



Original Research Article

Clinicopathological and immunohistochemical study of gastrointestinal stromal tumours: A series of 46 cases from a single institution

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ABSTRACT

Introduction: Mesenchymal tumours of gastrointestinal tract are a heterogeneous group of non-epithelial tumours of variable mesenchymal cell histogenesis. Gastrointestinal stromal tumour (GIST) is the most common primary mesenchymal tumour of the gastrointestinal tract and spans a clinical spectrum from benign to malignant. This study evaluated the clinicopathological morphological and immunohistochemical features of GIST of the intestinal tract.

Materials and Methods: All evaluable cases of mesenchymal tumors of the gastrointestinal tract were studied from January 2007 to December 2012. Tissue microarray was constructed and Immunohistochemical expression of CD 117, CD 34, DOG1, SMA, S100, Ki 67 were studied.

Results: Gastrointestinal stromal tumours were the most common mesenchymal tumours of the gastrointestinal tract accounting to 77.96%. The median age of presentation was 51 years with near equal sex preponderance with early clinical presentation. The gastric tumours were of significantly bigger size with increased expression of CD 34. Majority of tumors were of bigger size (>10 cm, 47%) thereby increased number of tumours in high risk category (76%). Spindle cell pattern was the most common morphology (69.5%). Epithelioid histology was significantly associated with pleomorphism and atypia (p value – 0.029). Inclusion of anatomic site into the risk category will help in better stratification. High risk tumours showed significant areas of necrosis when compared with low risk tumours (p value- 0.0156). Tumours of older age group (>40 years) showed significant mitotic activity (p value 0.04). DOG1 is a very sensitive (97.8%) marker for GIST. A wide immunopanel with emphasis on DOG 1 positivity for precise diagnosis of GIST and classification of mesenchymal tumors of the GI tract.

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1. Introduction

Mesenchymal tumours of gastrointestinal tract are a heterogeneous group of non-epithelial tumours of variable mesenchymal cell histogenesis. Gastrointestinal stromal tumour (GIST) is the most common primary mesenchymal tumour of the gastrointestinal tract and spans a clinical spectrum from benign to malignant. GIST are immunohistochemically positive for CD 117 and phenotypically paralleling Cajal – cell differentiation, the mesenchymal derived intestinal pacemaker cells.^{1–4} GIST demonstrates considerable morphological overlap and a diagnosis of

GIST is made by taking into account location of tumour, characteristic spindle /epithelioid morphology along with immunohistochemical positivity to CD117. Pathologically they are characterized by the presence of activating mutations affecting the c- *KIT* or the *PDGFRA* gene, and the presence of pathologically activated *KIT* and *PDGFRA* receptors leads to cell proliferation involving different downstream pathways.^{5–8}

Demonstration of a *KIT* or *PDGFRA* mutation is being used increasingly to supplement morphological and immunohistochemical assessment for diagnosing GIST, and has indeed been advocated as the gold standard for making a diagnosis of GIST. The prediction of the behaviour of GISTs is difficult and there are no absolute criteria

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for ascertaining the malignant potential. Many potential prognostic factors have been assessed in several studies, including tumour size, degree of cellularity, cellular atypia, mitotic count, cell type, IHC profile, location and the presence of c- *KIT* mutations. Tumour size >5 cm, mitotic rate >5/50 HPF and site (gastric versus Small intestine) have been found by several studies to be the most reliable predictors of malignancy.^{9,10} The molecular analysis which are being used increasingly nowadays still have limitations and are currently more time-consuming and expensive than immunohistochemistry. So immunohistochemistry still plays a crucial role in distinguishing GIST from its histological mimics in resource poor settings. The aim of this study was to classify mesenchymal tumors of the gastrointestinal tract based on histopathological and immunohistochemical features, to identify GIST with emphasis on the expression of novel marker DOG 1 (Discovered On GIST), and to evaluate the clinical, histopathological and immunohistochemical parameters in tumors classified as GISTs.

2. Materials and Methods

All evaluable cases of mesenchymal tumors of the gastrointestinal tract were retrieved from the database files of the Department of Pathology from January 2007 to December 2012. The clinical and pathological data of the patients were retrieved from the archives. Clinical data studied included patient age, gender, clinical presentation, investigations (laboratory, radiological, and endoscopic), operative details, tumor infiltration, recurrence and metastasis. The pathological data included tumor location, gross appearance- tumor size (maximum diameter in cm), consistency, ulcerations, infiltration into adjacent areas, areas of necrosis, haemorrhage, cystic change, and metastasis.

The formalin-fixed paraffin embedded tissue blocks and tissue sections of the 61 cases of mesenchymal tumors were retrieved and reviewed. Additional sections were cut and stained with hematoxylin and eosin, when required if there were storage artefacts.

The morphological details of the tumour were reviewed from the original H and E slides and various histopathological parameters such as cell pattern, pleomorphism, atypia, necrosis, myxoid change, hyalinization, lymphoid infiltration at periphery of tumour, calcification and cystic degeneration were studied. Mitotic rate was counted (expressed as the number of mitotic figures/ 50 high-power fields (HPFs) in the most mitotic area, using a x40 objective and a x10 ocular; field size 0.25mm²). Necrotic, haemorrhagic and paucicellular areas were avoided for mitosis counting purposes. Nuclear atypia was defined by nuclear enlargement, and pleomorphism. Necrosis was documented as coagulative, when ghosts of tumor cells were identified with inconspicuous cell membranes.

The risk stratification of GIST cases were done. The NIH Consensus classification, which proposed four risk categories (very low, low, intermediate and high), was adopted rapidly and widely in clinical practice. However, as was acknowledged in the NIH Consensus document itself, there were already emerging data suggesting that anatomical location of a GIST also had significant and independent prognostic relevance.¹¹ Subsequently, in 2006, Miettinen and Lasota² proposed their latest version of a modified classification (referred to commonly as the AFIP classification) with substratification according to anatomical location into six categories (benign, Very low malignant potential, Low to moderate malignant potential, Malignant potential and Uncertain potential). The cases were stratified according to both the classification systems and also compared.

Tissue Microarray (TMA): Representative areas were identified and marked on the corresponding tissue blocks after reviewing the original H& E sections of the tumour. Marked tissue was extracted from the donor block using Quick-Ray needle with 5mm tip. Two cores were taken per case. Tissue cores were delivered into corresponding holes of the recipient block. Recipient block was put in embedding mold with cutting section faced down and incubated at 60° C for 30 min. Section were cut at 4μ m thin and taken on to charged slides for H&E and for IHC studies.

Immunohistochemistry (IHC) was done by semi automated immunostainer. The paraffin sections of 4-5 micron thickness were subjected to immunohistochemistry by poly HRP polymer technique and all the antibodies were ready to use supplied by Biogenex. Sections were incubated at 37°C with primary antibody (CD 117, CD 34, DOG1, SMA, S100, Ki 67), followed by incubation with secondary antibody (superenhancer and poly HRP).

2.1. Statistical analysis

The comparison of groups for continuous variables and counts was done using Mann-Whitney U-test. The association among categorical variables was assessed using Pearson's Chi-square and Fisher's exact test. The correlation between the pairs of variables was tested using Pearson's bivariate analysis. The data were entered and analysed using SPSS v 17.

3. Results

A total of 61 cases of mesenchymal tumours of the gastrointestinal tract were diagnosed during the study period. Out of these 61 cases, 2 cases (3.27%) were excluded as tissue cores of these two cases were lost during TMA. Clinicopathological and immunohistochemical features of the remaining 59 cases were analyzed. In the present study we defined GIST based on location of tumour

with characteristic morphology along with CD117 and / or DOG 1 (Discovered on GIST1) immunohistochemical positivity, Figure 1. Out of the 59 cases of mesenchymal tumours studied, 41 cases were morphologically and immunohistochemically (CD117 and /or DOG1 positivity) categorized as GIST. Of the remaining 13 cases, morphology and IHC findings were consistent with schwannoma in 1 case (positive only for S100) Figure 2, leiomyoma in 2 cases (positive only for SMA) Figure 3, solitary fibrous tumour in one case (positive only for CD 34) Figure 4 and fibromatosis in 5 cases (negative for all markers but characteristic morphology and location) Figure 5. The remaining 5 cases were negative for all IHC markers but morphologically simulating GIST. There are subset of tumors that are typical for GIST both clinically and histologically lack apparent KIT expression, having low to undetectable KIT protein expression by immunohistochemistry and Western blot evaluations.^{12–14} With the identification of platelet-derived growth factor receptor alpha (*PDGFRA*) mutations as an alternative oncogenic mechanism in a small group of GISTs lacking *KIT* mutations, these 5 cases could represent the same, hence classified as GIST.

Only the 46 morphologically and immunohistochemically categorized cases of GIST were further analysed. The mean (range) age was 51 (10–78) years, predominantly seen in adults greater than 40 years (70%). There was near equal sex predilection. The presentation and symptoms varied according to site and tumour size. The most common presentation was pain abdomen which was seen in 28 cases (60.8%) followed by mass abdomen in 23 cases (50%). GI bleeding was seen in 18 cases (39.13%). The bleeding was most often insidious leading to anaemia in 3 (6.5%) patients and weakness / fatigue in 11 patients (23.9%).

Majority were located in stomach accounting to 45.6% followed by small intestine (43%), large intestine (8.6%).

3.1. Gross and microscopic features

The tumours were well demarcated, nodular, lobulated, often with bosselated appearance. On cut-section majority were solid, grey-pink to grey white with some showing haemorrhagic, necrotic areas. Large sized tumors showed cystic change. The consistency of the tumour varied but was typically firm in small and benign tumors, soft, fish-fleshy or gelatinous in malignant tumours and haemorrhagic in larger tumours. The tumor size varied from 0.3 cm to a maximum of 25 cm, tumor size < 2cm (4.3%), 2 to 5 cm (17%), 5 to 10cm (30%) and >10cm (47%). The tumours were also sub grouped based on size of tumour as <10 cm (24 cases, 52.14%) and ≥10 cm (22 cases, 47.8%).

3.2. Histopathological findings

The majority (n=32, 69.5%) of tumors were classified as spindle cell type (cellular, plump spindle cells, fascicles, moderate pleomorphism, varying mitosis) while 4 cases (8.6%) were classified as pure epithelioid type (cohesive polygonal cells, sheets, indistinct cell borders, pleomorphic), 7 cases (15.2%) as mixed- cell type and one case (2.1%) as sarcomatoid type (highly cellular, pleomorphic spindle cells, marked pleomorphism, high mitotic rate). The most common growth pattern was fascicular (n=39, 84.7%), followed by diffuse (11, 23.9%), hemangiopericytomatous (n=3, 6.5%), palisading (1, 2.1%) and pseudoangiomatous (n=1, 2.1%) patterns. Coagulative necrosis was present in 19 (56.2%) tumors, cellular atypia in 7 (15.2%), myxoid change in 11 (23.9%), areas of hyalinization in 4 (8.65%), lymphoid infiltration at periphery of tumor in 7 (15.2%) and calcification in 1 (2.1%). Figure 6

The presence of necrosis was not significantly associated with age, histologic pattern, site of tumour and mitotic rate. Occasionally, there was a lymphoid cuff at the periphery of the tumors similar to that more commonly seen in schwannoma.

Risk assessment for GIST cases was done by both the methods and compared. The assessment criteria proposed by Fletcher *et al* (size, mitotic index) and Miettinen *et al*.

(size, mitotic index and anatomic site) were done and cases grouped accordingly and both the methods were compared. Results are summarised in Table 1. Majority of tumours fell into high risk category mainly owing to bigger size of tumours (>10 cm).

The IHC results are summarised in Table 2.

The IHC results of *Kit* (CD117) positivity was detected in most the tumors 82.6% (38 out of 42 cases). Although *Kit* positivity was usually strong throughout the tumour, 4 tumors (8.6%) were negative. DOG 1 was found to be highly sensitive (97.8%) followed by CD117 (82.6%). Majority of gastric tumors showed significant CD 34 positivity when compared to small intestinal GIST. Table 3

Further analysis of subgroup of patients based on the age group was done (> and < 40 years). Fourteen patients were < 40 years (30%) and 32 were ≥ 40 years (70%). The mitotic rate was significantly higher in ≥40 year age group (p = 0.048) Table 4. Gastric GIST were of significantly larger size and they highly expressed CD34 positivity. Table 5

Based on mitotic index, tumours were divided as high and low mitotically active tumours. (<5/50 HPFs (16 cases, 34.7%) and ≥ 5 /50 HPFs (30 cases, 65.2%). The mitotic activity did not have any significance with respect to cell type, size of the tumor, site, presence or absence of necrosis, atypia or Ki67 index. The very low risk, low risk and intermediate risk groups were included as non-high risk group (11 cases, 23.9%), as compared to high risk group (35 cases, 76%). Tumors categorised

Table 1: Risk assessment of GIST cases

Risk Assessment of according Fletcher et al		Risk assessment according to Miettinen et al	
Very low risk	4.3% (2)	Benign	4% (2)
Low risk	8.6% (4)	Very low malignant potential	2% (1)
Intermediate risk	10.8% (5)	Low malignant potential	7% (3)
High risk	76% (35)	Low to moderate malignant potential	8.69 % (4)
		Malignant potential	78.2% (36)
		Uncertain	0

Table 2: Immunohistochemistry (IHC) results of GIST cases

CD 117	82.6% (38)
DOG1	97.8% (45)
CD34	67% (31)
SMA	47.8% (22)
S100	6.5% (3)

Table 3: Comparison of immunohistochemical positivity of markers with clinicopathological parameters

Variables	CD117 Pos	CD117 Neg	p value	DOG1 Pos	DOG1 Neg	p value	CD34 Pos	CD34 Neg	p value
n = 46	38	8		45	1		31	15	
Site									
Gastric	21	5	1.00	25	1	1.00	23	3	0.001
SI	17	3		20	0		8	12	
Risk category									
low	10	1	0.657	10	1	0.239	7	4	1.00
High	28	7		35	0		24	11	
Size									
<10 cm	22	2	0.127	23	1	1.00	15	9	0.328
>10 cm	16	6		22	0		16	6	
Mitotic rate									
<5/50 hpf	11	5	0.10	16	0	1.00	9	7	0.325
>5/50 hpf	27	3		29	1		22	8	
Cell type									
Spindle	27	5	1.00	31	1	1.00	21	11	1.00
Epithelioid	9	2		11	0		8	3	

Table 4: Comparison of clinicopathological features between age group less than and greater than 40 years

Variables	< 40 Years n = 14	> 40 Years n = 32	p value
Number of cases	14(30.4%)	32(69.5%)	1.00
Size>10cm	8(57.1%)	14(43.75%)	0.5252
High risk cases	10(71%)	25(78%)	0.713
Mitosis > 5/HPF	6(42%)	24(75%)	0.0482
Necrosis	5(35%)	14(43%)	1.00
Gastic	5(35%)	16(50%)	0.740
Small intestine	7(50%)	13(40%)	0.746

Table 5: Comparison of clinicopathological and IHC features of intestinal and gastric GIST

Parameters	Intestinal GIST (n = 20)	Gastric GIST (n = 21)	P value
Age in median (Mean±SD)	52 (47.75±14.93)	51.5 (52 ±15.56)	0.387
Size in Median (Mean±SD)	85mm (81mm ± 4.69)	125 mm (117±6)	0.0001
High risk (account size and mitosis)	13(65%)	18 (85.7%)	0.15
Mitosis >5/50HPF	11(55%)	15(71.4%)	0.34
Necrosis	10(50%)	9(42.8%)	0.76
Myxoid change	5(25%)	4(19%)	0.72
Spindle	13(65%)	14(66.6%)	1.00
Epithelioid	2(10%)	2(9.5%)	1.00
Mixed	2(10%)	4(19%)	0.66
CD 117 positivity	17(85%)	18(85.7%)	1.00
DOG 1	20(100%)	21(100%)	1.00
CD 34	8(40%)	20(95.2%)	0.0002

as high risk had significantly large areas of necrosis. (p value 0.0156). There was no specific association of cell type (Spindle /epithelioid type) with tumor site, size, risk category, presence or absence of necrosis. However there was significant cellular atypia in tumors exhibiting predominant epithelioid morphology.

Metastasis was observed in three cases. All three cases showed metastasis to liver, one case apart from liver it also showed metastasis to two lymph nodes.

Follow-up was available in ten cases (21.7%). Five cases are on imatinib 400mg with no recurrence. Three patients progressed in the form of recurrence of disease, liver metastases and peritoneal deposit respectively. In these cases the dose of imatinib was hiked to 600 mg.

4. Discussion

Gastrointestinal stromal tumours are the most common mesenchymal tumours of the gastrointestinal tract.¹ The precise diagnosis of GIST has become the utmost important because of the availability of first and second-generation tyrosine kinase inhibitor drugs (imatinib, sunitib, and others) specifically targeting the constitutional *Kit* activation by tumour-specific oncogenic mutations. In this regard, specific diagnosis enables delivery of potentially life-saving treatment to the right patients. In contrast, selection of the appropriate patient population for this very expensive treatment is part of health care resource optimization.

The diagnosis of these tumours can be done based on tumour location, histopathological pattern, morphology and by the CD 117 immunopositivity. There are situations in which *Kit* reactivity is negative or equivocal adding to difficulty in exact characterization. In these situations screening for mutations can be of help but taking into account the time and the cost of the test, this may be difficult in resource poor settings. We aimed to study the clinicopathological features of GIST and to study the

immunoprofile of GIST cases with emphasis on DOG1 expression.

Predicting the behaviour of GISTs is difficult, as there is a wide clinical spectrum of these neoplasms, ranging from small, incidental, benign nodules to larger, aggressive sarcomas. In general, tumours with >5 mitoses/10 HPF or size >5 cm (small intestine SI) or >10 cm (gastric) are likely to behave aggressively, and those <2 cm (SI) or <5 cm with 5 mitoses/10 HPF are likely to be benign; however, these are not absolute criteria, and there remains an intermediate group in which behaviour is unpredictable, and site-specific differences need to be taken into account.^{15,16}

4.1. Demographics. Frequency and tumour location

Among the 59 mesenchymal neoplasms of the gastrointestinal tract, GISTs were the commonest mesenchymal tumours of the GI tract accounting to 77.96%. The median age of presentation of GISTs was 51 years (range, 10-78 years). In a study of 66 cases of GISTs by Kaoy *et al*¹⁷ the median age at presentation was a decade later (median age, 61.5 years) than in our patients. Rabin *et al*¹⁸ in a review 93 cases of GISTs, reported a mean age of 62 years (range, 26 to 89 years) with M:F ratio of 1:1.3. The present study showed near equal sex predilection.

GISTs usually occur in older adults, and rarely in children and young adults. In the present study 30.4% (14/46) patients were < 40 years and 69.5% (32/46) patients (69.5%) > 40 years. In a study of 118 cases of GISTs by Yu Na Kang *et al*¹⁹ only 7.6 cases were younger than 40 years with 92.4 cases being older than 40 years. Our patients presented at a fairly younger age when compared to other similar studies.

Though majority of our tumours were located in stomach (45.6%), the percentage of intestinal tumors in our series was higher than that reported in literature. The other significant difference was our patients presenting with larger

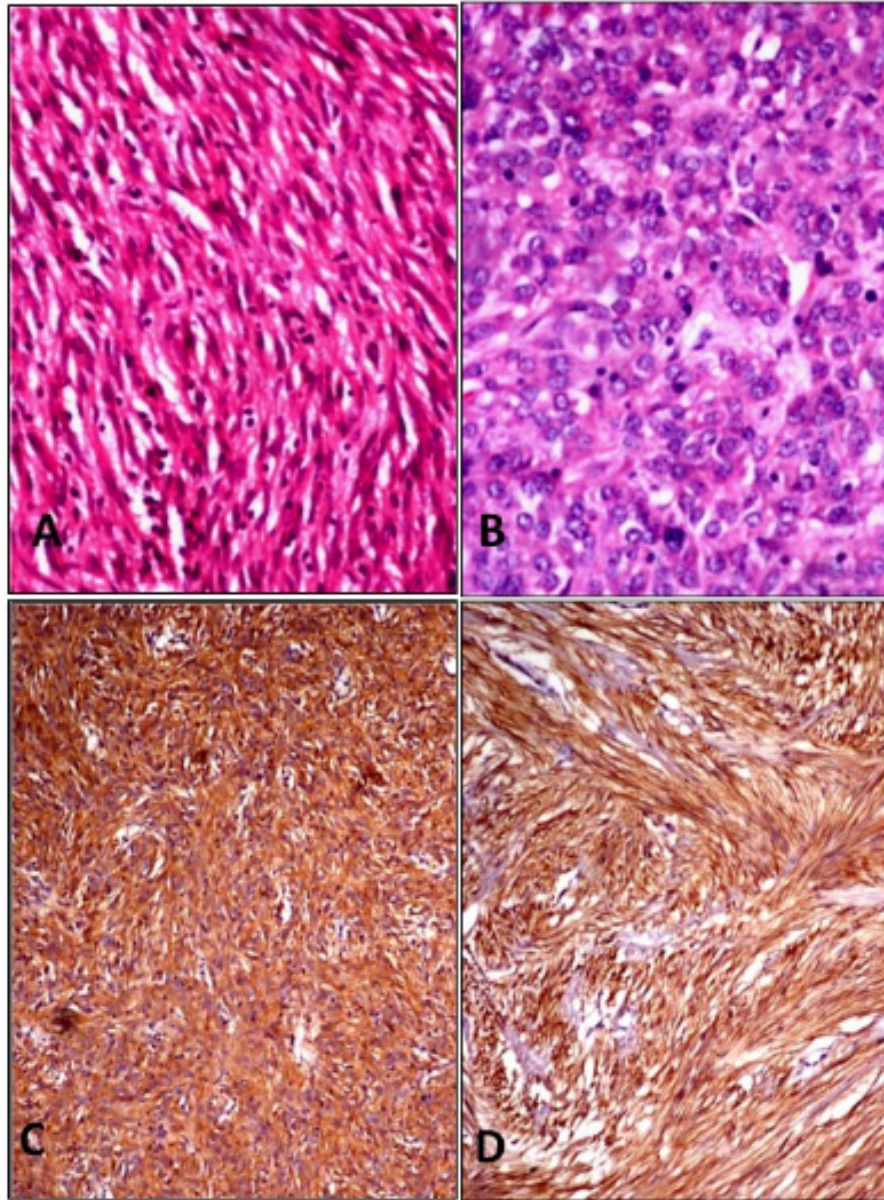


Fig. 1: Gastrointestinalstromal tumor (H&E; x400) (A, B): GIST With epithelioid morphology; (C): Diffuse CD 117 positivity; (D): Tumor cells show diffuse membranous andcytoplasmic positivity of DOG1

size tumors (47% >10 cm size) hence greater numbers falling in high risk category. This can be attributed to delay in seeking of medical attention by of our patients. The western literature documents GIST as being most prevalent in the stomach (50–60%), with much fewer being present in the small bowel (22–30%). Our cases were almost equally distributed between the intestinal (43.4%) and stomach (45.6%). Distribution of these tumours in our population may be different than western population. More studies from India are needed to substantiate our findings of relatively younger age at presentation and the tumour distribution in our population.

Anatomical location has been suggested to be an independent prognostic factor by some studies.²⁰ Generally, gastric GISTs have a better prognosis than GISTs of the SI, while most oesophageal and colonic GISTs are malignant. On comparing the clinicopathological features and IHC results of gastric and small bowel GIST, only size of the tumour had significant association, p value 0.0001. The age, sex, high mitosis, necrosis, myxoid change or type of cell did not have any significance with the site of tumour. The gastric GIST showed increased positivty to CD 34 than small intestinal GIST which was statistically significant (p<0.002).

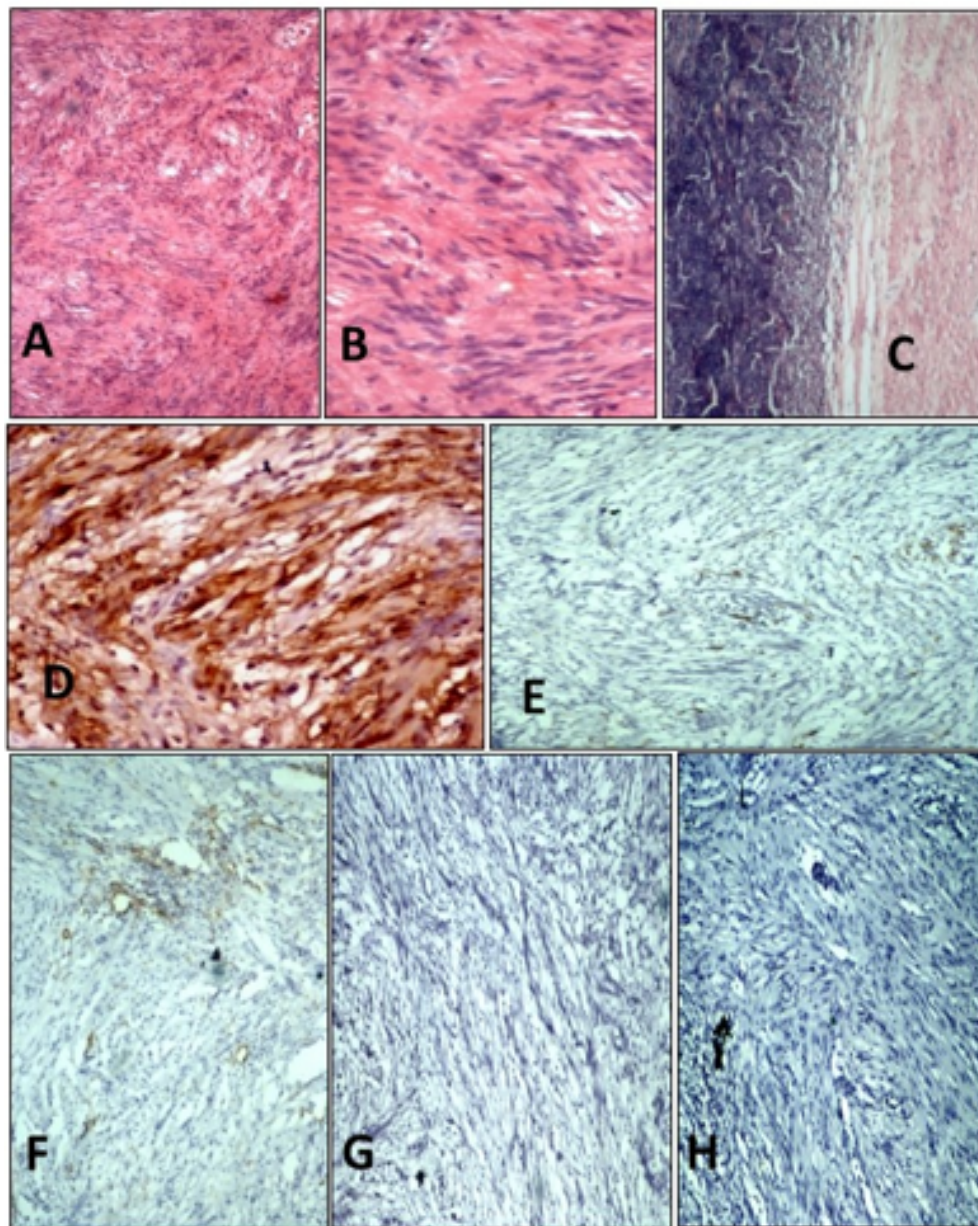


Fig. 2: Photomicrographs of case of Schwannoma (H&E; x100); (A, B): Cellular areas showing nuclear palisading with narrow elongated wavy nuclei; (C): Periphery showing prominent lymphoid cuff ; (D): S100-Strong diffuse cytoplasmic positivity; (E): CD 34-Negative; (F): SMA-Negative G) CD 117-Negative; (H): DOG 1- Negative

It has not been well documented whether GIST prognosis is age dependent. In the AFIP fascicle of intestinal tumours, intestinal GISTs below the fifth decade have been stated to be more often malignant than benign.

A comparative clinicopathological features between patients <40 and > 40 years was done and the results are provided in Table 5 and showed tumours in age group >40 years had higher mitotic rates and this was statistically significant (p value 0.0482). Site of tumour, size, presence of necrosis, risk category did not show any significance with

age.

4.2. Tumour characteristics

4.3. Significance of histologic subtypes

Spindle cell morphology was noted in 69.5% of cases, epithelioid morphology in 8.6%% of cases, and a combination of these in 15.2% of the cases. The subtypes described in this study are mainly intended to describe the consistent, recurrent histologic patterns that should be

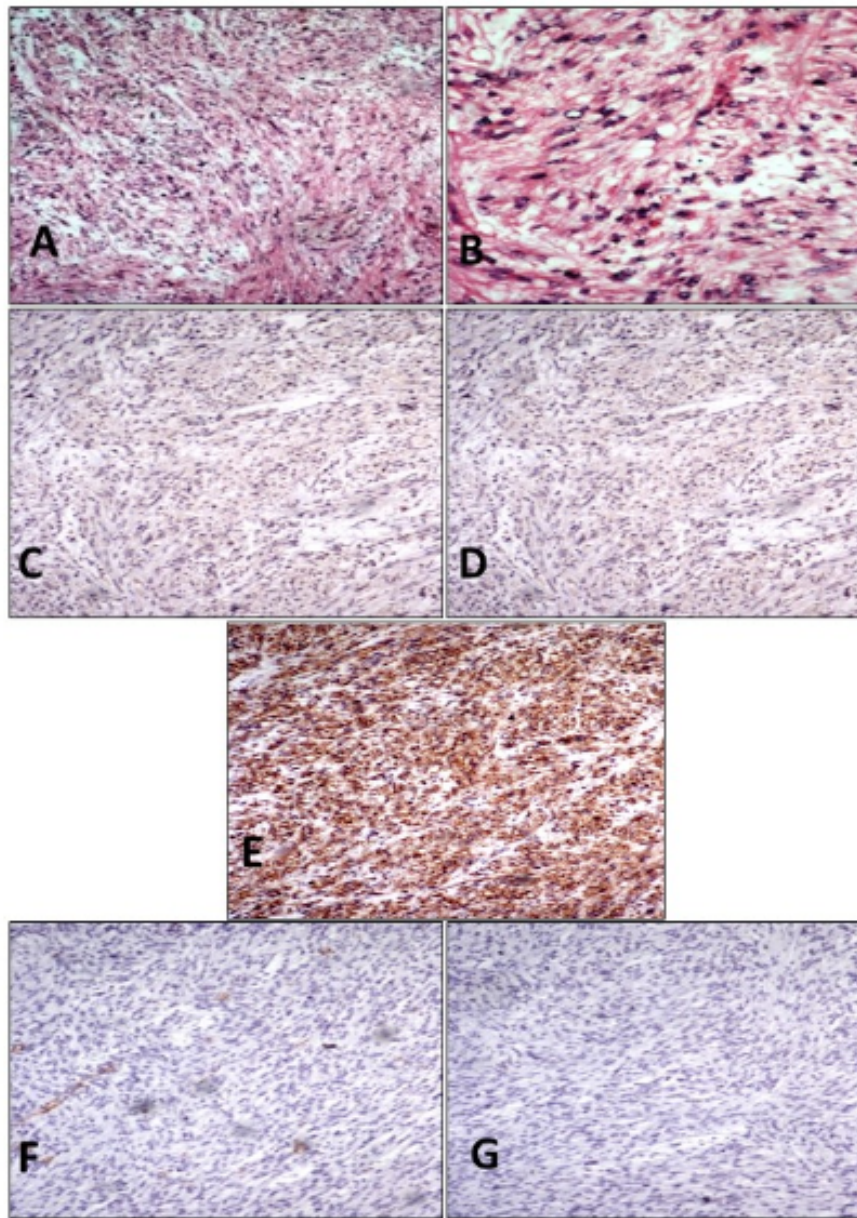


Fig. 3: Photomicrographs of case of Leiomyoma (H&E;x100); (A, B): Fascicular pattern of elongated smooth muscle cells with eosinophilic fibrillary cytoplasm; (C): CD117-negative; (D): DOG 1-negative; (E): SMA-intense cytoplasmic positivity; (F): CD 34-negative; (G): S100-negative

recognized as a part of the GIST spectrum. However, the spectrum from sclerosing, palisading-vacuolated, hypercellular to sarcomatous among spindle cell GISTs reflects increasing frequency of adverse outcome. The sarcomatous type differed significantly from the others by the presence of hypercellularity with marked pleomorphism and brisk mitotic activity. There were tumours which also showed focal hemangiopericytomatous pattern and angiectatic spaces. The cuff of lymphoid aggregates which are thought to be characteristic of schwannomas arising

from the GI tract were also seen in some of the GIST cases in our study.

Koay et al¹⁵ in their study have shown that size >10 cm, necrosis and pure epithelioid morphology were each significantly associated with adverse survival. In our study we also attempted to correlate histopathological cell type (spindle type vs epithelioid cell type) with the various clinicopathological and IHC features and there was no significant association of cell type with mitosis and size which are considered as important predictors of malignancy

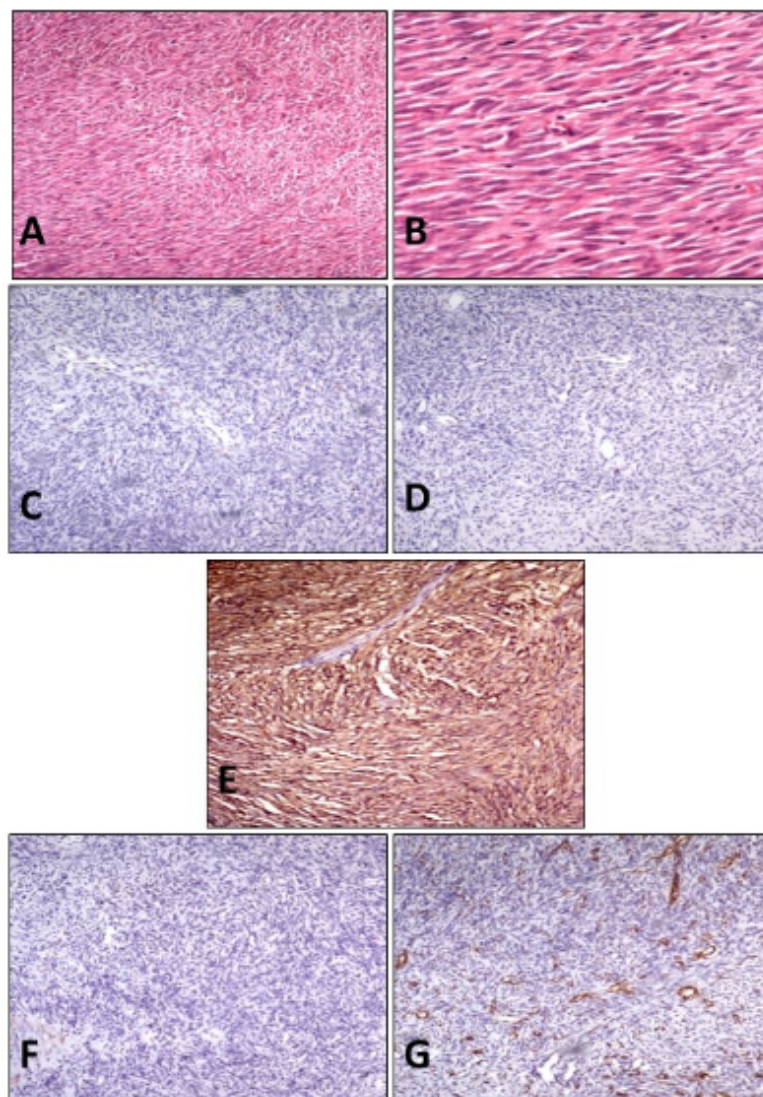


Fig. 4: Photomicrographs of case of solitary fibrous tumor (H&E); X100; **A, B**): Patternless pattern of bland uniform spindle cells with elongated nuclei and indistinct nucleoli; **(C)**: CD 117-negative; **(D)**: DOG 1- negative; **(E)**: CD 34- Intense cytoplasmic positivity; **(F)**: S100- Negative; **(G)**: SMA- Negative

in GIST cases. This might be explained by the relative low number of tumours with epithelioid morphology in the present study. However majority of the tumors which had epithelioid morphology exhibited high degree of atypia and pleomorphism (p value 0.029).

Atypia, ulceration and cellularity are parameters that have come under scrutiny as potential prognostic factors. Atypia and cellularity are difficult to standardise. Atypia is a subjective parameter that may be affected by the cellularity of a tumour. For instance, low tumour cellularity or oedema may lead to an underestimation of the degree of cellular atypia. Conversely, a low degree of atypia may be worrisome in the context of a highly cellular tumour. This problem can be minimised by having more than one

observer. This difficulty in assessing cellularity and atypia as prognostic factors has been noted by others, while some have felt confident in using cellularity as a parameter in defining malignancy, noting low cellularity as a favourable prognostic feature.

4.4. Histopathological parameters and mitotic index as prognostic markers

In our study, tumour size and mitotic activity are among the most powerful prognosticators, both of these parameters be carefully recorded in all pathology reports on GISTs. In several multivariate analysis, it was proved that tumour size to be the only independent prognostic factor. In another study of gastric GISTs, mitotic rate was found to be an

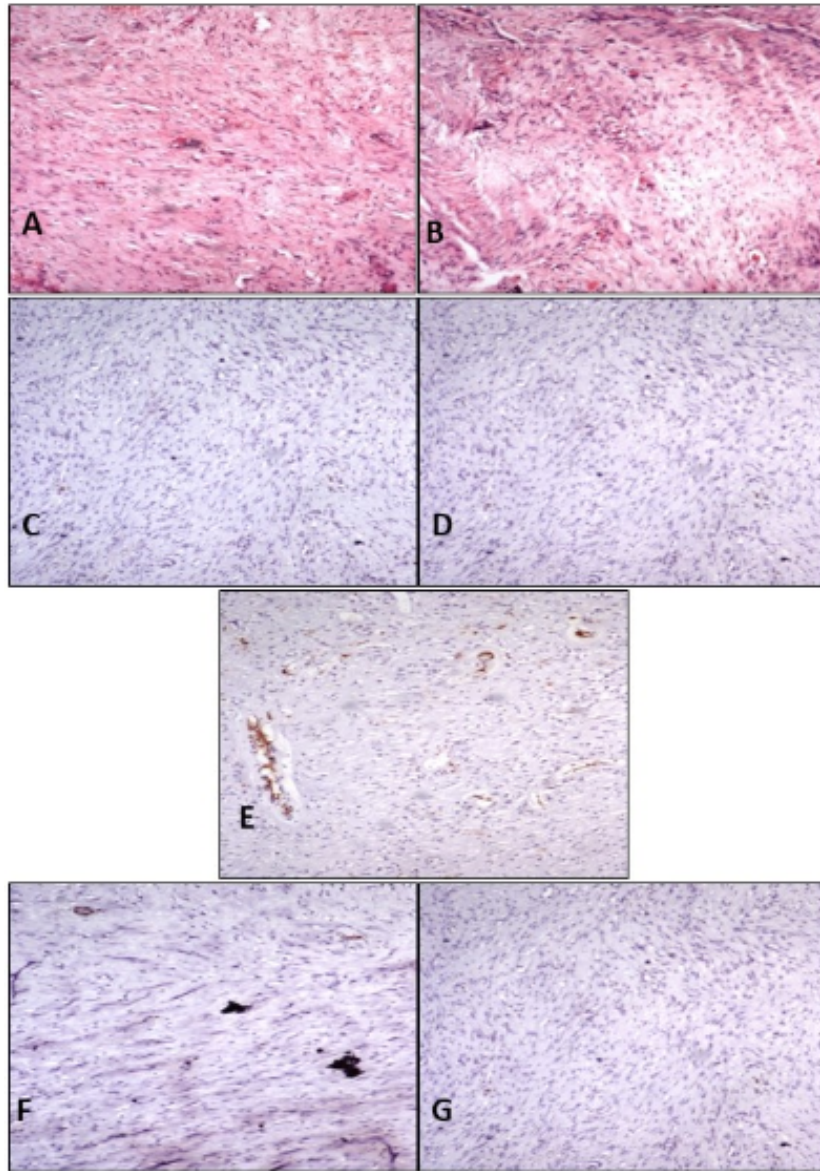


Fig. 5: Photomicrographs of case of Fibromatosis (H&E; x100) (A, B): Paucicellular fibrous proliferation with relatively uniform bland spindle cells intervening collagen bundles; (C): CD 117-negative; (D): DOG1-negative; (E): CD 34-negative; (F): S100- negative; (G): SMA-negative

independent factor in multivariate analysis. In the present study, high mitotic rate of tumour was (important risk factor for malignant potential) was not influenced by site, bigger size of tumour, increased age or type of cell. Our findings were similar to that done by Koay et al which also showed no correlation of high mitotic rate with age, size and site of tumour or cell type. However they found significant association of mitotic rate with necrosis, atypia and cellularity. In our study though presence of necrosis showed trend association, but it did not reach statistical significance (p value 0.085). Higher mitotic index did not correlate with the Ki 67 positivity as well. Few tumours

with low mitotic index had higher Ki 67 index and vice versa. Mitotic counts may be affected by variables such as tumour fixation, section thickness, selection of areas to be examined (whether random fields were examined, or the most cellular areas), tumour oedema, variability in field areas because of differing eyepiece field diameters, and interobserver variation.

Coagulative necrosis is usually associated with malignant tumour behavior of GISTs but it can also occur in benign tumours, perhaps representing tumor infarction. Necrosis creating a microcystic gross appearance is a very common microscopic change and also occurs in benign

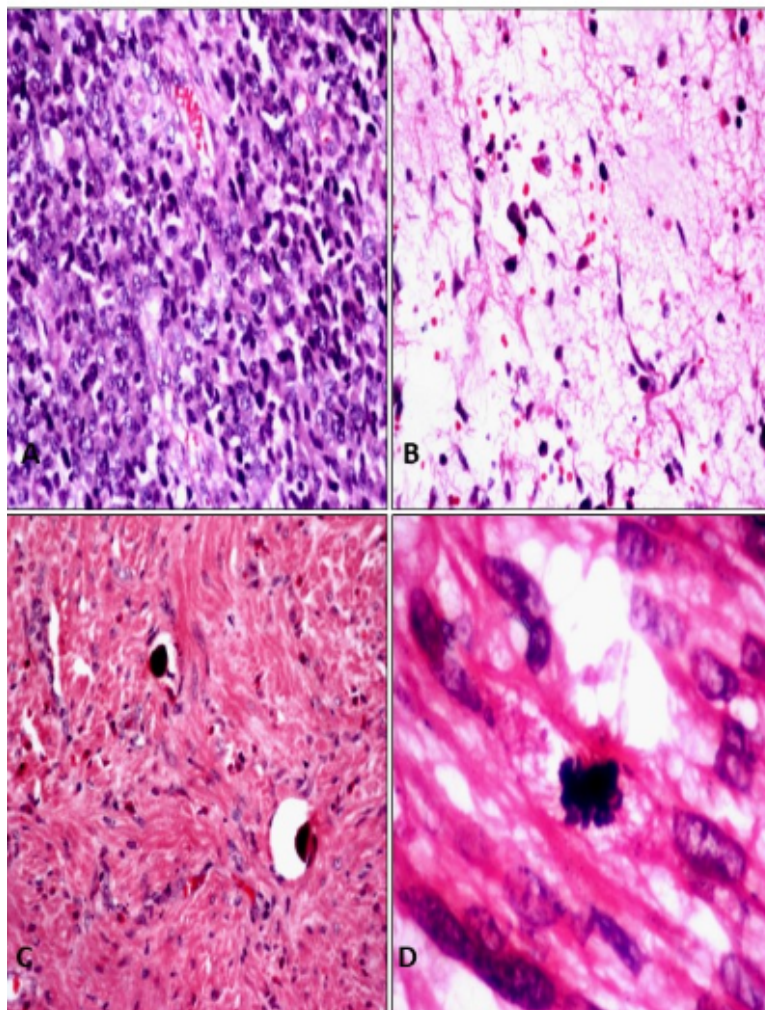


Fig. 6: Various histopathological features of GIST (H&E; x400) (A): Cellular pleomorphism; (B): Myxoid areas; (C): Calcifications; (D): Mitotic activity

GISTs; this change has no adverse significance. In our study presence of necrosis was significantly associated with high risk tumours (p value 0.0156).

4.5. Risk stratification

Histological assessment of malignancy is essentially based on mitotic counts and size of the lesion. Tumours less than 5 cm are usually benign. Different limits have been applied for low-grade malignant tumours. Mitotic activity and size of the tumour are considered independent prognostic variables. We have tried to risk stratify according to both Fletcher⁹ and Miettinen⁹ *et al.* Majority of our tumours fell in high risk category.

There were 3 discrepant cases when compared between two methods. The 3 cases which were of intermediate risk according to Fletcher's criteria were being upgraded to malignant potential according to Miettinen solely due to inclusion of site into the criteria. Among the three cases, one

case which was of smaller size (<5cm) and mitotically low showed metastasis to liver. This observation highlights the importance of site of tumour as an independent prognostic variable.

A previous study from the Armed Forces Institute of Pathology (AFIP), including 207 patients with small intestinal smooth muscle/ stromal tumours, found gastric tumors to have a more favorable outcome than the small intestinal ones.²¹ A recent series, including 84 jejunoileal and 170 gastric GISTs, found only a 10% higher risk for death from tumour for patients with intestinal versus gastric GISTs.²² Our study also highlights the incorporation of site into risk assessment for a better stratification of cases. However the sites included in the criteria are stomach and small intestine only. It does not take into account other sites such as large intestine, mesentery or pancreas.

The high risk tumours were compared with various clinicopathological and IHC results. High risk tumours

had more necrotic areas (p value 0.015). Age, sex, site of tumour, mitotic index, Ki 67 positivity did not correlate with high risk category. IHC results were not influenced by risk category of tumour.

4.6. Immunohistochemistry

Most GIST can be identified based on the combination of tumour location, histologic appearance, and the presence of *KIT* by immunohistochemistry. IHC stains such as CD34, smooth muscle actin (SMA) and S100, desmin, in addition to C-KIT (CD117), are necessary for making an accurate diagnosis of GIST and distinguishing from other mesenchymal tumours of the GI tract. In a significant proportion of GISTs (4% to 15%), *KIT* expression is equivocal or negative, leaving the diagnosis in question. In the NIH Consensus document several alternative immunohistochemical markers for GIST have been

proposed, including PDGFRA, nestin, protein kinase C theta, carbonic anhydrase II and Discoveted on GIST 1.^{23–28} DOG1 is a protein of unknown function that was found to be selectively expressed in GIST using gene expression profiling. We have demonstrated that DOG1 is a very sensitive (97.8%) marker for GIST that works in paraffin-embedded tissue. The use of DOG1 in clinical practice as either a backup to *KIT* or as a part of a panel can allow the identification of more GIST cases.

In our study, 7 cases were DOG1 positive and CD117 negative but only one case was DOG1 negative and CD117 positive, thus showing DOG1 to be a more sensitive marker than CD117. As a result of its localization in the cell membrane, its absence in the majority of normal tissue (with the exception of the myenteric plexus) and the presence in most of the GIST, DOG1 may be an additional target in the treatment of GIST.

Rabin et al.²⁹ reported 40% to 70% of GIST's were positive for CD34, 20% to 30% were positive for SMA, 10% were positive for S100 protein and <5% were positive for desmin. Our results were comparable to their study.

The IHC results for CD117, DOG1 and CD34 was correlated with the clinicopathological parameters (Table 4). CD 34 positivity was affected by site of tumour; gastric tumors showed higher positivity to CD 34 (p value -0.001). This finding of Gastric GIST be more commonly positive for CD34 as compared to small bowel GIST has been well observed in many studies.^{3,4}

However DOG 1 positivity was not influenced by site, size, or presence of necrosis.

5. Conclusion

We found Gastrointestinal stromal tumours were the most common mesenchymal tumours of the gastrointestinal tract accounting to 77.96%. The median age of presentation was 51 years with near equal sex predilection. Our

patients presented a decade earlier with majority in high risk groups. Inclusion of anatomic site into the risk category will help in better stratification of GIST cases. We recommend a wide immunopanel with emphasis on DOG 1 positivity for precise diagnosis of GIST and classification of mesenchymal tumors of the GI tract.

6. Source of funding

None.

7. Conflict of interest

None.

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