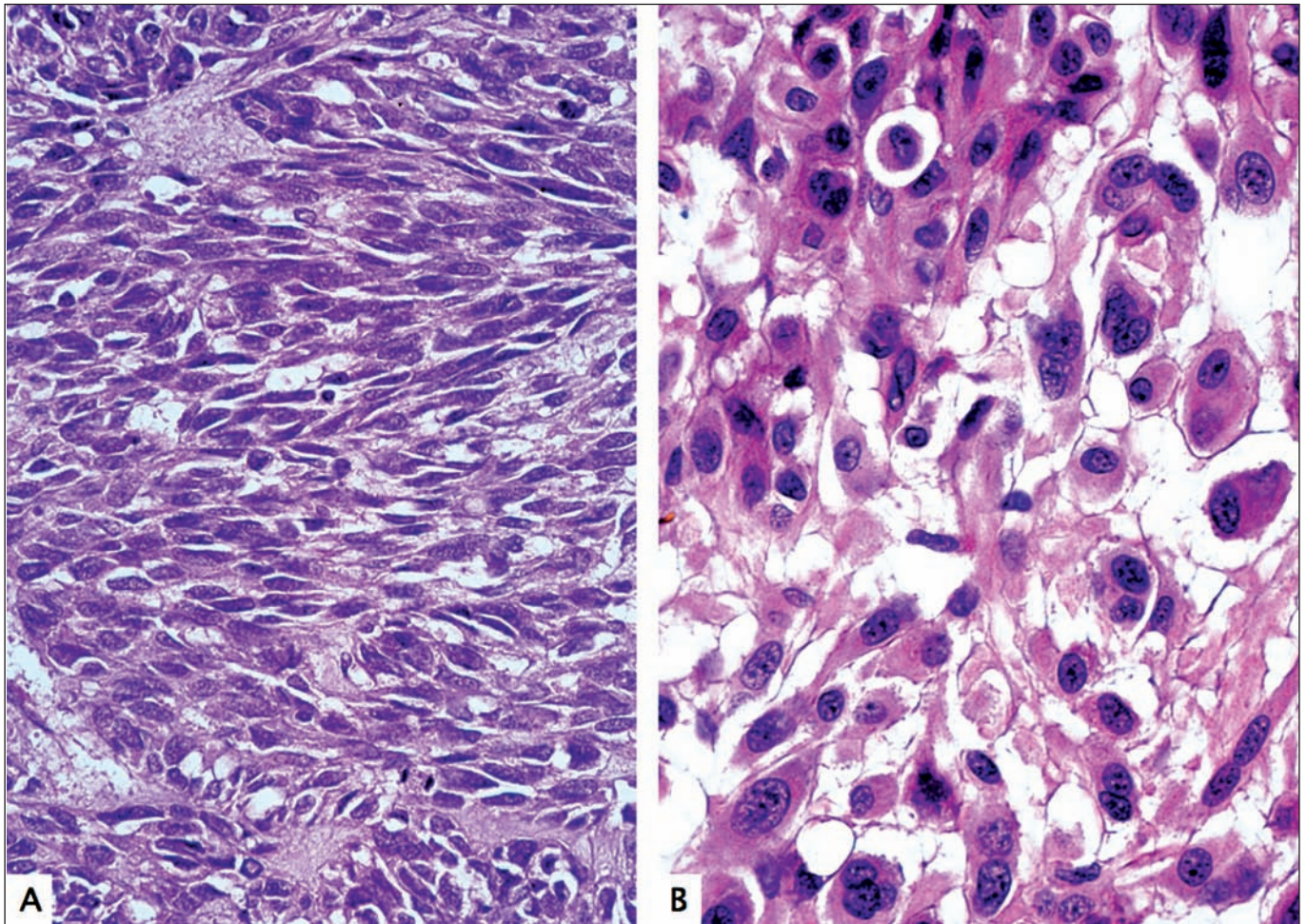


Fig. 2. Common histological patterns. A. Spindle cell morphology HE 40X. B. Epithelioid cell morphology. HE 60X.

Diagnosis of GIST requires a basic immunohistochemical examination including the following markers: CD117, CD34, smooth muscle actin, desmin and S100 protein; these markers, routinely used in histology laboratories, enable differential diagnosis with respect to morphologically similar tumors (mainly leiomyoma/leiomyosarcoma and schwannoma) (Fig. 4). Molecular analysis is recommended for the diagnosis of CD117-negative tumors, and also as the basis for targeted therapy, since patients with GISTs harboring *KIT* exon 9 mutations need to double the dosage of imatinib (20), while those displaying the *PDGFRA* exon 18 mutation D842V fail to respond to this drug (21,22). In view of its specialized nature, molecular analysis is currently performed by reference centers.

## MULTIPLE GASTROINTESTINAL STROMAL TUMORS

In very rare cases, multiple GISTs may be detected in one or more organs; this is not necessarily an indicator of

greater aggressivity. Digestive specialists need to be aware of these rare forms, whose prognosis and treatment differ from those of conventional GISTs. Multiple tumors may arise in three clinical contexts: sporadic tumor formation (in both pediatric and adult patients); familial GIST syndrome (autosomal dominant inheritance); or as an additional component of certain syndromes (Carney's triad, Carney-Stratakis syndrome and type I neurofibromatosis). The differential diagnosis of these syndromes is based mainly on clinical and genetic studies, rather than on morphological, immunohistochemical or molecular findings.

### Multiple sporadic GISTs in adults

Multiple sporadic GISTs are generally characterized by the presence of two or three lesions, at the same site or in different sites, showing different molecular alterations in the same gene (*KIT*) (23,24), or in *KIT* and *PDGFRA* genes (25,26). Agaimy et al. (27) recently analyzed 11 patients

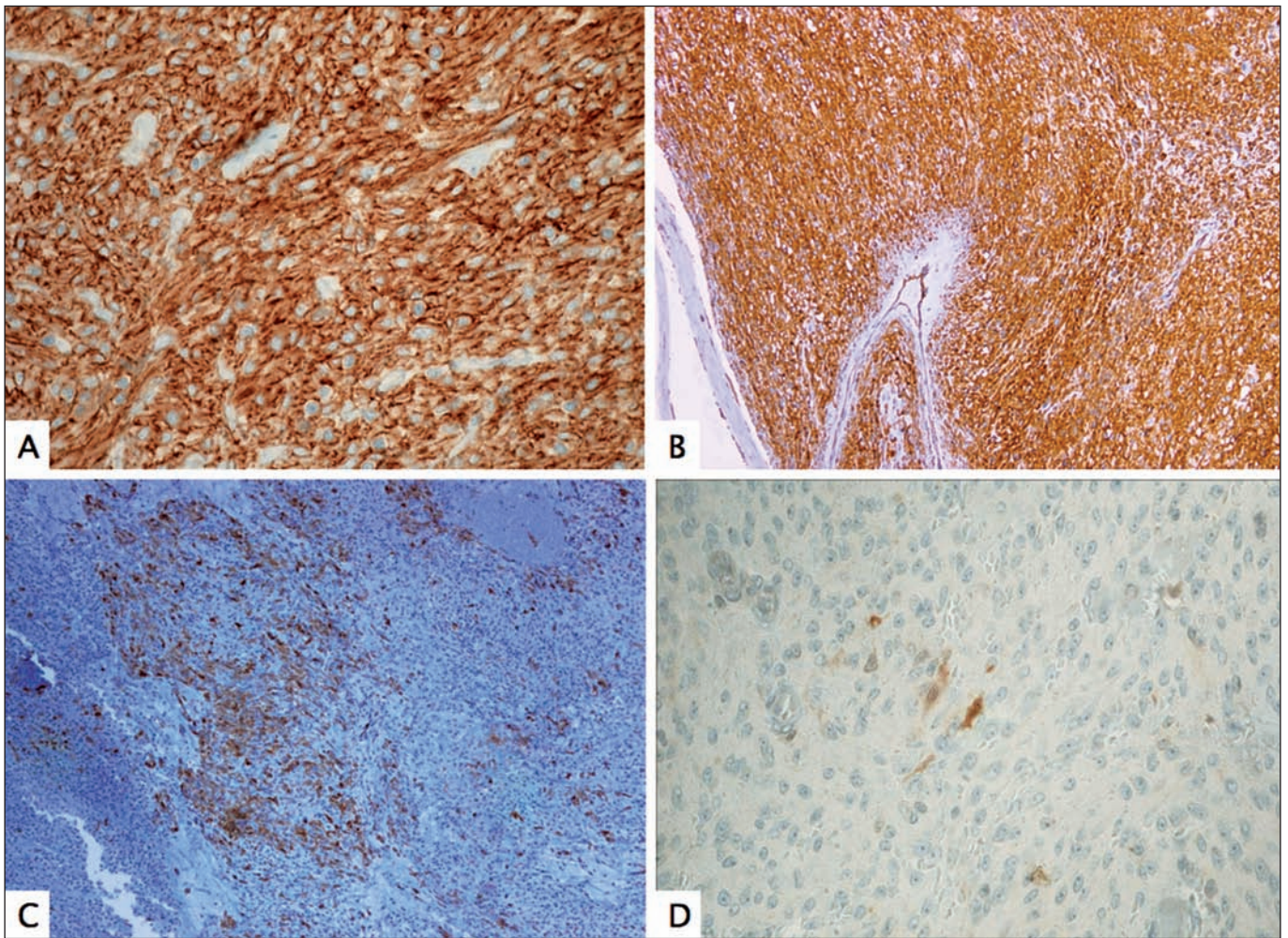


Fig. 3. A. CD117 positivity tends to be strong and diffuse, and may be detected in both membrane and cytoplasm. 40X. B. CD34 positivity is generally strong, and is detected in cell membrane. 20X. C. Smooth muscle actin expression tends to be focal and cytoplasmic. 20X. D. S100 protein positivity tends to be focal, and involves both the cytoplasm and the nucleus. 40X.

aged over 70, with small sclerosing tumors or tumorlets (2-4 each) in the proximal stomach; tumors were incidental findings at autopsy or during surgery. All displayed spindle-cell morphology, and all were CD117+ and CD34+. In all but one case, individual lesions from the same patient displayed different *KIT* mutations (Table I). The presence of multiple sporadic lesions in the same patient points to the existence of distinct subsets of interstitial cells of Cajal or their precursors in different locations (field carcinogenesis theory); molecular analysis is therefore essential in order to distinguish metastatic from non-metastatic lesions (27).

**Multiple sporadic GIST in children and adults under 30**

GISTs in pediatric and adolescent patients account for 1-2% of all GISTs, and differ in certain respects from

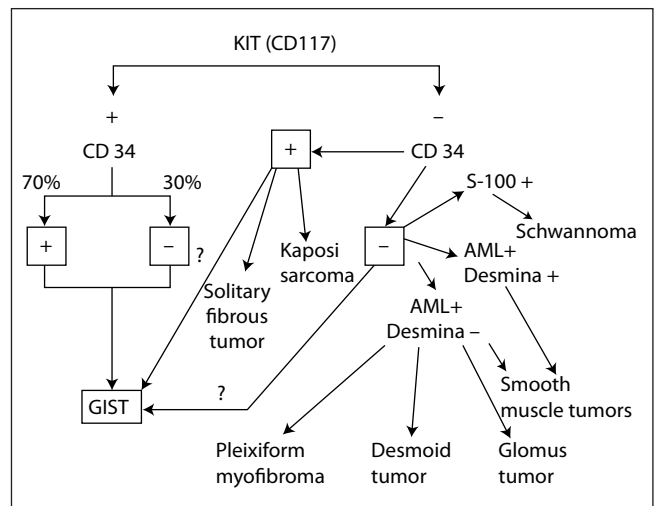


Fig. 4.

**Table I. Multiple sporadic GISTs in adults**

Elderly adults
Two or three synchronous lesions
Predilection for the stomach
Spindle cell morphology
CD117 and CD34 positive
Different mutations in <i>KIT</i> or in <i>KIT</i> and <i>PDGFRA</i>

those recorded in adults (28). Molecular analysis has been performed in around 75 cases, and *KIT/PDGFRA* mutations have been detected in 11-15% of these (28-30). Pediatric GISTs are more prevalent in females (70%), occur preferentially in the stomach (88%), tend to be multifocal (81%), display epithelioid or mixed cell morphology (82%), and generally lack both *KIT/PDGFRA* mutations (88%) and interstitial cell of Cajal hyperplasia (100%). By contrast, the few pediatric GISTs with *KIT* or *PDGFRA* mutations have very different features: prevalence is greater in males, lesions tend to be unifocal, and usually occur in extragastric locations, and spindle-cell morphology is found in all cases (28,29); in other words, they share many of the features of spontaneous adult tumors.

Immunohistochemically, pediatric GISTs consistently overexpress CD117 (28-30) and DOG1 (10). Unlike in adults, pediatric GISTs tend to follow an indolent course, and are associated with better survival rates, despite the high rate of intra-abdominal and even lymph-node metastasis (28-30). The transcriptional signature is distinct from that of adult GISTs; the top-ranked genes overexpressed in the pediatric subset are *CRLF1*, *BAALC*, *FGF4*, *PLAG1*, and *IGF1R*. Tumor progression to malignancy is also different, and probably results from gene promoter methylation; unlike in adults, sequential chromosomal deletions (at 14q and 22q) and LOH are not common; by contrast, chromosomal additions (X, 1q, 5p, 8q, 9p, 12p, 13q, 18p, 19q) and amplifications (1q and 19p) are more frequent (28,29). The major differences between adult and pediatric subsets are shown in table II.

Although GIST is a heterogeneous entity in adults aged under 30, most cases display the predominance of mutation and the clinicoanatomical features of the adult tumor (28).

### Familial GIST syndrome

This is an extremely rare disorder. To date, a clear autosomal dominant inheritance pattern associated with one or more GISTs affecting two or more family members

**Table II. Multiple sporadic GISTs in children and young adults. Differential features**

	Children	Adults
Frequency	89%	11%
Presentation	Stomach, multiple, >> female	Small bowel/stomach, solitary >>male
<i>KIT/PDGFRA</i> mutations	No	Yes
Histology	Epithelioid	Spindle/epithelioid
Clinical progress	Indolent	Progressive
ICC hyperplasia	No	Yes
Cytogenetics	Minimal changes (additions and amplifications)	Deletions (14q, 1p, 9p, 22q)
Transcriptional signature	<i>CRLF1</i> , <i>BAALC</i> , <i>FGF3</i> , <i>PLAG1</i> , <i>IGF1R</i> ...	<i>KIT</i> , <i>CD34</i> , <i>PKCθ</i> , <i>DOG-1</i> ,...
Carney's triad	Some	None

ICC: interstitial cells of Cajal.

has been reported in only 20 families (31-49) (Table III). The phenotypic syndrome varies considerably, and may include –in addition to multiple, generally benign GISTs appearing in middle age– hyperpigmentation, urticaria pigmentosa and dysphagia (32,33). Germline mutations generally affect the *KIT* gene (31-46), though cases of *PDGFRA* germline mutations have also been reported (47-49). Skin alterations are common in patients with *KIT* exon 8 (31) and exon 11 mutations (32-36,39). Dysphagia, caused by esophageal dysmotility related to ICC hyperplasia, is observed in patients with *KIT* exon 8 (31) and exon 17 mutations (44,45).

*KIT* exon 17 mutations, which are very rare in sporadic GIST, have been reported in three cases of famil-

**Table III. Familial gastrointestinal stromal tumor syndrome**

Gene	Exon	Mutation type (No. cases)	Clinical findings
<i>KIT</i>	Exon 8	Asp419del* (1)	GIST (31), achalasia, mastocytosis
		Trp557Arg (2)	Pigmentation, GIST (32,33)
	Exon 11	Val559Ala (4)	Pigmentation, urticaria pigmentosa, GIST (34-36)
		Val560Gly (1)	GIST (37)
		Val560del (1)	GIST (38)
		Gln575_Leu576dup (1)	Pigmentation, GIST (39)
		Asp579del (2)	GIST (40,41)
		Lys642Glu (2)	GIST (42,43)
	Exon 13	Asp820Tyr (2)	Achalasia, GIST (44,45) GIST (46)
		Asn822Tyr (1)	
<i>PDGFRA</i>	Exon 12	Tyr555Cys* (1)	GIST (47)
		Asp561Val (1)	Fibrous polyps, lipomas, GIST (48)
	Exon 18	Asp846Tyr (1)	GIST (49)

GIST: gastrointestinal stromal tumor. \*Mutations reported only in familial GIST.

ial GIST; two at codon 820 (Asp820Tyr) (44,45) and one at codon 822 (Asn822Tyr) (46). The specific type of mutation is generally the same as that found in sporadic tumors, with two notable exceptions: *KIT* (exon 8) Asp419del (31) and *PDGFRA* (exon 12) Tyr555Cys (47). Pasini et al. (48) recently reported the unusual case of a patient carrying a germline *PDGFRA* mutation Asp561Val who developed multiple fibrous polyps (> 100) in the intestine, stomach, duodenum and cecum, lipomas in the duodenum and jejunum and gastric GIST, with no associated ICC hyperplasia. Gastric and duodenal fibrous tumors were CD34+, CD117-, PKCθ- and *PDGFRA*+; whilst the two gastric GISTs were CD117+, CD34+, *PDGFRA*+, PKCθ+.

**GIST associated with Carney’s triad and the Carney-Stratakis syndrome**

Carney’s triad is a non-familial condition preferentially affecting women and combining the presence of multifocal epithelioid gastric GIST with the metachronous or synchronous occurrence of paraganglioma and pulmonary chondroma, although the conjunction of the three lesions is not necessary for diagnosis (50). Molecular studies to date have failed to reveal specific genetic alterations (51). Female predisposition, multifocal gastric location and wtKIT/*PDGFRA* genotype are findings which overlap considerably with clinicopathological features of pediatric GIST (28), suggesting that pediatric forms may represent a forme fruste of Carney’s triad; longer follow-ups appear to confirm this suspicion, at least in some cases (28).

The Carney-Stratakis syndrome is a similar condition, in that it combines gastric GISTs and paragangliomas; however, it differs from Carney’s triad in that it affects men and women with equal frequency, displays an autosomal dominant inheritance pattern and is not associated with pulmonary lesions; moreover, molecular analysis reveals germline mutations of the genes coding for SDH



Fig. 5. Type 1 neurofibromatosis. Resected small bowel specimen showing two independent tumors separated by a segment of normal tissue.

subunits B, C and D) (52,53). The main differential features of the two conditions are shown in table IV.

**GIST associated with type I neurofibromatosis**

The range of gastrointestinal lesions associated with type 1 neurofibromatosis (NF1) includes: a) hyperplasia of nerve plexuses; b) GIST; c) endocrine tumors of the duodenum and periampullary region (somatostatinoma); and d) other tumors of varied histogenesis (54). The incidence of GIST in patients with NF1 is 7% (55). Most reported cases occur in middle-aged or elderly patients; tumors are multiple and often microscopic, are located in the small intestine (ileum and jejunum), and are accompanied by hyperplasia of the interstitial cells of Cajal (Fig. 5). Histologically, tumors display spindle-cell morphology, with few mitotic figures and frequent skeinoid fibers; they are immunohistochemically positive for CD117, DOG-1 and PKCθ (10,56-62) (Fig. 6). However, immunostaining for S100 protein yields variable results: some authors report low expression (58,61,62) or no expression at all (54,57) whilst others have observed positivity for S100 in up to 64% of cases (57). Molecular analysis in the seven largest cohorts indicates an incidence of *KIT* and *PDGFRA* mutations of 4.1% (6/145) and del 1.7% (2/116), respectively; some of these may be random mutations, since they are not observed in sporadic GISTs (57). A recent study has reported loss of heterozygosity at 14q (87.5%) and at 22q (41.7%), together with activation of the Ras-MAPK pathway, probably associated with inactivation of the NF1 gene (62). The main features of GISTs associated with type 1 neurofibromatosis are shown in table V.

**Table IV. Carney’s triad and Carney-Stratakis syndrome. Differential features**

	<i>Carney’s triad</i>	<i>Carney-Stratakis syndrome</i>
Inheritance	Sporadic	Dominant autosomal
Sex	>>> women	Women and men
Synchronous/metachronous clinical signs	Gastric epithelioid GIST Paraganglioma Pulmonary chondroma	Gastric epithelioid GIST Paraganglioma
Molecular changes	Unknown	<i>SDH</i> mutations (subunits B, C, D)

>>>>: large majority. GIST : gastrointestinal stromal tumor.

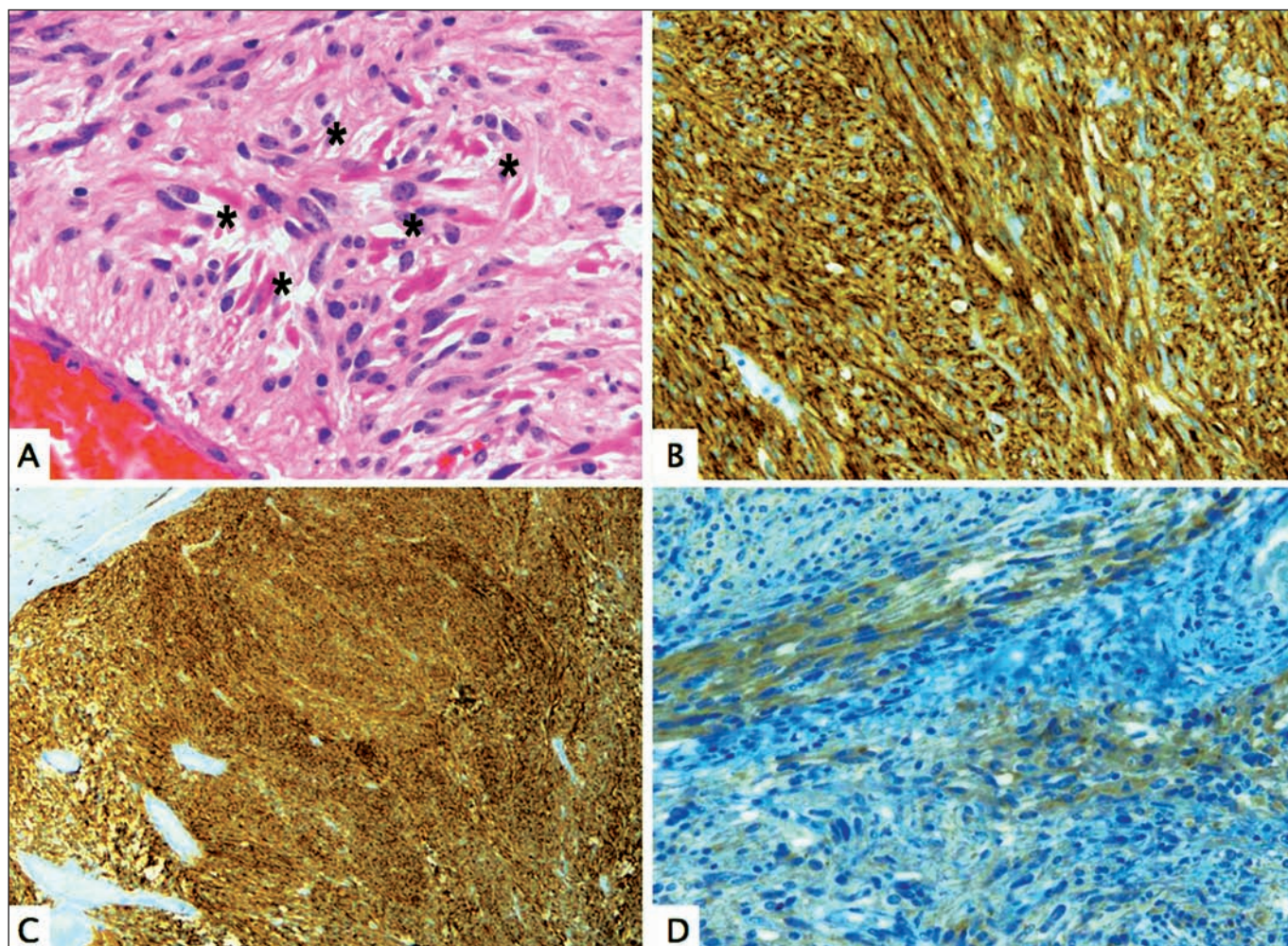


Fig. 6. Type 1 neurofibromatosis. A. Spindle-cell tumor containing numerous skeinoid fibers (asterisk) HE 40X. Strong, diffuse positivity for CD117 (B) DOG1 (C). 20X. Weak focal positive staining for PKC $\theta$ . 40X.

**Table V. General features of GISTs associated with type 1 neurofibromatosis**

Clinical features

Mean patient age: 49

Location: Small intestine (jejunum, ileum)

Morphology

Small and multiple

Spindle cell morphology, skeinoid fibers

ICC hyperplasia

Immunohistochemistry

CD117 (100%), S-100 (0-64%), CD34 (79%)

Molecular biology (86 cases)

*KIT/PDGFR*A mutations

Absent: 80 (93%)

Present: 7%\*

*KIT*: exon 11 (3); exon 13 (1)

*PDGFRA*: exon 12 (1); exon 18 (1)

\*Non-habitual mutations (SNIP).

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