

Received: 2020.11.01
Accepted: 2021.01.17
Available online: 2021.01.26
Published: 2021.03.24

Contribution of Interstitial Cells of Cajal to Gastrointestinal Stromal Tumor Risk

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABEG 1 **Qi Wang***
ACDEF 2 **Zhen-peng Huang***
B 3 **Yu Zhu**
B 1 **Fei Fu**
ABDG 1 **Lin Tian**

1 Department of Pathology, Renmin Hospital, Hubei University of Medicine, Shiyan, Hubei, P.R. China
2 Guangzhou Institute of Oncology, Affiliated Cancer Hospital and Institute of Guangzhou Medical University, Guangzhou, Guangdong, P.R. China
3 Department of Science and Education, Renmin Hospital, Hubei University of Medicine, Shiyan, Hubei, P.R. China

* Qi Wang and Zhen-peng Huang contributed equally

Corresponding Authors: Zhen-peng Huang, e-mail: huang_zhenpeng@126.com, Lin Tian, e-mail: 13872763897@163.com
Source of support: Departmental sources

Background: Gastrointestinal stromal tumors (GISTs), which originate from interstitial cells of Cajal (ICCs), are one of most common mesenchymal tumors of the gastrointestinal tract. This study explored the impact of ICCs and immunological markers on GIST risk.





Material/Methods: A total of 122 patients diagnosed with GISTs who underwent surgery were recruited for the study. Demographic and clinical information, including modified NIH criteria, sex, age, tumor site, and tumor size, of all patients were collected. GIST risk was assessed using the modified NIH risk classification for primary GISTs. Paraffin-embedded GIST specimens were evaluated by hematoxylin-eosin staining and ICCs immunohistochemistry.

Results: According to the modified NIH criteria, most GIST cases (44 cases, 36.07%) were at very low risk. Females had greater incidence of high-risk GISTs ($P < 0.05$). The mean age at GIST diagnosis was 58.69 ± 9.90 years and had no impact on GIST risk ($P > 0.05$). Most GISTs were located in the stomach (87 cases, 71.73%), and the size of the tumors varied (0.5-20 cm). CD117/c-kit and CD34 were specific immuno-markers for ICCs and GIST. Most patients with GIST were CD117-positive (115 cases, 94.26%), 111 cases (90.98%) were CD34-positive, and 109 cases (89.34%) were positive for both CD117/c-kit and CD34. With increasing GIST risk, CD117 (also named c-kit) and CD34 expression levels increased, as well as the number of ICCs (all $P < 0.05$).

Conclusions: ICCs have a great impact on GISTs incidence. CD117/c-kit and CD34 expression, as well ICCs levels, appear to affect GIST risk.

Keywords: **Gastrointestinal Neoplasms • Interstitial Cells of Cajal • Risk Assessment**

Full-text PDF: <https://www.medscimonit.com/abstract/index/idArt/929575>

 1883  4  2  31



Background

The term gastrointestinal stromal tumor (GIST) was first introduced by Mazur and Clark in 1983. It is among the most common mesenchymal tumors of the gastrointestinal tract, representing 0.1-3% of all gastrointestinal tumors, of which about 30% are malignant [1,2]. Most patients with GISTs lack typical clinical symptoms, with gastrointestinal bleeding being its most common clinical feature [3]. Genotypically, most GISTs are triggered by activating mutations in *KIT* and its platelet-derived growth factor receptor alpha (*PDGFRA*). Such mutations are known to promote early events of GIST by accelerating its transformation and progression. Immunophenotypically, they express specific markers such as CD117 (also named c-kit) and CD34 [4].

Interstitial cells of Cajal (ICCs) were first described in the gastrointestinal tract by Santiago Ramón y Cajal in 1893 [5]. In the past 100 years, studies have shown that ICCs are present in the esophagus, stomach, proximal duodenum wall, small and large bowel, ileum, appendix, and colon [6]. ICCs function in the digestive tract smooth muscle as power pacemakers, promoting gastrointestinal electrical activity, and mediating and regulating the gastrointestinal neurotransmitters [7]. Overall, these cells were shown to be involved in several functional gastrointestinal disorders [7]. Recently, several studies have also suggested that ICCs are implicated in GIST [8]; however, the relationship between GIST and ICCs remains unknown.

The present study aimed to examine the clinical, histopathological, and biomolecular features of ICCs and their potential contribution to the risk of GIST.

Material and Methods

Patients

A total of 122 patients diagnosed with GISTs who were admitted to the Renmin Hospital, Hubei University of Medicine, from January 2016 to June 2020, were included in the study. Demographic and clinical information of all patients, including sex, age, and tumor site and size, were collected at the earliest possible time after written informed consent was provided. Paraffin-embedded GIST specimens were obtained from all patients who underwent surgery. None of the patients included in the study had received radiation therapy or chemotherapy before surgery.

The study was conducted according with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and was approved by the Institutional Ethical Committee of the Renmin Hospital, Hubei University of Medicine.

GISTs risk assessment

According to the modified National Institutes of Health (NIH) risk classification for primary GIST, the risk of GIST was defined as very low-risk, low-risk, intermediate-risk, and high-risk, depending on the tumor size, mitotic index, and primary tumor site [9].

Hematoxylin-eosin Staining and Immunohistochemistry

Hematoxylin-eosin staining was performed in paraffin-embedded GIST specimens, coupled with diaminobenzidine staining, to detect pathological alterations within the tumor tissue.

GIST specimens were fixed using a 4% paraformaldehyde solution and embedded in paraffin. Paraffin-embedded tumor samples were sliced into 5- μ m-thick sections and mounted on glass slides. The sections were incubated with anti-CD117/c-kit rabbit monoclonal antibody (MXB Biotechnologies, Fuzhou, China) and anti-CD34 mouse monoclonal antibody (MXB Biotechnologies) at 4°C for 15 h. Afterwards, the slides were incubated with the respective HRP-conjugated anti-rabbit or anti-mouse secondary antibodies (BOSTER Biological Technology, Wuhan, China).

A bright-field microscope (Olympus BX53, Tokyo, Japan) was used to analyze the slides. Two pathologists independently observed the slides and scored the different samples according to the proportion of ICC-positive cells and staining intensity. Five representative regions were selected for analysis on each section. The samples were given a score of 0, 1, 2, 3, or 4 points as they presented <5%, 5-25%, 25-50%, 50-75%, or >75% of ICCs, respectively; and 0, 1, 2, or 3 points whereas they showed no color, light yellow, brown, or sepia marks, respectively. The final score of the field of vision was obtained by multiplying the 2 scores. The score of each sample was calculated as the average score of the 5 fields selected in the tissue slice. Lastly, the samples were divided into the following categories: a score of 0-2 points was defined as negative (-), 2-5 points was defined as (+), 5-8 points was defined as (++), and more than 8 points was defined as (+++) [10,11].

Statistical Analysis

Statistical analysis was performed using SPSS for Windows version 25.0 (IBM Corporation, Armonk, NY, USA). Continuous variables were expressed as mean \pm standard deviation (SD) and were compared using the *t* test. Categorical variables were represented as proportions and percentages. Categorical variables were compared using the chi-squared test. Differences with *P*<0.05 (two-sided) were considered statistically significant.

Table 1. The clinical and pathological characteristics of GIST patients.

	Number/ size	Percentage (%)/ mean/median		Number/ size	Percentage (%)/ mean/median
Modified NIH criteria			Age (years)*		
Very low risk	44	36.07	General	31-82	58.69±9.90
Low risk	29	23.77	Very low risk	37-77	57.11±9.69
Intermediate risk	16	13.11	Low risk	31-77	59.64±11.10
High risk	33	27.05	Intermediate risk	50-78	63.44±8.68
Gender#			High risk	40-82	58.69±9.90
General			Tumor site		
Male	58	47.54	Esophagus	3	2.46
Female	64	52.46	Stomach	87	71.31
Very low risk			Small intestine	7	5.74
Male	24	54.55	Colon and rectum	14	11.47
Female	20	45.46	Omentum and mesentery	10	8.20
Low risk			Liver	1	0.82
Male	19	65.52	Tumor size (cm)		
Female	10	34.48	Esophagus	0.5-2	0.7
Intermediate risk			Stomach	0.5-16	2.25
Male	6	37.5	Small intestine	3-14	8
Female	10	62.5	Colon and rectum	0.8-12	8
High risk			Peritoneum	7-20	14
Male	9	27.27	Liver	5	–
Female	24	72.73			

$\chi^2=2.4778$, $P=0.0132$; * $t=-0.990$, $P=0.327$. Most of GIST cases were very low-risk and low-risk. Male patients were more likely to have very low-risk and low-risk GISTs, whereas women were more likely to have high-risk GISTs ($\chi^2=2.4778$, $P=0.0132$). Age at GIST diagnosis ranged from 31 to 82 years (58.69±9.90), but was not significantly associated with GIST risk ($t=-0.990$, $P=0.327$). Most GISTs were located in the stomach. The size of tumors varied between 0.5 and 20 cm.

Results

Clinicopathological Features of GIST Patients

According to the modified NIH classification criteria, 44 of the 122 GIST cases (36.07%) were very low-risk, while 27.05% of patients (33 cases) were high-risk. In addition, male patients had very low-risk and low-risk GISTs, whereas women were more likely to have high-risk GISTs ($P<0.05$). Age at GIST diagnosis ranged between 31 and 82 years (58.69±9.90) and no significant differences in GIST risk were noted ($P>0.05$).

In our study, most GISTs were primarily located in the stomach (87 cases, 71.73%), followed by the colon and rectum

(14 cases, 11.47%). Fewer GISTs cases were affected in other organs of the gastrointestinal tract, including the esophagus (3 cases, 2.46%), small intestine (7 cases, 5.74%), peritoneum (10 cases, 8.20%), and liver (1 case, 0.82%). The size of tumors varied between 0.5 and 20 cm, depending on the tumor location (Table 1).

ICC-specific Immuno-markers in GIST Patients

CD117 (also named c-kit) and CD34 are specific immuno-markers for ICCs and GIST. Most patients with GIST were CD117- and CD34-positive, with 115 cases (94.26%) being CD117-positive, 111 cases (90.98%) were CD34-positive, and 109 cases (89.34%) were positive for both immuno-markers.

Table 2. ICCs-specific immuno-marker characteristics in GISTs patients.

Tumor site	CD117/c-kit positive [#]	CD34 positive [*]	CD117/c-kit and CD34 positive [§]
Esophagus	3 (100.00%)	3 (100.00%)	3 (100.00%)
Stomach	84 (96.55%)	81 (93.10%)	80 (91.95%)
Small intestine	10 (71.43%)	11 (78.57%)	10 (71.43%)
Colon and rectum	7 (100.00%)	6 (85.71%)	6 (85.71%)
Omentum and mesentery	10 (100.00%)	9 (90.00%)	9 (90.00%)
Liver	1 (100.00%)	1 (100.00%)	1 (100.00%)
Overall	115 (94.26%)	111 (90.98%)	109 (89.34%)

[#] $\chi^2=15.617$; $P=0.008$; ^{*} $\chi^2=3.751$; $P=0.586$; [§] $\chi^2=5.921$; $P=0.314$. The rate of CD117-positive cells was lower in tumors located in the small intestine than in tumors located in the colon and rectum, or stomach ($\chi^2=15.617$; $P=0.008$). The rate of CD34-positive cells was higher in tumors located in the stomach than in tumors located in the small intestine ($\chi^2=3.751$; $P=0.586$). The rate of both CD117/c-kit and CD34-positive cells was higher in tumors located in the esophagus and stomach than in tumors located in the small intestine ($\chi^2=5.921$; $P=0.314$). Moreover, the number of CD34-positive cells was lower as compare to the number of CD117-positive cells.

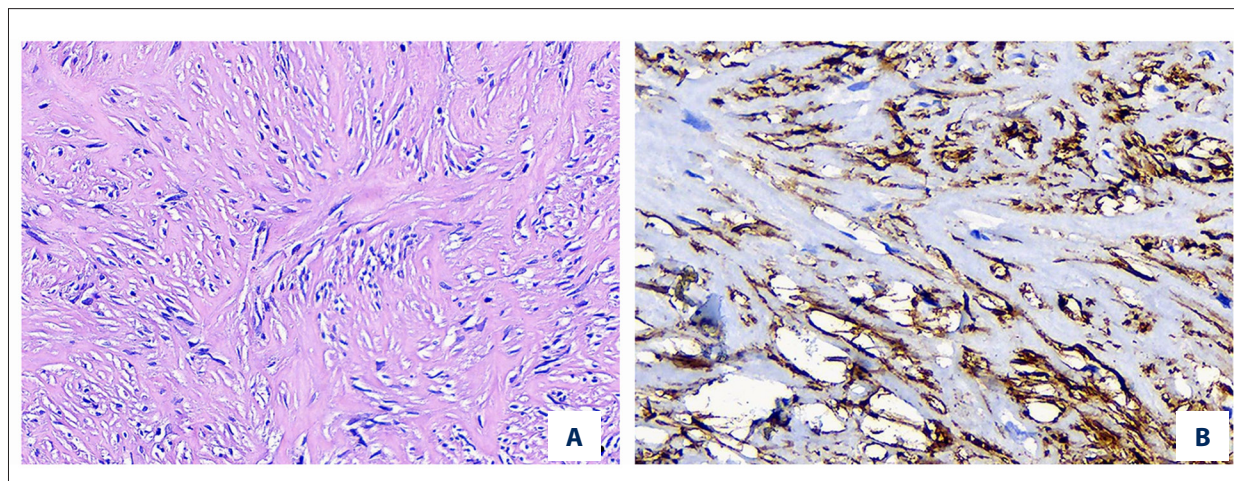
Expression of CD117 marker was found to be dependent on the location of the tumor. The rate of CD117-positive cells was lower in tumors located in the small intestine than in tumors located in the colon and rectum, or stomach ($P<0.05$). The rate of CD34-positive cells was higher in tumors located in the stomach than in tumors located in the small intestine ($P<0.05$). The rate of both CD117/c-kit and CD34-positive cells was higher in

tumors located in the esophagus and stomach than in tumors located in the small intestine ($P<0.05$). Moreover, the number of CD34-positive cells was lower than the number of CD117-positive cells (**Table 2**).

Table 3. ICCs and CD117-positive grade and GISTs risk.

NIH criteria	CD117/c-kit (-)	CD117/c-kit (+)	CD117/c-kit (++)	CD117/c-kit (+++)
Very low risk	2 (4.55%)	22 (50.00%)	11 (25.00%)	9 (20.45%)
Low risk	2 (6.90%)	14 (48.28%)	6 (20.69%)	7 (24.13%)
Intermediate risk	0 (0.00%)	5 (31.25%)	4 (25.00%)	7 (43.75%)
High risk	3 (9.0%)	6 (18.18%)	8 (24.24%)	16 (48.48%)

[#] $\chi^2=9.6482$, $P=0.0218$. Most high-risk GIST cases were highly positive for ICCs and had high expression of CD117 (+++). Nearly half of the very low-risk and low-risk GIST cases had low levels of ICCs and CD117 (+) ($\chi^2=9.6482$, $P=0.0218$).



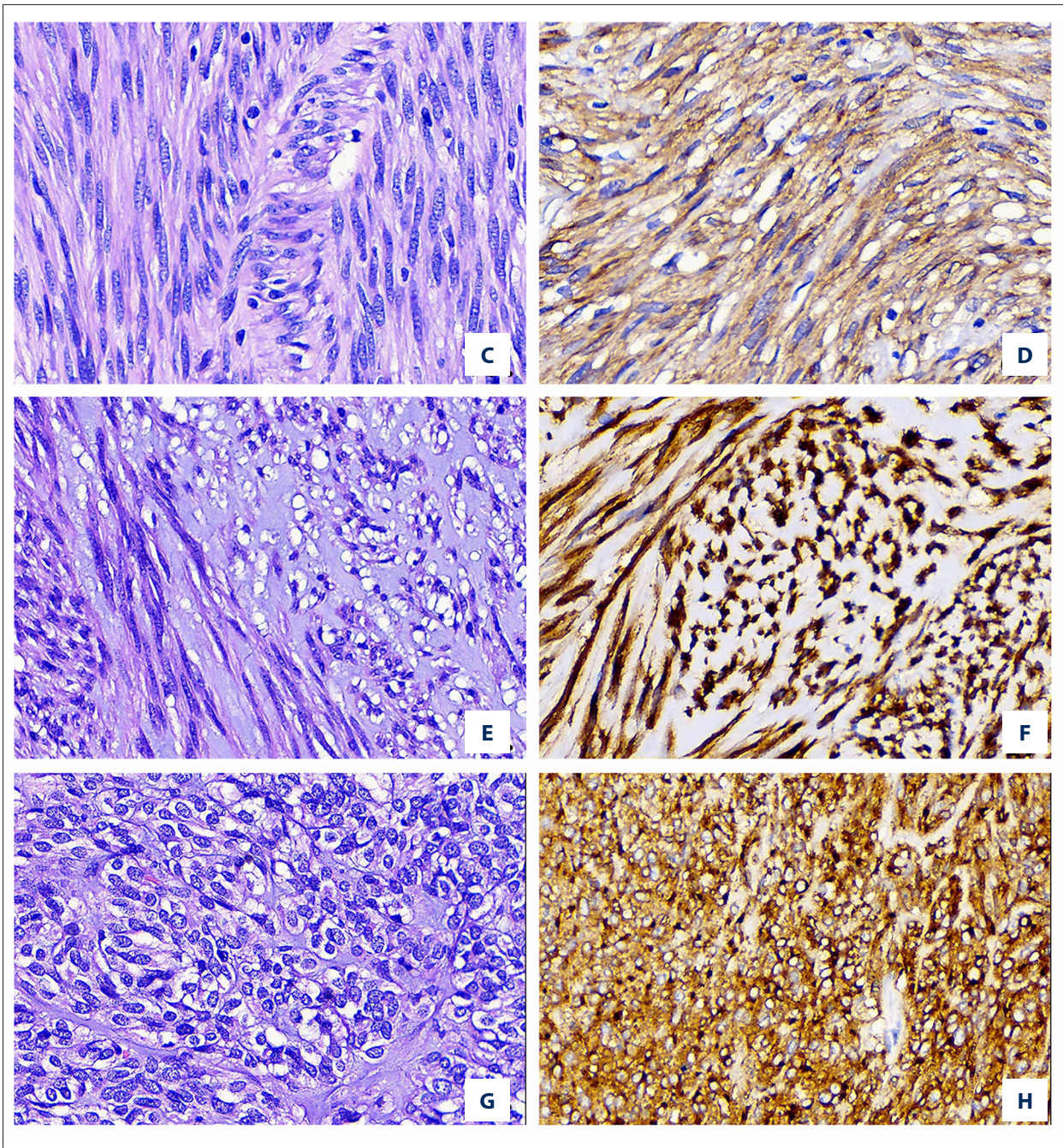
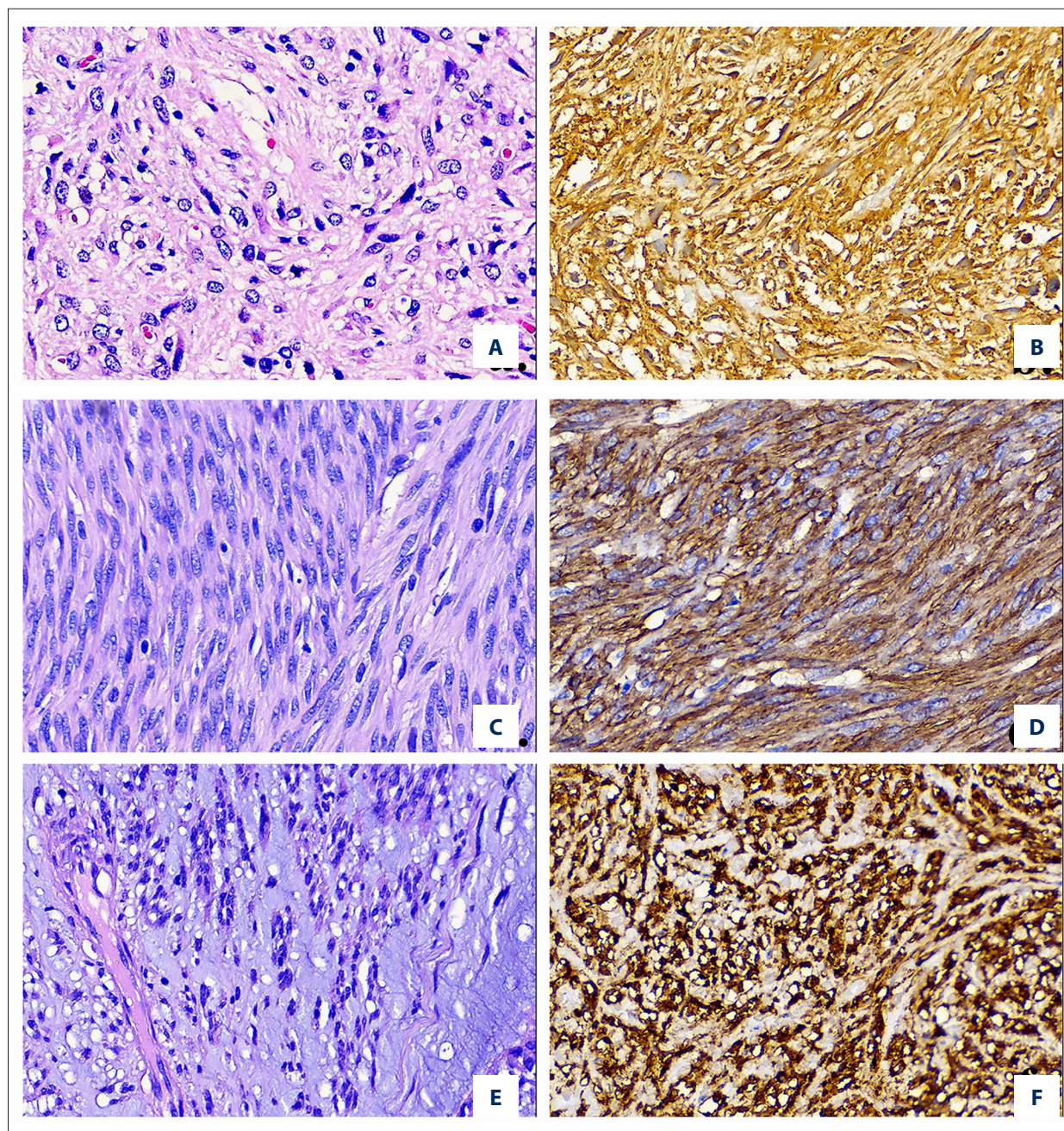


Figure 1. CD117/c-kit-positive grade and GIST risk. ICCs and CD117/c-kit-positive grade affected GISTs risk, the ICCs and CD117/c-kit positive grade were higher as the GIST risk level was higher (**A**, hematoxylin-eosin staining for the very low risk of stomach GIST; **B**, CD117/c-kit for the very low risk of stomach GIST; **C**, hematoxylin-eosin staining for the low risk of stomach GIST; **D**, CD117/c-kit for the very risk of stomach GIST; **E**, hematoxylin-eosin staining for the intermediate risk of stomach GIST; **F**, CD117/c-kit for the intermediate risk of stomach GIST; **G**, hematoxylin-eosin staining for the high intermediate risk of stomach GIST; **H**, CD117/c-kit for the high risk of stomach GIST).

Table 4. ICCs and CD34-positive grade and GISTs risk.

NIH criteria	CD34 (-)	CD34 (+)	CD34 (++)	CD34 (+++)
Very low risk	4 (9.09%)	24 (54.54%)	10 (22.73%)	6 (13.64%)
Low risk	3 (10.35%)	12 (41.38%)	10 (34.48%)	4 (13.79%)
Intermediate risk	0 (0.00%)	6 (37.5%)	5 (31.25%)	5 (31.25%)
High risk	4 (12.12%)	9 (27.27%)	8 (24.25%)	12 (36.36%)

$\chi^2=7.8485, P=0.0492$. Most high-risk GIST cases had very high levels of ICCs and CD34 (+++). Nearly half of the very low-risk and low-risk GIST cases had low levels of ICCs and CD34 (+) ($\chi^2=7.8485, P=0.0492$).



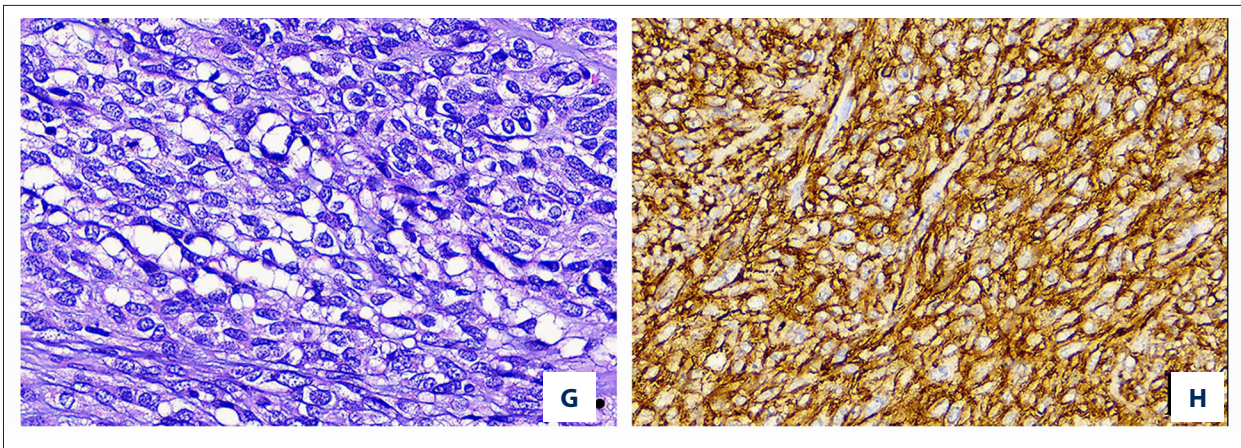


Figure 2. CD34-positive grade and GIST risk. ICCs and CD34-positive grade affected GISTs risk, and the ICCs and CD34 positive grade were higher as the GIST risk level was higher (A, hematoxylin-eosin staining for the very low risk of stomach GIST; B, CD34 for the very low risk of stomach GIST; C, hematoxylin-eosin staining for the low risk of stomach GIST; D, CD34 for the low risk of stomach GIST; E, hematoxylin-eosin staining for the intermediate risk of stomach GIST; F, CD34 for the intermediate risk of stomach GIST; G, hematoxylin-eosin staining for the high intermediate risk of stomach GIST; H, CD34 for the high risk of stomach GIST).

CD117-positive Cells, ICCs, and GIST Risk

Most high-risk GIST cases (16 cases, 48.48%) were highly positive for ICCs and had high expression of CD117 (+++). However, 9 (20.45%) and 7 (24.13%) patients who were classified as very low-risk and low-risk, respectively, had very high levels of ICCs and CD117 (+++). Nearly half of the very low-risk and low-risk GIST cases had low levels of ICCs and CD117 (+), and only 6 high-risk GIST patients (18.18%) had low ICCs and CD117 expression (+) ($P < 0.05$; **Table 3 and Figure 1**). Overall, these results suggest that the presence of ICCs and CD117 contribute to GIST risk.

CD34-positive Cells, ICCs, and GIST Risk

Similarly, CD34 expression may also have an impact on GIST risk. Herein, most high-risk GIST cases (12 cases, 36.36%) had very high levels of ICCs and CD34 (+++), while only 6 very low-risk (13.64%) and 4 low-risk (13.79%) GISTs cases had such high levels of ICCs and CD34 (+++). Nearly half of the very low-risk and low-risk GIST cases had low levels of ICCs and CD34 (+), and only 9 high-risk GIST cases (27.27%) had low numbers of ICCs and low CD34 expression (+) ($P < 0.05$; **Table 4 and Figure 2**).

Discussion

GISTs are specific mesenchymal tumors that affect any organ of the gastrointestinal tract. A recent study estimated that the annual incidence of GIST in Europe is around 1/100 000-1.5/100 000 [12]. Most GIST cases lack typical clinical symptoms and some patients experience gastrointestinal bleeding [3].

Most GISTs were benign and only 20%-30% of cases are malignant [13]. In our study, according to the modified NIH risk classification for primary GIST, over 60% of GIST cases were at very low-risk and low-risk. Previous studies reported that 27.05% of Chinese patients have high risk of GIST, similar to what was reported in Western countries [13]. Other reports have also suggested that the incidence of GISTs is similar in males and females, but increased predisposition in males was suggested [14]. Our results are in accordance with these reports; however, we also found that females were more likely to have high-risk GIST. The mean age at GIST diagnosis was about 60 years old. GISTs are occasionally detected in young adults, but it is extremely rare in children [15]. Our study revealed several cases of young adults who were diagnosed with GIST. This finding highlights the need for close attention to potential GIST incidence in the younger population. Most GISTs are located in the stomach (4-70%), small intestine (20-40%), colon and rectum (5-15%), and esophagus (5%) [16]. However, we also identified 10 cases (8.20%) in which the omentum and mesentery tissues were affected, which is a higher incidence than that of GIST in the small intestine (7 cases, 5.74%). GISTs can vary greatly in size, ranging from less than 1 cm to over 20 cm in diameter [17]. We also found that the size of GISTs may depend on the location of the tumor.

GISTs are known to originate from ICCs and they can express stemness-related markers, such as CD117 (c-kit) and CD34 [18]. ICCs act as pacemakers of gastrointestinal motility [19]. Reduction, injury, or loss of function of gastrointestinal tract ICCs may be associated with impaired gastrointestinal motility [20]. ICCs undergo apoptosis over time, and maintenance of ICCs networks is required for adequate regeneration

of healthy tissues [21]. The tyrosine kinase receptor c-kit (also named CD117) and its ligand stem cell factor (SCF) can have a significant promoter effect on the development, maturation, and phenotypic maintenance of ICCs [22].

Recent studies suggested that about 95% of GISTs are positive for CD117 (also named c-kit) and that 40-70% are positive for CD34 [23,24]; in agreement with these rates, the present study found that 115 (94.26%) and 111 GISTs cases (90.98%) were positive for CD117 and CD34, respectively, and that 109 cases (89.34%) were positive for both markers. Moreover, higher GIST risk was associated with a higher number of ICCs.

c-kit is a growth factor transmembrane receptor that is encoded by the proto-oncogene *KIT* [25]. Activation of c-kit by its ligands, such as SCF, enhances the development and growth of ICCs, but it can also contribute to several steps of the oncogenic process, including proliferation, adhesion, apoptosis, and differentiation, which are also involved in GIST development and growth [26,27].

CD34 was also found to be a specific marker in ICCs, with CD34-positive cells being common in GISTs. In our study, most GISTs were CD34-positive. The CD34 expression level is affected by the GIST location and kinase mutation status [28,29]. In GISTs, spindle variants usually lead to the expression of CD34 [30,31].

There are several limitations in our study. This research is a single-center clinical retrospective study, and its results need to be confirmed by multicenter clinical studies in the future.

Conclusions

Taken together, the results of the present study confirm that CD117/c-kit and CD34 are specific markers for GISTs and ICCs; and that CD117/c-kit and CD34 affect GIST risk. Moreover, higher GIST risk was associated with higher levels of ICCs.

Acknowledgments

We all thank for the staff in the Department of Pathology, Renmin Hospital, Hubei University of Medicine for assistance with this study.

References:

- Mazur MT, Clark HB. Gastric stromal tumors. Reappraisal of histogenesis. *Am J Surg Pathol*, 1983;7:507-19
- Burch J, Ahmad I. Cancer, gastrointestinal stromal (GIST). In: StatPearls. Treasure Island (FL): StatPearls Publishing, June 9, 2020
- Miettinen M, Lasota J. Gastrointestinal stromal tumors: Review on morphology, molecular pathology, prognosis, and differential diagnosis. *Arch Pathol Lab Med*, 2006;130:1466-78
- Vannucchi MG, Traini C. Interstitial cells of Cajal and telocytes in the gut: Twins, related or simply neighbor cells? *BioMol Concepts*, 2016;7:93-102
- Sanders KM, Ward SM. Interstitial cells of Cajal: A new perspective on smooth muscle function. *J Physiol*, 2006;576:721-26
- Foong D, Zhou J, Zarrouk A, et al. Understanding the biology of human interstitial cells of Cajal in gastrointestinal motility. *Int J Mol Sci*, 2020;21:4540
- Vanner S, Greenwood-Van Meerveld B, Mawe G, et al. Fundamentals of neurogastroenterology: Basic science. *Gastroenterology*, 2016 [Online ahead of print]
- Badalamenti G, Rodolico V, Fulforo F, et al. Gastrointestinal stromal tumors (GISTs): Focus on histopathological diagnosis and biomolecular features. *Ann Oncol*, 2007;18:vi136-40
- Joensuu H. Risk stratification of patients diagnosed with gastrointestinal stromal tumor. *Hum Pathol*, 2008;39(10):1411-19
- Sinicropo FA, Ruan SB, Cleary KR, et al. bcl-2 and p53 oncoprotein expression during colorectal tumorigenesis. *Cancer Res*, 1995;55:237-41
- Tanaka K, Iwamoto S, Gon G, et al. Expression of survivin and its relationship to loss of apoptosis in breast carcinomas. *Clin Cancer Res*, 2000;6:127-34
- Casali PG, Abecassis N, Aro HT et al, ESMO Guidelines Committee and EURACAN. Gastrointestinal stromal tumours: ESMO-EURACAN Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*, 2018;29:iv68-78
- Schaefer IM, Mariño-Enríquez A, Fletcher JA. What is new in gastrointestinal stromal tumor? *Adv Anat Pathol*, 2017;24(5):259-67
- Hamzaoui L, Medhioub M, Bouassida M, et al. Gastrointestinal stromal tumours (GISTs): A descriptive study on 29 cases. *Arab J Gastroenterol*, 2016;17:185-87
- Corless CL, McGreevey L, Haley A, et al. KIT mutations are common in incidental gastrointestinal stromal tumors one centimeter or less in size. *Am J Pathol*, 2002;160:1567-72
- Rubin BP, Singer S, Tsao C, et al. KIT activation is a ubiquitous feature of gastrointestinal stromal tumors. *Cancer Res*, 2001;61:8118-21
- Judson I, Bulusu R, Seddon B, et al. UK clinical practice guidelines for the management of gastrointestinal stromal tumours (GIST). *Clin Sarcoma Res*, 2017;7:6
- Bai C, Liu X, Xu J, et al. Expression profiles of stemness genes in gastrointestinal stromal tumor. *Hum Pathol*, 2018;76:76-84
- Wei R, Parsons SP, Huizinga JD. Network properties of interstitial cells of Cajal affect intestinal pacemaker activity and motor patterns, according to a mathematical model of weakly coupled oscillators. *Exp Physiol*, 2017;102:329-46
- Foong D, Zhou J, Zarrouk A, et al. Understanding the biology of human interstitial cells of Cajal in gastrointestinal motility. *Int J Mol Sci*, 2020;21:4540
- Gibbons SJ, Giorgio RD, Fausone-Pellegrini MS, et al. Apoptotic cell death of human interstitial cells of Cajal. *Neurogastroenterol Motil*, 2009;21:85-93
- Rich A, Miller SM, Gibbons SJ, et al. Local presentation of Steel factor increases expression of c-kit immunoreactive interstitial cells of Cajal in culture. *Am J Physiol Gastrointest Liver Physiol*, 2003;284(2):G313-20
- Koo DH, Ryu MH, Kim KM, et al. Asian consensus guidelines for the diagnosis and management of gastrointestinal stromal tumor. *Cancer Res Treat*, 2016;48:1155-66
- Nishida T, Blay JY, Hirota S, et al. The standard diagnosis, treatment, and follow-up of gastrointestinal stromal tumors based on guidelines. *Gastric Cancer*, 2016;19:3-14
- Corless CL. Gastrointestinal stromal tumors: What do we know now? *Mod Pathol*, 2014;27:S1-16
- Hirota S, Isozaki K, Moriyama Y, et al. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science*, 1998;279:577-80
- Wu CE, Tzen CY, Wang SY, Yeh CN. Clinical diagnosis of gastrointestinal stromal tumor (GIST): From the molecular genetic point of view. *Cancers (Basel)*, 2019;11:679

28. Bure I, Braun A, Kayser C, et al. The expression of hematopoietic progenitor cell antigen CD34 is regulated by DNA methylation in a site-dependent manner in gastrointestinal stromal tumours. *Int J Cancer*, 2017;141:2296-304
29. Miettinen M, Virolainen M, Maarit-Sarlomo-Rikala. Gastrointestinal stromal tumors-value of CD34 antigen in their identification and separation from true leiomyomas and schwannomas. *Am J Surg Pathol*, 1995;19:207-16
30. Miettinen M, Lasota J. Gastrointestinal stromal tumors: Pathology and prognosis at different sites. *Semin Diagn Pathol*, 2006;23:70-83
31. Miettinen M, Sobin LH, Lasota J. Gastrointestinal stromal tumors of the stomach: A clinicopathologic, immunohistochemical, and molecular genetic study of 1765 cases with long-term follow-up. *Am J Surg Pathol*, 2005;29:52-68