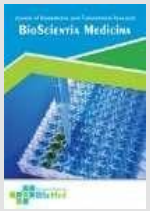
eISSN (Online): 2598-0580



Bioscientia Medicina: Journal of Biomedicine & Translational Research

Journal Homepage: www.bioscmed.com

Differential Roles of CD117 and Ki67 in Gastrointestinal Stromal Tumors: Diagnostic Utility Versus Prognostic Power

Fitri Nur Handriyani[1*], Noza Hilbertina[1], Henny Mulyani[2], Loli Devianti[3], Avit Suchitra[4], Husna Yetti[5]

[1]Department of Anatomical Pathology, Faculty of Medicine, Universitas Andalas/Dr. M. Djamil General Hospital, Padang, Indonesia

[2]Department of Anatomical Pathology, Faculty of Medicine, Universitas Andalas, Padang, Indonesia

[3]Department of Anatomical Pathology, Dr. Achmad Mochtar Regional General Hospital/Department of Anatomical Pathology, Faculty of Medicine, Universitas Andalas, Padang, Indonesia

[4]Department of Surgery, Faculty of Medicine, Universitas Andalas/Dr. M. Djamil General Hospital, Padang, Indonesia

[5]Department of Public Health Sciences and Community Medicine, Faculty of Medicine, Universitas Andalas, Padang, Indonesia

ARTICLE INFO

Keywords:
c-KIT
CD117
Gastrointestinal stromal tumor
GIST
Ki67 labeling index

***Corresponding author:**
Fitri Nur Handriyani

E-mail address:
fitri.n.handriyani@gmail.com

All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.37275/bsm.v9i7.1337

A B S T R A C T

Background: Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal neoplasms of the digestive tract, primarily driven by mutations in KIT or PDGFRA genes. CD117 (c-KIT) expression is a key diagnostic marker, while the Ki67 labeling index reflects cellular proliferation. Risk stratification, often using modified NIH criteria based on tumor size, mitotic rate, and location, guides prognosis and treatment. This study investigated the distinct roles of CD117 and Ki67 expression in relation to risk stratification in GIST patients. **Methods:** This cross-sectional analytical study examined 27 GIST cases diagnosed between January 2021 and December 2024 from three Indonesian hospitals. Formalin-fixed paraffin-embedded tissues were analyzed using immunohistochemistry for CD117 (clone YR145) and Ki67 (clone K2). CD117 positivity was defined as ≥5% tumor cell staining, and high Ki67 expression as >10% nuclear staining. Risk stratification utilized the modified NIH criteria. The Chi-square test assessed correlations ($p<0.05$ significance). **Results:** The cohort predominantly comprised patients >50 years (66.7%), males (59.3%), with gastric tumors (51.9%), large tumor size (>5cm in 96.3%), spindle cell morphology (77.8%), and high mitotic rates (74.1%). Most cases (85.2%) were classified as high-risk. CD117 was positive in 81.5% (22/27) of cases but showed no significant correlation with risk stratification ($p=0.561$). High Ki67 expression was found in 74.1% (20/27) of cases and demonstrated a significant positive correlation with high-risk stratification ($p=0.002$). The combination of CD117 and Ki67 status also showed a significant association with risk stratification ($p=0.001$). **Conclusion:** While CD117 expression remains a cornerstone for GIST diagnosis and targeted therapy selection, it did not correlate significantly with risk stratification in this cohort. Conversely, a high Ki67 labeling index was significantly associated with high-risk GIST, underscoring its potential as a valuable prognostic marker alongside established risk stratification parameters.

1. Introduction

Gastrointestinal stromal tumors (GISTs) represent the most prevalent mesenchymal neoplasms originating within the gastrointestinal (GI) tract, accounting for approximately 1-3% of all GI malignancies and up to 5% of sarcomas overall. First distinctly characterized by Mazur and Clark in 1983, these tumors are understood to arise from the interstitial cells of Cajal (ICC) or their pluripotential mesenchymal precursors. ICCs are specialized

pacemaker cells distributed throughout the muscularis propria of the GI tract, crucial for regulating gut motility. GISTs can occur anywhere along the digestive tube, from the esophagus to the rectum, and occasionally arise in extragastrointestinal sites such as the omentum, mesentery, or retroperitoneum. The stomach (60-65%) and small intestine (20-35%) are the most common locations, followed by the colorectum (3-5%) and esophagus (<1%).[1,2]

The incidence of GIST varies globally, with estimates generally ranging from 7 to 15 cases per million population per year. Studies in Western countries report incidences around 10-15 per million, while Asian countries like China and Korea may have rates exceeding 10 per million. Data from Indonesia remains limited, though institutional reports suggest an increasing recognition of the disease. GISTs typically occur in older adults, with a median age at diagnosis between 60 and 65 years, although they can arise at any age, including in pediatric populations, often associated with specific genetic syndromes. A slight male predominance is often observed.[3,4]

The molecular pathogenesis of GIST was revolutionized by the discovery of activating mutations in the receptor tyrosine kinase (RTK) genes KIT (encoding CD117) and Platelet-Derived Growth Factor Receptor Alpha (PDGFRA), found in approximately 85-90% of cases. These mutually exclusive mutations, most commonly located in KIT exon 11 (60-70%), KIT exon 9 (9-10%), PDGFRA exon 18 (8%), or PDGFRA exon 12 (2%), lead to ligand-independent activation of the kinase. This constitutive signaling drives cell proliferation and survival primarily through downstream pathways like PI3K-AKT-mTOR and RAS-RAF-MEK-ERK. A smaller subset of GISTs, termed "wild-type" (WT), lack KIT/PDGFRA mutations and may harbor alternative driver alterations, including deficiencies in the succinate dehydrogenase (SDH) complex (associated with syndromic GISTs like Carney-Stratakis syndrome or Carney triad), mutations in NF1 (neurofibromatosis type 1), BRAF, or, rarely, alterations in NTRK or FGFR genes.[5,6]

Histologically, GISTs exhibit a spectrum of morphologies, broadly classified into spindle cell type (70-80%), epithelioid type (20-30%), or mixed type (10%). Accurate diagnosis relies heavily on immunohistochemistry (IHC) due to morphological overlap with other mesenchymal tumors. The expression of CD117 (c-KIT protein) is detected in approximately 95% of GISTs and is considered the diagnostic hallmark. The discovery of KIT mutations and the high prevalence of CD117 expression paved the way for targeted therapy with tyrosine kinase inhibitors (TKIs) like imatinib, dramatically improving outcomes for patients with advanced or metastatic GIST. Discovered on GIST 1 (DOG1/ANO1) is another sensitive and specific marker, particularly useful for identifying the ~5% of GISTs that are CD117-negative, often those harboring PDGFRA mutations.[7,8]

While CD117 is crucial for diagnosis and predicting response to specific TKIs, its role as a prognostic marker independent of other factors is less clear. The biological behavior of GISTs is highly variable, ranging from indolent lesions with minimal malignant potential to aggressive sarcomas prone to recurrence and metastasis. Predicting this behavior is critical for clinical management, particularly for deciding on adjuvant therapy after surgical resection. Risk stratification systems have been developed to estimate the likelihood of recurrence, integrating key clinicopathological parameters: tumor size, mitotic count (typically per 5 mm^2 or 50 high-power fields [HPFs]), and tumor location (gastric GISTs generally having a better prognosis than those arising in the small intestine or rectum). Commonly used systems include the original NIH consensus criteria (Fletcher criteria), the modified NIH criteria (Joensuu criteria), and the Armed Forces Institute of Pathology (AFIP) criteria. These systems categorize GISTs into risk groups (very low, low, intermediate, high).[9,10]

Cellular proliferation rate is a fundamental aspect of tumor aggressiveness. The Ki67 protein is a nuclear antigen expressed during all active phases of the cell cycle (G1, S, G2, M) but absent in quiescent (G0) cells, making the Ki67 labeling index (LI), assessed by IHC,

a widely used marker of proliferation. High Ki67 LI has been associated with poorer prognosis in various cancers. In GIST, several studies have explored the prognostic value of Ki67 LI, often finding a correlation with higher risk stratification categories, increased recurrence rates, and reduced survival. However, its independent prognostic value beyond mitotic count and its precise role in risk stratification remain debated, partly due to variations in study methodologies and chosen cut-off values for defining "high" Ki67 expression.

Given the established diagnostic and therapeutic relevance of CD117 and the emerging prognostic significance of Ki67, understanding their distinct and potentially complementary roles in the context of established risk stratification parameters is crucial for refining GIST management. CD117 primarily reflects the underlying pathogenetic mechanism (KIT activation) and guides therapy, while Ki67 reflects the tumor's proliferative activity, a key determinant of aggressive behavior.

Despite extensive research on GIST biomarkers, the distinct contribution of CD117 expression versus Ki67 proliferation index specifically in relation to standard clinicopathological risk stratification within the same patient cohort, particularly in Southeast Asian populations like Indonesia, remains less explored. Previous studies often focus on one marker's correlation with outcome or risk, but directly comparing their relationship to established risk parameters (like modified NIH criteria) is crucial for clarifying their differential clinical utility. The novelty of this study lies in its direct comparative analysis of both CD117 and Ki67 expression against comprehensive risk stratification within an Indonesian GIST cohort, evaluating not only their individual associations but also their combined status. This approach allows for a clearer delineation of CD117's established diagnostic/therapeutic role versus Ki67's potential as a key prognostic indicator reflecting tumor biology beyond baseline KIT activation. Therefore, this study aimed to scientifically and rigorously analyze the relationship between immunohistochemical expression of CD117 (c-KIT) and the Ki67 labeling index with modified NIH risk stratification in GIST patients, thereby elucidating their distinct diagnostic versus prognostic power in this specific clinical context.

2. Methods

This study employed an observational, cross-sectional analytical design. The study population comprised all patients diagnosed with GIST via histopathology at the Anatomical Pathology Laboratories of Dr. M. Djamil General Hospital Padang, Andalas University Hospital, and Dr. Achmad Mochtar Regional General Hospital Bukittinggi between January 2021 and December 2024. A total of 41 cases were initially identified from records. Samples were included if they met the following criteria: Confirmed GIST diagnosis based on histopathology and IHC, availability of complete clinicopathological data from medical records (age, gender, tumor location, tumor size derived from radiology/pathology reports), availability of adequate formalin-fixed paraffin-embedded (FFPE) tissue blocks for further IHC staining, patient underwent biopsy or surgical resection, positive expression of CD117 (c-KIT) or, if CD117-negative, positive expression of DOG1, and no prior history of TKI therapy (imatinib) before tissue sampling. Cases with incomplete data or insufficient tissue were excluded. Based on these criteria and a sample size calculation aiming for 95% confidence and 10% precision, using an estimated CD117 positivity rate of 92%, a minimum sample size of 24 was determined. A total of 27 cases satisfying the inclusion criteria were ultimately included in the final analysis using a simple random sampling approach from the eligible pool. The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Andalas, Padang (Approval No: 226/UN.16.2/KEP-FK/2025) and the Ethics Committee of Dr. M. Djamil General Hospital, Padang. As the study utilized archived biological materials and retrospective data, patient confidentiality was strictly maintained by anonymizing

all data.

Clinicopathological data, including patient age at diagnosis, gender, primary tumor location, and maximum tumor diameter (from imaging or gross pathology reports), were retrieved from the medical and pathology records. Age was categorized as ≤50 years or >50 years. Tumor location was classified as gaster, small intestine, large intestine (colon/rectum), or extragastrointestinal (mesentery, retroperitoneum). Tumor size was categorized according to standard risk stratification cut-offs: ≤2 cm, >2-≤5 cm, >5-≤10 cm, and >10 cm. Archived hematoxylin and eosin (H&E)-stained slides from the selected cases were retrieved and re-evaluated by two experienced pathologists to confirm the diagnosis and assess histomorphological features according to the WHO Classification of Tumours, Digestive System Tumours, 5th Edition (2019). Morphological type was classified as spindle cell, epithelioid cell, or mixed. The mitotic count was determined by counting mitotic figures in the most active areas of the tumor over an area of 5 mm^2 (equivalent to 50 HPFs using a standard microscope field diameter, typically 0.50-0.55 mm at 400x magnification). The mitotic rate was categorized as low (≤5 mitoses/5 mm^2) or high (>5 mitoses/5 mm^2). If original slides were unavailable or suboptimal, new H&E sections were prepared from the corresponding FFPE blocks. The risk of progressive disease (recurrence or metastasis) for each primary GIST was assessed using the modified NIH consensus criteria proposed by Joensuu. This system incorporates tumor size, mitotic rate, and primary tumor location (gastric vs. non-gastric). Cases were initially assigned to very low, low, intermediate, or high-risk categories. For statistical analysis, these were further grouped into two broader categories: Low/Intermediate Risk (encompassing original very low, low, and intermediate categories) and High Risk. Tumor rupture, if documented, automatically places a case into the high-risk category.

IHC staining was performed on 3-4 µm-thick sections cut from the FFPE blocks. The staining procedure followed a standard protocol using the Streptavidin-Biotin Complex (SBC) method manually. Briefly, sections were deparaffinized in xylene and rehydrated through graded alcohols. Antigen retrieval was performed using heat-induced epitope retrieval (HIER) by immersing slides in 10 mM sodium citrate buffer (pH 6.0) and heating in a pressure cooker/decloaking chamber (95°C for 10-20 minutes). Endogenous peroxidase activity was blocked using 0.3-3% hydrogen peroxide in methanol for 10-30 minutes. Non-specific binding was blocked using a normal serum block (1.5%). Rabbit monoclonal anti-CD117 (c-KIT), clone YR145 (CellMarque, Paramount kit). Dilution used in the original thesis was 1:150, though 1:20 was mentioned elsewhere - standard lab protocols would apply. Rabbit monoclonal anti-DOG1 (ANO1), clone 244R-14 (CellMarque, Paramount kit) (Used for confirmation in CD117-negative cases). Rabbit monoclonal anti-Ki67, clone K2 (Leica reagent). Incubation was typically performed overnight at room temperature or 4°C according to manufacturer/laboratory protocols. Following primary antibody incubation, sections were washed and incubated with a secondary antibody (biotinylated universal link) and then with the Streptavidin-HRP complex (TrekAvidin HRP). The reaction was visualized using 3,3'-Diaminobenzidine (DAB) chromogen, resulting in a brown precipitate at the site of antigen localization. Slides were counterstained with Mayer's hematoxylin, dehydrated, cleared in xylene, and mounted with a permanent mounting medium. Appropriate positive controls (known GIST tissue, normal kidney tubules for CD117, tonsil or breast carcinoma for Ki67) and negative controls (omitting primary antibody) were included in each run.

CD117 (c-KIT) staining was evaluated based on the presence of brown reaction product in the cytoplasm and/or cell membrane of tumor cells. A semi-quantitative scoring system assessing intensity and proportion: Score 0 (<5% weak staining), Score 1+ (≥5-25% weak), Score 2+ (>25-50% moderate), Score 3+ (>50% strong). For analysis correlating with risk, CD117 expression was dichotomized into Negative (<5% staining) and Positive (≥5% staining). DOG1

staining (membranous/cytoplasmic) was similarly assessed in CD117-negative cases to confirm GIST diagnosis. Ki67 Labeling Index (LI) was determined by assessing the percentage of tumor cell nuclei showing distinct brown staining. Counting was performed manually under high magnification (400x) in areas with the highest density of positive cells ("hot spots"), evaluating at least 500-1000 tumor cells where possible, or using image analysis software (ImageJ was mentioned in the thesis for assessment). The index was calculated as (Number of Ki67-positive nuclei / Total number of tumor nuclei counted) x 100%. For statistical analysis, the Ki67 LI was categorized as Low (≤10%) or High (>10%) based on the commonly accepted, albeit debated, cut-off used in several GIST studies and adopted in the source thesis.

Data were compiled and analyzed using IBM SPSS Statistics software, Version 24. Descriptive statistics (frequencies, percentages, means, medians, ranges) were used to summarize the clinicopathological characteristics, risk stratification distribution, and biomarker expression patterns. The relationship between categorical variables – specifically, CD117 expression (Positive/Negative) and Ki67 LI (High/Low) as independent variables, and Risk Stratification (Low-Intermediate/High) as the dependent variable – was assessed using Pearson's Chi-square test or Fisher's exact test where appropriate (if expected cell counts were <5). A p-value less than 0.05 was considered statistically significant.

3. Results

Table 1 shows a detailed breakdown of the clinicopathological characteristics of the 27 GIST patients included in this study cohort. Demographically, the cohort skewed towards an older population, with two-thirds (66.7%, n=18) of patients being over 50 years old. The mean age at diagnosis was 51.59 years, with a wide range spanning from 26 to 71 years, indicating that while GIST is more common in later life, it can affect younger adults as well. A slight male predominance was observed, with males constituting 59.3% (n=16) of the cohort compared to 40.7% (n=11) females. Regarding tumor location, the stomach (gaster) was the most frequent primary site, accounting for just over half of the cases (51.9%, n=14). This was followed by the small intestine (18.5%, n=5), large intestine (14.8%, n=4), and extragastrointestinal sites (14.8%, n=4), aligning with known GIST distribution patterns where the stomach is the most common location due to the higher concentration of interstitial cells of Cajal. Pathologically, the tumors in this cohort were predominantly large at presentation. A significant majority (96.3%) measured over 5 cm in maximum diameter, with more than half (55.6%, n=15) exceeding 10 cm. Only one tumor (3.7%) was in the 2-5 cm range, and notably, no tumors smaller than 2 cm were identified. This finding suggests that patients in this cohort generally presented with sizable lesions. Histologically, the spindle cell morphology was the dominant type observed (77.8%, n=21), consistent with typical GIST presentations, while epithelioid and mixed types were less common (11.1% each, n=3). Furthermore, a high mitotic rate (>5 mitoses/5 mm^2), a key indicator of aggressive potential, was prevalent, identified in nearly three-quarters of the cases (74.1%, n=20). Collectively, the characteristics detailed in Table 1 portray a GIST cohort predominantly comprising older adults presenting with large tumors, often located in the stomach, exhibiting spindle cell morphology, and demonstrating a high proliferative activity based on mitotic rate. These features inherently suggest a cohort weighted towards more advanced or aggressive disease profiles at the time of diagnosis.

Table 2 shows the distribution of risk stratification among the 27 GIST patients, classified according to the modified NIH criteria. The data strikingly reveals a cohort heavily skewed towards a higher potential for aggressive behavior. A vast majority of the cases, 23 out of 27 (85.2%), were categorized into the High-Risk group. Conversely, only 4 cases (14.8%) fell into the combined Low/Intermediate Risk category. Breaking this down further into the specific modified NIH categories provides additional granularity. Notably, no

cases were classified as Very Low Risk or Low Risk in this cohort. All 4 cases classified as non-high risk actually belonged to the Intermediate Risk category. This complete absence of the lowest risk categories further emphasizes the advanced nature of the GISTs studied. This pronounced predominance of high-risk GISTs (85.2%) directly correlates with the clinicopathological features observed in Table 1, namely the high frequency of large tumor sizes (>5 cm in 96.3%) and high mitotic rates (>5/5mm² in 74.1%). These are key parameters driving the risk assessment in the modified NIH system. Such a distribution has significant clinical implications, suggesting that the patient population encountered in these centers often presents with tumors that inherently carry a substantial risk of recurrence or metastasis following surgical resection alone. This underscores the critical importance of accurate risk assessment in this population for guiding clinical decision-making, particularly regarding the potential benefit of adjuvant tyrosine kinase inhibitor therapy to mitigate the high inherent risk. The findings presented in Table 2 set the stage for understanding how biomarkers like CD117 and Ki67 correlate within these distinct risk groups.

Table 3 showed a comprehensive analysis of CD117 (c-KIT) immunohistochemical expression within the GIST cohort (N=27) and explored its relationship with the modified NIH risk stratification categories. The results confirm the high prevalence of CD117 expression typically associated with GIST, with 22 out of 27 cases (81.5%) demonstrating positive staining (defined as ≥5% tumor cell staining). Consequently, only 5 cases (18.5%) were CD117-negative, likely representing GISTs driven by alternative pathways (PDGFRA mutations or wild-type) confirmed by other means (like DOG1 staining, as per study criteria). Among the 22 CD117-positive tumors, a semi-quantitative assessment revealed a spectrum of staining patterns. Moderate intensity staining (Score 2+, >25-50% positive cells) was the most common pattern, observed in half of the positive cases (n=11, 50.0%). Weak staining (Score 1+, ≥5-25%) was seen in just over a third (n=8, 36.4%), while strong, diffuse staining (Score 3+, >50%) was less frequent (n=3, 13.6%). This distribution highlights the variability in CD117 protein expression levels even among positive cases. The central finding presented in Table 3, however, relates to the correlation analysis between CD117 status and risk stratification. Despite the high rate of CD117 positivity, the analysis revealed no statistically significant association between CD117 expression (Positive vs. Negative) and the assigned risk category (High vs. Low/Intermediate). The Chi-square test yielded a p-value of 0.561, which is well above the conventional threshold for statistical significance ($p<0.05$). While the majority of CD117-positive cases (18 out of 22) were indeed high-risk, this largely mirrored the overall high-risk prevalence within the cohort (85.2%). Importantly, the 4 low/intermediate risk cases identified were all CD117-positive. Furthermore, all 5 CD117-negative cases fell into the high-risk category, though this number is too small for definitive conclusions. Therefore, the interpretation drawn from Table 3 is that while CD117 expression is a crucial diagnostic marker confirming GIST lineage in the vast majority of cases within this cohort, its presence or absence did not significantly differentiate between tumors classified as high-risk versus low/intermediate risk based on established clinicopathological criteria (tumor size, mitotic rate, location). This supports the understanding that CD117's primary clinical utility lies in diagnosis and prediction of response to KIT-targeted therapies, rather than serving as an independent prognostic factor for risk stratification itself in this patient population.

Table 4 showed the analysis of the Ki67 labeling index (LI), a marker of cellular proliferation, and its association with risk stratification in the GIST cohort (N=27). The Ki67 LI values varied considerably across the samples, ranging from a minimum of 3.15% to a maximum of 51.48%, with a mean value of 20.54% and a median of 17.64%. This wide range indicates diverse proliferative activity among the tumors studied. Using a pre-defined cut-off of >10% to categorize tumors as having high proliferative activity,

the data revealed that a substantial majority, 20 out of 27 cases (74.1%), exhibited a high Ki67 LI. Correspondingly, 7 cases (25.9%) were classified as having a low Ki67 LI (≤10%). This prevalence of high Ki67 LI mirrors the high proportion of cases with high mitotic rates observed in Table 1, providing further evidence of significant proliferative activity within this GIST cohort. Most importantly, Table 4 demonstrated a statistically significant and strong positive correlation between the Ki67 LI category and the modified NIH risk stratification. The Chi-square test yielded a p-value of 0.002, well below the threshold for significance. This association was remarkably clear: all 20 cases (100%) with a high Ki67 LI (>10%) were classified into the High-Risk group. Conversely, the 7 cases with a low Ki67 LI (≤10%) were distributed across risk categories, with 4 (57.1%) falling into the Low/Intermediate Risk group and 3 (42.9%) still classified as High Risk, likely driven by other factors like large tumor size or non-gastric location in those specific cases. The interpretation derived from Table 4 strongly suggests that the Ki67 labeling index, reflecting the tumor's proliferative fraction, is a powerful indicator of biological aggressiveness that aligns significantly with established GIST risk stratification criteria in this cohort. Unlike CD117 expression (Table 3), which primarily served a diagnostic role, a high Ki67 LI (>10%) was a robust marker associated almost exclusively with high-risk classification. This finding supports the growing body of evidence suggesting Ki67 LI is a valuable prognostic tool that can complement, or potentially refine, risk assessment based on traditional parameters like mitotic count, tumor size, and location, offering clinicians additional insights into the potential behavior of the tumor.

Table 5 showed the detailed analysis of the combined expression patterns of CD117 and Ki67 in relation to modified NIH risk stratification among the 27 GIST patients. This combined biomarker approach revealed distinct subgroups and reinforced the significant association with risk, as evidenced by a Chi-square p-value of 0.001. The largest subgroup identified was composed of cases that were CD117 positive and exhibited a high Ki67 Labeling Index (LI) (>10%). This group accounted for over half of the cohort (15 out of 27 cases, 55.6%). Critically, all 15 cases within this CD117 Positive / Ki67 High subgroup were classified as High Risk. This finding underscores that even within the diagnostically typical CD117 positive GISTs, high proliferative activity, as indicated by Ki67, strongly correlates with a high-risk profile. The second largest group consisted of cases that were CD117 Positive but had a low Ki67 LI (≤10%), representing 7 out of 27 cases (25.9%). This particular subgroup was unique as it contained all 4 of the Low/Intermediate Risk cases identified in the entire study cohort. The remaining 3 cases in this CD117 Positive / Ki67 Low group were still classified as High Risk, likely due to other overriding risk factors such as large tumor size or specific tumor location. Interestingly, the 5 cases (18.5% of the cohort) that were CD117 Negative all exhibited a high Ki67 LI (>10%). Similar to the CD117 Positive / Ki67 High group, all of these CD117 Negative / Ki67 High cases were also classified as High Risk. This suggests that when CD117 is not expressed (or below the threshold for positivity), a high Ki67 LI remains a strong indicator of a high-risk tumor phenotype. Finally, the study found no cases (0.0%) that were both CD117 Negative and had a low Ki67 LI. The highly significant p-value of 0.001 for the association between these combined biomarker profiles and risk stratification highlights the value of considering these markers in conjunction. The data indicates that a high Ki67 LI consistently aligns with high-risk GISTs, irrespective of CD117 status in this cohort. Conversely, the combination of CD117 positivity with a low Ki67 LI was the only profile that identified patients within the Low/Intermediate risk category. These patterns suggest that a combined immunohistochemical assessment offers a more nuanced approach to evaluating the biological aggressiveness and risk profile of GISTs compared to assessing each marker in isolation.

Table 1. Clinicopathological characteristics of the gastrointestinal stromal tumor (GIST) patient (N=27).

Characteristic	Category	Frequency (n)	Percentage (%)
Demographics			
Age (Years)	Mean ± SD	51.59 ± 11.5	-
	Range	26 - 71	-
	≤ 50	9	33.3
	> 50	18	66.7
Gender	Male	16	59.3
	Female	11	40.7
Primary tumor location	Stomach	14	51.9
	Small Intestine	5	18.5
	Large Intestine	4	14.8
	Extragastrointestinal	4	14.8
Tumor pathology			
Tumor size (cm)	< 2	0	0.0
	2 - ≤ 5	1	3.7
	> 5 - ≤ 10	11	40.7
	> 10	15	55.6
Histological type	Spindle Cell Type	21	77.8
	Epithelioid Cell Type	3	11.1
	Mixed Cell Type	3	11.1
Mitotic rate (/5 mm²)	Low (≤ 5)	7	25.9
	High (> 5)	20	74.1

Table 2. Distribution of gastrointestinal stromal tumor (GIST) risk stratification using modified NIH criteria (N=27).

Risk category (Modified NIH criteria)	Frequency (n)	Percentage (%)
Detailed categories		
Very low risk	0	0.0
Low risk	0	0.0
Intermediate risk	4	14.8
High risk	23	85.2
Grouped categories for analysis		
Low/Intermediate risk	4	14.8
High risk	23	85.2
Total	**27**	**100.0**

Table 3. CD117 (c-KIT) expression and correlation with modified NIH risk stratification in GIST patients (N=27).

Feature	Category	Frequency (n)	Percentage (%)
Overall CD117 expression status	Positive (≥5% staining)	22	81.5
	Negative (<5% staining)	5	18.5
	Total	**27**	**100.0**
Semi-quantitative score (CD117 positive cases, n=22)	Score 1+ (Weak, ≥5-25%)	8	36.4
	Score 2+ (Moderate, >25-50%)	11	50.0
	Score 3+ (Strong, >50%)	3	13.6
	Total Positive Cases	**22**	**100.0**
Correlation: CD117 status vs. risk stratification			
CD117 expression	**Risk Stratification**		
	High Risk (n)	Low/Int Risk (n)	Total (n)
Positive	18	4	22
Negative	5	0	5
Total	**23**	**4**	**27**
Statistical significance (Chi-Square Test)	**p-value**	**0.561**	

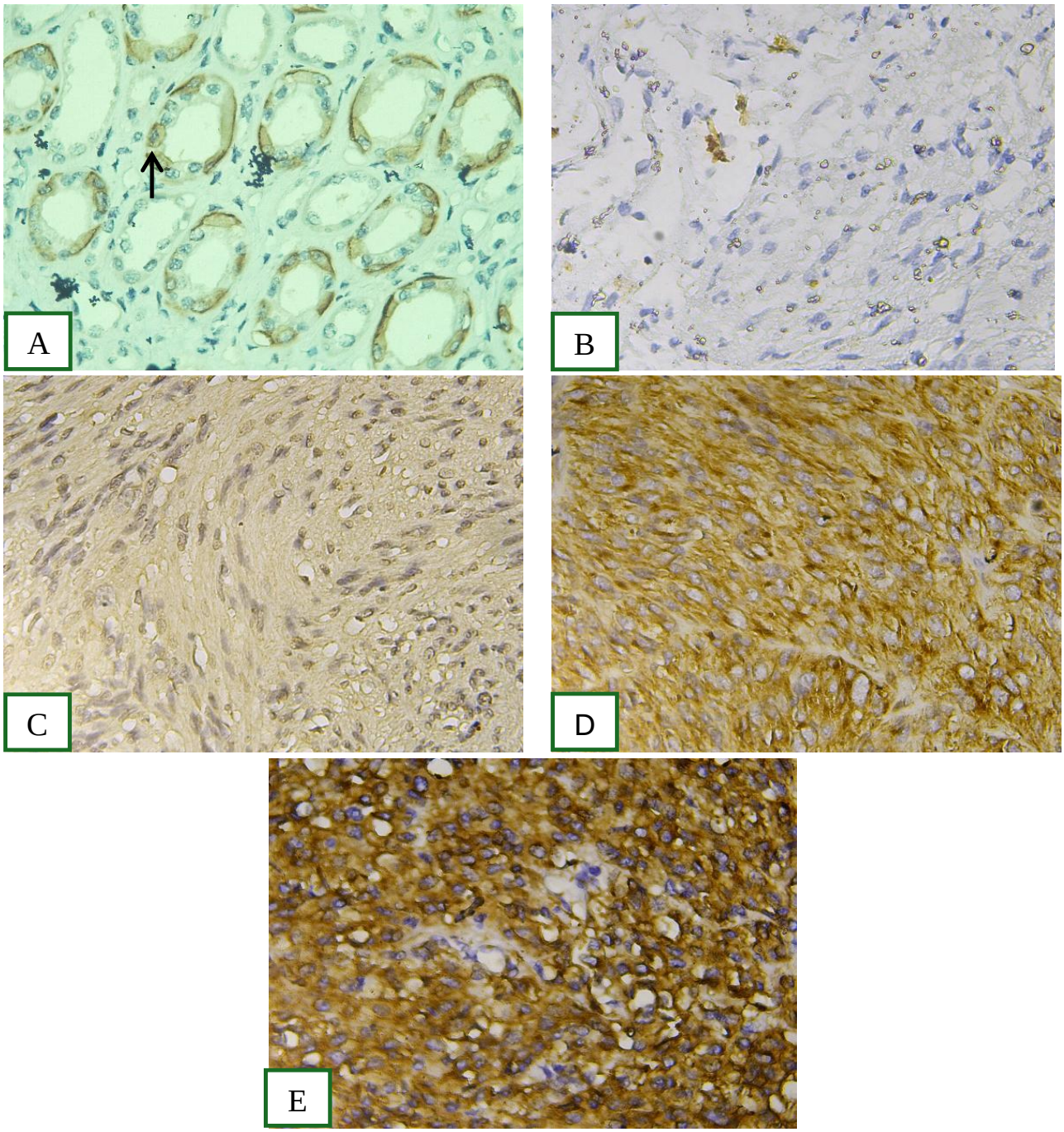


Figure 1. Immunohistochemical expression of CD117 (c-KIT). (A) CD117 expression in the cytoplasm and membrane (black arrow) of normal kidney tubules, serving as a positive control (IHC, 400x magnification). (B) Tumor cells negative for CD117 staining (IHC, 400x magnification). (C) Positive CD117 expression score 1+ in the membrane and cytoplasm of tumor cells (IHC, 400x magnification). (D) Positive CD117 expression score 2+ in the membrane and cytoplasm of tumor cells (IHC, 400x magnification). (E) Positive CD117 expression score 3+ in the membrane and cytoplasm of tumor cells (IHC, 400x magnification).

Table 4. Ki67 labeling index (LI) expression and correlation with modified NIH risk stratification in GIST patients (N=27).

Feature	Category	Value / Frequency (n)	Percentage (%)
Ki67 labeling index distribution	Mean	20.54%	-
	Median	17.64%	-
	Range	3.15% - 51.48%	-
Ki67 expression category (Cut-off: >10%)	High (>10%)	20	74.1
	Low (≤10%)	7	25.9
	Total	**27**	**100.0**
Correlation: Ki67 category vs. risk stratification			
Ki67 category	**Risk Stratification**		
	High Risk (n)	Low/Int Risk (n)	Total (n)
High (>10%)	20	0	20
Low (≤10%)	3	4	7
Total	**23**	**4**	**27**
Statistical significance (Chi-Square Test)	**p-value**	**0.002**	

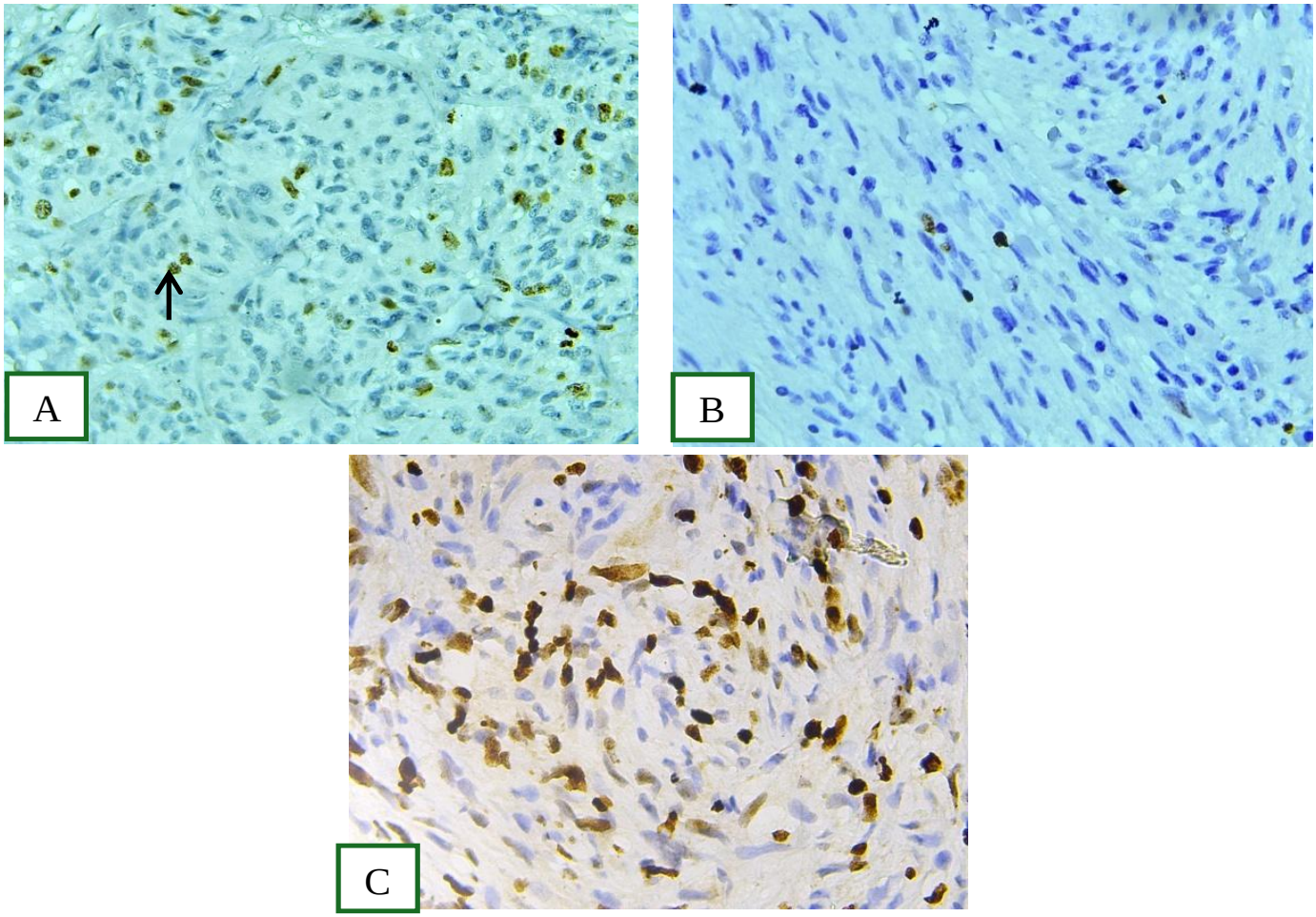


Figure 2. Immunohistochemical expression of Ki67 labeling index. (A) Ki67 expression in a positive control (malignant breast tumor), showing nuclear staining in tumor cells (black arrow, IHC, 400x magnification). (B) Low Ki67 expression in tumor cell nuclei (IHC, 400x magnification). (C) High Ki67 expression in tumor cell nuclei (IHC, 400x magnification).

Table 5. Correlation between combined CD117 & Ki67 expression status and modified NIH risk stratification in GIST patients (N=27).

Combined CD117 & Ki67 expression status	High risk (n, % of total cohort)	Low/Intermediate Risk† (n, % of total cohort)	Total (n, % of total cohort)
CD117 positive & Ki67 high (>10%)	15 (55.6%)	0 (0.0%)	15 (55.6%)
CD117 positive & Ki67 low (≤10%)	3 (11.1%)	4 (14.8%)	7 (25.9%)
CD117 negative & Ki67 high (>10%)	5 (18.5%)	0 (0.0%)	5 (18.5%)
CD117 negative & Ki67 low (≤10%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Total	**23 (85.2%)**	**4 (14.8%)**	**27 (100.0%)**
Statistical significance (Chi-square test)	**p-value**	**0.001**	

4. Discussion

This study investigated the relationship between the expression of two key biomarkers, CD117 (c-KIT) and Ki67, and established risk stratification criteria in a cohort of 27 GIST patients from Indonesia. The findings highlight the differential roles of these markers: CD117 remains pivotal for diagnosis but shows no independent correlation with risk category, whereas the Ki67 labeling index demonstrates a significant association with high-risk GIST, supporting its prognostic relevance.[11,12]

The clinicopathological characteristics of our cohort generally align with known GIST epidemiology, showing a predominance in patients over 50, a slight male majority, and the stomach as the most common site. However, our cohort was notably skewed towards larger tumors (>5 cm in 96.3%) and high mitotic rates (74.1%), consequently leading to a very high proportion (85.2%) of cases being classified as high-risk according to the modified NIH criteria. This high prevalence of advanced features might reflect referral patterns to tertiary centers or delays in diagnosis in the study setting, potentially influencing the correlative analyses compared to population-based studies with a broader risk spectrum.[13,14]

CD117 expression was detected in 81.5% of cases, a rate slightly lower than the typically reported ~95%, but still confirming its high sensitivity for GIST diagnosis. The five CD117-negative cases were presumably confirmed as GIST based on morphology and likely DOG1 positivity. Crucially, our analysis revealed no statistically significant association between CD117 expression status (positive vs. negative) and risk stratification (p=0.561). While CD117 positivity reflects the underlying KIT pathway activation crucial for pathogenesis and targeted therapy response, its mere presence or absence, or even semi-quantitative level, does not appear to reliably predict the degree of clinical aggressiveness as defined by standard risk parameters. This finding is consistent with several previous studies that also reported a lack of significant correlation between CD117 expression and risk stratification or other prognostic factors. Although some studies have suggested potential links between specific CD117 staining patterns or intensity and outcome, or correlation with mutation type, which does have prognostic implications, CD117 expression is primarily a diagnostic and predictive (for TKI response) marker, not a strong independent prognosticator of recurrence risk beyond the established criteria.[15,16]

In stark contrast, the Ki67 labeling index demonstrated a strong and statistically significant association with risk stratification (p=0.002). A high Ki67 LI (>10%) was almost exclusively found in high-risk GISTs (20 out of 20 high Ki67 cases were high-risk), while low/intermediate-risk cases predominantly had low Ki67 LI (4 out of 7 low Ki67 cases were low/intermediate risk). This finding strongly supports the role of Ki67 as a marker of

tumor proliferation that correlates well with the parameters defining malignant potential in GIST (size, mitotic rate, location). It aligns with numerous studies and meta-analyses indicating that a higher Ki67 LI is associated with higher risk categories, increased likelihood of recurrence or metastasis, and poorer overall survival in GIST patients. While mitotic count is already a key component of risk stratification, Ki67 provides a broader assessment of the cell population actively cycling, potentially capturing proliferative activity beyond visible mitoses, especially in tumors with lower mitotic rates or where counting is challenging. Some researchers argue Ki67 might even be a more robust or reproducible measure of proliferation than mitotic counting in certain contexts. The optimal cut-off for Ki67 LI in GIST remains debated (common values include 5%, 8%, 10%), but our use of >10% effectively segregated the high-risk group. The significant correlation observed reinforces the potential value of incorporating Ki67 assessment, perhaps alongside or even refining mitotic count, in prognostic evaluation and guiding decisions about adjuvant therapy, particularly in intermediate or high-risk cases.[17,18]

The combined analysis of CD117 and Ki67 further illustrated these differential roles. The largest group (CD117+/Ki67 high) consisted entirely of high-risk patients, highlighting that even within the diagnostically typical CD117+ GISTs, high proliferation signifies high risk. Conversely, the CD117+/Ki67 low group contained all the low/intermediate risk patients found in the study, suggesting that low proliferation in a CD117+ GIST correlates with lower risk categorization. Interestingly, all CD117-negative GISTs in this cohort displayed high Ki67 expression and were categorized as high-risk. While the number is small (n=5), this observation warrants further investigation, as it might suggest that CD117-negative GISTs (often PDGFRA-mutant or WT) in this population may inherently possess higher proliferative activity or aggressive features, although larger studies are needed to confirm this.[19,20]

This study has several limitations, as acknowledged in the original thesis. Firstly, the sample size (N=27) is relatively small, limiting the statistical power to detect weaker associations and potentially impacting the generalizability of the findings. Secondly, the cohort was heavily weighted towards high-risk cases, which may obscure relationships that might be apparent in a population with a more balanced distribution across risk categories. Thirdly, this study focused on biomarker expression by IHC and its correlation with risk stratification parameters (size, mitosis, location), not directly with clinical outcomes like recurrence-free survival (RFS) or overall survival (OS) due to the cross-sectional design and likely lack of long-term follow-up data. Fourthly, the underlying genetic mutations (KIT, PDGFRA) were not assessed; correlating biomarker expression with specific mutation types could provide deeper insights, as mutation status itself carries significant prognostic and therapeutic implications. Lastly, while IHC protocols were outlined, inter-observer variability in assessing staining and mitotic counts remains a potential factor, although mitigated by evaluation by experienced pathologists.

Despite these limitations, the study provides valuable data from an Indonesian cohort, reinforcing the established diagnostic utility of CD117 and strongly supporting the prognostic significance of the Ki67 labeling index in GIST. The significant correlation between high Ki67 LI (>10%) and high-risk stratification suggests that Ki67 assessment can serve as a valuable adjunct to standard risk assessment, helping to identify tumors with greater malignant potential.

Future research should involve larger, prospective cohorts with long-term clinical follow-up to definitively establish the independent prognostic value of Ki67 LI compared to or combined with mitotic count and other risk factors for predicting actual patient outcomes (RFS, OS). Investigating the correlation between Ki67 LI, specific mutation types (KIT exon 11 deletion vs. insertion, exon 9, PDGFRA D842V vs. others, WT subtypes), and response to TKI therapy would also be

highly informative. Standardization of Ki67 assessment methodology and interpretation, including consensus on optimal cut-off values, remains an important goal for its reliable implementation in routine clinical practice.

5. Conclusion

In this cohort of GIST patients, immunohistochemical expression of CD117 (c-KIT), while positive in the majority of cases (81.5%) and essential for diagnosis, did not show a significant correlation with the modified NIH risk stratification categories. This underscores its primary role as a diagnostic and therapy-predictive marker rather than an independent prognostic indicator of risk level. Conversely, a high Ki67 labeling index (>10%), observed in 74.1% of cases, was strongly and significantly associated with high-risk GIST classification (p=0.002). These findings highlight the differential utility of these biomarkers: CD117 confirms GIST lineage and potential TKI sensitivity, while Ki67 reflects proliferative activity and provides significant prognostic information that correlates with established parameters of tumor aggressiveness. Routine assessment of Ki67 LI, alongside standard clinicopathological features and risk stratification, may enhance the prognostic evaluation of GIST patients and potentially aid in refining therapeutic strategies, particularly for those in intermediate or high-risk groups. Further studies with larger cohorts and clinical outcome data are warranted to solidify the prognostic role of Ki67 in diverse GIST populations.

6. References

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