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Differential diagnosis of gastrointestinal stromal tumors versus leiomyomas by special stains

Shiwei Zhang[1], Pan Qin[1] and Hongliang Ji[1*]

Abstract

The objective of the study was to investigate whether special stains can differentiate gastrointestinal stromal tumors (GISTs) and gastrointestinal leiomyomas (GILs). In this retrospective study, 39 cases of GISTs (diameter, 0.2–8.8 cm) and 75 cases of GILs (diameter, 0.2–4.5 cm) were recruited, all biopsy specimens were obtained by endoscopic submucosal dissection (ESD) and endoscopic mucosal resection (EMR) excision, and the depth of excision included the whole mucosa, mucosal myometria, and most submucosa. GISTs and GILs were the most common types of mesenchymal tumors found anywhere along the gastrointestinal (GI) tract, from the esophagus to the rectum. GISTs were often associated with a higher risk of malignancy. In this study, the gender, age of onset, size and sites of the lesions, together with the number of mucosal or lamina propria lesions all have significant differences, nevertheless, there was no significant difference in cell morphology of GISTs and GILs tested by hematoxylin eosin (H&E) stain, and all showed low echo areas by EUS examination. In this retrospective study, the GISTs and GILs had been diagnosed by immunohistochemistry combined with clinical morphology. Subsequently, special stains including Masson's trichrome (MT) stain, Alcian blue periodic acid-Schiff (AB-PAS) stain (pH 2.5), Wright-Giemsa (W-G) stain and periodic acid-Schiff (PAS) combined with diastase periodic acid-Schiff (D-PAS) stains were also applied in the diagnosis, the retrospective study results showed that 92.3% GISTs were stained blue with MT stain, 97.3% GILs were stained red with MT stain ($P < 0.01$), almost all GISTs were PAS-negative (light purple), in contrast, all GILs were PAS-positive (rose red) ($P < 0.01$), all of these experiments set control using the blood vessels stained by MT and AB-PAS stains. Nevertheless, there was no significant difference between GISTs and GILs stained by W-G stain. These obvious and meaningful differential results were also confirmed in the detection of new GISTs and GILs cases using MT and AB-PAS stains. In conclusion, MT and AB-PAS stains could also identify GISTs and GILs cases, particularly, AB-PAS was more sensitive and more specific, providing a more cost-effective, simple, and high sensitivity and specificity inspection methods, which should be noticed and widely used in the future, especially in resource-limited grass-roots testing institution or in cases with inconclusive immunostains or insufficient material.

Keywords Gastrointestinal stromal tumor, Gastrointestinal leiomyoma, Malignancy, Special stain

Introduction

Nowadays, Gastrointestinal stromal tumors (GISTs) have become increasingly common. The incidence of GISTs worldwide is estimated to be around 7 to 19 cases per million annually [1]. GISTs usually originate from the muscularis mucosa or muscularis propria, which are malignant or potentially malignant subepithelial tumors

*Correspondence:
Hongliang Ji
jinhl90@163.com
[1] Department of Pathology, Hubei Provincial Hospital of Integrated Chinese and Western Medicine, Wuhan 430000, China

in the gastrointestinal (GI) tract. This category also includes non-GIST subepithelial tumors such as leiomyomas, schwannomas, leiomyosarcomas, and others [2]. GISTs are believed to derive from the interstitial cells of Cajal (ICCs), which are spindle-shaped mesenchymal cells found in the muscle layer of the GI tract [3–5].

It has been reported that GISTs, along with gastrointestinal leiomyomas (GILs), can be found anywhere from the esophagus to the rectum in the GI tract. However, the distribution is not equal, with more than half of GISTs occurring in the stomach, followed by the small intestine. GISTs in the colon, rectum, and esophagus are quite rare, they also include extraintestinal GISTs such as primary GISTs of the prostate gland [6–8]. Our current research is consistent with these well-established reports, for example, we found many peritoneal tubercles were GISTs in our daily diagnostic work and we also found that both lesions occur in different parts of the gastrointestinal tract as shown in Table 1. GISTs have the potential to become malignant, therefore, it seriously threatens human life and health [9, 10]. Researchers have identified two oncogene mutations, namely the tyrosine kinase receptor KIT and/or platelet-derived growth factor receptor-α (PDGFR-α), which are thought to play a major role in the development of GISTs [11–17]. There are also many relevant studies on the treatment of corresponding stromal tumors.

The most common symptoms of GISTs include gastrointestinal bleeding, such as acute melena and hematemesis, as well as abdominal pain, distension, and discomfort [18]. However, a small portion of GISTs patients may be asymptomatic and are only discovered incidentally during postmortem autopsy or surgery for other conditions. While endoscopic ultrasonography (EUS) can detect GISTs, they could only provide a reference, yet not a definitive diagnosis. Recently, surgical resection has been recommended for GISTs larger than 2.0 cm or all GISTs, due to their malignant potential. Therefore, techniques such as endoscopic submucosal dissection (ESD) and

Table 1 Clinical data of the gastrointestinal stromal tumors (GISTs) and gastrointestinal leiomyomas (GILs) cases

	GISTs ($n=39$)	leiomyomas ($n=75$)	Statistical analysis
Gender			*
Male (n)	14	44	
Female (n)	25	31	
Age rage, median (years)	38—86, 60	33—79, 54	***
Lesion size range, median (cm)	0. 2—8.8, 1.2	0.2—4.5, 0.6	***
Lesion site of digestive tract			***
Esophagus (n)	0	42	
Cardia (n)	1	4	
Stomach (n)	37	15	
Pars descendens duodeni (n)	0	1	
Lower digestive tract (n)	1	13	
Form of organization			ns
Spindle cell (n)	38	75	
Epithelioid cell(n)	1	0	
Lesion location			***
Lamina musculi propria	37	8	
Muscularis mucosae	2	67	
MT stain			***
Blue	36	2	
Red	3	73	
AB-PAS (pH 2.5) stain			***
PAS negative (light purple)	39	0	
PAS positive (rose red)	0	75	
Immunohistochemical experiments			***
CD117 & DOG1 (+)	39	0	
CD117 & DOG1 (-)	0	75	

"ns" indicates no significant difference when $P>0.05$, "*" indicates $P<0.05$, "**" indicates $P<0.01$, and "***" indicates $P<0.001$ with significant difference, "+" means positive, "-" means negative

endoscopic mucosal resection (EMR) are widely used in clinical treatments to enhance diagnostic accuracy.

Pathologically, it is still challenging to definitively distinguish GISTs from other gastrointestinal mesenchymal tumors, particularly with GILs when just using histopathological examination alone with hematoxylin and eosin (H&E) stain. Immunohistochemistry (IHC) is essential for the diagnosis and involves testing for markers such as CD117 (KIT), DOG1, Desmin, CD34, S-100 or smooth muscle actin (SMA). While H&E stain is the most commonly used method worldwide due to its simplicity, as a matter of fact, other histological stains can also be used to highlight different tissue components and provide additional diagnostic information [19]. For example, Masson's trichrome (MT) stain can identify collagen fibers and muscle fibers, and Alcian blue periodic acid-Schiff (AB-PAS) stain can show glycogen and intestinal metaplasia. Additionally, periodic acid-Schiff (PAS) combined with diastase periodic acid-Schiff (D-PAS) stains can identify glycogen and neutral mucus. Wright-Giemsa (W-G) stain can be used for cytological stain and diagnosis of benign and malignant cells.

Given that endoscopic examination and computed tomography (CT) have the characteristics of low specificity [20], and IHC combined with pathogenic gene analysis are too expensive with time-consuming, the accurate and timely diagnosis of GISTs versus GILs is extremely crucial for the prompt treatment of patients. In our study, we proposed a new detection approach using special stains for the differential diagnosis of GISTs versus GILs based on our daily work experience accumulation and experimental verification. This approach is cost-effective, simple, and has high sensitivity and specificity, making it a valuable inspection method for accurate diagnosis.

Materials and Methods

Study subjects

Up to 114 patients bearing GISTs and GILs were recruited from December 2014 to October 2023 in the Department of Pathology, Hubei Provincial Hospital of Integrated Chinese and Western Medicine, and all the diseases were diagnosed according the diagnostic guides [21] by two experienced diagnosticists. All biopsy specimens were obtained by endoscopic forceps, the GISTs group included 14 men and 25 women ranged from 38 to 86 years old (mean 61.6 ± 10.9 years). The GILs group included 44 men and 31 women ranged from 33 to 79 years old (mean 54.3 ± 10.0 years) in Table 1. In addition to retrospective trials, there were confirmatory trials, including 28 GISTs and 26 GILs cases from November 2023 to October 2024 as shown in supplementary material Table 1.

The criteria of diagnose for GISTs and GILs were as follows: i) patients with hypoechoic tumor below the mucosal layer of the gastrointestinal tract by EUS examination, and all biopsy specimens were obtained by ESD and EMR excision, and the depth of excision included the whole mucosa, mucosal myometria, and most submucosa.; ii) patients with typical symptoms of acute melena and hematemesis abdominal pain, distension, and discomfort; iii) GISTs patients with positive c-kit (CD117) and/or DOG1 antibodies; iv) GILs patients with positive SMA antibody, negative for CD117 and DOG1 antibodies; v) patients with schwannoma, leiomyosarcoma, et al. were excluded.

Biopsy specimens

All biopsy specimens were surgically resected by ESD or EMR, and fixed by 10% neutral buffered formalin (NBF) immediately, then made tissue wax blocks through dehydration and embedded in paraffin successively. Tissue section was 3 microns thickness attaching to adhesive slides and stained with H&E, IHC, MT, AB-PAS, W-G, and PAS, D-PAS respectively.

Antibodies and special stains

The primary antibodies in this study were ready-to-use type and all were purchased from Fu Zhou Mai Xin company, including CD117 (YR145); DOG1 (SP31); SMA (1A4); and S-100 (Rabbit polyclonal antibody). The secondary antibodies labeled with horseradish peroxidase (HRP) were from Xia Men Tong Ling company named blockers (Yellow) and polymers (Red) (DD23-3, #III). Special stains including MT (C230801), AB-PAS (C231001), W-G (C221011), and amylase reagent (ready-to-use type) were all purchased from Zhu Hai Baso® company.

Experimental procedures

H&E stain process

The H&E stain processes were following the general operating instructions: 3 microns thickness paraffin sections were dewaxed to water after heating for 0.5 h at 65 ℃ and soaked in gradient alcohol, hematoxylin stained for 10 min, running it under water for 2 min, then 0.5% hydrochloric acid alcohol differentiation for 2 s, rinsing under running water for a few minutes then eosin stained for 1.5 min, gradient alcohol dehydration (95% and anhydrous ethanol), transparency and seal was performed for the final microscopic examination.

IHC stain process

A two-step IHC technique (iVision method invented by Fujian Xiamen Tongling company) was used in this study, paraffin sections were dewaxed to water after heating

for 1 h at 65 ℃, heat mediated antigen retrieval was performed with citrate buffer (pH 6.0), then 3% Hydrogen Peroxide blocked endogenous peroxidase for 10 min at room temperature, after that, primary antibodies mentioned above were incubated for 50 min at 37 ℃. The secondary antibodies were incubated for 20 min at room temperature respectively, each steps were washed with 1 × phosphate buffer adding tween 20 (PBST, 1:2000, pH 7.4—7.6) buffer, 3,3'-diaminobenzidine (DAB) treated as the substrate stained for 5–6 min and terminated the reaction with running water, cell nuclei was restained with hematoxylin for 1–2 min, Finally, the sections were dehydrated with anhydrous ethanol and sealed with neutral gu

MT, AB-PAS, W-G, PAS and D-PAS stain process

Special stains had the properties of different dyes adsorbing to various tissue components to distinguish between different molecules. For instance, MT stain having the positive and negative charge characteristics can be used to distinguish collagen fibers (blue, GISTs) versus muscle fibers (red, GILs), AB-PAS (pH 2.5) stain could also differentiate GISTs (PAS negative, light purple) from GILs (PAS positive, rose red) by different adsorption.

MT stain 1) Paraffin sections were dewaxed to water after heating for 0.5 h at 60 ℃, mixed equal proportions of weigert hematoxylin A and B before dyeing for 5—10 min. 2) Washed away the dye using running water, and processed with 0.5% hydrochloric acid and alcohol for a few seconds, then ran the water for a few minutes. 3) The ponceau acid fuchsin solution dyed for 10 min, then flushed with running water. 4) The phosphomolybdic acid dyed for 5 min, shaked off the dye and do not wash with water. 5) Dyed with aniline blue for 3—5 min, and treated directly with 1% glacial acetic acid until no blue came out. 6) Gradient alcohol dehydration and neutral gum seal were performed in the end.

AB-PAS stain 1) Paraffin sections were dewaxed to water after heating for 0.5 h at 60 ℃. 2) Alcian blue (pH 2.5) dye solution, periodic acid and schiff were processed each for 10 min, rinsed with distilled water and dried with cold air before dyeing. 3) Mayer hematoxylin dyed for 5 min, running water washed and gradient alcohol dehydration and neutral gum seal were performed in the end.

W-G stain 1) Paraffin sections were dewaxed to water after heating for 0.5 h at 60 ℃. 2) Dropwise add Wright-Giemsa stain A 0.5—0.8 ml for 1 min. 3) The volume of Wright-Giemsa stain A called phosphate buffer (pH 6.8) was two or three times of A, well mixed A and B then stained for 5—10 min. 4) Dehydrated and transparent, sealed with neutral gum.

PAS and D-PAS stain 1) Paraffin sections were dewaxed to water after heating for 0.5 h at 60 ℃. 2) One tissue slice was incubated by diastase, the other one was incubated by PBS buffer, then incubate at 37 ℃ for 30 min. 3) PBS washed for a few seconds, and the subsequent dyeing step was the same as the periodic acid dyeing of the AB-PAS dyeing process.

The control of MT and AB-PAS stain were operated using the blood vessels of a gastric tissue. The special stain results and IHC analysis were interpreted strictly in accordance with the guidelines by at least two diagnosticians, and the data analysis of the verification experiments using Graphpad Prism 9.5, including two-way analysis of variance (two-way ANOVA), Fisher's exact test for nonparametric difference test, such as gender, lesion location, etc. T test for the difference test of parameters conforming to normal distribution, for example, age, size of lesion.

Results

Clinical data analysis

GISTs and GILs may occur anywhere in the digestive system, and the clinical symptoms were diverse from each other, for instance, several patients may feel difficulty in swallowing, hemorrhage of digestive tract, chest pain et al., some even have no symptoms of discomfort. The size of GISTs ranged from 0.2 to 8.8 cm (mean 2.0 ± 2.0). One case occurred in the cardia, thirty-seven of lesions in the stomach, including body, angle, fundus and antrum of stomach. one case occurred in the lower digestive tract, such as small intestine, however, no cases have occurred in the esophagus. For GILs, the size raged from 0.2 to 4.5 cm (mean 0.8 ± 0.8). Forty-two cases occurred in the esophagus, four cases occurred in the cardia, fifteen cases occurred in the stomach, including body, angle and fundus of stomach. One occurred in the pars descendens duodeni, thirteen cases occurred in the lower digestive tract, including duodenum and colorectum. The gender, age of onset, the lesions size and sites, together with the number of mucosal or lamina propria lesions all have significant differences, nevertheless, there was no significant difference in cell morphology of tumor tissue as shown in Table 1.

Histopathological diagnosis of GISTs versus GILs

No matter where the lesions occurred, EUS showed low echo areas for GISTs and leiomyomas. Almost all GISTs originated from the musculi propria, on the contrary, most GILs originated from the mucosal muscle layer [22].

Histologically, 39 patients were diagnosed with GISTs, 75 patients were diagnosed with GILs, there was no significant difference between the two lesions stained by H&E, almost all GISTs were diffuse strong positive for CD117 and DOG1, but negative for S-100 as shown in Fig. 1, the special marker molecules for schwannomas. Almost all GILs were diffuse strong positive for SMA, but negative for CD117 and DOG1 as shown in Fig. 2.

Special stains diagnosis of GISTs versus GILs

The GISTs approximately account for 92.3% were stained blue with MT stain ($P<0.01$) and all of the GISTs cases were PAS negative (light purple) for AB-PAS ($P<0.01$) an shown in Fig. 3. In contrast, 97.3% of GILs were stained red with MT stain ($P<0.01$) and almost all were PAS positive (rose red) for AB-PAS stain ($P<0.01$) as shown in Fig. 4. The blood vessels of a gastric tissue was used as control in MT and AB-PAS stain as shown in Fig. 4. PAS and D-PAS stain showed the rose red (PAS positive) was glycogen, a characteristic component of leiomyomas as shown in figure S1, however, the W-G stain was indistinguishable between GISTs and GILs as shown in Figs. 3 and 4.

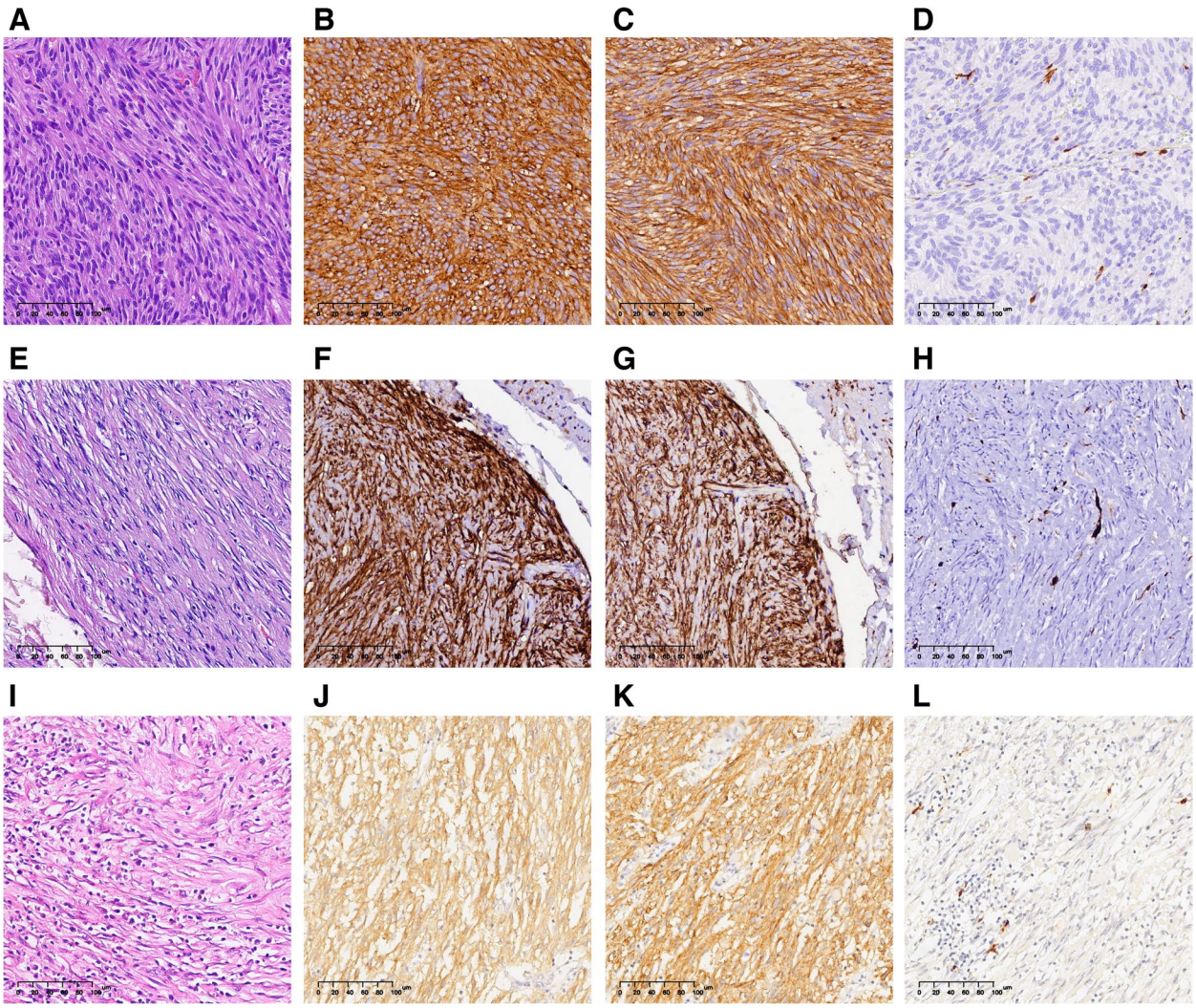

Fig. 1 Hematoxylin eosin stain and Immunohistochemical diagnosis GISTs. **A** H&E staining in cardia of stomach GIST. **B**-**D** The characteristic markers of stromal tumors such as CD117 (**B**) and DOG1 (**C**) were diffuse strong positive, but negative for S-100 (**D**) in cardia of stomach GIST. **E** H&E staining in fundus of stomach GIST. **F**-**H** CD117 (**F**) and DOG1 (**G**) were diffuse strong positive, but negative for S-100 (**H**) in fundus of stomach GIST. **I** H&E staining in small intestine GIST. **J**-**L** CD117 (**J**) and DOG1 (**K**) were diffuse strong positive, but negative for S-100 (**L**) in small intestine GIST. (Magnification: 200 ×).

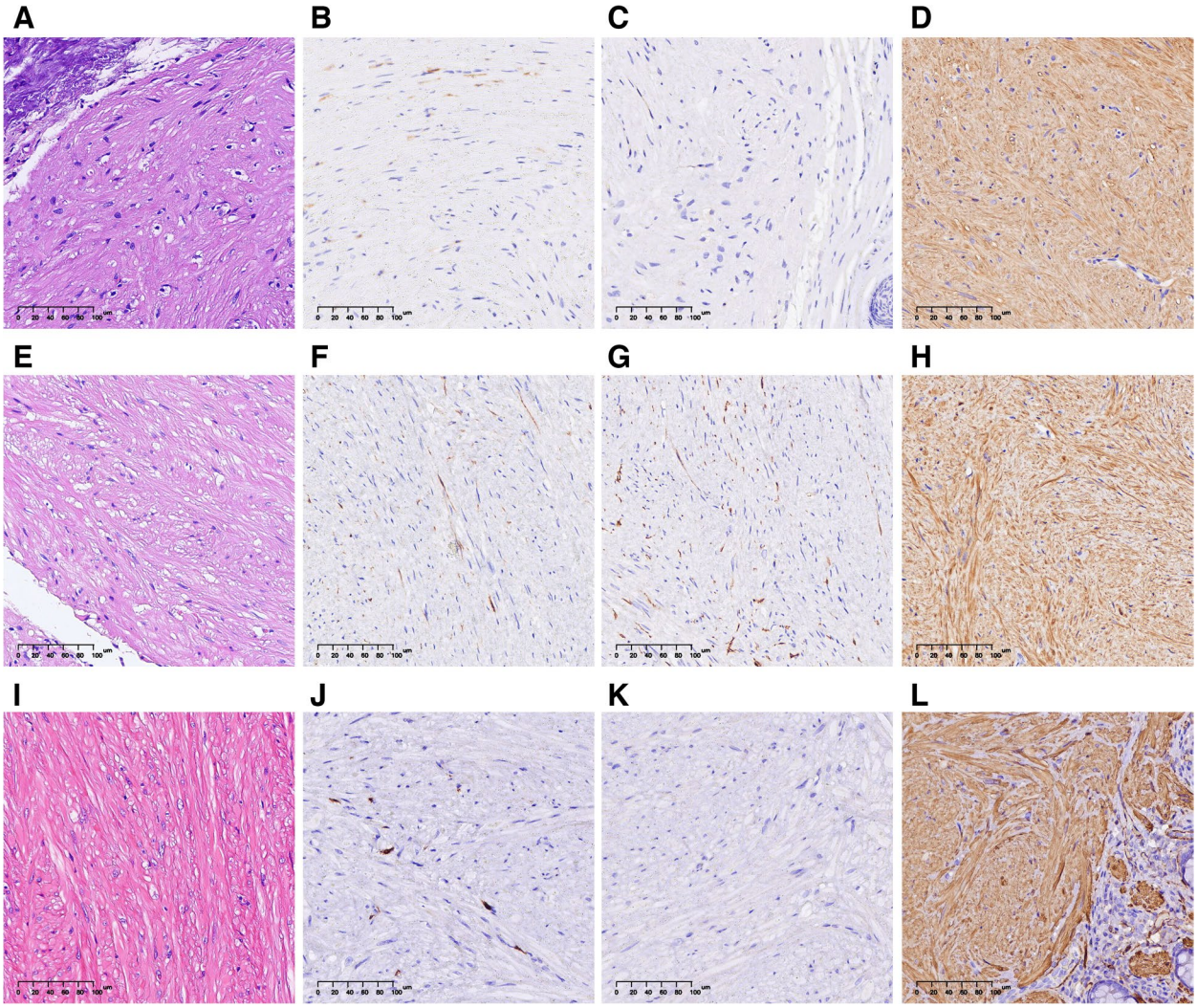

Fig. 2 Hematoxylin eosin stain and Immunohistochemical diagnosis GILs. **A** H&E staining in esophagus GIL. **B**-**D** The characteristic markers of smooth muscle such as SMA (**D**) were diffuse strong positive, but were negative for CD117 (**B**) and DOG1 (**C**). **E** H&E staining in body of stomach GIL. **F**-**H**). CD117 (**F**) and DOG1 (**G**) were negative, but diffuse strong positive SMA (**H**) in body of stomach GIL. **I** H&E staining in rectum GIL. **J**-**L** CD117 (**J**) and DOG1 (**K**) were negative, but diffuse strong positive SMA (**L**) in rectum GIL. (Magnification: 200 ×).

Statistical analysis of the results of special stain and IHC diagnosis of GISTs versus GILs.

It has been verified by repeated experiments that the GISTs approximately account for 92.3% were stained blue, 97.3% of GILs were stained red with MT stain ($P < 0.01$), all of the GISTs cases were PAS negative (light purple), and almost all GILs were PAS positive (rose red) ($P < 0.01$). Almost all GISTs were diffuse strong positive for CD117 and DOG1, on the contrary, CD117 and DOG1 were negative in almost all GILs as shown in Table 1 and Fig. 5.

Discussion

Among the gastrointestinal mesenchymal tumors, GISTs and GILs were the most common submucosal lesions worldwide [23]. They could happen anywhere in the digestive tract and GISTs possessed malignant transformation potence, whereas, GILs were benign lesion, the difference in size and shape of them were significant, and the incidence was not associated with age or sex. With the increasing update of detection technologies, endoscopic ultrasonography (EUS) and computed tomography (CT) were always the dominated detection methods,

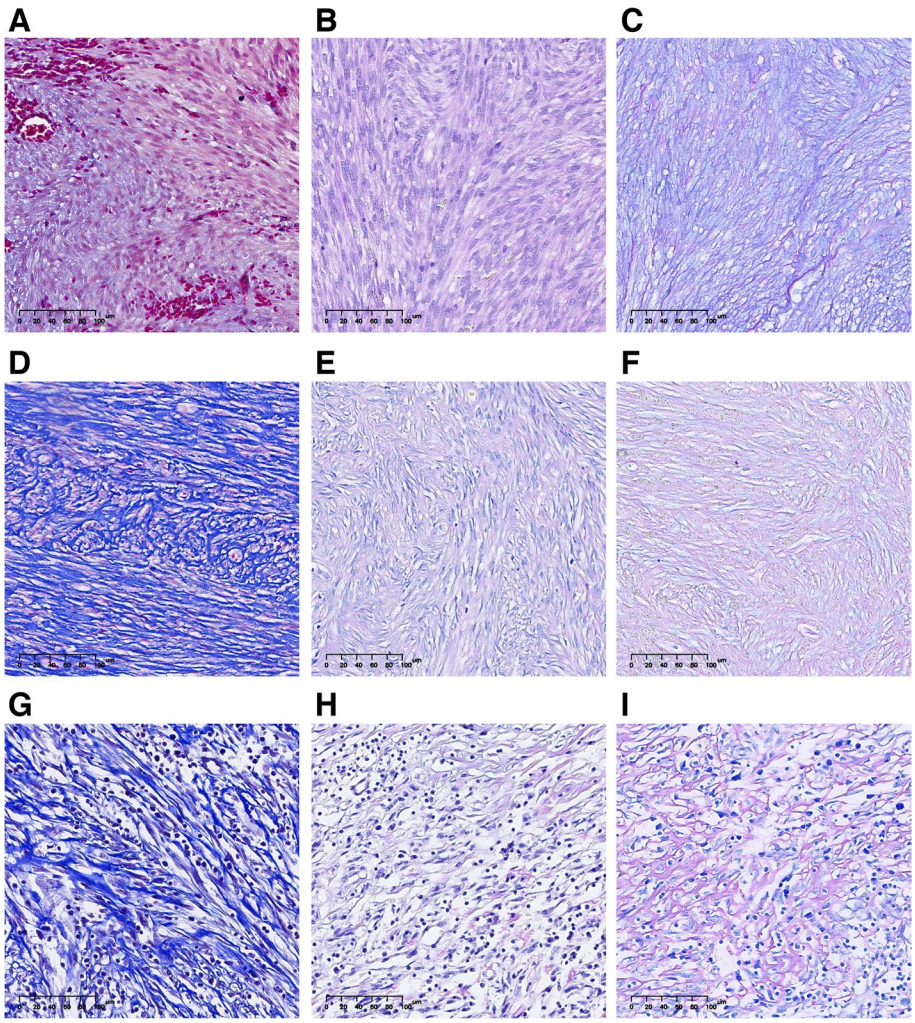

Fig. 3 Special stains including MT, AB-PAS, W-G diagnosis GISTs. **A**-**C** GIST in cardia was stained red (**A**) by MT stain, AB-PAS stain was PAS-negative (**B**), (**C**) was W-G stain. **D**-**F** GIST in fundus of stomach was stained blue (**D**) by MT stain, AB-PAS stain was PAS-negative (**E**), (**F**) was W-G stain. **G**-**I** GIST in small intestine was stained blue (**G**) by MT stain, AB-PAS stain was PAS-negative (**H**), (**I**) was W-G stain. (Magnification: 200 ×).

even combined with AI-assisted diagnostic technology [24]. The pathological diagnosis was usually morphology together with IHC and genetic testing [25], however, the economic and time cost were relatively higher.

Immunohistochemical experiments with multiple indicators need to cut multiple tissue sections, especially when the number of fine needle puncture tissues or cytology specimens is relatively small, it is not guaranteed to continuously cut sections with the same target cells. In this case, special staining can play an advantage, because it usually only needs a single section to diagnose the disease. Nowadays, the incidence of GISTs and GILs was still increasing, the morbidity were 34.2% (39/114) and 65.8% (75/114) in our current research as shown in Table 1, respectively, new GISTs and GILs cases are still on the rise, thus, timely and accurate diagnosis is particularly important for treatment, the primary treatment option has always been surgical removal [26], whereas, GISTs had postoperative recurrence risk.

The basic principle of special stains to identify different components

In the present study, a new detection technique, special stains, was used to distinguish GISTs and GILs, which was easily overlooked, because of IHC analysis and molecular examinations were the dominanted methods. On account of the main components of GISTs (collagen fibers secreted by stromal cells) and GILs (smooth muscle fibers) were significant different, which were rarely reported in domestic and foreign researches, special stain technology was to dye different components into different colors by the specific adsorption of different

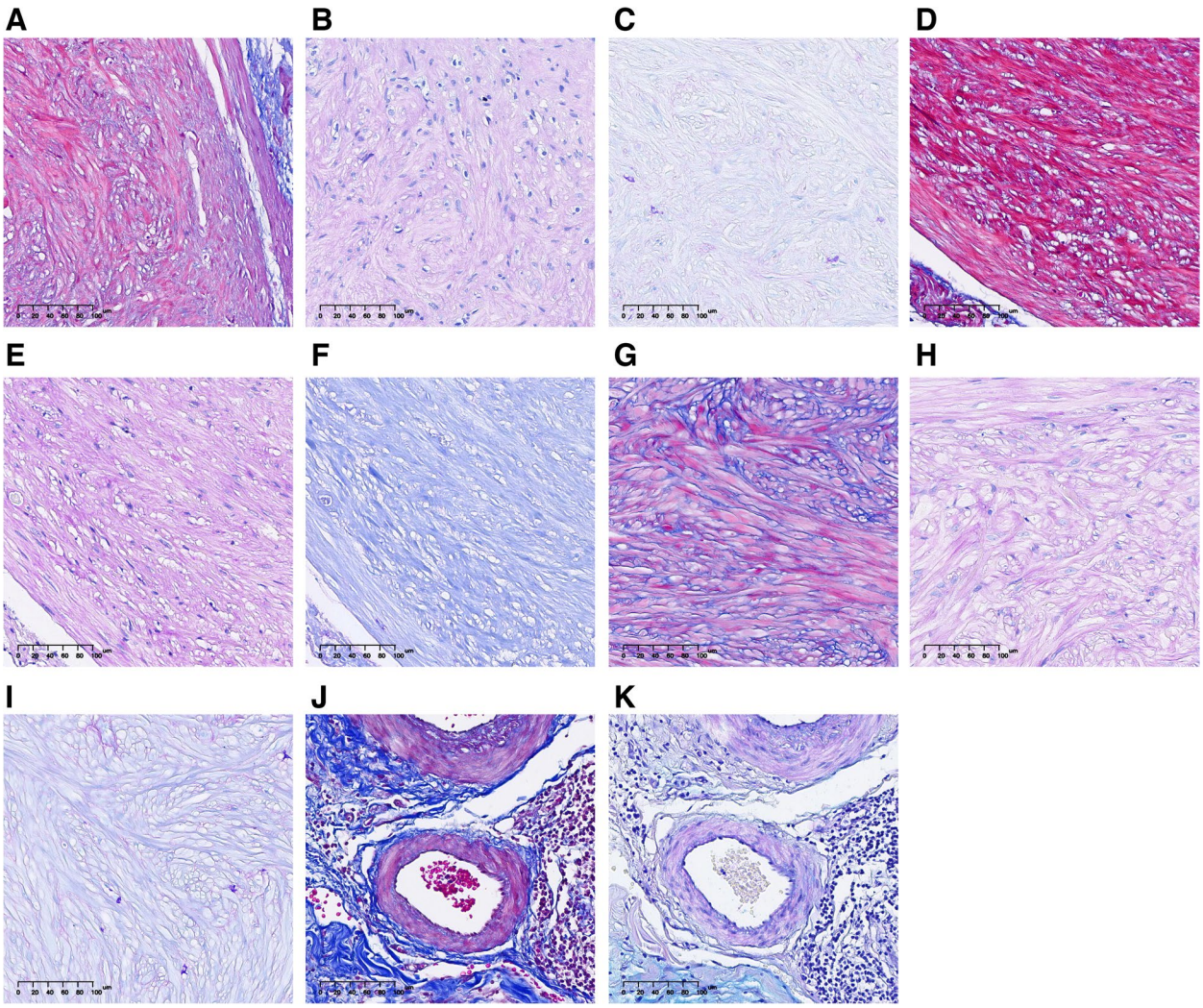

Fig. 4 Special stains including MT, AB-PAS, W-G diagnosis GILs. **A**-**C** GIL in esophagus was stained red (**A**) by MT stain, AB-PAS stain was PAS-positive (**B**), (**C**) was W-G stain. **D**-**F** GIL in body of stomach was stained red (**D**) by MT stain, AB-PAS stain was PAS-positive (**E**), (**F**) was W-G stain. **G**-**I** GIL in rectum was stained red (**G**) by MT stain, AB-PAS stain was PAS-positive (**H**), (**I**) was W-G stain. **J**-**K** The control of muscle fibers of the blood vessels are stained red by MT stain (**J**), PAS positive in (**K**), all as shown by the red arrow. The collagen fibers around the blood vessels are stained blue by MT stain as shown by the black arrow in (**J**) and PAS negative as shown by the black arrow in (**K**). (Magnification: 200 ×).

stains to distinguish them, and there is no report at home or abroad about this new finds from the daily pathological diagnosis.

The basic principle of MT stain

MT stain was related to the size of anionic dye molecules and tissue penetration: small molecular weight like ponceau dye, can easily penetrate the dense structure and low permeability of the tissue to be red, like muscle fibers, while large molecular weight can only enter the loose structure and high permeability of the tissue such as collagen fibers. On account of the molecular weight of aniline blue was very large, and contained alkaline amino acids, the collagen fibers appeared blue by aniline blue of MT stain. Consequently, the end results were that 92.3% (36/39) GISTs were stained blue and 97.3% (73/75) GILs were stained red ($P<0.01$), individual differences in results were due to collagen fibrogenesis or collagen fibrogenesis. To sum up, GISTs were mainly composed of stromal cells, which can secrete both collagen fibers and muscle fibers and ultimately lead to the particularity of individual cases.

The basic principle of AB-PAS stain

AB-PAS stain was a method to detect the distribution and content of glycogen in cells, the dyeing principle was

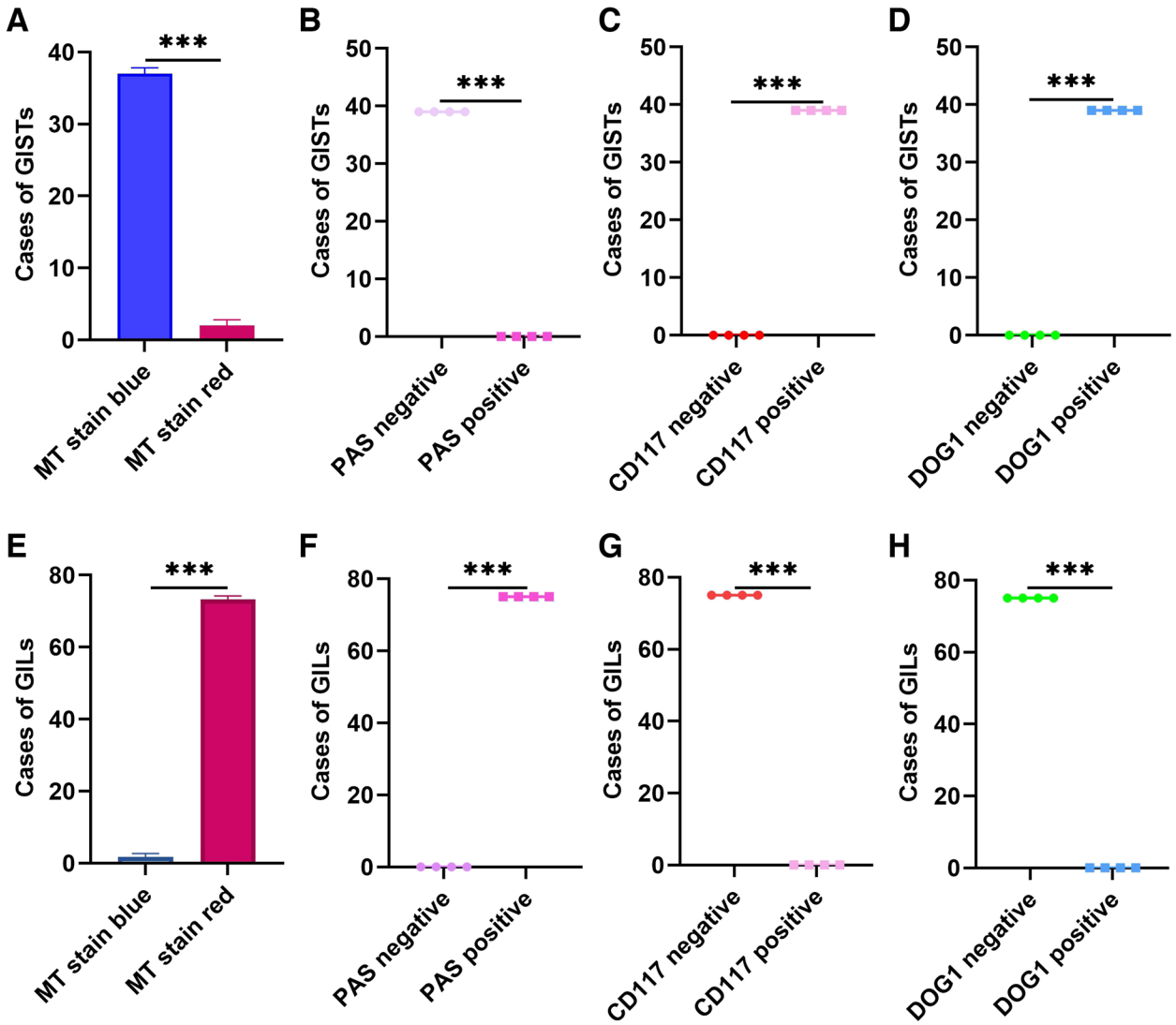

Fig. 5 Statistical analysis of the results of special stain and IHC diagnosis. **A** The bar chart showed about 92.3% of GISTs were stained blue with MT stain. **B** All of the GISTs were negative for AB-PAS stain. **C**-**D** CD117 (**C**) and DOG1 (**D**) were diffuse strong positive in almost all GISTs. **E** 97.3% of GILs were stained red with MT stain. **F** Almost all GILs were positive for AB-PAS stain. **G**-**H** CD117 (**G**) and DOG1 (**H**) were negative in all GILs. ("***" indicates P < 0.001 with significant difference.)

that AB-PAS stain consisted of Alcian Blue and Periodic Acid-Schiff (PAS) stain. Alcian Blue was an acidic stain mainly used to stain acidic polysaccharides such as heparin sulfate and acid mucopolysaccharide. PAS stain was a selective stain method used to detect the presence of glycogen in tissues. Because GILs were rich in smooth muscle, and their contraction requires the consumption of a lot of energy released by glycogen, the glycogen rich in them can eventually be stained rose red by PAS, but GISTs were absence of this specificity, it was verified by PAS and D-PAS stain as shown in figure S1. Consequently, AB-PAS stain positive 100% (75/75) ($P<0.01$) in GILs cases, in contrast, GISTs were AB-PAS negative (light purple) for all the cases studied. It should be added that the special stain results were consistent between GISTs in different parts of the digestive tract, and the special stain results were also consistent between GILs in different parts of the digestive tract.

The basic principle of W-G stain

W-G stain was a modified Romanowsky staining technique used to stain blood and bone marrow smears. Cell staining involves physical adsorption and chemical affinity that allows the stain to penetrate and remain inside the cell. Due to the different chemical composition of each cell and its components, their affinity for acidic

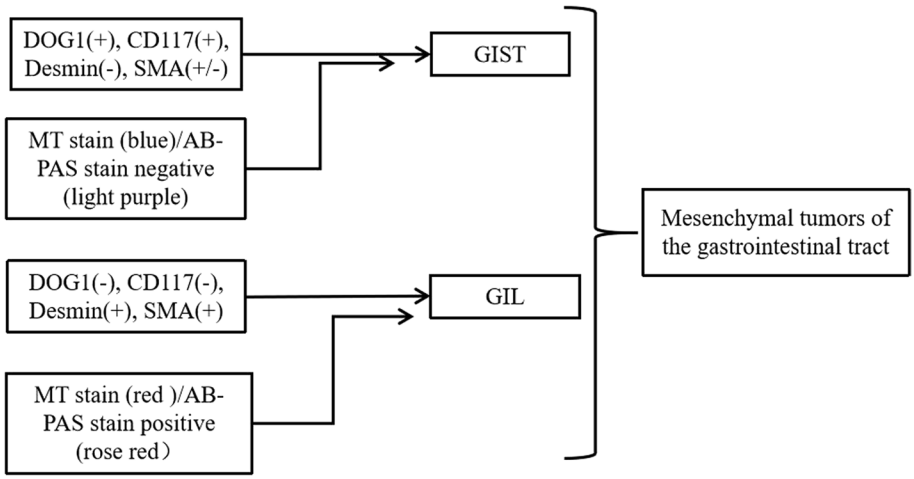

Fig. 6 The flow chart for differential diagnosis GISTs versus GILs by IHC and special stains, including MT and ABPAS stains. Generally, GISTs were DOG 1 and CD117 diffuse strong positive, yet, Desmin was absolute negative, and SMA was negative or positive in several GISTs cases, for GILs, Desmin and SMA were diffuse strong positive, yet, DOG 1 and CD117 were absolute negative. Furthermore, New diagnostic methods, GISTs were blue by MT stain, and AB-PAS negative (light purple), on the contrary, GILs were red by MT stain, and AB-PAS positive (rose red). MT and AB-PAS stain all could can be identified GISTs and GILs instead of IHC stain, individually. Whereas, AB-PAS stain might be much more sensitive than MT stain.

staining (eosin) and basic staining (methylene blue) for this kit varies greatly. In this study, there was no significant difference in staining between GISTs and GILs, but it could stain mast cells and distinguish between tumor and non-tumor stroma.

In conclusion

To sum up this retrospective study present a new method for the diagnosis of GISTs and GILs, MT and AB-PAS stains could also diagnose these two tumors, furthermore, AB-PAS stain was more sensitive and more specific, which were also confirmed in the detection of new GIST and GIL cases as shown in supplementary material Table 1. The research achievement could provide more cost-effective, simple, and high sensitivity and specificity inspection methods, which may instead of IHC analysis in the future, especially in resource-limited grass-roots testing institution, that is, MT and AB-PAS (much more specificity) could be a pre-diagnosis of GISTs and GILs in the absence of immunohistochemical conditions, the diagnostic flow chart showed as Fig. 6, it should be noted that the ultimate accurate diagnosis should must combine with clinical signs and symptoms.

The special staining reagents MT and AB-PAS (pH 2.5) used in this study are existing commercial reagents, which have differences in the diagnosis of gastrointestinal stromal tumor and leiomyoma. We hope to communicate with the developer of staining reagents in the future to see if the staining ingredients or pH value can be improved to make these differences more significant. When the experimental conditions are relatively backward or the specimens are too small for immunohistochemical or molecular detection, it is entirely possible to consider the use of special staining techniques for disease diagnosis.

In spite of this study presents a new detection method for the identification of GISTs and GILs, after all, this is only a retrospective study, the conclusion still needs to be further verified in daily work until it became a commonly used clinical diagnostic method. In the future, special stain technology will be combined with artificial intelligence assisted diagnosis technology to contribute to the precision diagnosis and treatment of gastrointestinal tumors.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12876-024-03511-5.

Supplementary Material 1 .

Supplementary Material 2.

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Authors' contributions

Thanks for the support of Hubei Provincial Hospital of Integrated Chinese and Western Medicine. None of the authors have a conflict of interest, Shiwei Zhang designed the research idea and afforded guidance, Pan Qin participated in the revision of the first draft of the paper, and Hongliang Ji mainly responsible for related experiments and analysis, mapping, typeset the resulting pictures, and wrote this article.

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Data availability
Data is provided within the manuscript or supplementary information files, and experimental data can be obtained from Hongliang Ji, E-mail:jinhl90@163.com.

Declarations

Ethics approval and consent to participate
All the recruits of this present study were informed and have written informed consent to participate the research, meanwhile, this present study was approved by the ethics committee of Hubei Provincial Hospital of Integrated Chinese and Western Medicine.

Consent for publication
All participants were informed and consented to the publication of the results of the study. This present manuscript has not been published or accepted for publication, and the department of pathology, Hubei Provincial Hospital of Integrated Chinese and Western Medicine was fully aware of this submission, and was consent for publication.

Competing interests
The authors declare no competing interests.

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