

Article

Reappraising Cytoplasmic WT1 in Gastrointestinal Stromal Tumors: Immunophenotypic Patterns and Diagnostic Context

Mahi Balcı *, Zeynep Kurt and Merva Aydemir Akkaya

Department of Pathology, Faculty of Medicine, Kırıkkale University, Kırıkkale 71450, Türkiye; zeynepkurt@yahoo.com (Z.K.); mervademirr@gmail.com (M.A.A.)

* Correspondence: mahibalci@gmail.com

Abstract

Background/Objectives: Gastrointestinal stromal tumors (GISTs) are primarily identified using KIT and DOG1 immunohistochemistry, although staining may be absent or heterogeneous in some cases. Cytoplasmic Wilms tumor 1 (WT1) expression has been reported in GISTs, but its immunohistochemical characteristics and distribution among other mesenchymal tumors remain incompletely characterized. This study aimed to evaluate cytoplasmic WT1 expression in GISTs, compare its staining profile with established GIST-associated markers, and examine its distribution in a non-GIST mesenchymal comparison cohort. **Methods:** Twenty-eight GISTs were evaluated for WT1, KIT, DOG1, CD34, and h-caldesmon expression. Staining intensity and extent were assessed using an immunoreactivity score (IRS; 0–12). The comparison cohort comprised 17 non-GIST mesenchymal lesions: eight leiomyomas, six schwannomas, two inflammatory fibroid polyps, and one desmoid-type fibromatosis. IRS distributions were analyzed using the Friedman test, followed by Wilcoxon signed-rank tests with Bonferroni correction. Kruskal–Wallis and Mann–Whitney U tests were used for comparisons within the non-GIST cohort. **Results:** All 28 GISTs exhibited strong, diffuse cytoplasmic WT1 immunoreactivity, with a maximal IRS of 12. In contrast, IRS values varied for KIT (median, 8; range, 0–12), DOG1 (median, 12; range, 4–12), CD34 (median, 12; range, 0–12), and h-caldesmon (median, 9; range, 0–12). Overall, IRS distributions differed significantly across the five markers (Friedman $\chi^2(4) = 31.18$, $p < 0.001$), with WT1 showing higher scores than each comparator marker in post-hoc analyses. Five GISTs were KIT-immunonegative, including three spindle-cell and two epithelioid tumors; all retained DOG1 expression and demonstrated maximal WT1 scores. Among non-GIST lesions, all six extra-gastrointestinal schwannomas exhibited maximal cytoplasmic WT1 immunoreactivity, whereas leiomyomas, inflammatory fibroid polyps, and desmoid-type fibromatosis were WT1-negative. WT1 scores were higher in GISTs than in the overall non-GIST cohort and the non-neural mesenchymal subgroup (both $p < 0.001$), whereas GISTs and schwannomas showed identical IRS distributions. Additional WT1 immunoreactivity was observed in myenteric plexus regions and vascular-associated structures, while striated muscle fibers exhibited a characteristic sarcomeric staining pattern. **Conclusions:** Strong, diffuse cytoplasmic WT1 immunoreactivity was consistently observed in this GIST series, including KIT-immunonegative and epithelioid tumors. However, comparable expression in extra-gastrointestinal schwannomas indicates that cytoplasmic WT1 staining is not specific for GIST. Its potential complementary diagnostic value requires further validation and should be interpreted alongside morphology and established immunohistochemical markers.

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Keywords: gastrointestinal stromal tumor; WT1; cytoplasmic staining; immunohistochemistry; KIT-immunonegative GIST; DOG1; schwannoma

1. Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal neoplasms of the gastrointestinal tract and are characterized in most cases by KIT (CD117) and/or DOG1 expression [1,2]. KIT remains a key immunohistochemical marker in their diagnostic evaluation, with positivity reported in approximately 95% of cases [1]. Nevertheless, a small but diagnostically relevant subset lacks detectable KIT expression [3]. DOG1 provides high complementary sensitivity, including in many KIT-immunonegative tumors, although staining intensity may vary [2]. CD34 is also expressed in a substantial proportion of GISTs but is less consistently positive [4]. Thus, variation in the immunophenotypic profile of GISTs remains relevant in diagnostically challenging cases and provides a rationale for evaluating additional markers that may complement established panels.

The immunophenotype of GISTs also reflects their complex mesenchymal differentiation. GISTs are closely related to interstitial cells of Cajal (ICCs) and their precursors, a relationship supported by shared morphologic and immunophenotypic features, particularly KIT expression [5,6]. A subset of ICCs also expresses CD34, further supporting phenotypic overlap between GISTs and their proposed cellular counterparts [6]. In addition, h-caldesmon, traditionally associated with smooth-muscle differentiation, is frequently expressed in GISTs and therefore lacks specificity for smooth-muscle lineage in this setting [7]. These overlapping profiles emphasize the importance of interpreting individual markers within a broader morphologic and immunohistochemical context.

Wilms tumor 1 (WT1) is a zinc-finger transcription factor involved in development, tissue homeostasis, and tumor biology [8]. Although classically associated with nuclear localization, immunohistochemical studies have documented cytoplasmic WT1 staining in a variety of neoplasms, including soft-tissue tumors [9]. Cytoplasmic WT1 has also been reported in GISTs, with previous series demonstrating frequent immunoreactivity across tumors with diverse clinicopathologic characteristics [10,11]. However, its staining profile relative to established GIST-associated markers and its distribution among other mesenchymal tumors remain incompletely characterized.

WT1 immunoreactivity is not restricted to GISTs and has been described in other soft-tissue tumors [10] and in a variety of vascular lesions [12]. This broader distribution is relevant when considering its potential diagnostic utility and raises questions regarding the specificity and cellular context of cytoplasmic WT1 staining. Evaluation of non-GIST mesenchymal tumors, together with non-neoplastic cellular components, may therefore help define both the diagnostic limitations and the broader tissue distribution of this immunophenotypic finding.

Accordingly, the present study aimed to characterize the subcellular localization and immunoreactivity of WT1 in a series of GISTs and to compare its staining profile with those of KIT, DOG1, CD34, and h-caldesmon using a standardized immunoreactivity score. Particular attention was given to KIT-immunonegative and epithelioid tumors. WT1 staining was additionally assessed in a comparison cohort of non-GIST mesenchymal tumors and in non-neoplastic cellular components encountered in the examined sections. Through these complementary analyses, we sought to define the diagnostic context of cytoplasmic WT1 staining in GISTs and provide a basis for its interpretation alongside established immunohistochemical markers.

2. Materials and Methods

2.1. Study Design and Case Selection

This retrospective, single-center study included 28 gastrointestinal stromal tumors (GISTs) from 28 individual patients diagnosed between 2015 and 2024 at the Department of Pathology, Kırıkkale University Faculty of Medicine. One tumor per patient was included. Formalin-fixed, paraffin-embedded (FFPE) tissue sufficient for histopathological and immunohistochemical evaluation was available in all cases. The cohort comprised 28 surgical resection specimens, including 26 institutional cases and two cases surgically resected at external institutions and subsequently referred to our department for pathological consultation.

Twenty-seven tumors represented primary GISTs, whereas one surgical resection specimen represented a peritoneal metastasis of GIST.

The diagnosis of GIST was based on characteristic histomorphological features and the corresponding immunohistochemical profile, including KIT (CD117) and DOG1. Molecular testing for KIT and PDGFRA mutations was not performed. None of the patients had received neoadjuvant tyrosine kinase inhibitor therapy before tissue sampling.

Clinicopathological data, including tumor site, size, histological type, and risk category, were retrieved from the pathology records. For the two consultation cases, information not directly assessable from the submitted resection material, including tumor size and risk classification, was obtained from the corresponding original pathology reports. Tumors were categorized according to the Fletcher/NIH risk classification [13].

2.2. Non-GIST Comparison Group

A comparison cohort of 17 non-GIST mesenchymal lesions was retrospectively retrieved from the pathology archives and comprised eight gastrointestinal leiomyomas, six extra-gastrointestinal schwannomas, two gastrointestinal inflammatory fibroid polyps (Vanek tumors), and one abdominal intramuscular desmoid fibromatosis. All diagnoses were established on the basis of characteristic histomorphological features supported by immunohistochemical evaluation. Schwannomas exhibited S100 and/or SOX10 immunoreactivity, whereas leiomyomas demonstrated smooth muscle differentiation confirmed by SMA and desmin expression. The diagnoses of inflammatory fibroid polyps and desmoid-type fibromatosis were based on characteristic histomorphological findings, supported by comprehensive immunohistochemical assessment and exclusion of relevant diagnostic mimics. Representative formalin-fixed, paraffin-embedded (FFPE) tissue blocks containing sufficient lesional tissue were selected for WT1 immunohistochemistry.

2.3. Immunohistochemistry

All immunohistochemical findings were evaluated by direct microscopic examination of stained tissue sections. When the original immunohistochemical slides were unavailable, the corresponding stains were repeated on archival formalin-fixed, paraffin-embedded (FFPE) tissue sections.

Sections measuring 4 µm in thickness were prepared from representative FFPE tissue blocks and mounted on adhesive-coated slides. Immunohistochemical staining was performed using automated Ventana BenchMark staining platforms (Ventana Medical Systems, Tucson, AZ, USA).

The immunohistochemical panel evaluated in the GIST cohort included WT1 (clone 6F-H2, catalog no. 760-4397), KIT/CD117 (clone EP10, catalog no. 790-7061), DOG1 (clone SP31, catalog no. 760-4590), CD34 (clone QBEnd/10, catalog no. 790-2927), and h-caldesmon (clone E89, catalog no. 760-4375), all supplied as ready-to-use antibodies (Ventana/Roche Diagnostics). No manual antibody dilutions were performed.

WT1 immunohistochemistry was performed on a BenchMark GX automated staining platform using Cell Conditioning 1 (CC1) for antigen retrieval, with an initial 8 min conditioning step followed by 30 min of mild CC1 treatment. The primary antibody was incubated for 32 min, and immunoreactivity was visualized using the ultraView Universal DAB detection system. Sections were counterstained with hematoxylin for 8 min, followed by bluing for 4 min. The remaining immunohistochemical stains were performed on automated Ventana BenchMark staining platforms using the manufacturer's recommended antibody-specific protocols, including the corresponding antigen retrieval, incubation, and detection procedures.

Appropriate staining controls were included. Cryptorchid testicular tissue served as a positive control for WT1, demonstrating the expected nuclear immunoreactivity in Sertoli cells (Supplementary Figure S1).

In the non-GIST comparison cohort, WT1 immunohistochemistry was performed using the same antibody clone and staining protocol as in the GIST cohort.

2.4. Immunohistochemical Evaluation

Immunohistochemical staining was jointly re-evaluated by three pathologists using a multiheaded microscope, and final scores were assigned by consensus. Evaluation was initially performed on immunostained sections from one representative tumor block per case. In cases with equivocal or difficult-to-interpret staining, the relevant immunohistochemical stains were repeated on sections from a second tumor block. The entire evaluable tumor tissue on each immunostained slide was examined for staining intensity and extent, without restricting the assessment to selected microscopic fields. Necrotic, hemorrhagic, and technically inadequate areas were excluded from evaluation. As the histopathological diagnoses had been established before study inclusion, the pathologists were aware of the diagnostic classification during reassessment. Independent observer scores were not recorded.

Staining intensity was scored semiquantitatively as 0 (negative), 1 (weak), 2 (moderate), or 3 (strong). In cases exhibiting heterogeneous or mixed staining intensities, the predominant staining intensity across the entire evaluable tumor area was used to assign the intensity score. The extent of staining was scored according to the proportion of positive lesional cells as 0 (0%), 1 (<25%), 2 (25–50%), 3 (51–75%), or 4 (>75%). An immunoreactivity score (IRS), based on the Remmele–Stegner method [14], was calculated by multiplying the staining intensity and extent scores, yielding a total score ranging from 0 to 12.

The same IRS system was applied to WT1, KIT, DOG1, CD34, and h-caldesmon in the GIST cohort. WT1 staining was additionally assessed according to subcellular localization as nuclear, cytoplasmic, or combined nuclear and cytoplasmic. The same WT1 scoring criteria were applied to the non-GIST comparison group.

WT1 staining in non-neoplastic cellular and tissue components present in the examined sections was additionally recorded descriptively.

2.5. Statistical Analysis

Categorical variables were summarized as frequencies and percentages, and continuous variables as means, standard deviations, medians, and ranges, as appropriate. Immunohistochemical scores were treated as ordinal variables, and 95% confidence intervals for proportions were calculated using the exact binomial (Clopper–Pearson) method.

IRS values for WT1, KIT, DOG1, CD34, and h-caldesmon were compared using the Friedman test, followed by Wilcoxon signed-rank tests for four prespecified comparisons of WT1 with the other markers. Zero differences were excluded (zero_method = "wilcox"), tied absolute differences were assigned average ranks, and no continuity correction was

applied. P-values were calculated using SciPy's automatic method selection (method = "auto") and adjusted for the four comparisons using the Bonferroni method.

WT1 IRS distributions across the four non-GIST diagnostic groups were compared using the Kruskal–Wallis test. Mann–Whitney U tests were used for comparisons of schwannomas with the remaining non-GIST tumors and of GISTs with the overall non-GIST cohort and its neural and non-neural subgroups.

Given the small sample sizes and frequent tied observations, particularly in the non-GIST comparison cohort, between-group analyses were interpreted as exploratory. All tests were two-sided, with statistical significance set at $p < 0.05$. Statistical analyses were performed using Python (version 3.11; Python Software Foundation) and the SciPy statistical library (version 1.11.4).

3. Results

3.1. Clinicopathological Characteristics of the GIST Cohort

The mean patient age was 66.8 ± 13.8 years (median, 68 years; range, 34–90 years), and 17 of 28 patients (60.7%) were male. Among the 27 primary GISTs, tumors were located in the stomach ($n = 19$), jejunum ($n = 4$), ileum ($n = 3$), and colon ($n = 1$). Tumor size was available for 27 of the 28 GISTs. The mean tumor size was 6.2 ± 5.5 cm (median, 3.5 cm; range, 0.5–25.0 cm).

Histologically, 21 tumors (75.0%) showed spindle-cell morphology and seven (25.0%) showed epithelioid morphology. According to the Fletcher/NIH classification, three tumors were categorized as very low risk, nine as low risk, five as intermediate risk, and ten as high risk. One peritoneal metastatic GIST was designated as not assessable because the recorded tumor size corresponded to the metastatic lesion rather than the original primary tumor. The clinicopathological characteristics of the cohort are summarized in Table 1.

Table 1. Clinicopathological characteristics of the GIST cohort ($n = 28$).

Characteristic	Value
Age (years)	
Mean $\pm$ SD	66.8 $\pm$ 13.8
Median (range)	68 (34–90)
Sex, n (%)	
Male	17 (60.7)
Female	11 (39.3)
Case source/specimen type, n (%)	
Resection specimen	28 (100.0)
Institutional case	26 (92.9)
Consultation case	2 (7.1)
Tumor/specimen site, n (%)	
Stomach	19 (67.9)
Jejunum	4 (14.3)
Ileum	3 (10.7)
Colon	1 (3.6)
Peritoneal metastasis	1 (3.6)
Tumor size (cm)	

Mean $\pm$ SD	6.2 $\pm$ 5.5
Median (range)	3.5 (0.5–25.0)
Histological type, <i>n</i> (%)	
Spindle	21 (75.0)
Epithelioid	7 (25.0)
Fletcher/NIH risk category, <i>n</i> (%)	
Very low	3 (10.7)
Low	9 (32.1)
Intermediate	5 (17.9)
High	10 (35.7)
Not assessable	1 (3.6)

Abbreviations: GIST, gastrointestinal stromal tumor; SD, standard deviation. Note: Risk-category percentages were calculated using the full cohort (*n* = 28). The peritoneal metastatic GIST was classified as not assessable for Fletcher/NIH risk stratification.

3.2. Immunohistochemical Findings in GISTs

WT1 showed exclusively cytoplasmic staining in all 28 GISTs (28/28, 100%; 95% CI, 87.7–100%), with an IRS of 12 in every case; no nuclear WT1 staining was identified. In comparison, IRS values were more variable for KIT (median, 8; range, 0–12), DOG1 (median, 12; range, 4–12), CD34 (median, 12; range, 0–12), and h-caldesmon (median, 9; range, 0–12) (Table 2). Representative histomorphological and immunohistochemical findings from the GIST cohort are shown in Figure 1.

Table 2. Immunohistochemical findings in the GIST cohort (*n* = 28).

Marker	IRS 0, <i>n</i> (%)	IRS 1–4, <i>n</i> (%)	IRS 5–8, <i>n</i> (%)	IRS 9–12, <i>n</i> (%)	Median IRS (Range)
WT1	0 (0.0)	0 (0.0)	0 (0.0)	28 (100.0)	12 (12–12)
KIT	5 (17.9)	2 (7.1)	9 (32.1)	12 (42.9)	8 (0–12)
DOG1	0 (0.0)	2 (7.1)	7 (25.0)	19 (67.9)	12 (4–12)
CD34	5 (17.9)	1 (3.6)	3 (10.7)	19 (67.9)	12 (0–12)
h-caldesmon	1 (3.6)	2 (7.1)	10 (35.7)	15 (53.6)	9 (0–12)

Abbreviations: GIST, gastrointestinal stromal tumor; IRS, immunoreactivity score.

IRS values differed significantly among the five immunohistochemical markers (Friedman $\chi^2(4) = 31.18$, $p < 0.001$). Post-hoc pairwise comparisons using the Wilcoxon signed-rank test with Bonferroni correction showed higher WT1 IRS values than KIT ($p < 0.001$), DOG1 ($p = 0.018$), CD34 ($p = 0.008$), and h-caldesmon ($p = 0.001$) (Table 3).

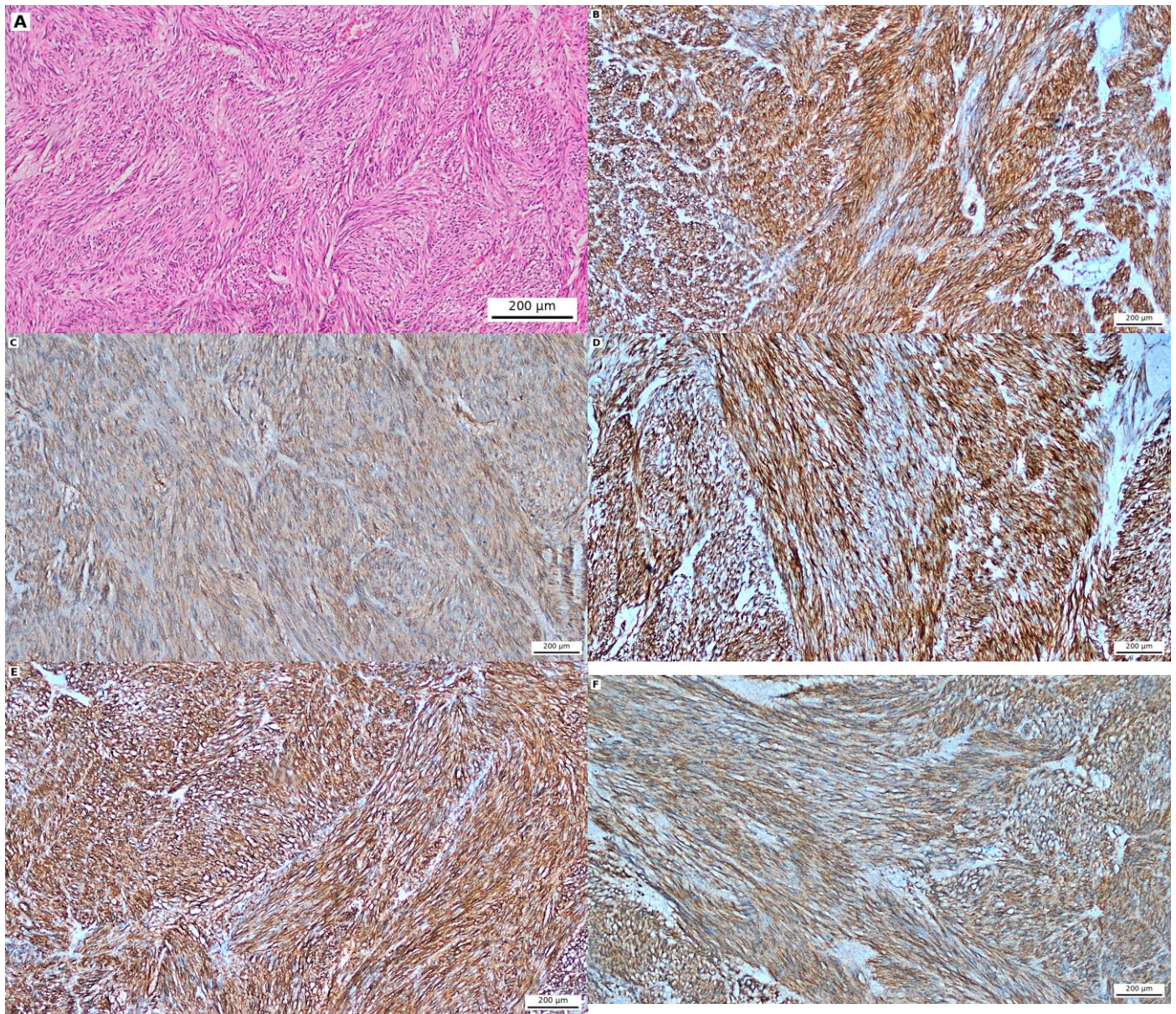


Figure 1. Histomorphological and immunohistochemical features of a representative spindle-cell gastrointestinal stromal tumor. (A) Spindle-cell morphology with intersecting fascicles on H&E (×100). (B) Diffuse and strong KIT (CD117) expression (×100). (C) Weak DOG1 expression (×100). (D) Diffuse and strong cytoplasmic WT1 expression (×100). (E) Diffuse and strong CD34 expression (×100). (F) Moderate h-caldesmon expression (×100).

Table 3. Post hoc pairwise comparisons of WT1 IRS with other immunohistochemical markers in the GIST cohort.

Pairwise Comparison	Adjusted <i>p</i> -Value
WT1 vs. KIT	<0.001
WT1 vs. DOG1	0.018
WT1 vs. CD34	0.008
WT1 vs. h-caldesmon	0.001

Note: Overall Friedman test: $\chi^2(4) = 31.18$, $p < 0.001$. Pairwise comparisons were performed using the Wilcoxon signed-rank test with Bonferroni correction.

3.3. KIT-Immunonegative and Epithelioid GISTs

Five of the 28 GISTs were KIT-immunonegative (5/28, 17.9%; 95% CI, 6.1–36.9%), including three spindle-cell and two epithelioid tumors. All five retained DOG1 expression (IRS range, 4–12) and showed strong, diffuse cytoplasmic WT1 immunoreactivity with an IRS of 12. Their clinicopathological and immunohistochemical characteristics are detailed in Table 4. Representative histomorphological and immunohistochemical findings of KIT-immunonegative GISTs are shown in Figure 2.

Table 4. Clinicopathological and immunohistochemical characteristics of KIT-immunonegative gastrointestinal stromal tumors (GISTs).

Case	Age	Sex	Tumor Site	Size (cm)	Histo-logic Type	Risk	WT1 IRS	DOG1 IRS	CD34 IRS	h-Caldesmon IRS
1	68	M	Stomach	3.5	Spindle	Low	12	12	12	12
2	54	M	Stomach	8.5	Epithelioid	Intermediate	12	4	6	6
3	76	M	Stomach	14.0	Epithelioid	High	12	12	12	12
4	66	M	Stomach	8.0	Spindle	Intermediate	12	12	12	12
5	88	M	Stomach	11.0	Spindle	High	12	6	12	6

Abbreviations: GIST, gastrointestinal stromal tumor; IRS, immunoreactivity score; M, male.

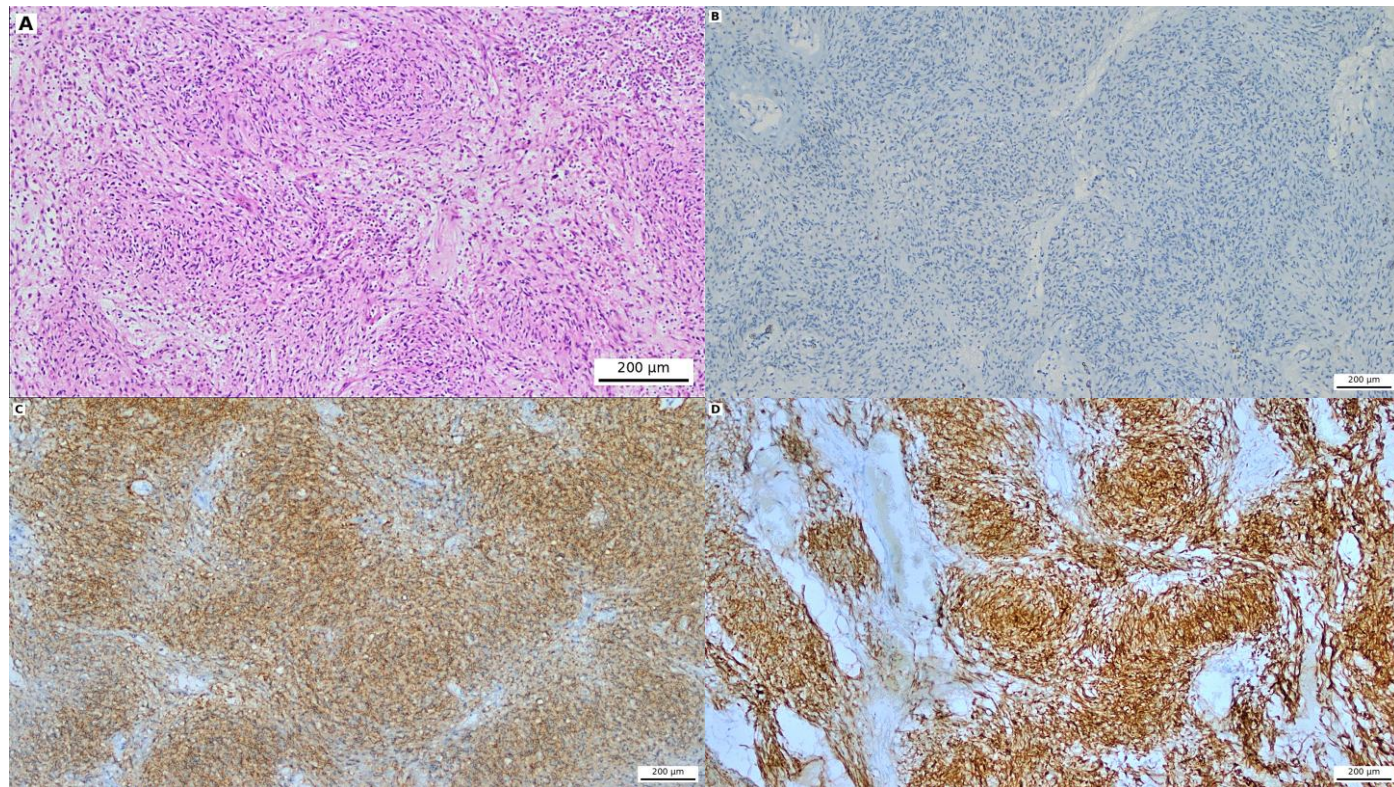


Figure 2. Histomorphological and immunohistochemical features of a representative KIT-immunonegative spindle-cell gastrointestinal stromal tumor. (A) Spindle-cell morphology with a whorled growth pattern on H&E (×100). (B) Absence of KIT (CD117) immunoreactivity in the tumor cells (×100). (C) Diffuse and strong DOG1 expression with focal membranous staining (×100). (D) Diffuse and strong cytoplasmic WT1 expression (×100).

Among the 28 GISTs, seven showed epithelioid morphology (7/28, 25.0%; 95% CI, 10.7–44.9%). Of these seven tumors, two were KIT-immunonegative, whereas five retained KIT expression. DOG1 expression was present in all seven tumors, with IRS values ranging from 4 to 12. All seven epithelioid GISTs showed strong, diffuse cytoplasmic WT1 immunoreactivity with an IRS of 12, irrespective of KIT expression. Their clinicopathological and immunohistochemical characteristics are summarized in Table 5. Figure 3 illustrates the histomorphological and immunohistochemical features of epithelioid GISTs.

Table 5. Clinicopathological and immunohistochemical characteristics of epithelioid gastrointestinal stromal tumors (GISTs).

Case	Age	Sex	Tumor Site	Size (cm)	Risk Category	WT1 IRS	KIT IRS	DOG1 IRS	CD34 IRS	h-Caldesmon IRS
1	77	M	Stomach	15.0	High	12	4	4	9	12
2	80	M	Jejunum	13.0	High	12	12	12	3	6
3	34	M	Jejunum	2.5	Low	12	6	12	0	4
4	54	M	Stomach	8.5	Intermediate	12	0	4	6	6
5	76	M	Stomach	14.0	High	12	0	12	12	12
6	56	F	Stomach	3.0	Low	12	6	8	0	4
7	68	M	Stomach	3.5	Intermediate	12	6	6	12	6

Abbreviations: GIST, gastrointestinal stromal tumor; IRS, immunoreactivity score; M, male; F, female.

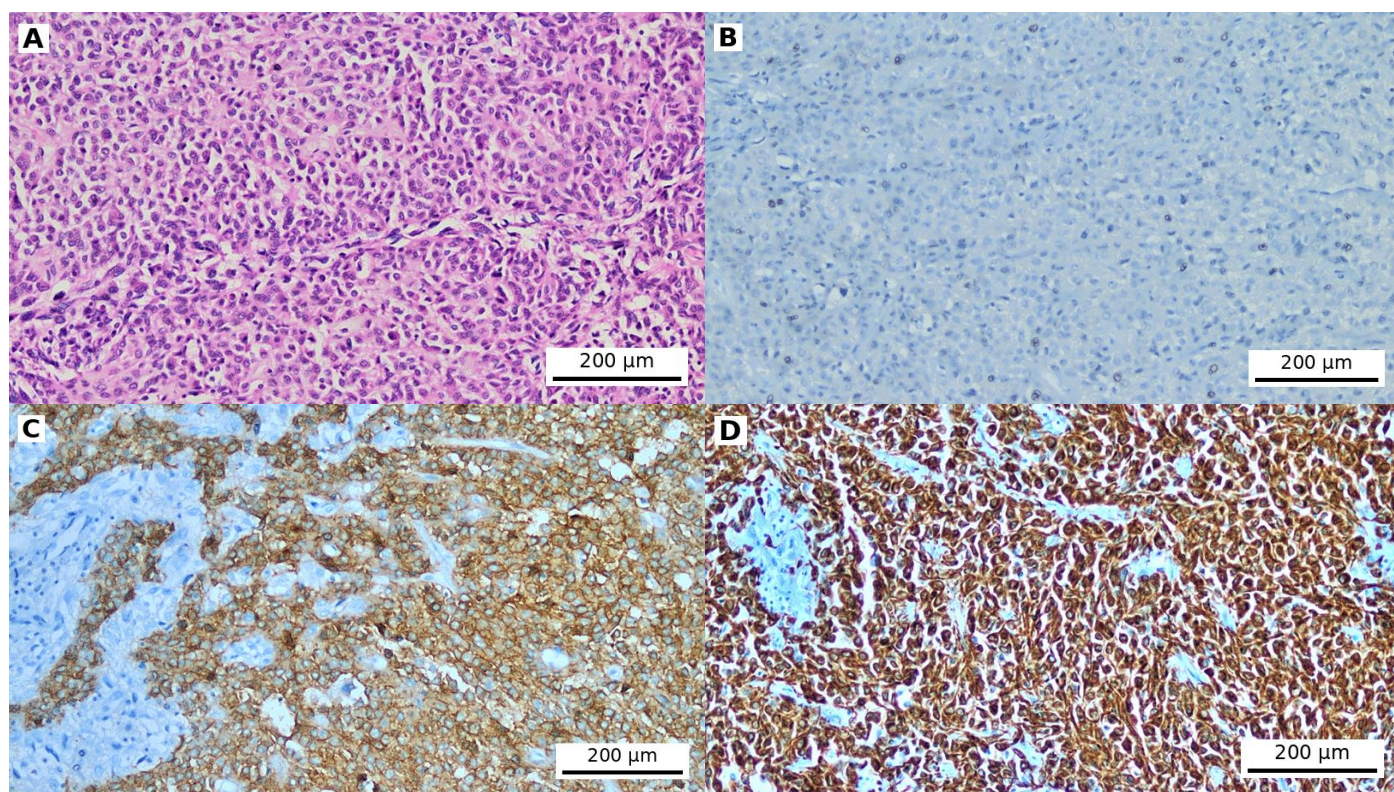


Figure 3. Histomorphological and immunohistochemical features of a representative KIT-immunonegative epithelioid gastrointestinal stromal tumor. (A) Epithelioid tumor morphology on

H&E (×100). (B) Absence of KIT (CD117) immunoreactivity in the tumor cells (×100). (C) Diffuse and strong DOG1 expression (×100). (D) Diffuse and strong cytoplasmic WT1 expression (×100).

3.4. WT1 Expression in Non-GIST Mesenchymal Lesions

The non-GIST comparison group comprised 17 mesenchymal lesions, including eight leiomyomas, six extra-gastrointestinal schwannomas, two inflammatory fibroid polyps, and one desmoid fibromatosis. The clinicopathological characteristics of the comparison group are summarized in Table 6.

Table 6. Clinicopathological characteristics of the non-GIST comparison group ($n = 17$).

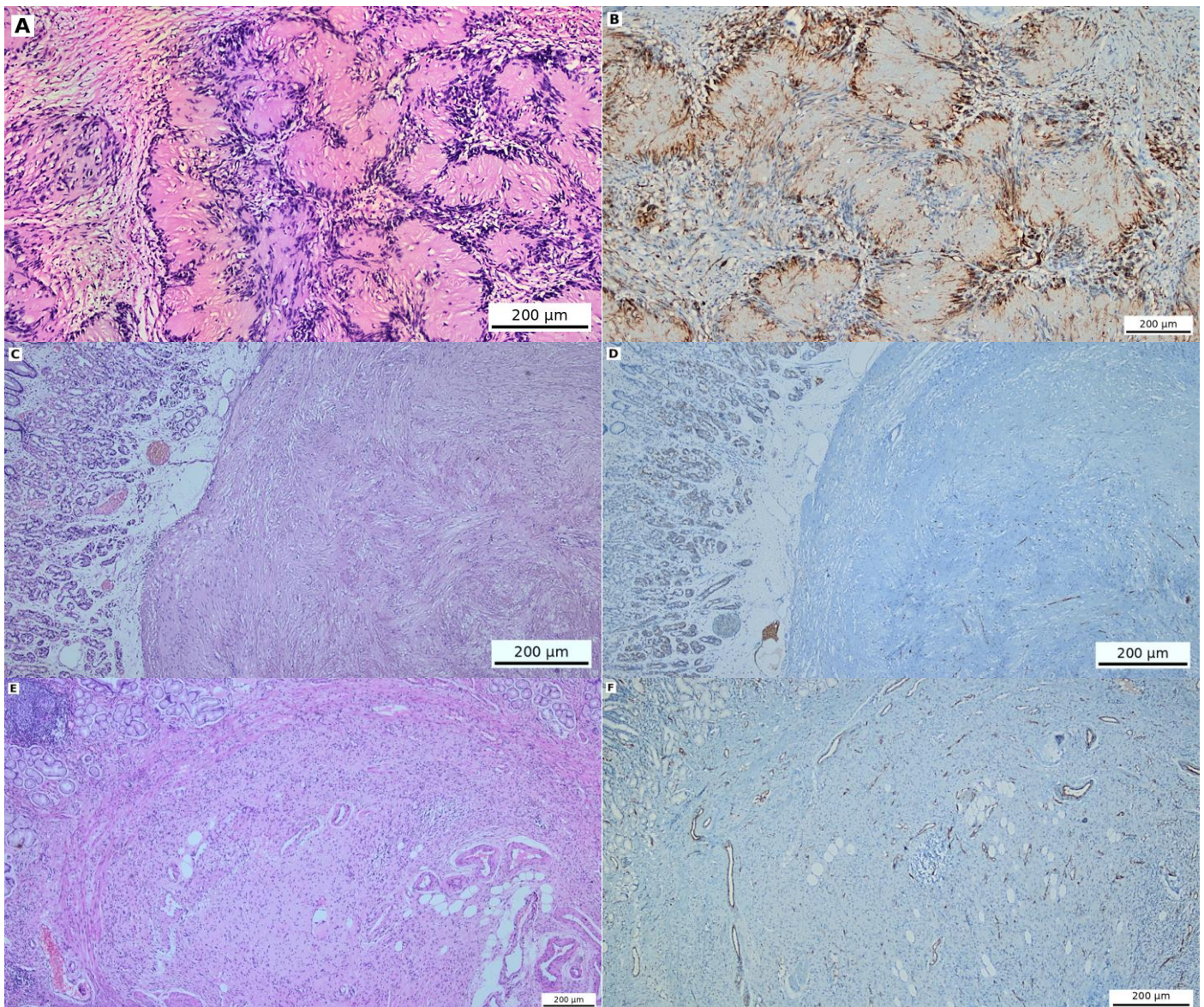
Characteristic	Value
Age (years)	
Mean $\pm$ SD	48.6 $\pm$ 19.4
Median (range)	58 (19–71)
Sex, n (%)	
Female	11 (64.7)
Male	6 (35.3)
Diagnosis, n (%)	
Leiomyoma	8 (47.1)
Schwannoma	6 (35.3)
Inflammatory fibroid polyp	2 (11.8)
Desmoid fibromatosis	1 (5.9)
Anatomic site, n (%)	
Gastrointestinal	
Stomach	8 (47.1)
Colon	2 (11.8)
Extra-gastrointestinal	
Cervical region (neck/postauricular)	1 (5.9)
Nasal cavity (nasal base)	1 (5.9)
Eyebrow	1 (5.9)
Eyelid	1 (5.9)
Foot (dorsum)	1 (5.9)
Spine	1 (5.9)
Abdominal (intramuscular)	1 (5.9)
Tumor size (cm)	
Mean $\pm$ SD	1.7 $\pm$ 1.8
Median (range)	1.0 (0.2–6.8)

All six schwannomas showed strong, diffuse cytoplasmic WT1 immunoreactivity (6/6, 100%; 95% CI, 54.1–100%), with an IRS of 12. In contrast, WT1 immunoreactivity was absent in all eight leiomyomas, two inflammatory fibroid polyps, and the single desmoid fibromatosis (IRS = 0) (Table 7). WT1 IRS values differed significantly among the four diagnostic groups (Kruskal–Wallis $H(3) = 16.00$, $p = 0.001$). Schwannomas showed significantly higher WT1 IRS values than the remaining non-GIST lesions combined (Mann–Whitney $U = 66.0$, $p < 0.001$). Representative WT1 immunostaining patterns in the non-GIST comparison group are shown in Figure 4.

Table 7. WT1 immunoreactivity in the non-GIST comparison group ($n = 17$).

Diagnosis	<i>n</i>	IRS 0, <i>n</i> (%)	IRS 1–4, <i>n</i> (%)	IRS 5–8, <i>n</i> (%)	IRS 9–12, <i>n</i> (%)	Median IRS (Range)
Leiomyoma	8	8 (100)	0 (0)	0 (0)	0 (0)	0 (0–0)
Schwannoma	6	0 (0)	0 (0)	0 (0)	6 (100)	12 (12–12)
Inflammatory fibroid polyp	2	2 (100)	0 (0)	0 (0)	0 (0)	0 (0–0)
Desmoid fibromatosis	1	1 (100)	0 (0)	0 (0)	0 (0)	0 (0–0)
Total	17	11 (64.7)	0 (0)	0 (0)	6 (35.3)	0 (0–12)

Note: Percentages for individual diagnostic categories are calculated within each diagnostic group.



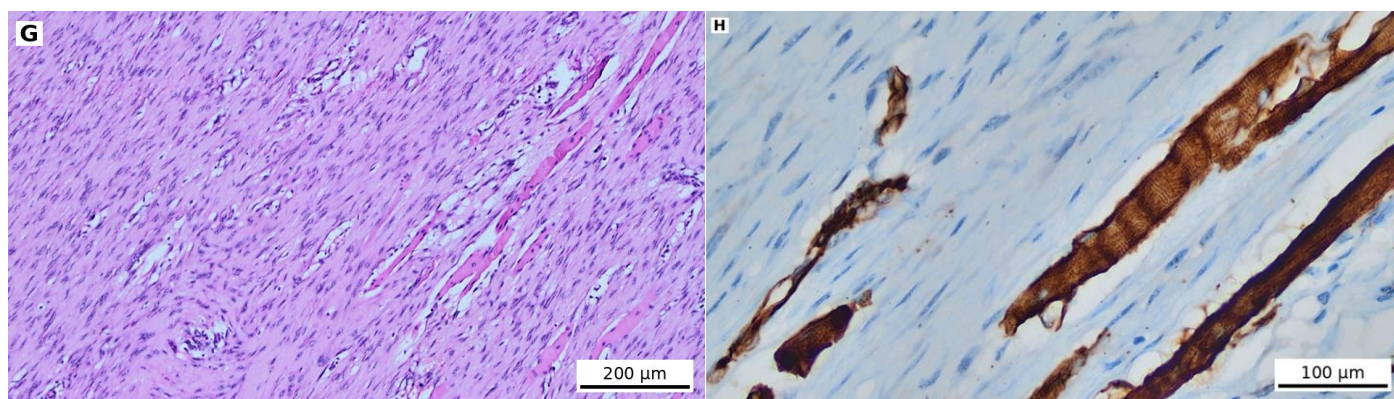


Figure 4. Histomorphological features and WT1 immunoreactivity in the non-GIST mesenchymal comparison group. (A) Schwannoma demonstrating characteristic Verocay bodies on H&E (×100). (B) Diffuse and strong cytoplasmic WT1 immunoreactivity in the cellular component of the schwannoma (×100). (C) Gastrointestinal leiomyoma showing intersecting fascicles of bland smooth muscle cells on H&E (×100). (D) Absence of WT1 immunoreactivity in the lesional smooth muscle cells (×100). (E) Inflammatory fibroid polyp showing a bland spindle-cell proliferation accompanied by eosinophilic leukocytes on H&E (×100). (F) Absence of WT1 immunoreactivity in the lesional spindle cells, with WT1 staining confined to endothelial cells (×100). (G) Peripheral area of desmoid-type fibromatosis showing infiltration of adjacent skeletal muscle fibers by the bland spindle-cell proliferation on H&E (×100). (H) Absence of WT1 immunoreactivity in the lesional spindle cells, with prominent cytoplasmic WT1 staining showing a sarcomeric striated pattern in entrapped skeletal muscle fibers (×200).

3.5. Comparison of WT1 Expression Between GISTs and Non-GIST Tumors

WT1 IRS values were significantly higher in GISTs than in the overall non-GIST group (Mann–Whitney $U = 392.0$, $p < 0.001$) (Table 8). All 28 GISTs showed an IRS of 12, whereas the non-GIST group had a median IRS of 0 (range, 0–12).

Table 8. Comparison of WT1 immunoreactivity between GISTs and non-GIST tumors.

Comparison	Group	<i>n</i>	Median WT1 IRS (Range)	Mann–Whitney <i>U</i>	<i>p</i> -Value
GIST vs. all non-GIST tumors	GIST	28	12 (12–12)	392.0	<0.001
	All non-GIST tumors	17	0 (0–12)		
GIST vs. non-neural non-GIST tumors*	GIST	28	12 (12–12)	308.0	<0.001
	Non-neural non-GIST tumors*	11	0 (0–0)		
GIST vs. schwannoma	GIST	28	12 (12–12)	84.0	1.000
	Schwannoma	6	12 (12–12)		

*Non-neural non-GIST tumors comprised eight leiomyomas, two inflammatory fibroid polyps, and one desmoid fibromatosis.

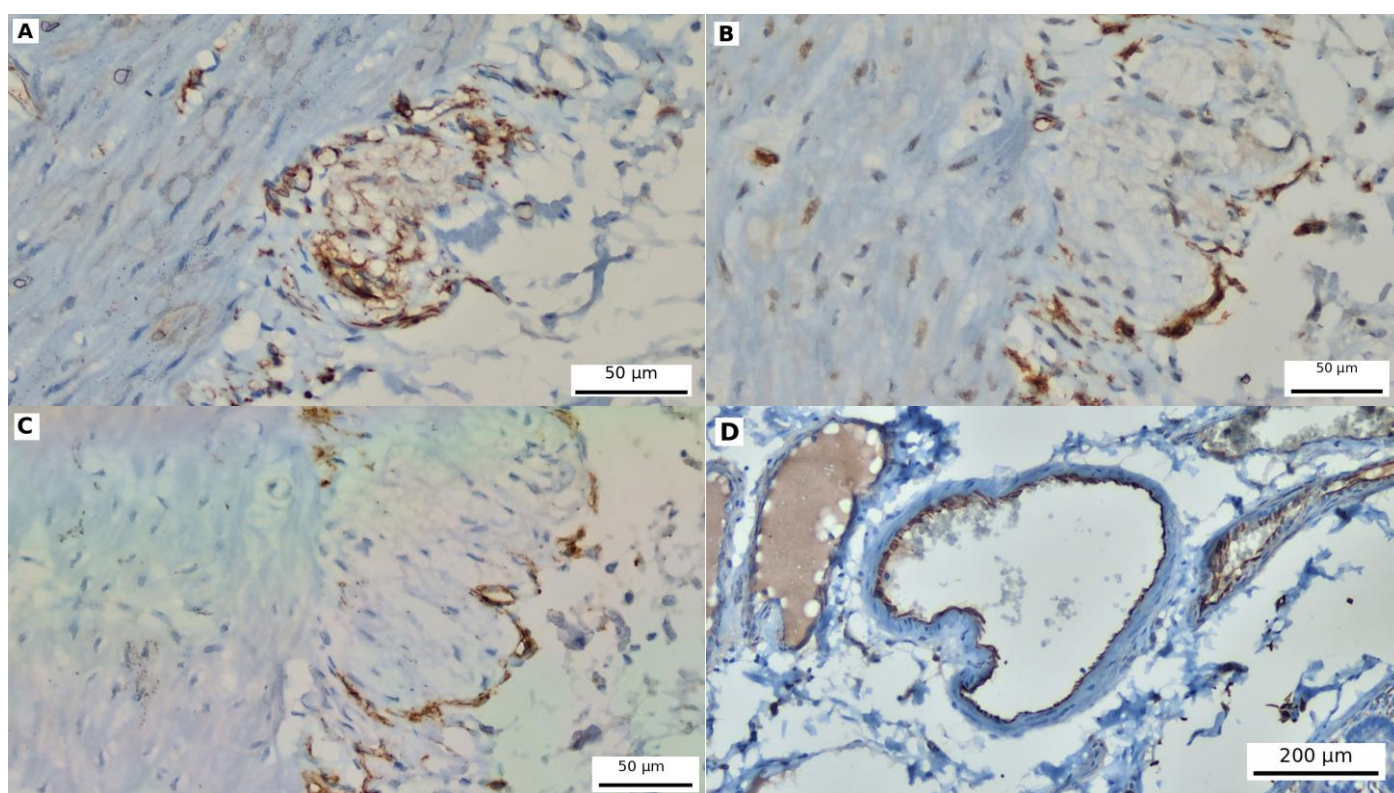
When GISTs were compared specifically with non-neural non-GIST tumors, comprising leiomyomas, inflammatory fibroid polyps, and desmoid fibromatosis, WT1 IRS values were also significantly higher in GISTs (Mann–Whitney $U = 308.0$, $p < 0.001$). In contrast, GISTs and schwannomas showed identical WT1 IRS distributions (median, 12; range, 12–12 in both groups), with no significant difference between the two groups ($U = 84.0$, $p = 1.000$) (Table 8).

3.6. WT1 Immunoreactivity in Non-Neoplastic Tissues

WT1 immunoreactivity was also observed in selected non-neoplastic tissue components. As tissue blocks were primarily selected for tumor evaluation, non-neoplastic components were not uniformly represented across cases. Accordingly, these components were assessed descriptively whenever present and evaluable, rather than through systematic compartment-specific quantification.

In the myenteric plexus regions, WT1 exhibited a complex cytoplasmic staining pattern involving both central neural and peripheral structures, whereas DOG1 and CD117 immunoreactivity was restricted to peripheral regions, sparing the central neural component. Cytoplasmic WT1 staining was additionally identified in vascular-associated elements. In skeletal muscle fibers, WT1 showed a distinctive cytoplasmic staining pattern with sarcomeric striations, as illustrated in Figure 4H.

These staining patterns were observed in the examined sections containing the corresponding evaluable non-neoplastic components. Representative comparative WT1, DOG1, and CD117 staining patterns in the myenteric plexus regions, together with WT1 immunoreactivity in vascular-associated elements, are shown in Figure 5.



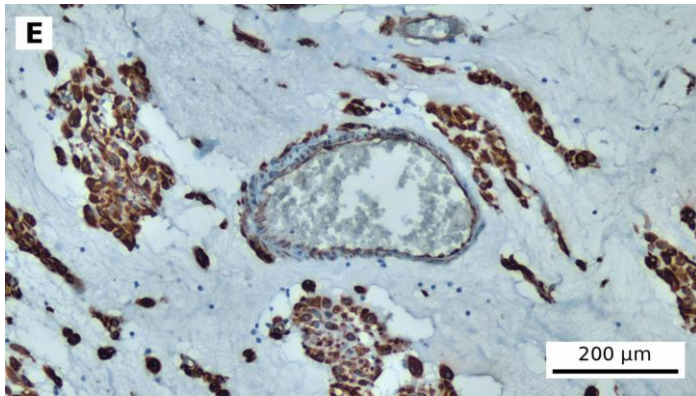


Figure 5. WT1 immunoreactivity and comparative CD117 and DOG1 staining patterns in selected non-neoplastic tissue components. (A) WT1 immunoreactivity showing a mixed cytoplasmic staining pattern involving both central neural and peripheral structures within the myenteric plexus region ($\times 400$). (B) CD117 immunoreactivity highlighting peripheral spindle cells within the myenteric plexus region ($\times 400$). (C) DOG1 immunoreactivity showing a distribution similar to that of CD117, predominantly highlighting peripheral spindle cells within the myenteric plexus region ($\times 400$). (D) WT1 immunoreactivity in endothelial cells lining a vascular structure ($\times 100$). (E) Pericytic WT1 staining surrounding a vascular structure, with diffuse and strong cytoplasmic WT1 immunoreactivity in the adjacent epithelioid GIST cells ($\times 100$).

4. Discussion

The present study demonstrates a highly consistent pattern of cytoplasmic WT1 expression across GISTs, with all 28 tumors showing diffuse, strong cytoplasmic staining corresponding to the maximum IRS of 12. This consistency was maintained across different anatomic sites, histologic phenotypes, and KIT-expression profiles, including both KIT-immunonegative and epithelioid tumors. Bing et al. reported WT1 expression in all 28 GISTs examined, with predominantly cytoplasmic and generally strong staining, although the extent of immunoreactivity varied among tumors [10]. Similarly, Kang et al. demonstrated WT1 positivity in all 97 GISTs in a larger series, but staining intensity ranged from weak to strong, with strong staining observed in 60.8% of cases [11]. This striking uniformity suggests that cytoplasmic WT1 may represent a particularly robust immunophenotypic feature of GIST, although differences in antibody clones, staining protocols, scoring methods, and cohort characteristics may partly explain the variability reported across studies.

KIT and DOG1 remain the principal immunohistochemical markers for the diagnosis of GIST, although their expression may be variable in diagnostically challenging tumors [2,3]. CD34 is less consistently expressed [4], whereas h-caldesmon may be expressed in GISTs despite its traditional association with smooth muscle differentiation [7]. Such immunophenotypic variability may reflect the biological heterogeneity of GISTs, encompassing distinct morphological phenotypes, anatomical distributions, and molecular subtypes. Differences in KIT expression may be associated with epithelioid morphology and specific molecular backgrounds, while heterogeneous staining within individual tumors may reflect regional differences in cellular differentiation or phenotypic states. In our cohort, KIT showed the greatest variability, ranging from complete absence to strong expression, while DOG1, CD34, and h-caldesmon also demonstrated variable IRS values. Against this heterogeneous immunophenotypic background, the uniformity of cytoplasmic WT1 staining raises the possibility that its expression may be less dependent on the phenotypic differences influencing conventional GIST-associated markers, although this hypothesis remains to be investigated.

KIT-immunonegative and epithelioid GISTs are particularly relevant to this potential diagnostic context. KIT-immunonegative GISTs constitute a small but clinically relevant subset and may exhibit either spindle-cell or epithelioid morphology [2,3]. DOG1 provides important complementary diagnostic information in this setting and is retained in most KIT-immunonegative GISTs [2,3], although its staining intensity and distribution may vary. In our cohort, all five KIT-immunonegative tumors retained DOG1 expression, with IRS values ranging from 4 to 12. These observations do not establish diagnostic superiority over DOG1 but suggest that cytoplasmic WT1 may provide additional immunophenotypic support in selected diagnostically challenging GISTs. This potential complementary role requires further validation.

The identical WT1 staining pattern observed in GISTs and schwannomas is a noteworthy finding of the present study. All six extra-gastrointestinal schwannomas in our comparison cohort showed strong, diffuse cytoplasmic WT1 expression, closely paralleling the pattern observed in GISTs. This finding is consistent with Schittenhelm et al., who demonstrated cytoplasmic WT1 expression in all 69 schwannomas examined, as well as in neurofibromas and malignant peripheral nerve sheath tumors, and also identified WT1 expression in Schwann cells in the majority of normal peripheral nerves [15]. More recently, Cepeda-Emiliani et al. demonstrated cytoplasmic WT1 expression in both myelinating and non-myelinating human Schwann cells, including localization within Cajal bands and Schmidt–Lanterman incisures, and proposed potential roles for WT1 in Schwann-cell development, maintenance, plasticity, and cytoplasmic organization [16]. Although Schwann cells and interstitial cells of Cajal (ICCs) have distinct embryological origins, their biology is linked to neural–mesenchymal interactions. ICCs are mesenchymal in origin and are regarded as the putative cells of origin of GISTs [5,6], while developmental studies have demonstrated close reciprocal interactions between mesenchymal ICC precursors and neural crest-derived cells during the formation of the enteric neuromuscular compartment [17]. These observations raise the possibility that the shared cytoplasmic WT1 phenotype in GISTs and schwannomas may reflect aspects of cellular differentiation or protein localization that extend beyond a single tumor lineage, although this interpretation remains hypothetical.

In contrast to schwannomas, WT1 immunoreactivity was absent in all non-neural mesenchymal tumors examined in our cohort, the majority of which were leiomyomas. This finding is consistent with the absence of WT1 expression in leiomyomas reported by Kang et al. [11], although WT1 expression in smooth muscle neoplasms appears to be more complex. Cytoplasmic WT1 expression has been documented in leiomyosarcomas [10,11], and Coosemans et al. demonstrated WT1 expression in the majority of uterine leiomyosarcomas not only by immunohistochemistry but also at the mRNA and protein levels, with predominantly cytoplasmic protein localization [18]. These findings support WT1 expression in leiomyosarcoma as a genuine biological phenomenon, although its functional significance and the mechanisms underlying its cytoplasmic localization remain uncertain. Heterogeneity of cytoplasmic WT1 expression is also evident among other mesenchymal neoplasms. Magro et al. demonstrated strong cytoplasmic expression in several young-type fibromatoses and infantile fibrosarcoma, whereas nodular fasciitis and desmoid-type fibromatosis were largely negative [19]. Such differences fit within the broader ontogenetic framework proposed by Salvatorelli et al., in which WT1 expression and subcellular localization vary according to cellular and developmental context and may, in some neoplasms, recapitulate patterns present during tissue development [20]. Collectively, these observations suggest that the contrasting WT1 staining patterns among mesenchymal neoplasms may be related to differences in cellular lineage and differentiation, although this relationship cannot be established from immunohistochemical findings alone.

The distribution of cytoplasmic WT1 immunoreactivity in non-neoplastic tissues provides an additional perspective on its potential biological significance. Within the myenteric plexus regions, WT1 staining involved both central neural and peripheral structures, whereas DOG1 and KIT immunoreactivity was predominantly confined to the peripheral components. This overlapping yet distinct distribution is of particular interest given the close developmental and functional relationships between enteric neural elements and interstitial cells of Cajal [5,6,17]. Although the precise cellular identity of the stained elements remains to be established, these findings suggest that WT1 expression may extend across interacting cellular compartments of the gastrointestinal neuromuscular apparatus. Cytoplasmic WT1 immunoreactivity was also observed in vascular-associated structures and striated muscle fibers, the latter displaying a distinctive sarcomeric staining pattern. Previous studies have documented WT1 expression in vascular tissues and skeletal muscle in developmental and neoplastic contexts [12,20,21]. Together, these observations support a broader, tissue-dependent distribution of cytoplasmic WT1 beyond neoplastic cells, while leaving its functional significance open to further investigation.

Although WT1 is classically recognized as a nuclear transcription factor, its subcellular localization and potential functions extend beyond the nucleus. WT1 undergoes nucleocytoplasmic shuttling and has been shown to associate with ribonucleoprotein particles and actively translating polysomes, suggesting possible roles in RNA metabolism and translational regulation [22]. Its cytoplasmic expression has also been documented in a broad spectrum of normal and neoplastic tissues, with localization patterns that may vary according to cellular and developmental context [9,20]. These observations provide a potential biological framework for interpreting the cytoplasmic WT1 phenotype observed in GISTs, although its underlying mechanisms remain unresolved. The 6F-H2 clone used in this study can also demonstrate nuclear WT1 immunoreactivity in established WT1-expressing tumors, including Wilms tumor, mesothelioma, and ovarian serous tumors [9]. Nevertheless, antibody-dependent and methodological differences may influence WT1 staining patterns, and the exclusively cytoplasmic immunoreactivity observed in our series was not independently verified using a second antibody clone or an orthogonal method. Further investigations into WT1 isoform expression, transcript levels, and subcellular protein localization are needed to elucidate the molecular basis of this phenotype in GIST.

Beyond its immunophenotypic context, WT1 has also attracted interest as a potential therapeutic target. WT1-directed approaches, including peptide- and dendritic cell-based strategies, have been investigated in several hematologic and solid malignancies [23]. Although the therapeutic implications of cytoplasmic WT1 expression in GIST remain unexplored, its consistent detection in our cohort provides a rationale for future studies examining the molecular characteristics and potential functional relevance of this phenotype.

This study has several limitations, primarily related to its retrospective, single-center design and cohort size. The uniformly maximal WT1 staining scores, while representing a central finding, did not permit an assessment of potential associations with clinicopathological features, tumor aggressiveness, or prognosis; thus, the possibility of such relationships remains open. The absence of KIT and PDGFRA mutational data also limited molecular-immunophenotypic correlations, although the diagnosis of KIT-immunonegative GISTs was supported by characteristic morphology and retained DOG1 expression. Furthermore, while immunohistochemical scoring was established by consensus among three pathologists, interobserver reproducibility could not be independently evaluated. The non-GIST comparison cohort was limited by the small representation of certain diagnostic categories, particularly the absence of gastrointestinal schwannomas in the available archival material. Nevertheless, the inclusion of extra-gastrointestinal schwannomas offered a biologically relevant comparison with peripheral nerve sheath tumors, although the

findings cannot be directly generalized to gastrointestinal schwannomas. Within these boundaries, the study provides a systematic assessment of cytoplasmic WT1 expression in GIST, including KIT-immunonegative and epithelioid tumors, alongside established diagnostic markers and selected mesenchymal mimics. Further investigation in larger, molecularly characterized cohorts may clarify the clinicopathological significance and diagnostic applicability of this distinctive immunophenotypic pattern.

5. Conclusions

The present study demonstrates remarkably uniform, strong cytoplasmic WT1 immunoreactivity across GISTs, including KIT-immunonegative and epithelioid tumors. Notably, WT1 staining was more homogeneous than DOG1 in KIT-immunonegative cases, despite retained DOG1 expression in all tumors. The contrasting expression patterns among the mesenchymal neoplasms examined suggest that cytoplasmic WT1 may provide supportive immunophenotypic information in distinguishing GIST from selected smooth muscle and fibroblastic tumors, while its comparable expression in schwannomas limits its discriminatory value in this setting. Beyond these diagnostic considerations, the shared cytoplasmic WT1 phenotype of GISTs and schwannomas raises the possibility of immunophenotypic convergence across distinct cellular lineages, a hypothesis of particular interest in the context of the enteric neuromuscular apparatus. Further studies are warranted to clarify the biological basis and potential diagnostic and clinicopathological relevance of cytoplasmic WT1 expression in GIST.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/biomedicines14112439/s1>. Figure S1. Positive and internal negative cellular controls for WT1 immunohistochemistry in cryptorchid testicular tissue. (A) Nuclear WT1 immunoreactivity in Sertoli cells, with adjacent spermatogonia remaining negative (×100). (B) Higher-magnification view of the staining pattern (×200). Table S1. De-identified case-level clinicopathological and immunohistochemical data of the GIST cohort.

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Informed Consent Statement: Patient consent was not required due to the retrospective nature of the study and the use of anonymized archival formalin-fixed, paraffin-embedded tissue specimens.

Data Availability Statement: The de-identified case-level clinicopathological and immunohistochemical data supporting the findings of this study are provided in Supplementary Table S1. Additional data are available from the corresponding author upon reasonable request.

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