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# The Diagnostic and Prognostic Utility of DOG1 Expression on Gastrointestinal Stromal Tumors

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## ABSTRACT

***Objective:*** We aimed to review our archives in order to evaluate both the diagnostic and prognostic significance of DOG1 on gastrointestinal stromal tumors (GISTs), and add further insight about those issues to the current literature including some conflicting results.

***Material and Method:*** DOG1 was evaluated in 100 cases of GISTs, immunohistochemically. Immunostaining index was counted for each antibody by using both the intensity and extent of staining. The association between immunostaining index of DOG1 and CD117, CD34, SMA, desmin, S-100, and Ki-67 index and clinicopathological features were analyzed.

***Results:*** Ninety cases were positive for DOG1, and 89 were positive for CD117. All CD117-negative tumors were positive for DOG1. High-risk group was directly correlated with tumor diameter, cellularity, necrosis, nuclear pleomorphism, mitotic count and Ki-67 index, by univariate analysis. The association between high-risk group and tumor diameter, mitotic count, and Ki-67 index was proved by multivariate analysis. Immunostaining index of DOG1, Ki-67 index, mitotic count, ulceration and hemorrhage were inversely correlated with overall survival by univariate analysis. The adverse impact of DOG1 ISI and mitotic count on overall survival were supported by multivariate analysis.

***Conclusion:*** DOG1 positivity was detected in most of GISTs and all in CD117-negative cases as a result underlining its diagnostic utility. Additionally, DOG1 overexpression was related with adverse prognosis. Thus, we suggest that immunostaining index of DOG1 should routinely be used while diagnosing GIST, and DOG1 might be considered as a potential prognostic tool and a target for novel therapies.

***Key Words:*** DOG1, Gastrointestinal stromal tumor, Immunohistochemistry

## INTRODUCTION

Gastrointestinal stromal tumor (GIST) is the most common mesenchymal neoplasm in gastrointestinal tract (1). It is considered to be originated from the interstitial cells of Cajal (2). Most GISTs have a mutation of c-KIT (CD117) proto-oncogene that encodes the CD117 protein, a transmembrane tyrosine kinase receptor (2). However, approximately 4-5% of the GISTs miss this mutation, and show immunonegativity for CD117 (3). The exact diagnosis of CD117-negative GISTs is crucial because more than two-thirds of them are still sensitive for imatinib-a small-molecule of tyrosine-kinase inhibitor-an effective targeted therapy for GIST (3). Thus, some molecules alternative for CD117 such as DOG1 (discovered on GIST-1) have been reported recently to be positive in especially CD117-negative GISTs (3).

DOG1 is a calcium-dependent chloride channel protein that is encoded by a gene called TMEM16A (TMEM16, FLJ10261, ANO1, ORAOV2, and AOS2) located on chromosome 11q13 (3). DOG1 has many significant functions such as regulation of the cholinergic activity of gastrointestinal smooth muscle (4-6), and regulation of both the survival and proliferation of cells (7). In addition, DOG1 activates alternative signals downstream of the RAS/RAF/MEK/ERK and the insulin-like growth factor (IGF)- dependent pathways (4, 8-9). These findings suggest that DOG1 may play a role in GIST development and progression, regardless of KIT and platelet-derived growth factor receptor alpha (PDGFRA) activation. DOG1 has been demonstrated to be positive in 89% of GISTs that have not CD117 or PDGFRA mutations (3). In addition, DOG1 is claimed to be more sensitive and specific than CD117 in many studies, with some contradictory results in the literature (10-12). About one third to one half of CD117-negative GISTs are reported to be positive for DOG1 (3). Although the diagnostic utility of DOG1 for accurate GIST diagnosis is being widely investigated, its prognostic role is little evaluated in the literature. A few recent studies suggest that DOG1 expression affect the prognosis with some conflicting results (4, 10-15).

The goals of the present study were to review our archives in order to evaluate both the diagnostic and prognostic

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significance of DOG1 on GISTs, and achieve further data to clarify those issues in the current literature containing some contradictory results.

## MATERIAL and METHOD

After obtaining Bozok University Ethic Committee approval, 100 cases of GIST diagnosed between 2008 and 2014 were included in the study. Ninety-six cases were from the archives of Department of Pathology, Gazi University School of Medicine, and 4 cases were retrieved from the archives of Department of Pathology, Bozok University School of Medicine. The clinicopathological features [age, gender, risk group, mitotic count in 50 high power fields (HPFs), tumor size, tumor location, growth pattern, cellularity, nuclear pleomorphism, ulceration, hemorrhage, necrosis, cell type, surgical/biopsy procedure type, and Ki-67 proliferation index] were achieved from the original pathology reports. Risk-groups had been established and adopted according to the criteria of Fletcher et al. (16) based on tumor size and mitotic count in 50 HPFs. In the present study, the cases were divided into two groups as the "lower-risk group" and "high-risk group" to predict the prognosis of GISTs. Very low-risk, low-risk, and intermediate-risk groups according to Fletcher et al. were considered as "lower-risk group", and high-risk group according to Fletcher et al. was considered as "high-risk group" in the study. Paraffin blocks were cut into 4-μm sections, deparaffinized and dehydrated according to standard protocols. Then, immunohistochemistry was performed using the streptavidin-biotin-peroxidase method for DOG1 (ready to use, mouse anti-human monoclonal antibody, clone K9, Leica Biosystems, United Kingdom) in an automated stainer (Leica Bond-Max, Leica Biosystems, United Kingdom). Cytoplasmic staining was considered as positive for DOG1. Besides DOG1, the slides of CD117, CD34, SMA, desmin, S-100 performed at the time of initial diagnosis were re-evaluated for both extent and intensity of staining. Five random HPFs were examined to count immunoreactive cells under light microscope. Extent of staining was scored as: score 0=no staining, score 1=<10%, score 2=10-60%, score 3=61-100%. Lower than 10% staining was considered as negative, ≥10% was considered as positive regardless from the intensity. Intensity of staining was scored as follows: score 0=no staining, score 1=mild, score 2=moderate, score 3=strong. Then, an immunostaining index (ISI) for each stain was calculated by multiplying the scores of extent of staining and intensity similar to the study of Wang et al. (ISI=extent X intensity scores) (17). The ISI of the antibodies ranged from 0 to 9. The ISI was considered as a feature indicating the expression of each stain in the study. The cases were divided into two groups according to Ki-67 proliferation index as <10% and ≥10%. ISI's of DOG1, CD117, CD34, SMA, desmin, and S-100 were correlated with each other and the clinicopathological parameters (age, gender, risk group, tumor location, tumor size, mitotic count, cell type, cellularity, nuclear pleomorphism, necrosis, hemorrhage, ulceration, and growth pattern), and Ki-67 proliferation index statistically. Follow-up and survival data were retrieved from the hospital records. Patients with severe diseases during follow-up were excluded from the survival data.

### Statistical Analysis

All data were analyzed using PASW Statistics version 18.0 (SPSS Inc. Chicago. IL. USA). The demographic variables were detected using descriptive statistics. The compliance of data with normal distribution was evaluated with the Kolmogorov-Smirnov and Shapiro-Wilk tests. Independent Samples t-test (t test for independent groups) and One-Way ANOVA tests were used in order to investigate the quantitative data with normal distribution. Mann-Whitney U and Kruskal-Wallis H tests were used in the evaluation of the data that did not show normal distribution. The Tukey HSD test was applied in order to determine from which group the difference was originated. The Chi-squared test, Fisher's exact tests, Pearson and Spearman's Rho correlation analysis were used for investigating the association between ISI's of antibodies and the clinicopathological parameters. The effects of associated variables were studied by multiple linear regression analysis using backward method. P-value <0.05 was considered as significant.

## RESULTS

### Clinicopathological Findings

We examined specimens from 100 patients (53 women and 47 men) with a mean age of 58.3±12.4 years (range 21 to 84). The tumors ranged from 0.4 to 25 cm (mean=6.06±4.24 cm) in diameter. Mitotic count varied from 0 to 80 (mean=7.4±15.3) per 50 high-power fields (HPFs). Four tumors were from esophagus, 60 tumors were from stomach, 7 were from duodenum, 11 were from jejunum, 3 were from ileum, 3 were from colon, and 12 were from mesentery/omentum. According to the criteria of Fletcher et al., 70 (70%) cases were classified as lower-risk group (15 were *very low*-, 37 were *low*-, 18 were *intermediate-risk* group), and 30 (30%) cases as high-risk group. The follow-up time ranged from 1 to 94 months (mean=45.2±23.9 months). Thirteen of the 100 cases were deceased, and 87 cases were alive when the follow-up was finished. It

was detected during follow-up that 2 cases died due to renal cell carcinomas, and the other 2 died due to colon adenocarcinomas, so those 4 cases were excluded from the survival analysis. Overall survival (OS) ranged from 1 to 94 months (mean=82.2±2.9 months). The clinicopathological features and their correlation with DOG1 expression of 100 GIST's are summarized in Table I.

**Immunohistochemical Findings**

Ninety of 100 cases were positive for DOG1, 89 were positive for CD117, 77 were positive for CD34, 22 were positive for SMA, 10 were positive for desmin, and one was positive for S-100 .The detailed immunohistochemical findings underlining the correlation of DOG1 with other markers are given in Table II and Table III.

All CD117-negative GISTs (11 cases) were positive for DOG1 (Figure 1A-C). Eight of them were positive for CD34, while 3 of them were negative for CD34. One of them was positive for SMA, while 10 of them were negative for SMA. All CD117-negative GISTs were negative for both desmin and S-100 . Male/female ratio in CD117-negative GISTs were 3/8. The mean age was 62.9±1.8 (range: 50-83). Nine cases were in lower-risk group, 2 cases were in high-risk group. Nine tumors were from stomach, one was from sigmoid colon, and one was from mesentery. They were ranged from 0.4 to 11 cm in diameter. All of them had expansive growth pattern. Seven of them were composed of spindle cells, 3 were of epithelioid cells, and remaining one was of mixed (spindle+epithelioid) cells. Six showed mild cellularity, 3 showed moderate cellularity, and 2 showed high cellularity. Mild cellular atypia was present in 6 cases, moderate cellular atypia was found in 2 cases, high cellular atypia was found in 2 cases, and no significant atypia was found in one case. Only one case showed ulceration. Hemorrhage was not present in any case. The mean mitotic count was 1.81±2.08 in 50 HPFs. Ki-67 proliferation index varied from 0% to 20% (mean=3.09±5.75%).

During follow-up, we detected that 3 of 10 DOG1 negative cases were deceased, while remaining 7 were alive. Ten of 90 DOG1 positive cases were found to be deceased. Six of those cases were deceased due to GISTs, 2 of those cases were deceased due to RCCs, and 2 of those were deceased due to colon carcinomas. Remaining 80 of DOG1 positive cases were found to be alive. There was no statistically significant correlation with DOG1 expression and current status of the patients (p=0.092). Mean OS was 52.4±33.2 months in DOG1 negative cases, while it was 44.5±22.8 in DOG1 positive cases. There was no statistically significant correlation between DOG1 positivity and OS (p=0.1). However, we detected that when ISI of DOG1 increased, OS decreased, and that was statistically significant both by univariate (p=0.023) and multivariate analysis (p=0.006, β=-0.269, t=-2.819). The data about the mean OS of the cases according to the ISI of DOG1 is given in Table IV.

Sixty-seven cases showed <5 mitoses/50 HPFs with a mean OS of 48.1±24.3 months, while remaining 33 cases showed ≥5 mitoses/50 HPFs with a mean OS of 39.5±22.4 months. According to those data, OS was detected to be inversely correlated with mitotic count by both univariate (p=0.012) and multivariate analysis (p=0.003, β=-0.289, t=-3.032). Additionally, OS was found to be negatively correlated with Ki-67 proliferation index, ulceration and hemorrhage by univariate analysis (p=0.039, p=0.043, p=0.043, respectively), but those findings were not supported by univariate analysis. The results of univariate and multivariate analysis of clinicopathological and immunohistochemical features are summarized in Table V.

High-risk group was directly correlated with tumor diameter, cellularity, necrosis, cellular pleomorphism,

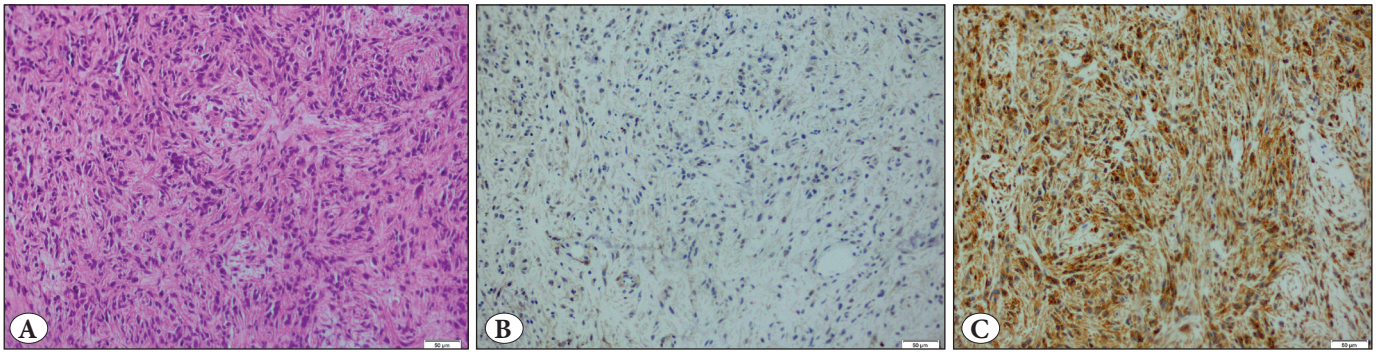


**Figure 1:** Photomicrographs of a case of CD117-negative GIST. **A)** The tumor was composed of both epithelioid and spindle cells (H&E; x200). **B)** CD117 immunonegativity of the tumor cells (CD117; x200). **C)**Diffuse and strong immunopositivity of the tumor cells for DOG1 (DOG1; x200).

**Table I:** The clinicopathological features of GISTs (n: 100)

| Patient Characteristics | Cumulative Population | DOG1 Positive | DOG1 Negative |
|---|---|---|---|
| **Age (mean, months)** | 58.3±12.4 | 59.4±11.09 | 48±18.6 |
| **Gender (female/male)** | | | |
| Female | 53 | 49 | 4 |
| Male | 47 | 41 | 6 |
| **Risk groups** | | | |
| **Lower risk group** | | 14 | 1 |
| Very low-risk | 15 | 34 | 3 |
| Low-risk | 37 | 15 | 3 |
| Intermediate-risk | 18 | | |
| **High-risk group** | | | |
| High-risk | 30 | 27 | 3 |
| **Location** | | | |
| Esophagus | 4 | 2 | 2 |
| Stomach | 60 | 57 | 3 |
| Small intestine | 21 | 17 | 4 |
| Colon | 3 | 2 | 1 |
| Mesentery/omentum | 12 | 12 | 0 |
| **Tumor size (cm)** | 6.0±4.24 (0.4-25) | | |
| <2 cm | 10 | 9 | 1 |
| 2-5 cm | 34 | 31 | 3 |
| 5-10 cm | 41 | 36 | 5 |
| >10 cm | 15 | 14 | 1 |
| **Mitotic count (in 50 HPFs)** | 7.4±15.3 (0-80) | | |
| <5 | 67 | 60 | 7 |
| ≥5 | 33 | 30 | 3 |
| **Cell type** | | | |
| Spindle | 73 | 65 | 8 |
| Epithelioid | 6 | 4 | 2 |
| Mixed | 21 | 21 | 0 |
| **Cellularity** | | | |
| Mild | 33 | 27 | 6 |
| Moderate | 23 | 22 | 1 |
| High | 44 | 41 | 3 |
| **Nuclear pleomorphism** | | | |
| Mild | 62 | 54 | 8 |
| Moderate | 11 | 11 | 0 |
| High | 16 | 14 | 2 |
| Absent | 11 | 11 | 0 |
| **Necrosis** | | | |
| Present | 30 | 26 | 4 |
| Absent | 70 | 64 | 6 |
| **Hemorrhage** | | | |
| Present | 3 | 3 | 0 |
| Absent | 97 | 87 | 10 |
| **Ulceration** | | | |
| Present | 15 | 14 | 1 |
| Absent | 85 | 76 | 9 |
| **Growth pattern** | | | |
| Expansive | 15 | 14 | 1 |
| Infiltrative | 85 | 76 | 9 |
| **Operation** | | | |
| Tumor resection | 48 | 44 | 4 |
| Radical surgery | 52 | 46 | 6 |

**GISTs:** Gastrointestinal stromal tumors, **HPF**: High power field.

**Table II:** The correlations of DOG1 and other immunohistochemical markers (n: 100)

| Immohistochemical Markers | Cumulative Population (n) | DOG1 Positive (n) | DOG1 Negative (n) |
|---|---|---|---|
| **CD117** | | | |
| Positive | 89 | 79 | 10 |
| Negative | 11 | 11 | 0 |
| **CD34** | | | |
| Positive | 77 | 72 | 5 |
| Negative | 23 | 18 | 5 |
| **SMA** | | | |
| Positive | 22 | 20 | 2 |
| Negative | 78 | 70 | 8 |
| **Desmin** | | | |
| Positive | 10 | 9 | 1 |
| Negative | 90 | 81 | 9 |
| **S100** | | | |
| Positive | 1 | 0 | 1 |
| Negative | 99 | 90 | 9 |
| **Ki-67** | | | |
| <10% | 81 | 74 | 7 |
| ≥10% | 19 | 16 | 3 |

mitotic count and Ki-67 proliferation index (p=0.000, p=0.004, p=0.019, p=0.000, p=0.005, p=0.000, respectively), by univariate analysis. The association between high-risk group and tumor diameter, mitotic count and Ki-67 proliferation index was supported by multivariate analysis (p=0.000, each).

No statistically significant association was detected between expression of DOG1 and CD117, CD34, desmin, S-100, Ki-67 proliferation index, mitotic count, age, gender, risk group, tumor size, growth pattern, cellularity, nuclear pleomorphism, ulceration, hemorrhage, and necrosis (Table V).

## DISCUSSION

The diagnosis of CD117-negative GISTs is still problematic in the literature (3). Recently, DOG1 has been suggested to be an alternative fruitful molecule for establishing GIST diagnosis, particularly for CD117-negative GISTs (11). As a result of wide review of the literature, CD117 and DOG1 positivity rate were found to be 91% and 93%, respectively, in about 3000 GIST cases (18). In parallel to the literature, we have documented CD117 and DOG1 positivity rate as 89% and 90%, respectively. In addition, the rate of DOG1

**Table III:**Immunostaining results of GISTs (n: 100)

| | DOG1 (%) | CD117 (%) | CD34 (%) | SMA (%) | Desmin (%) | S-100 (%) |
|---|---|---|---|---|---|---|
| **Extent of staining** | | | | | | |
| **Range** | 0-100 | 0-100 | 0-100 | 0-100 | 0-100 | 0-100 |
| **Mean** | 77.9±33.9 | 78.7±31.4 | 69.8±40.6 | 17.0±33.8 | 5.95±18.4 | 1.3±10.0 |
| **Score 0** | 9 | 3 | 18 | 58 | 72 | 92 |
| **Score 1** | 1 | 8 | 5 | 20 | 18 | 7 |
| **Score 2** | 13 | 8 | 8 | 7 | 7 | 0 |
| **Score 3** | 77 | 81 | 69 | 15 | 3 | 1 |
| **Intensity of staining** | | | | | | |
| **Score 0** | 9 | 3 | 18 | 58 | 72 | 92 |
| **Score 1** | 17 | 19 | 7 | 29 | 20 | 7 |
| **Score 2** | 24 | 14 | 2 | 4 | 2 | 0 |
| **Score 3** | 50 | 64 | 73 | 9 | 6 | 1 |
| **Immunostaining index (ISI)** | | | | | | |
| **Range** | 0-9 | 0-9 | 0-9 | 0-9 | 0-9 | 0-9 |
| **Mean** | 6.8±3.09 | 6.2±3.19 | 6.48±3.67 | 1.26±2.19 | 0.73±1.83 | 0.16±0.92 |
| **0** | 9 | 3 | 18 | 58 | 72 | 92 |
| **1** | 1 | 5 | 2 | 18 | 17 | 7 |
| **2** | 7 | 8 | 2 | 2 | 3 | 0 |
| **3** | 10 | 9 | 6 | 12 | 1 | 0 |
| **4** | 5 | 2 | 0 | 2 | 2 | 0 |
| **6** | 19 | 10 | 8 | 4 | 2 | 0 |
| **9** | 49 | 63 | 64 | 4 | 3 | 1 |

**Table IV:** The overall survival of cases according to ISI (immunostaining index) of DOG1

| ISI of DOG1 | Number of Cases | Overall Survival (Mean, months) | Standard Deviation |
|---|---|---|---|
| 0 | 9 | 49.3333 | 33.76389 |
| 1 | 1 | 80.0000 | . |
| 2 | 7 | 55.1429 | 22.40111 |
| 3 | 9 | 51.1000 | 22.14322 |
| 4 | 5 | 42.0000 | 23.80126 |
| 6 | 18 | 40.3684 | 22.20163 |
| 9 | 47 | 39.4490 | 22.51487 |

positivity in CD117-negative GISTs range from 20% to 100%, in the literature (18). This discrepancy might be attributed to the clinicopathological differences between the study groups. One of those differences may be the clone of DOG1 used in the study. In the literature, the sensitivity of clone K9 of DOG1 is suggested to be superior to other clones (10). In our study, we have used clone K9 and found that all CD117-negative cases (100%) were positive for DOG1 by immunohistochemistry. However, it should be noted that DOG1 is not pathognomonic for GIST (19-20). The data about the specificity of DOG1 is controversial in the literature (18). DOG1 may also be positive in some nonneoplastic tissues such as gastric epithelium, breast, testis, salivary gland, gallbladder, liver, lung, prostate, stomach, pancreas, urinary bladder, sweat glands, endometrium, and renal tubules (19). In addition, germ cell tumors, melanomas, some mesenchymal tumors and carcinomas are described to be positive for DOG1 (3, 20). In order to make differential diagnosis and establish accurate GIST diagnosis, a panel composed of CD117, CD34, SMA, desmin, and S-100 is routinely used in many laboratories to differ its mimickers, similar to ours. We suggest that DOG1 might be added this panel, since 90% of GISTs showed positivity for DOG1 in our study. In addition, some researchers recommend using a first step immunohistochemical panel composed of only CD117 and DOG1 may be more useful and reliable for the diagnosis of GISTs, especially for the CD117-negative cases (3). Additional immunohistochemical antibodies including CD34, SMA, desmin, and S-100 are suggested to be performed in the cases that are negative for either CD117 or DOG1 (3). The rate of CD34, SMA, desmin, and S-100 are reported to be as 72-78%, 19-57%, 4.1-5%, and 6-28% in various studies (12). In the present study the rate of positivity for CD34, SMA, desmin, and S-100 were as 77%, 22%, 10%, and 1%.

**Table V:** Statistically significant associations between immunohistochemical and clinicopathologic characteristics (p<0.05)

| | Univariate Analysis | | | Multivariate Analysis | | |
|---|---|---|---|---|---|---|
| | **DOG1 (ISI)** | **High-risk Group** | **OS** | **DOG1 (ISI)** | **High-risk Group** | **OS** |
| **DOG1 (ISI)** | | - | p=0.023, inv | | - | p=0.006, inv<br>β= -0.269<br>t= -2.819 |
| **Ki-67 index** | - | p=0.000, dir | p=0.039, inv | - | p=0.000, dir<br>β= 0.465<br>t= 3.711 | - |
| **Mitotic count** | - | p=0.005, dir | p=0.012, inv | - | p=0.000, dir<br>β= 0.454<br>t= 3.653 | p=0.003, inv<br>β= -0.289<br>t= -3.032 |
| **Tumor size** | - | p=0.000, dir | - | - | p=0.000, dir<br>β= 0.437<br>t= 4.923 | - |
| **Necrosis** | - | p=0.019, dir | - | - | - | - |
| **Ulceration** | - | - | p=0.043, inv | - | - | - |
| **Hemorrhage** | - | - | p=0.043, inv | - | - | - |
| **High cellularity** | - | p=0.004, dir | - | - | - | - |
| **Nuclear pleomorphism** | - | p=0.000, dir | - | - | - | - |

**Dir:** Directly correlated, **Inv:** Inversely correlated, **ISI**: Immunostaining index, **OS:** Overall survival

Beside those immunohistochemical markers, performing Ki-67 is strongly recommended while diagnosing a GIST, since high Ki-67 proliferation index is widely considered as an indicator of poor outcome in the literature (12, 18). Recently, some studies have suggested that Ki-67 proliferation index over than 10% indicates poor outcome (12, 21-24). Similar to the literature, Ki-67 proliferation index more than 10% was found to be associated with low OS by univariate analysis in our study. We have also found a direct association with higher mitotic count and low OS and high-risk group by univariate analysis, similar to Ki-67 labeling index. Nevertheless, we have demonstrated an inverse correlation between OS and higher mitotic count, but not with Ki-67 proliferation index by multivariate analysis. This result might be contributed to the fact that mitotic count reflects the M phase of mitotic cycle, while Ki-67 indicates the proliferative cells in G1, S, and G2 phases (21). Therefore, we think that higher mitotic count is still more reliable prognostic indicator than Ki-67 for GIST, and future studies should be conducted to clarify this issue.

Some recent studies have indicated that DOG1 might have potential prognostic affect in GIST (10,12,13), while some others have reported that it has only a diagnostic utility but not a prognostic value (25). Sözütek et al. claimed that DOG1 negativity in GISTs may indicate poor prognosis, however their result was not statistically significant (12). Jung et al. have proposed that DOG1 negativity is significantly correlated with recurrence and/or metastasis (13). However, Rizzo et al. have recently suggested that DOG1 overexpression might be used to predict poor prognosis of GISTs, since they have found low relapse-free survival in the cases with DOG1 overexpression than the cases with lower expression (4). Similar to Rizzo et al., we have demonstrated an inverse correlation between OS and ISI of DOG1 by both univariate and multivariate analysis. In parallel to our and Rizzo et al.'s study (4), Li et al. (15) have documented that higher levels of DOG1 expression in peripheral blood mononuclear cells of GIST patients indicate poor prognosis and might be used for monitoring recurrence and investigating efficacy of imatinib therapy for GIST patients. In addition, DOG1 is known to intervene the receptor-activated chloride current and modulate the cell proliferation by influencing the retinoblastoma (Rb) tumor activating the MEK/ERK pathway (8). Additionally, xenograft DOG1−/− models of GISTs exhibit an impaired cell proliferation as a result of the reduced IGF binding protein-5 levels that inhibit IGF-mediated downstream signals by trapping both IGF1 and IGF2 (9, 26). Thus, these data are likely that DOG1 overexpression might supply a proliferative advantage to malignant stromal cells, and elevated levels of DOG1 might adversely affect prognosis. However, we have not shown any association between ISI of DOG1 and Ki-67 proliferation index and mitotic count. That contradictory result might have been obtained due to other unknown signaling mechanisms related with DOG1 that should be clarified.

In the literature, there are some risk group classifications established for predicting the prognosis and malignant potential of GISTs (27-28). In this study, we have used the risk assessment of Fletcher et al., due to its simplicity and widely use. Mitotic count, tumor size, anatomic location, tumor necrosis, and nuclear pleomorphism have also been shown to be the prognostic parameters for GIST (27-29). Similar to the literature, necrosis, high mitotic count, high cellularity, greater tumor size and high nuclear pleomorphism were also detected to be associated with high-risk group in the present study. Thus, we suggest that these features should be noted in the pathology reports as indicators of poor prognosis.

In summary, this study has showed that DOG1 is a reproducible and reliable marker for GIST diagnosis, particularly for CD117-negative GISTs. We also strongly recommend that adding DOG1 in the routine immunohistochemical panel of GIST differential diagnosis (CD117, CD34, SMA, desmin, S-100) would aid establishment of accurate diagnosis of GIST. In addition, we have documented that DOG1 overexpression is related with poor outcome by both univariate and multivariate analysis. Thus, DOG1 ISI, referring the score of multiplication of staining intensity and extent, seems to be a useful prognostic tool as well as an ancillary diagnostic method. We claim that more comprehensive future studies including higher number of patients and longer follow-up might clarify the potential role of DOG1 on pathogenesis and prognosis of GISTs.

## CONFLICT of INTEREST

The authors declare no conflict of interest.

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