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Gastrointestinal autonomic nerve tumours and their separation from other gastrointestinal stromal tumours: an ultrastructural and immunohistochemical study of seven cases

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Abstract Gastrointestinal stromal tumours (GIST) represent a heterogeneous group whose classification frequently requires ultrastructural and immunohistochemical studies. In a retrospective study of the ultrastructural findings of 24 gastrointestinal stromal tumours, whose light microscopic study has yielded ambiguous results and in which accurate diagnosis had required ultrastructural support, seven were found to have the characteristics of gastrointestinal autonomic nerve (GAN) tumours. In all of them the diagnosis was based on the presence of dendritic processes with dense neuroendocrine granules. Immunohistochemically, the seven tumours were negative for smooth-muscle markers. All stained positively for vimentin. NSE, chromogranin, and synaptophysin were positive in most of them, while S-100 protein was positive only in two cases. We present the ultrastructural and immunohistochemical features of seven GANT against the background of the GISTs of our series. We conclude that GAN tumours cannot be diagnosed by light microscopy alone but this tumour group displays characteristic electron microscopic and immunohistochemical features and appears to represent a distinct type of GIST.

Key words Gastrointestinal autonomic nerve tumours · Ultrastructure

Introduction

Gastrointestinal stromal tumours (GIST) comprise several different entities whose differentiation by light microscopy can prove difficult. In 1984 Herrera et al. [8] described a neoplasm with ultrastructural characteristics suggesting neuroectodermic differentiation. More recently, Lauwers et al. [11] reported twelve new cases with identical characteristics. These tumours, known as gastrointestinal autonomic nerve (GAN) tumours or plexosarcoma, display histological images very similar to those of some smooth-muscle tumours, and their definitive diagnosis thus far requires ultrastructural and immunohistochemical study [8, 14]. This study presents seven cases which match the GAN ultrastructural criteria and immunohistochemical features and which were found among 24 GIST whose histological findings were not diagnostic.

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Materials and methods

A search of the Ultrastructural Section files of the Pathology Department at "12 de Octubre" Hospital was made for all GIST studied from 1984 to 1993. We found 24 GIST, the light microscopic assessment of which had yielded ambiguous results and in which accurate diagnosis had required ultrastructural support. In all 24 tumours, before the ultrastructural study, a smooth-muscle origin was suggested: leiomyosarcoma in 18 and leiomyoblastoma in six cases. The 24 patients included 13 males and eleven females ranging in age from 43 to 71 years (mean 58.2). Anatomic sites were oesophagus (one), stomach (16), jejunum (five), large intestine (one), mesentery (one).

For light microscopic examination tumour specimens were fixed in 4% buffered formaldehyde; paraffin sections were prepared and stained with haematoxylin and eosin (H & E).

For ultrastructural examination small fresh samples were fixed in Karnovsky's reagent and embedded in Epon 12 resin. Sections 1 μm thick were stained with alcoholic toluidine blue and representative areas were selected. Ultrathin sections were stained with uranyl acetate and lead citrate [18] and examined with an electron microscope. The GAN tumour diagnostic criteria applied were the presence of dendritic processes with more than occasional membrane-bound neurosecretory granules displaying eccentric or central electron-dense cores and no evidence of thin filaments or dense bodies [8]. The cases displaying isolated granules, lack of prolongations, or showing exclusively perinuclear granules were not considered as GAN tumours.

Immunohistochemical study was conducted in all tumours with the antibodies listed in Table 1, using the avidin-biotin-peroxidase complex method [10]. Appropriate negative (using buffer solution instead of primary antibodies) and positive controls from normal tissues were also examined.

Clinical information was obtained from patient charts or by contacting clinicians.

All patients with GAN tumours were treated surgically. Radical surgery (gastrectomy with Billroth II) was performed in those cases in which the tumour was located in stomach. The other pa-

Table 1 Antibodies used in immunohistochemical reactions (*M*, monoclonal; *P*, polyclonal)

Antibody	M/P	Dilution	Company
NSE	P	1:500	Biomeda, Foster City CA
Desmin (D33)	M	1:100	DAKO, Glostrup, Denmark
Vimentin (V9)	M	1:10	DAKO, Glostrup, Denmark
Synaptophysin	P	1:50	DAKO, Glostrup, Denmark
S-100	P	1:2000	DAKO, Glostrup, Denmark
Actin (HHF-35)	M	1:5000	ENZO, New York, NY
Chromogranin (A3)	M	1:100	DAKO, Glostrup, Denmark

tients underwent segmental resection with end-to-end anastomosis when the tumour affected small intestine. None of the patients was treated with adjuvant chemotherapy. Clinical follow-up ranged from six months to 10 years (mean duration 14 months). At present, all patients are free of tumour.

Results

Of the 24 GIST, seven of the tumours fulfill the ultrastructural criteria for GANT, two of which had been previously diagnosed as leiomyosarcoma and the other five as leiomyoblastomas. The seven patients (Table 2) included three males and four females ranging in age from 54 to 70 years (mean 62 years). Presenting symptoms were rectal bleeding (two cases), melaena (three cases), abdominal pain (one case), and vomiting (one case). One case was an incidental finding in a patient with von Recklinghausen's disease suffering a rectal adenocarcinoma. Four of the tumours were located in the stomach, two in the small intestine, and one in the mesentery of the small bowel.

Macroscopically, five of the tumours were solid and two cystic. The solid tumours ranged in size from 1 to 6 cm and were intramural with luminal invasion (Fig. 1). The two predominantly cystic lesions measured 17 and 26 cm in greatest diameter and were found in the stomach and on the mesenteric edge of the small bowel. The solid tumours were whitish, soft and had imprecisely defined margins on the cut surfaces. The cystic masses were thick-walled, with some solid areas and abundant necrotic and haemorrhagic foci (Fig. 2). One case (case 6) displayed hepatic metastases at the time of diagnosis.

Microscopically, all seven tumours had a similar appearance, with spindle and epithelioid cells arranged in poorly defined lobules in variable patterns (Fig. 3). The most frequent cellular arrangement was large fascicles made up of imprecisely limited cells with oval-shaped smooth-contoured nuclei (Fig. 4). Four cases showed cells with clear, vacuolated cytoplasm randomly nested among the spindle cells (Fig. 5). Pleomorphism was significant in only one case displaying bizarre-looking multinucleated cells, some with intracytoplasmic vacuoles. The cells were arranged in a highly vascularized loose stroma. Frequent areas of haemorrhage were seen in all but one tumour (Fig. 6). Extensive areas of necro-

Table 2 Clinicopathological data of 23 gastrointestinal tumours examined by electron microscopy (*EM*, Electron microscopy diagnosis; *GIST, NOS*, GIST, not otherwise specified; *NA*, not available)

Case	Age/Sex	Symptoms	Localization diagnosis	Size	E.M.
1	70 F	Vomiting	Stomach	3×2×1 cm	GAN
2	58 M	Melaena	Stomach	6×5, 5×3,5 cm	GAN
3	54 F	Rectal bleeding	Jejunum	3×2, 5×2,3 cm	GAN
4	71 M	Abdominal pain	Stomach	26×26×12 cm	GAN
5	63 F	Rectal bleeding	Jejunum	5×4, 5×4 cm	GAN
6	64 M	Melaena	Jejunum	17×15 cm	GAN
7	56 F	Melaena	Stomach	6×5, 8×4,5 cm	GAN
8	59 M	None	Stomach	NA	Leiomyosarcoma
9	48 M	Vomiting	Stomach	6×3,5 cm	Leiomyosarcoma
10	60 M	None	Stomach	3 cm	Leiomyosarcoma
11	67 F	Abdominal pain	Stomach	4×1,5×2 cm	GIST, NOS
12	61 M	NA	Stomach	NA	Leiomyosarcoma
13	70 F	Abdominal distension	Stomach	4×3,3×2 cm	Leiomyosarcoma
14	52 F	NA	Stomach	NA	GIST, NOS
15	57 M	Abdominal mass	Stomach	7×3×2 cm	Leiomyoblastoma
16	49 F	Abdominal distension	Stomach	10×4×1 cm	GIST, NOS
17	43 M	Dysphagia	Oesophagus	3,5×2,5×2 cm	Leiomyosarcoma
18	44 F	Rectal bleeding	Large intestine	6×6×3,5 cm	Leiomyosarcoma
19	57 M	Melaena	Stomach	5 cm	Leiomyosarcoma
20	67 F	Rectal bleeding	Jejunum	7,5×3×3 cm	GIST, NOS
21	NA M	NA	Stomach	NA	Leiomyosarcoma
22	61 M	Melaena	Stomach	7×8×3 cm	GIST, NOS
23	50 F	Abdominal distension	Mesentery	15×15×6 cm	Leiomyosarcoma

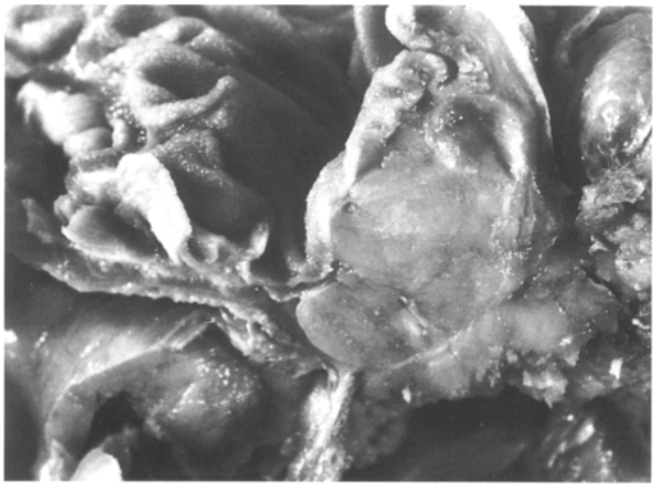

Fig. 1 A solid submucosal tumour in the small intestine

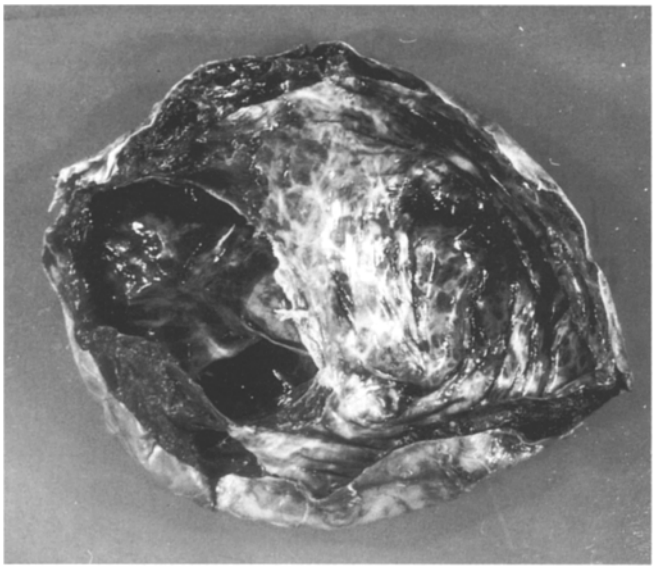

Fig. 2 Cystic tumour with haemorrhagic solid areas in the mesentery of small intestine

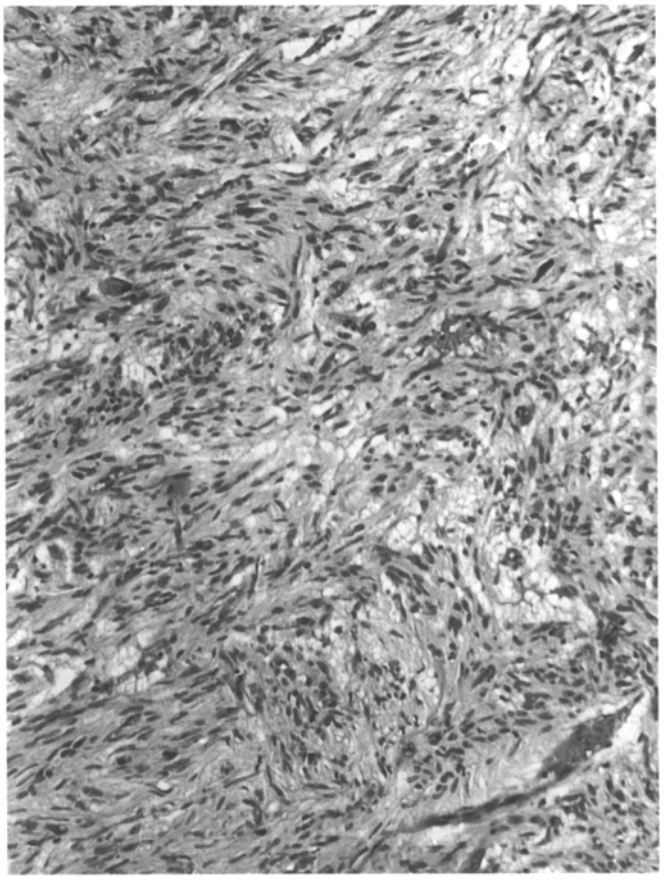

Fig. 3 Tumour composed predominantly of spindle and vacuolated cells arranged in irregular fascicles. (H & E ×100)

sis were observed in three cases. Four cases demonstrated mitosis (1×10 HPF).

The remaining GIST showed a similar picture and consisted of coalescent bundles of elongated spindle cells, with eosinophilic cytoplasm; nuclei were mostly oval to spindle shaped with prominent nucleoli. Ten tumours showed an even mixture of epithelioid and spindle areas, and the remaining seven cases had a predominantly spindle cell morphology. All were characterized by marked cellularity, high mitotic rate, and necrosis.

The ultrastructural picture for all the cases diagnosed as GAN was similar. All seven cases displayed a star-shaped cell pattern, with long processes branching away from the cell body, intertwined with the neighboring elements (Fig. 8). In other areas the cells were arranged in a loose matrix and two cases displayed a discontinuous external lamina (Fig. 9). Cell surfaces were found to contain rudimentary cell junctions (Fig. 10). Occasional pinocytotic vesicles were observed in two cases, though less consistently than in smooth-muscle tumour cells. The cell cytoplasm had a moderate amount of organelles and, as a constant and characteristic feature, all seven cases displayed spherical electron dense granules surrounded by membranes having a thin peripheral halo (Fig. 11). The number of granules was variable, both in the cell bodies and in the peripheral prolongations (Fig. 12). Synaptic vesicles were not seen in any of the cases. The nuclei were ovoid, with scarce heterochromatin and with the nucleolus visible in 25% of the sections.

The remaining 17 GIST showed the following ultrastructural features: in eleven cases we found cells variable shaped immersed in a non-specific matrix with more than occasional fine filaments with dense bodies, and subplasmalemmal dense plaques. Pinocytotic vesicles were occasionally found. All these findings were considered compatible with the diagnosis of leiomyosarcoma (ten cases) and leiomyoblastoma (one case). Five cases showed spindle shaped tumour cells lacking features of smooth muscle, Schwann cells or GAN differentiation and were diagnosed as GIST without other specification. The remaining case was deleted for ultra-

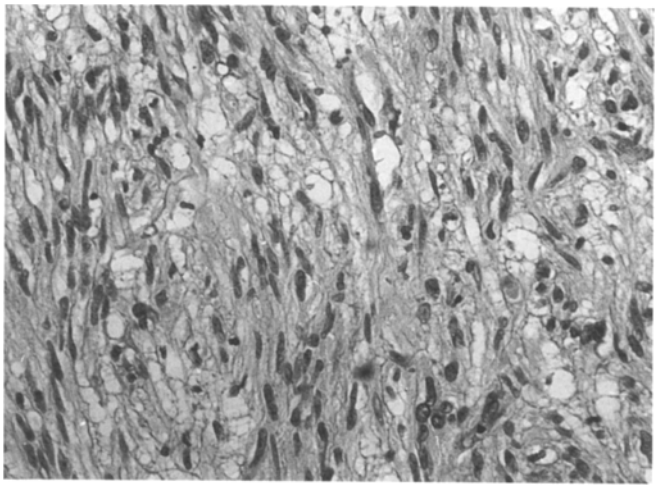

Fig. 4 The fusiform cells showed ill-defined outline and elongated nuclei. (H & E ×400)

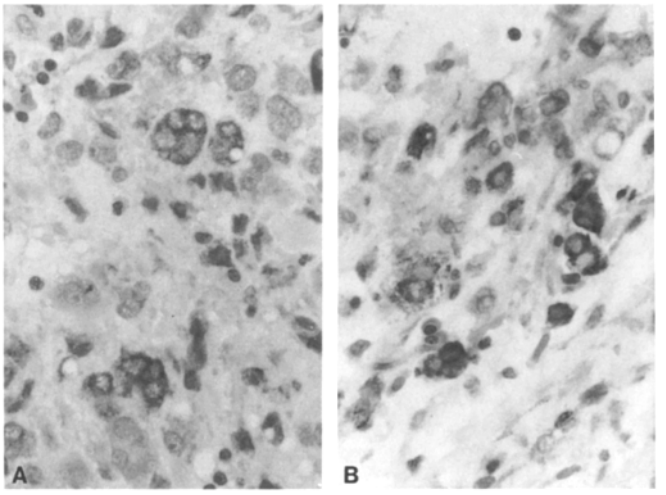

Fig. 7 A Immunohistochemical staining for NSE was focal (×400). **B** Focal staining for synaptophysin was observed in neoplastic cells (×400)

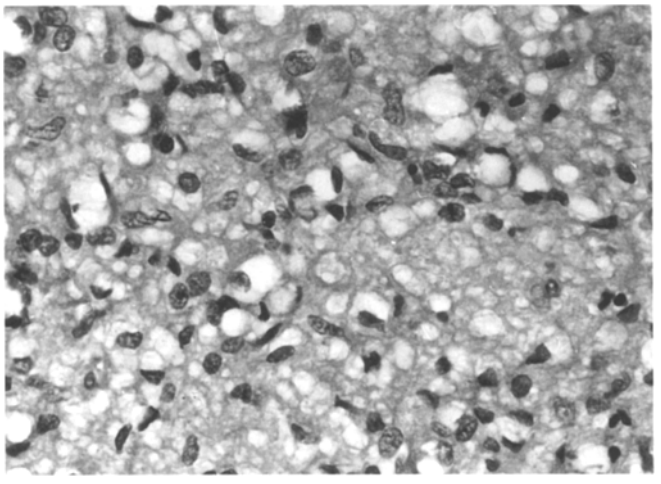

Fig. 5 Note the prominent vacuolated cytoplasm and vesicular nuclei in a loose stroma. (H & E ×400)

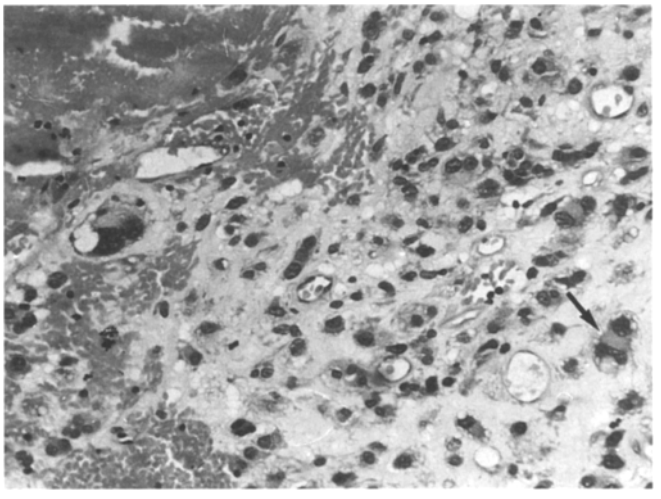

Fig. 6 Some of the areas of the neoplasm reveal foci of haemorrhage and necrosis. Note the cellular pleomorphism (*arrow*). (H & E ×400)

structural study because of poor fixation. All these cases failed to show specific dense core granules.

Immunohistochemical findings of the tumours ultrastructurally diagnosed as GAN are shown in Table 3. Vimentin was consistently positive in all seven cases; NSE and synaptophysin were focally positive in six (Fig. 7a, b) and there was also focal staining for S-100 protein and chromogranin A in two cases. Desmin and α-smooth muscle actin were consistently negative in all seven cases. The immunoreactivity for each marker in the remaining cases is summarized in Table 3. Each of the ten cases diagnosed as leiomyosarcoma after electron microscopic study showed a positive reaction for vimentin. Eight of them stained diffusely with muscle-specific antibodies. Three tumours showed focal NSE positivity. All leiomyosarcomas were negative for synaptophysin, chromogranin A and S-100 protein. The case diagnosed as leiomyoblastoma was positive for vimentin, α-smooth muscle actin and NSE. The remaining case, considered unsatisfactory for electron microscopic study because of poor fixation, was deleted. The five cases without specific electron microscopic findings were diffusely immunoreactive for vimentin and negative for desmin, synaptophysin and chromogranin A. Two cases were positive for α-smooth muscle actin (one reacted also with NSE) and another case showed positive reaction for S-100 protein.

Discussion

GIST are a group of non-epithelial neoplasms which include leiomyomatous tumours (leiomyoma, leiomyosarcoma and leiomyoblastoma) and malignant and benign nerve sheath tumours. There is a poorly differentiated group of stromal tumours which has been classically diagnosed as leiomyosarcoma, leiomyoblastoma or malig-

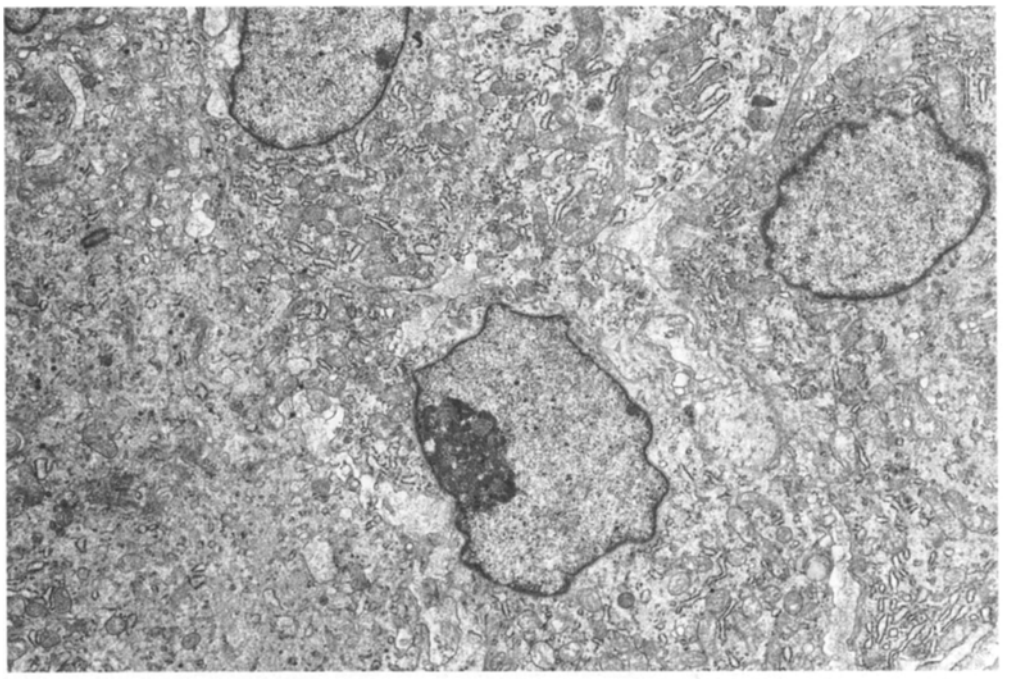

Fig. 8 Panoramic view that demonstrated irregular cells with interdigitating processes and scarce matrix (ME ×5500)

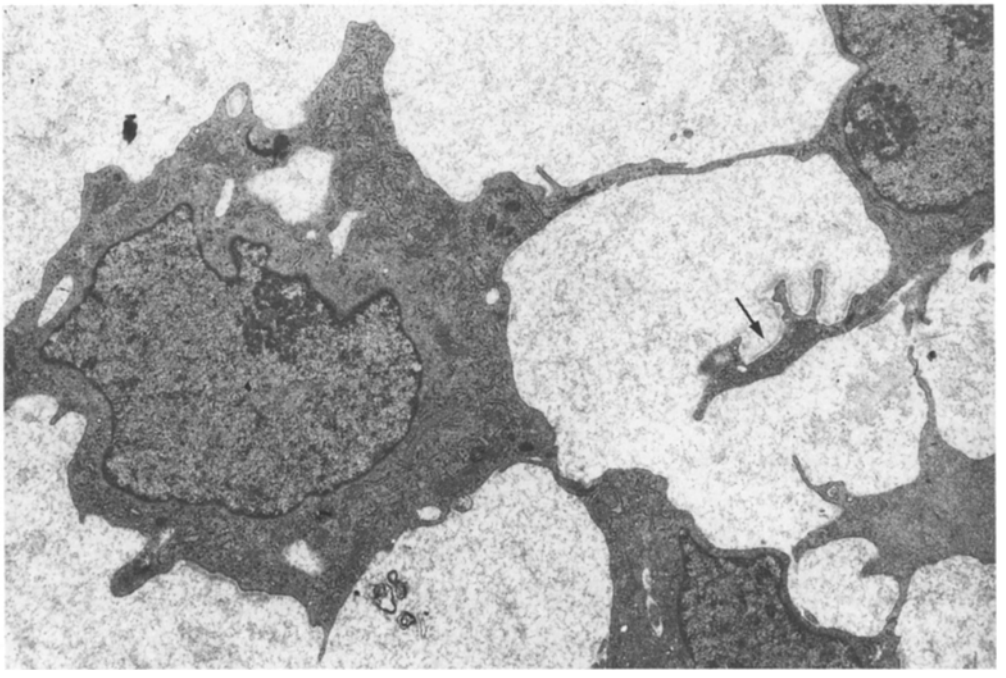

Fig. 9 Stellate cells in a loose matrix. Discontinuous external lamina can be observed (*arrow*) (ME ×9500)

nant schwannoma but whose histological and even ultrastructural features are ambiguous.

In 1984 Herrera et al. [8] described a tumour characterized by neurosecretory granules, for which they assumed a neural plexus origin and therefore termed plexomas or plexosarcoma, depending on its aggressiveness. This type of neoplasm has more recently come to be designated as GANT [9, 25]. McLeod and Tsokos [14] and Pinedo-Moraleda et al. [17] later described other cases with identical characteristics. After Herrera's initial description most authors have agreed that these tumours derive from myenteric or autonomic plexuses.

Distinction of GAN tumours from other GIST usually requires ultrastructural study [1]. A search of our Ultrastructural Section files showed 24 GIST whose light microscopic study has yielded ambiguous results; in all of them the light microscopical diagnosis of smooth muscle differentiation was suggested; from these, seven cases showed those features on electron microscopic study that fulfilled the ultrastructural diagnostic criteria of GAN tumours [8]. All seven cases displayed a star-shaped cell

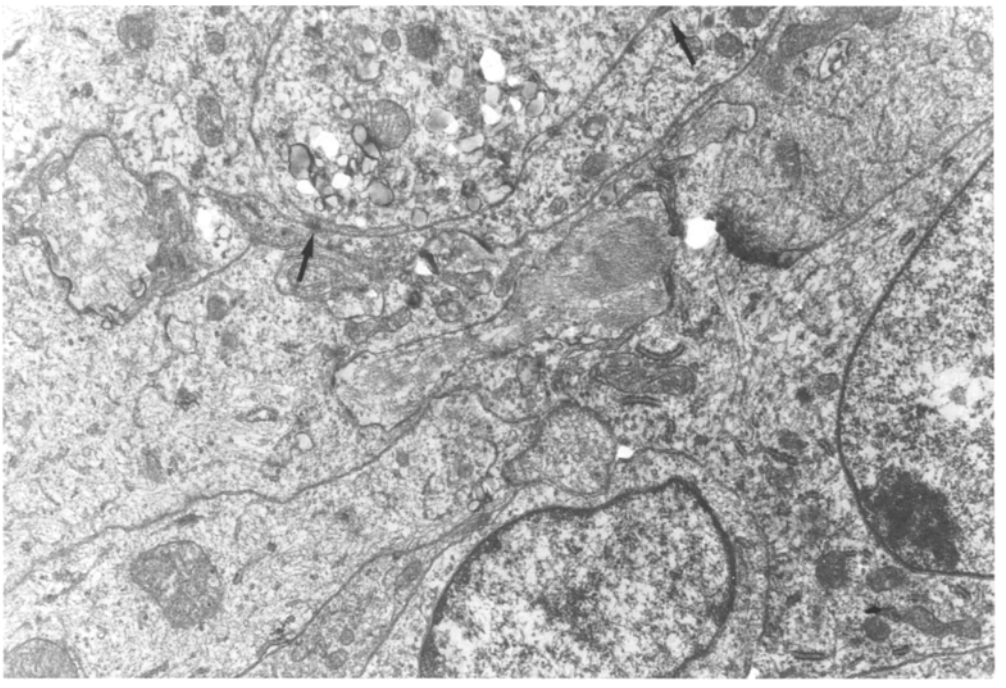

Fig. 10 Multiple interdigitating processes with primitive junctions (*arrows*) (ME ×13500)

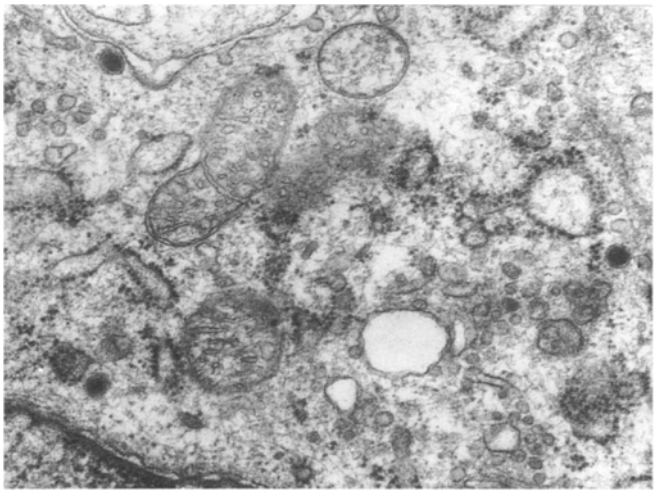

Fig. 11 Detail of the cytoplasm of a neoplastic cell with numerous dense core granules. Some pinocytotic vesicles can be observed (ME ×42000)

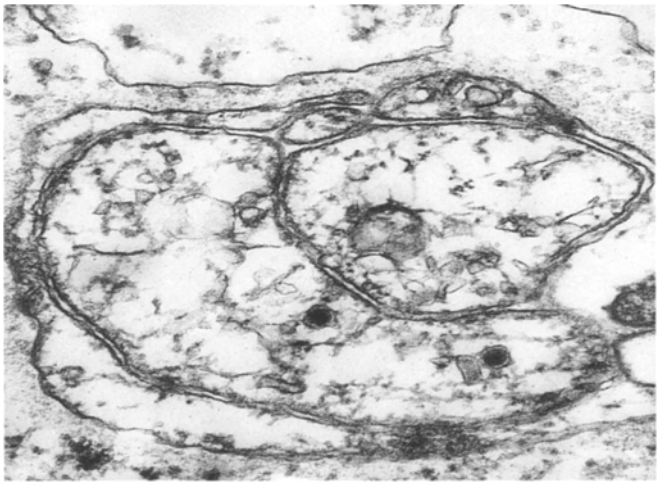

Fig. 12 Detail of peripheral prolongation with dense core granules (ME ×40500)

pattern, with long prolongations intertwined with the neighboring elements. Cell surfaces were found to contain rudimentary cell junctions and occasional pinocytotic vesicles. In a compatible context, the key condition for identifying a tumour as GANT is the presence of more than occasional secretory granules in the neoplastic cells, and necessarily in the periphery or in the prolongations of the cytoplasm. Synaptic vesicles were not found in any of our cases, but this feature is not indispensable for identifying neural differentiation in a tumour [13]. One case, the subject of a previously published report, demonstrated discontinuous external lamina, which is also compatible with neural tumours [17]. GAN tumours usually lack smooth muscle features [8].

The immunophenotype of GIST remains controversial issue. Typing of the intermediate filaments and other structural elements within GIST by immunohistochemistry has been used increasingly, with variable results [1, 4, 20]. In a recent report Newman et al. [16] have examined the immunophenotype of 60 cases of GIST and identified 36% of the cases as showing neural differentiation; 31% appeared smooth muscular, 20% manifested bidirectional differentiation, and 13% were negative for the markers used. In this report nine of 60 cases reacted with NSE and/or PGP 9.5. They stated that histological appearances do not reliably reflect immunophenotype. More recently, Franquemont and Frierson [5] studied 46 cases of GIST and found that the majority showed im-

Table 3 Immunohistochemical results of 23 gastrointestinal stromal tumours including 7 GANT (EM Diagnosis, Electron microscopic Diagnosis; *GIST, NOS,* Gastrointestinal stromal tumour, not otherwise specified; NSE, neuron specific enolase; (−), negative; (+), positive (±), focal)

EM Diagnosis	Vimentin	NSE	Synaptophysin	Chromogranin A	S-100 Protein	α-smooth muscle actin	Desmin
Leiomyosarcoma	+	−	−	−	−	+	+
Leiomyosarcoma	+	−	−	−	−	+	+
Leiomyosarcoma	+	−	−	−	−	+	+
Leiomyosarcoma	+	+	−	−	−	+	+
Leiomyosarcoma	+	−	−	−	−	+	+
Leiomyosarcoma	+	+	−	−	−	+	+
Leiomyosarcoma	+	−	−	−	−	+	−
Leiomyosarcoma	+	+	−	−	−	+	−
Leiomyosarcoma	+	−	−	−	−	−	+
Leiomyosarcoma	+	−	−	−	−	−	−
Leiomyoblastoma	+	+	−	−	−	+	−
GIST, NOS	+	−	−	−	−	−	−
GIST, NOS	+	+	−	−	−	+	−
GIST, NOS	+	−	−	−	−	−	−
GIST, NOS	+	−	−	−	+	−	−
GIST, NOS	+	−	−	−	−	+	−
GANT	+	±	±	−	−	−	−
GANT	+	±	+	+	±	−	−
GANT	+	±	±	−	−	−	−
GANT	+	±	±	±	−	−	−
GANT	+	−	+	±	−	−	−
GANT	+	±	±	−	−	−	−
GANT	+	±	−	−	±	−	−

munophenotypic evidence of smooth muscle differentiation, with 85% of the tumours reacting with α-smooth muscle actin or desmin. Most reacted with at least two antibodies. All of the cases were negative for synaptophysin and only one showed reactivity for chromogranin (α-smooth muscle actin and vimentin positive). Ultrastructural studies were not available in either report.

S-100 protein positivity must be evaluated carefully, particularly if it is focal and scattered, and there are no ultrastructural findings that support a neural origin. It has also been detected in a variety of nonneural neoplasms especially with the use of monoclonal antibodies [24]. It also might represent entrapped nonneoplastic neural tissue [5].

Few GANTs have been studied immunohistochemically, but most have been immunoreactive for vimentin, NSE, and synaptophysin, with variable results for S-100 protein and chromogranin. Desmin and α-smooth muscle actin were always negative [11]. Our immunohistochemical findings were similar, and the remaining GIST of our series failed to show such phenotype. In our opinion, the negativity for muscle markers and the presence of NSE, synaptophysin or chromogranin A strongly suggest the diagnosis of GANT, specially if supported by the appropriate ultrastructural findings. In the majority of our cases the ultrastructural and immunohistochemical findings allow us the distinction between GAN and smooth muscle tumours; a source of difficulty is to differentiate between them and Schwann cell tumours. The presence of electron dense granules in dendritic prolongations indicates neuroaxonal differentiation [27]. Our cases do not display features which are considered to be hallmarks of schwannian differentiation such as continuous basal lamina, mesoaxonal structures and long-spaced collagen. However, Yagigashi et al. [27] describe a distinctive neurogenic tumour of the stomach composed of neuroaxonal and schwannian elements suggesting they probably arise from a combination of Schwann cells and ganglion cells. These tumours display mesoaxonal structures containing microtubules, microfilaments and small electron-dense granules (some of them considered to be lysosomes). Min [15] also describe other stromal tumours with "skeinoid fibers"-considered to be a neurogenic marker and which were also found in a plexosarcoma described by the same author. Worth noting among the cases reported here is the fortuitous discovery of a GAN tumour in a patient suffering from von Recklinghausen's disease. Gastrointestinal involvement in von Recklinghausen's disease is well recognized in 11 to 25% from cases [21]. Classically, it takes the form of various stromal tumours [19] including diffuse ganglioneuromatosis [22]. Some authors have suggested these tumours derive from the Auerbach's plexus [26] given their similar ultrastructural characteristics to those described for GANT.

Assuming the existence of GIST with both schwannian and neuroaxonal differentiation, we believe the demonstration of cytoplasmic prolongations with more than occasional dense core granules along with the positivity for NSE, synaptophysin and chromogranin A, are distinctive of derivation from autonomic nerve and serve to distinguish these neoplasms from tumours of smooth-muscle and Schwann cell origin.

Five cases from our series failed to show evidence of smooth-muscle or Schwann cell derivation, and no con-

clusive diagnosis was raised on immunohistochemical grounds, although two cases were α-smooth muscle actin-positive. Possibly they may represent undifferentiated mesenchymal cells, but the possibility of a Schwann cell origin must be kept in mind, since the observation that malignant peripheral nerve sheath tumours frequently show non-specific ultrastructural findings [3, 6].

Thus far 24 cases of GANT have been reported [11], and this study describes seven additional cases with similar miscrocopic, immunohistochemical and ultrastructural characteristics those previously observed.

The disorder is more common in adults over the age of 40 and in males. The usual tumour site is stomach, small intestine and retroperitoneum [8, 9, 11,13, 25] and thus far no cases have been described in the oesophagus, duodenum, appendix or large intestine. The reported tumour sizes vary from 5 to 20 cm and, in general, the larger tumour masses are predominantly cystic and located in the mesentery and retroperitoneum of the small intestine [11], as with two of our cases. Hepatic metastases have also been described in other published cases [9, 14, 25].

Light microscopic study always reveals a spindle and epithelioid cell pattern, and clear vacuolated cell cytoplasm in a richly vascularized stroma with abundant areas of necrosis and/or haemorrhage in many cases, which Lauwers et al. [11] regards as more frequent than in other stromal tumours. One of the cases reported in this study displayed significant pleomorphism, with a large number of bizarre-looking multinucleated cells in part of the tumour. We detected a small number of mitoses, whereas substantial mitotic activity has been reported in other series [11]. Most authors agree that light microscopy is not sufficient for establishing a GANT diagnosis [14, 25] and distinguishing these tumours from other stromal tumours, and from smooth-muscle tumours in particular.

The biological behaviour of GAN tumours is uncertain. Lauwers et al. [11] reported that 58% of their cases developed metastases or recurrence in a mean follow-up time of 26 months. Herrera et al. [9] reported high mortality (4/6), with metastases and recurrence. In a recent report Lerma et al. [12] analysed the prognostic value of the pathological features in 33 patients with GIST and stated that local invasion, pathological grade, mitotic index, and necrosis are related to poor prognosis, whereas cellularity and nuclear pleomorphism are considered less important by other authors [7]. Herrera et al. [9] and Lauwers et al. [11] have used chemotherapy after surgery with variable results. All our patients received radical surgical treatment only and remain free of tumour after mean follow-up of 14 months (range from 6 months to 10 years). This result is probably related to the short follow-up.

The histogenesis of GAN tumours appears to be from submucosal and muscular nervous plexuses, supported by ultrastructural findings and, in part, by the immunohistochemical results. Lauwers [11] has suggested that the GAN tumour can be included as a unique subgroup of tumours arising from tissue with neural crest lineage, given that the neurons and satellite cells of the enteric plexuses are derived from migrating neural crest cells.

There are some clinical associations with GANT. We have commented on the association with von Recklinghausen's disease elsewhere in the text; another one is with the so-called Carney's triad [23], originally described by Carney in 1977 [2] as a combination of non-epithelial gastric tumours, pulmonary chondromas and extra-adrenal paragangliomas.

Finally, our approach does not allow us to draw conclusions about the frequency of GANT among GIST, as we have only studied GIST cases in which material for ultrastructural study was available and light microscopic study was ambiguous. We may nonetheless speculate that GAN tumours are probably more common than previously thought. Adequate ultrastructural and immunohistochemical study of all GIST with uncertain light microscopic findings is needed for conclusive diagnosis.

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