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A clinical and immunohistochemical study of gastrointestinal stromal tumours

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Abstract

Aim: To study the clinical features, histology and immunohistochemical properties of gastrointestinal stromal tumours (GISTs); and establish any parameters that can help prognosticate the malignant potential. *Methods:* Twenty-six patients with GISTs who were seen in Sultanah Aminah Hospital Johor, Malaysia from 1999 to 2003 were selected for study. Patient, clinical characteristics and outcome based on surgical records were analysed. Tumour variables (tumour size, cellularity, mitotic count, necrosis and haemorrhage) were compared between very low to low risk groups and intermediate to high risk groups. The immunohistochemical properties of GISTs were also studied. *Results:* Patients with GISTs presented mainly with pain, palpable mass or gastrointestinal tract bleeding. The tumours were seen in stomach (50%) followed by small intestine (38.5%) and rectum (11.5%). In the period of study, six patients had metastasis, mainly in the liver or peritoneum. Immunoreactivity for CD117, CD34, vimentin, S100, neuron specific enolase, alpha-smooth-muscle-actin and desmin were observed in 100%, 76.9%, 61.5%, 46.1%, 80.8%, 11.5% and 0% of tumours respectively. The behaviour of GISTs was largely dependent on tumour size and number of mitosis. Necrosis and haemorrhage were seen in tumours with high risk potential.

Keywords: Gastrointestinal stromal tumour, CD117, immunohistochemistry, high risk potential.

INTRODUCTION

Although relatively rare, gastrointestinal stromal tumour (GIST) is the most common mesenchymal tumour of the gastrointestinal tract. Until recently, diagnosis and prognosis of this tumour was often complex and confusing. Of late, recent advances and studies have been able to bring some understanding and uniformity in diagnosing this tumour. It is now thought that gastrointestinal stromal tumours differentiated along the lines of interstitial cells of Cajal, the pacemaker cells of the gut. Along with this understanding, the majority of GISTs was found to be reactive to the immunohistochemical marker, CD117 (c-kit), a growth factor receptor with tyrosine kinase activity. Following a GIST workshop in April 2001 held at the National Institutes of Health (NIH)[1], a consensus was reached to define GISTs as mesenchymal neoplasms in the gastrointestinal tract displaying CD117 (c-kit) immunopositivity with very rare exceptions[2]. Immunohistochemistry is now mandatory to make the diagnosis of GIST and differentiate it from other mesenchymal tumours of the gastrointestinal tract.

We retrospectively reviewed 26 GISTs to study their clinical features, histology and immunohistochemical properties. The study also aimed to correlate any parameters that can help indicate the malignant potential of GISTs.

MATERIALS AND METHODS

Twenty-six cases of gastrointestinal stromal tumours were selected from 33 cases of mesenchymal tumours of the gastrointestinal tract from the files of the Department of Pathology, Sultanah Aminah Hospital Johor, Malaysia in the 5-year period from 1999 to 2003, based on immunohistochemical positivity for CD117 (c-kit) and histological appearance of the tumours. Seven cases excluded in the study were either leiomyoma or leiomyosarcoma of the gastrointestinal tract which were CD 117-negative.

The GISTs specimens were from patients who had excision of the tumour with or without clear surgical margins. Clinical information, including demographic data, clinical presentation and outcome, were obtained from the surgical records of the patients. Four patients were lost to follow-up. The other patients were followed-up for between 6.5 months and 3 years 5 months

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with a mean of 1 year 5 months.

The tumour size was measured as the largest diameter (in centimetre) of the tumour. Tumour slides stained with Haematoxylin and eosin (H&E) from 26 GISTs were reviewed by one of the authors (JJK). For each tumour, a total of five groups of 10 high-power fields (HPF) were observed to determine the mitotic activity and tumour cellularity. The number of mitosis per 50 HPF was recorded. The cellularity of the tumour was described as low, intermediate and high while cellular atypia was noted as mild, moderate or severe. In each case, tumour necrosis and tumour haemorrhage were recorded as present if moderate to severe amounts were seen or minimal if mild or none were noted.

A new approach was proposed by Fletcher *et al*[2] where gastrointestinal stromal tumours were ranked into various risk categories based on tumour size and mitotic count. In this study, the tumours were grouped into 2 main groups with this proposed risk assessment, namely, Group 1: very low to low risk and Group 2: intermediate to high risk (Table 1). Age, sex and tumour variables: size, location, cellularity, mitotic count, presence of necrosis and haemorrhage; were compared between Group 1 (very low to low risk group) and Group 2 (intermediate to high risk group). They were statistically evaluated using the unpaired t-test or chi square. $P < 0.05$ was considered statistically significant.

The immunohistochemical properties of GISTs were studied. Antibodies to the following antigens were used: CD117 (c-kit proto-oncogene product, polyclonal, 1:50, DAKO), CD34 (QBEnd-10, monoclonal, 1:50, DAKO), vimentin (Vim 3B4, monoclonal, prediluted, DAKO), S100 protein (polyclonal, prediluted, DAKO), neuron specific enolase (BBS/NC/VI-H14, monoclonal, prediluted, DAKO), alpha-smooth-muscle-actin (1A4, monoclonal, prediluted) and desmin (DE-R-11, monoclonal, prediluted, DAKO). Immunohistochemistry was performed with EnVision staining technique followed by incubation with 3, 3'-diamnobenzidine chromogen solution and counterstained with haematoxylin. Appropriate positive and negative controls were used.

RESULTS

Patient and clinical characteristics

Of the 26 cases of GISTs, there were 13 females (50%) and 13 males (50%). The age range was 26 years to 86.4 years with a mean age of 58.3 years. Age had a unimodal distribution, with a median of 60.9 years (Fig.1). The patients were predominantly Malays (84.6%) with only 15.4 % Chinese. There were no records of Indian patients with this tumour in our study.

The most common presentation was pain (14 patients), with palpable masses (6 cases) and or bleeding (1 case). Eleven other patients had gastrointestinal bleeding with or without palpable masses (Fig.2). In addition, other constitutional symptoms such as loss of appetite, weight loss, fever, dyspepsia and lethargy were present in four patients. Only 7 patients (26.9%) were clinically suspected to have GIST preoperatively based on gross endoscopic findings without histological confirmation. All 7 cases had tumours in the stomach.

Six patients were overtly metastatic at presentation or developed metastasis during the period of follow-up. Five patients had liver and/or peritoneal metastasis, one of whom also had bone and lymph nodes metastasis and succumbed to the disease fourteen months later. Another

TABLE 1. Categorising GISTs based on proposed risk assessment[2].

Major groups with risk levels		Size (cm)	Mitotic count (per 50 HPF†)
GROUP 1:	Very low to low risk	⩽ 5	< 5
GROUP 2:	Intermediate risk	< 5	6-10
		5-10	< 5
	High risk	>5	>5
		>10	Any number
		Any size	>10

† high power field

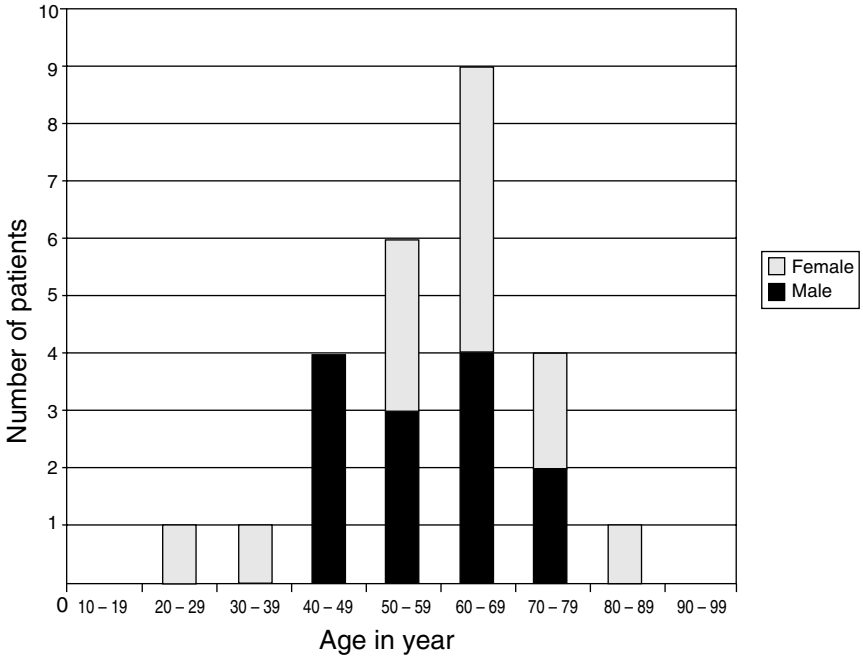

FIG. 1: Age and sex distribution of patients with GIST (n=26)

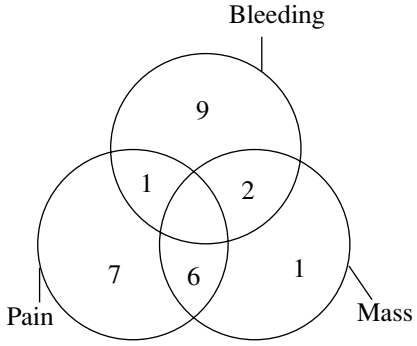

FIG. 2: Various presentations seen in patients with GISTs (n=26)

patient had metastatic lymph node involvement (Fig.3) at the time of surgery. Altogether two patients died in the period of study: one from disseminated disease as mentioned and another from septicaemia due to perforation of tumour.

Tumour features

Thirteen tumours were located in the stomach (50.0%), 10 in the small intestine (38.5%) including 2 in the Meckel's diverticulum, and 3 in the rectum (11.5%). There were no tumours in the oesophagus in this study. The size of tumour ranged between 1.2 and 21.5 cm, with a mean of 8.4 cm.

Microscopically, the tumours were either purely spindle-cell tumours, a mixture of spindle cell and epithelioid cell tumours or predominantly epithelioid tumours. The spindle cells formed interlacing bundles, whorls or storiform pattern with varying amounts of interstitial collagen. The cells appeared elongated or spindle-shaped with eosinophilic cytoplasm and blunt-ended nuclei (Fig.4). Occasional nuclear palisading, reminiscent of nerve sheath differentiation were seen (Fig.5). Epithelioid components of GISTs consisted of sheets of round to polygonal cells with eosinophilic or clear cytoplasm (Fig.6).

The majority of the tumours (96.1%) showed mild to moderate cellular atypia. Only one case showed severe cellular atypia (Fig.7). Four cases of GISTs had low cellularity while 22 cases had moderate to high cellularity. In 5 GISTs mitotic figures were not observed. All these 5 cases had tumour size less than 5 cm. The tumours in Group 1 (very low to low risk group) had mitotic rate ranging from 0-4 per 50HPF, mean of 1.8 per 50HPF while those in Group 2 (intermediate to high risk) had a range of 9-60 per 50HPF, mean of 19.6 per 50HPF. There were 9 tumours with moderate to severe necrosis and 8 with moderate to severe haemorrhage. These were in the intermediate to high risk group.

Table 2 compared various parameters between the two groups. There were no significant

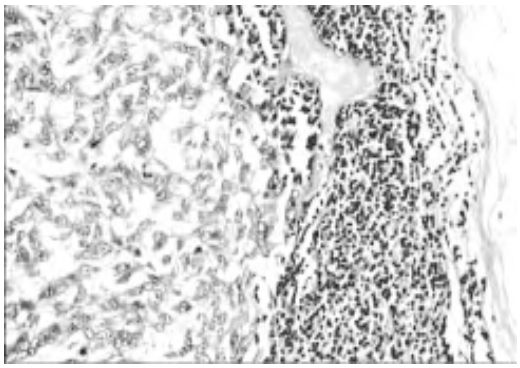

FIG. 3: Metastatic GIST in lymph node. This is a very rare occurrence. (H&E stain, original magnification × 80).

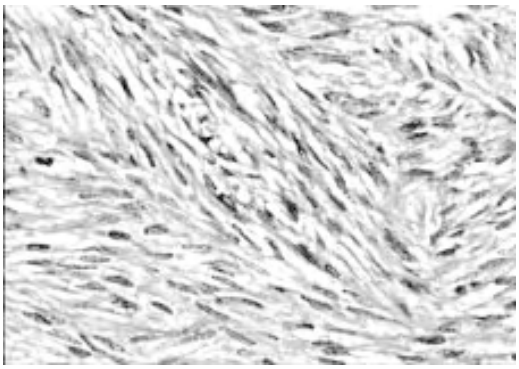

FIG. 4: Spindle cell GIST (H&E stain: original magnification × 80).

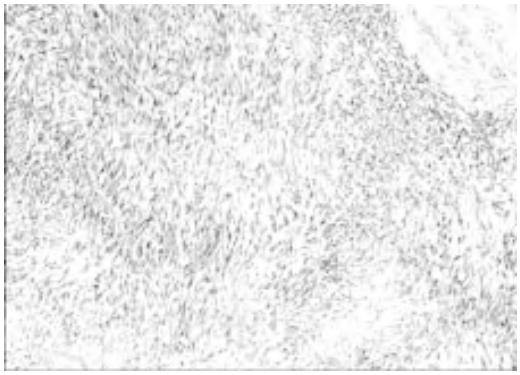

FIG. 5: GISTs with serpiginous neural palisades (H&E stain: original magnification × 16).

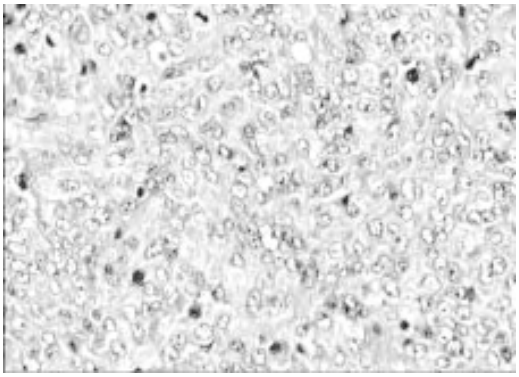

FIG. 6: Epithelioid GIST showing sheets of round to polygonal cells with minimal nuclear pleomorphism (H&E stain: original magnification × 80).

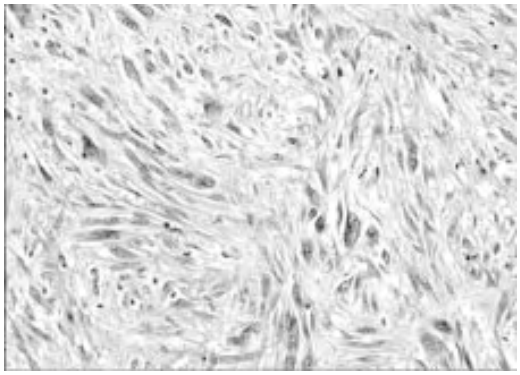

FIG. 7: Severe nuclear atypia was rare but was seen in a case of gastric GIST (H&E stain: original magnification × 40).

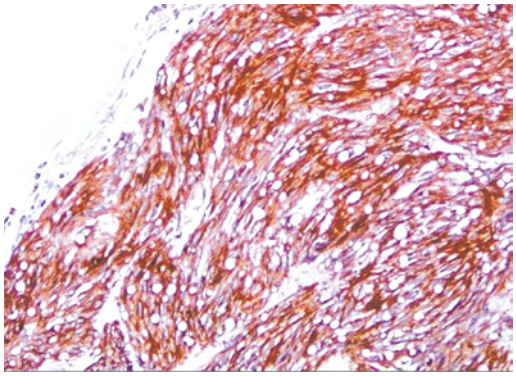

FIG. 8: Positive expression for c-kit (CD117) in tumour cells of GIST (immunohistochemical staining: Envision system, original magnification × 80).

TABLE 2. Clinical and histomorphological features of tumour and follow-up results

	Group 1 (n=9) (very low to low risk group)	Group 2 (n=17) (intermediate to high risk group)	*P* value
Age (years)	58.6	58.1	NS (*P*=0.9318)
Male: female	4:5	9:8	NS (*P*=1.000)
Mean tumour size (cm)	3.7	10.9	*P*=0.0015
Location (S:SI:LI)‡	5:4:0	8:6:3	S vs others, NS (*P*=1.000) SI vs others, NS(*P*=0.974)
Cellularity (low:moderate+high)	3:6	1:16	NS (*P*=0.203)
Number of Mitosis (per 50 HPF)	1.8	19.6	*P*=0.0029
Necrosis (moderate to severe)	0/9	9/17	*P*=0.024
Haemorrhage (moderate to severe)	0/9	8/17	*P*=0.044
Presence of metastasis	0/9	6/17	-
Alive:dead§	7:0	15:2	-

NS= not significant, *P*>0.05
‡S=stomach, SI=small intestine, LI=large intestine
§Follow-up results were only available for 22 cases.

TABLE 3. Immunohistochemical results in 26 cases of GIST

	Number of cases (%)		
Antigen (type)	Stomach (n=13)	Non-stomach (n=13)	Total (n=26)
CD117 (c-kit)	13 (100%)	13 (100%)	26 (100%)
CD34	12 (92.3%)	8 (61.5%)	20 (76.9%)
Vimentin	8 (61.5%)	8 (61.5%)	16 (61.5%)
S100	3 (23.1%)	9 (69.2%)	12 (46.1%)
Neuron-specific enolase	12 (92.3%)	9 (69.2%)	21 (80.8%)
Alpha-smooth-muscle-actin	0 (0%)	3 (23.1%)	3 (11.5%)
Desmin	0 (0%)	0 (0%)	(0%)

differences between the two groups for age, sex, cellularity and site of the tumours. However, tumour size, number of mitosis, presence of necrosis and haemorrhage were found to be significantly different between the two main groups. Tumours with intermediate to high risk were larger, with higher mitotic rates and showed presence of significant necrosis and haemorrhage compared with tumours in the very low to low risk group. All 6 cases with metastasis including both deaths came from the intermediate to high risk group.

Table 3 summarised the immunohistochemical profiles of GISTs in the study. All the tumours showed diffusely positive expression for CD117 (Fig.8) while 20 (76.9%) also showed co-expression with CD34 (diffusely). Nearly all tumours (92.3%) in the stomach showed reactivity to CD34 while only 61.5% of tumours in small and large intestine showed reactivity to CD34. 11.5% of cases were reactive to α-SMA, all of which were small intestinal lesions. None of the stomach or rectal lesions stained for α-SMA.

Eighteen GISTs showed reactivity for neuron specific enolase (NSE) with another 3 showing focal positivity, making a total of 80.8% of tumours reactive for NSE. However, only 61.5% were reactive for vimentin and 46.1% for S100 protein. All tumours were negative for desmin.

DISCUSSION

Gastrointestinal stromal tumours, defined as mesenchymal tumours arising in the gastrointestinal tract with immunoreactivity to CD117 (c-kit), occurred predominantly in the older age group with the median age varying between 55 and 65 years. In most series GISTs showed an equal sex incidence. The most common site was in the stomach (60% to 70%), followed by small intestine (25% to 35%), colon and rectum (5%) and oesophagus (<2%)[3]. The main clinical presentations were abdominal pain, gastrointestinal bleeding and palpable mass. Other constitutional symptoms included weight loss, anorexia and discomfort.

GISTs could not always be confidently categorised into benign and malignant neoplasms. Frequently, it was advised that the term "benign" be left out in diagnosing GISTs. Instead, as suggested by Franquemont[4] and in a recent consensus document[2] various parameters were used to rank the risk levels of GISTs, rather than dividing the tumours into benign and malignant lesions. Various clinical and histological parameters have been studied to predict the biological behaviour of GISTs, but with varying results.

Age and sex had not been found to show any correlation with the malignant potential of this tumour in most studies. However, some studies showed that tumours in the oesophagus had a benign behaviour while those in the small intestine showed recurrent and metastatic behaviour[5]. Our study had not been able to dispute or support this findings. This was probably due to the small sample size of tumours in each location and also that there were no oesophageal GISTs in our study. However, in our study, tumour size was found to be significantly correlated to the risk level of the tumour. In a recent study of 171 patients with GISTs, Hasegawa *et al*[6] found that tumours more than 10 cm were associated with a poor outcome. DeMatteo *et al*[7] showed that the relative risk became 4.4 (confidence interval 2.0-9.8) when the tumour size was >10 cm.

Amongst the histological criteria studied, mitotic index has been found to be the most reliable predictor of malignant potential. In the past, most literature used a cut-off mitotic index of >5/50 HPF for distinguishing benign and malignant cases. Our results support the contention that mitotic count was predictive of malignant potential of GISTs. It also makes sense that mitotic count is used to assess the risk of the tumour as it is a convenient method that is both practical and reproducible amongst pathologists.

The presence of haemorrhage and necrosis may be unreliable predictors of malignancy but has been shown in a few studies[5] including our study to be generally associated with higher risk level. On the other hand, severe cellular atypia was not a feature of malignant GISTs.

Immunostaining for CD 117 is not specific for GISTs. It has been noted to be expressed in a variety of normal tissues, acute myeloid leukaemia, mast cell disease and various types of carcinoma (such as breast, lung, ovary, thyroid and endometrium)[8-11]. CD117, however, is a sensitive marker for GISTs and combined with the morphological appearance of the tumour, has been said to be mandatory for making a diagnosis[2]. However, recent research[12-13] had demonstrated a small group of GIST that were CD117 negative but were positive for platelet derived growth factor receptor α (PDGFRα). PDGFRα is another tyrosine kinase receptor with activity similar to CD117. This finding

may explain for the small group of tumours in the exceptional categories with CD117 negativity, discussed in the GIST workshop[1] and presented in the consensus approach of GIST[2]. This may mean adding PDGFRα as an additional stain to CD117 for making a confirmatory diagnosis of GIST.

In our study, CD117 was diffusely positive in gastrointestinal stromal tumours and seen in all the cases. However, CD34 was not seen in all cases of GISTs. In our study, the percentage of CD34 positivity was high in stomach lesions but low in non-stomach lesions; mainly small intestinal GISTs. In contrast, α-SMA reactivity was seen only in small intestinal lesions and not found in any of the gastric or rectal lesions. This observation that α-SMA showed a reciprocal relationship with CD34 expression was noted in other series[6,11] as well. In addition, our study showed S100 reactivity was present more frequently in small intestinal lesions. On the other hand, NSE reactivity was fairly high in all the lesions, especially the lesions located in the stomach. None of the cases were desmin positive but almost 2/3 of all the cases were vimentin positive. These findings concurred with many series studying the immunohistochemical properties of GISTs. Combined immunostaining for CD117, CD34, α-SMA, desmin and S100 for mesenchymal tumours in the gastrointestinal tract, thus would be helpful to differentiate GISTs from other tumours viz. leiomyoma, leiomyosarcoma, inflammatory fibroid polyps or gastrointestinal (GI) schwannomas. True leiomyomas were negative for CD117 and CD34 but react to desmin and α-SMA. GI schwannomas were negative for CD117 but positive for S100. These were in agreement with a series of mesenchymal tumours of the GI tract studied by Miettinen *et al*[11] where the immunohistochemical features of GISTs were compared with tumours that entered into the differential diagnoses of GIST.

Malignant GISTs have a high risk for diffuse peritoneal spread and liver metastasis. These were the two most common modes of dissemination found in most series. Five of six patients who had metastasis in our study showed either peritoneal or hepatic metastasis. Extra-abdominal spread (to bone or lung) were relatively rare and lymph nodes metastasis were much more infrequent.

In summary, our study confirmed that the behaviour of GISTs was largely dependent on tumour size and number of mitosis. Necrosis and haemorrhage were frequently seen in tumours with high risk potential. GISTs were stromal tumours that expressed CD117 but also had a high co-expression for CD34, vimentin, NSE and S100. There was inverse expression of CD34 and α-SMA while desmin was not expressed in these tumours.

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