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Prognostic impact of Ki-67 in patients with gastric cancer—the importance of depth of invasion and histologic differentiation

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Abstract

Ki-67 protein is a cellular marker for proliferation. The role of Ki-67 as a prognostic biomarker has not been established in gastric cancer. The present study was performed to investigate the significance of Ki-67 expression as a biomarker in early gastric cancer (EGC).

With tissue microarray for 320 patients with gastric cancer, we performed immunohistochemical staining for Ki-67. Its clinical significance was analyzed with adjustment via the propensity score-matching. For validation, we performed bootstrap resampling.

The median follow-up duration was 72 months (range: 3–120 months). Ki-67-high group showed worse prognosis than Ki-67-low group in EGC (5-YSR, 78.9% vs 92.0%, $P = .018$), but not in advanced gastric cancer (AGC) (5-YSR, 58.5% vs 59.2%, $P = .951$). Interestingly, in the patients with well-differentiated histology, prognosis for Ki-67-high group was considerably worse than that for Ki-67-low group (5-YSR, 67.0% vs 94.4%, $P = .012$), but not in those with moderately differentiated ($P = .504$) and poorly differentiated histology ($P = .905$). In this cohort, there was a strong correlation between the proportion of EGC and well-differentiated histology ($r = 0.215$, $P = .002$). Multivariate analysis also revealed that the high-Ki-67 expression serves as a poor prognostic factor in EGC (HR 4.346, 95% CI 1.397–13.515, $P = .011$), especially in the well-differentiated histology, but not in all the patients ($P = .171$). Bootstrap resampling internally validated this result ($P = .011$).

This study suggests that Ki-67 expression may be a good biomarker for prognosis prediction for EGC with well-differentiated histologic type.

Abbreviations: AGC = advanced gastric cancer, CI = confidence interval, EGC = early gastric cancer, HR = hazard ratio, IHC = immunohistochemical staining, LN = lymph node, M/D = moderately differentiated, OS = overall survival, P/D = poorly differentiated, TMA = tissue microarray, W/D = well-differentiated, 5-YSR = 5-year survival rate.

Keywords: advanced gastric cancer, early gastric cancer, Ki-67, prognosis

1. Introduction

Gastric cancer ranks as the fourth most prevalent cancer worldwide and has the second highest prevalence among cancers in Korea.[1–3] Its treatment outcome, although improved by regular gastroscopic examination, is still unsatisfactory, and there are few prognostic biomarkers available. Up to now, pathologic TNM staging still serves as one of the best prognostic markers for gastric cancer. However, there is a large variation even within the same TNM stage; some stage I early gastric cancers (EGCs) relapse with distant metastasis even after radical gastrectomy. Therefore, a new biomarker needs to predict prognosis of the gastric cancers within the same TNM stage.

Recently, many researchers have been performing biomarker studies to identify a new molecular biomarker involved in carcinogenesis, invasion, or metastasis of gastric cancer. Among identified markers, Ki-67 protein is widely used as a cellular marker for proliferation.[4] It is strictly associated with cell proliferation and is detected in cells in the active phases of the cell cycle (i.e., G1, S, G2, and mitosis) but not in resting cells (G0),[5] so determining the percentage of Ki-67-expressing cells (Ki-67 labeling index; Ki-67 LI) has become the standard procedure to assess the proliferative activity of cancer cells.[6,7] In addition, a high Ki-67 LI is associated with high grade, advanced stage, and poor prognosis of cancers.[8–10] To confirm this finding, researchers have investigated the clinical significance of the Ki-67 LI in gastric cancer, but the role of the Ki-67 LI as a prognostic

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GHK and S-IG contributed equally to this work.

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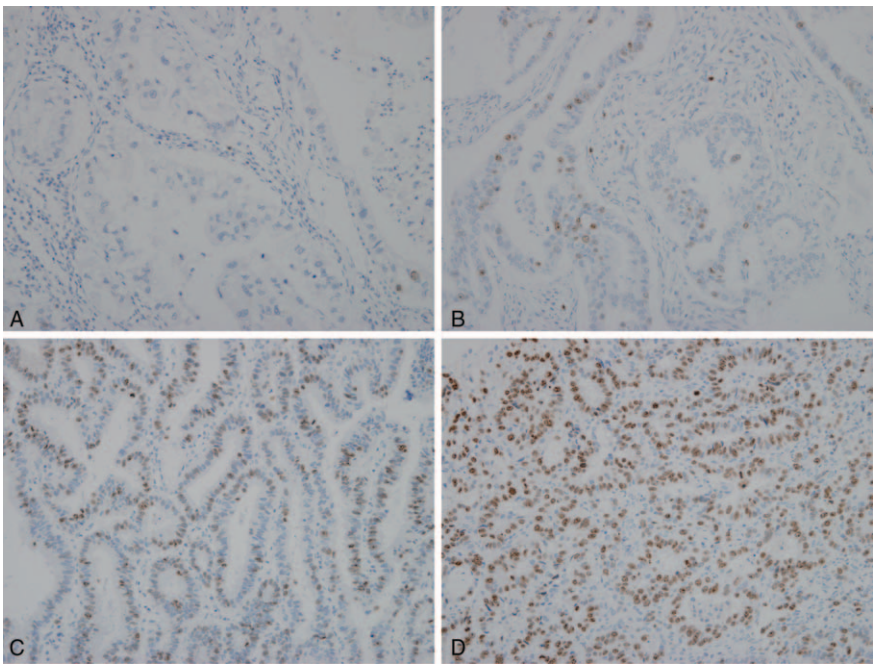

Figure 1. The representative findings of immunohistochemical staining for Ki-67 in various gastric tissues. Nuclei of gastric carcinoma cells were immunohistochemically stained for Ki-67. A, Ki-67 score 0. B, Ki-67 score 1. C, Ki-67 score 2. D, Ki-67 score 3.

factor in gastric cancer has not been established.[11,12] Our previous study suggested that it is much harder to find a single prognostic biomarker for advanced gastric cancer (AGC) than for early gastric cancer (EGC) because AGC has a more heterogeneous histologic subtype.[13] Additionally, AGC has more complicated genetic abnormalities than EGC.[14] Hence, we hypothesized that the role of the Ki-67 LI in EGC is different from that in AGC and that it could serve as a biomarker to predict the prognosis in EGC. Therefore, we conducted this study to determine whether Ki-67 protein serves as a prognostic factor in EGC.

2. Materials and methods

2.1. Clinical specimens and patients

Tissue microarray samples were used; they contained 329 gastric cancer tissues from the patients with gastric cancer who had received radical gastric resection from 1999 to 2007. All available hematoxilin and eosin (H&E)-stained slides of surgical specimens were initially reviewed, and a representative paraffin block for each case was selected. From each case of formalin-fixed and paraffin-embedded gastric cancer tissues, 2 mm diameter core tissue biopsies were obtained, and arranged in new recipient paraffin blocks. Each tissue core contained the invasive front of gastric cancer. Institutional Review Board of Gyeongsang National University Hospital permitted us to use these samples for this study (GNUHIRB-2009-19).

2.2. Immunohistochemical staining (IHC)

Briefly, a 4-um-thick paraffin-embedded tissue microarray (TMA) on each slide was deparaffinized and rehydrated. Then, the slides were incubated in 3% H_2O_2 for 10 min to prevent nonspecific background staining, and heated for 20 min in 10 mmol/L citrate buffer (pH 6.0) in a microwave oven (700 W) for epitope retrieval. After additional incubation with Ultra V Block (Lab Vision Corporation, Fremont, CA) for 10 min at room temperature to further block background staining, slides were incubated with a primary monoclonal antibodies specific to Ki-67 (DAKO, dilution 1:2000). Antibody binding was detected by the UltraVision LP Detection System (Lab Vision Corporation). Color development was performed with 3–3'-diaminobenzidine and counterstained with hematoxylin. The immunohistochemical staining process was performed by an automatic staining machine (BenchMark XT, VENTANA, Tucson, AZ).

Antigen expression was examined by pathologists with blindness to the clinical data of the patients. For Ki-67, staining intensity was scored as 0 when less than 5% of tumor cells were stained, 1 when 5% to 19% of tumor cells were stained, 2 when 20% to 49% of tumor cells were stained, and 3 when 50% or more of tumor cells were stained. Scores of 0 and 1 were interpreted as Ki-67-low, and 2 and 3 as Ki-67-high. Histological type and tumor stage were classified according to the WHO[15] and Lauren classification,[16] and the American Joint Committee on Cancer TNM system,[17] respectively. The representative figures of Ki-67 IHC are shown in Fig. 1.

2.3. Propensity score matching

We performed the propensity score matching between patients with Ki-67-high and Ki-67-low to reduce the selection bias, using the MatchIt package in the R statistical software version 3.1.3 (The R Foundation for Statistical Computing, Vienna, Austria). Of the original 329 patients, 9 patients with missing values were excluded from the matching process. With remaining 320 patients (123 patients with Ki-67-low and 197 patients with Ki-67-high), Ki-67 status was regressed through a logistic regression analysis on the following variables: age, gender, primary tumor location, types of operation, number of lymph node dissection, depth of invasion, TNM stage, tumor size, histological differentiation, and Lauren classification. Among

Table 1

Baseline characteristics of patients.

	Total population (n = 320)			Propensity score-matched population (n = 246)		
	Low Ki-67 (n = 123)	High Ki-67 (n = 197)	*P*	Low Ki-67 (n = 123)	High Ki-67 (n = 123)	*P*
Median age, y	61 (range: 32– 81)	66 (range: 24– 85)	.008	61 (range: 32– 81)	62 (range: 24 – 81)	.907
Sex			.872			.893
Male	81 (65.9%)	128 (65.0%)		81 (65.9%)	80 (65.0%)	
Female	42 (34.1%)	69 (35.0%)		42 (34.1%)	43 (35.0%)	
Location			.137			.479
Upper	15 (12.2%)	24 (12.2%)		15 (12.2%)	12 (9.8%)	
Middle	31 (25.2%)	32 (16.2%)		31 (25.2%)	25 (20.3%)	
Lower	77 (62.6%)	141 (71.6%)		77 (62.6%)	86 (69.9%)	
Operation			.487			.713
Subtotal gastrectomy	85 (69.1%)	138 (70.1%)		85 (69.1%)	84 (68.3%)	
Total gastrectomy	30 (24.4%)	48 (24.4%)		30 (24.4%)	34 (27.6%)	
Proximal gastrectomy	5 (4.1%)	10 (5.1%)		5 (4.1%)	4 (3.3%)	
Wedge resection	3 (2.4%)	1 (0.5%)		3 (2.4%)	1 (0.8%)	
Number of LN dissection			.790			.724
< 15	18 (14.6%)	31 (15.7%)		18 (14.6%)	20 (16.3%)	
≥ 15	105 (85.4%)	166 (84.3%)		105 (85.4%)	103 (83.7%)	
Depth of invasion			.530			1.000
EGC	58 (47.2%)	100 (50.8%)		58 (47.2%)	58 (47.2%)	
AGC	65 (52.8%)	97 (49.2%)		65 (52.8%)	65 (52.8%)	
TNM Stage			.784			.956
I	65 (52.8%)	111 (56.3%)		65 (52.8%)	67 (54.5%)	
II	24 (19.5%)	38 (19.3%)		24 (19.5%)	24 (19.5%)	
III	34 (27.6%)	48 (24.4%)		34 (27.6%)	32 (26.0%)	
Tumor size, cm	4 (range: 1.0–17.0)	4 (range: 0.5–15.0)	.884	4 (range: 1.0– 17.0)	4 (range: 1.0 – 15.0)	.541
WHO classification			.636			.898
W/D	25 (20.3%)	41 (20.8%)		25 (20.3%)	23 (18.7%)	
M/D	35 (28.5%)	65 (33.0%)		35 (28.5%)	38 (30.9%)	
P/D and others*	63 (51.2%)	91 (46.2%)		63 (51.2%)	62 (50.4%)	
Lauren classification			.008			.700
Intestinal	76 (61.8%)	153 (77.7%)		76 (61.8%)	82 (66.7%)	
Diffuse	40 (32.5%)	36 (18.3%)		40 (32.5%)	34 (27.6%)	
Mixed	7 (5.7%)	8 (4.1%)		7 (5.7%)	7 (5.7%)	

AGC = advanced gastric cancer, EGC = early gastric cancer, LN = lymph node, M/D = moderately-differentiated, P/D = poorly-differentiated, W/D = well-differentiated.

* Undifferentiated and mucinous adenocarcinoma, and signet-ring cell carcinoma were included.

these, the statistically insignificant variables were removed and the propensity score was calculated from a logistic regression model regarding age and Lauren classification of which variable showed statistically significant unbalanced proportion between Ki-67-high and Ki-67-low expression in the initially included 320 patients. With the calculated propensity score, patients were matched using a 1:1 nearest neighbor matching algorithm.

2.4. Statistical analysis

All further statistical analyses were performed by SPSS version 21.0 software (SPSS, Chicago, IL). A two-sided *P* value <.05 was considered statistically significant. Baseline characteristics were compared between the two groups with χ^2 and Fisher exact tests for categorical variables and Mann–Whitney *U* test for continuous variables. The Spearman rank test was used to calculate the correlation coefficient. Overall survival (OS) was defined as the time from surgery to death from any cause or last follow-up. OS according to Ki-67 status was plotted with the Kaplan–Meier method and the difference was analyzed with the log-rank test. All the variables moderately associated with survival ($P < .10$) in the univariate analyses were included in the Cox regression model to identify independent prognostic factors. The final model was internally validated by bootstrap resampling (1000 replications).

3. Results

3.1. Baseline characteristics before and after the propensity score matching

The baseline characteristics in unmatched and matched cohorts are presented in Table 1. In an unmatched cohort of 320 patients, the median age was 65 years (range: 24–85 years), and there was a male predominance of 2:1. Two hundred eighteen patients (68.1%) had a primary tumor located in the lower third of stomach; thus, subtotal gastrectomy was the most commonly performed operation (n = 223, 69.7%). The ratio of EGC and AGC was nearly even (0.98, 158/162), and 176 patients (55.0%) had stage I disease. Before matching, the median age and the distribution of the Lauren classification were significantly different ($P = .008$ and .008, respectively) between the Elevated Ki-67 and Ki-67-low groups. After the propensity score matching (123 pairs), all the variables were balanced between the 2 groups.

3.2. The role of Ki-67 as a prognostic biomarker

Survival was compared between the Elevated Ki-67 and Ki-67-low groups in 123 matched pairs. The median follow-up duration was 72 months (range: 3–120 months). There was no difference in OS between the Elevated Ki-67 and Ki-67-low groups [5-year survival rate (5-YSR), 68.0% vs 74.9%, $P = .235$; Fig. 2]. In the

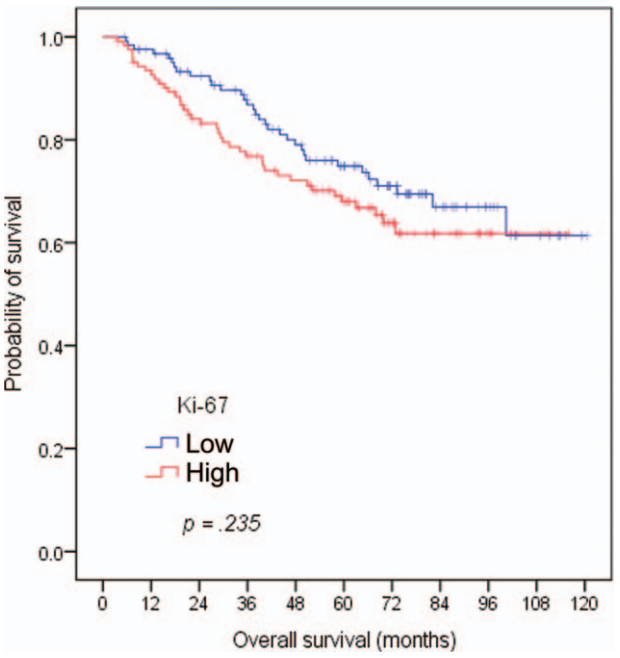

Figure 2. Kaplan–Meier curves for OS according to Ki-67 expression in 246 gastric cancer patients. OS = overall survival.

subgroup analysis, the Elevated Ki-67 group showed a lower 5-YSR than the Ki-67-low group in EGC (5-YSR, 78.9% vs 92.0%, $P = .018$; Fig. 3A) but not in AGC (5-YSR, 58.5% vs 59.2%, respectively; $P = .951$; Fig. 3B). Interestingly, in patients with well-differentiated histology, the 5-YSR for the Elevated Ki-67 group was considerably lower than that for the Ki-67-low group (5-YSR, 67.0% vs 94.4%, respectively; $P = .012$; Fig. 4A), but this difference in the 5-YSR was not observed when comparing patients with moderately differentiated ($P = .504$; Fig. 4B) and poorly differentiated histology ($P = .905$; Fig. 4C). In this cohort, there was a significant correlation between the depth of invasion and histological differentiation ($r = 0.215$, $P = .002$). To clearly elucidate the prognostic influence of these factors on OS, we compared OS between the Elevated Ki-67 and Ki-67-low groups by stratifying the groups according to both the depth of invasion and histological differentiation. There was a statistically significant difference in OS between the Elevated Ki-67 and Ki-67-low groups only in EGC patients with well-differentiated histology (5-YSR, 75.3% vs 100.0%, respectively; $P = .029$; Fig. 5A). These findings suggest that Ki-67 expression might better serve as a prognostic biomarker in EGC with well-differentiated histology than in either EGC or well-differentiated histology alone.

Further subgroup analysis was performed to identify other factors that influenced the impact of Ki-67 status on OS (Fig. 6). Elevated Ki-67 expression was associated with poor prognosis in patients with primary tumors located in the lower third of the stomach [hazard ratio (HR) 2.039, 95% CI 1.116–3.726, $P = .020$] and in male patients (HR 1.800, 95% confidence interval (CI) 1.033–3.138, $P = .038$). However, there were no correlations between either of these factors and the depth of invasion (tumor location and depth of invasion, $r = 0.054$, $P = .397$; gender and depth of invasion, $r = 0.050$, $P = .433$, respectively) or histological differentiation (tumor location and histologic differentiation, $r = 0.039$, $P = .539$; gender and histologic differentiation, $r = 0.009$, $P = .888$, respectively). To clearly elucidate the influence of tumor location and gender on OS, we combined these factors with the depth of invasion or histological differentiation and compared OS between the Elevated Ki-67 and Ki-67-low groups based on the combined stratification. Regarding tumor location and depth of invasion, elevated Ki-67 expression was associated with poor prognosis in EGC patients with primary tumors in the lower third of the stomach (5-YSR, Elevated Ki-67 vs low, 76.7% vs 93.9%, respectively; $P = .010$; Supplementary Fig. S1A, http://links.lww.com/MD/B747). However, the 5-YSRs were not different between Elevated Ki-67 and Ki-67-low groups in EGC patients with primary tumors in the upper two-thirds of the stomach ($P = .849$; Supplementary Fig. S1B, http://links.lww.com/MD/B747) or in AGC patients irrespective of the primary tumor location (lower third, $P = .287$; upper two-thirds, $P = .151$). Regarding the tumor location and histological differentiation, there was a tendency suggesting that patients with well-differentiated histology in the Elevated Ki-67 group had a worse prognosis regardless of the tumor location, although there was no statistical significance (Supplementary Fig. S1C and S1D, http://links.lww.com/MD/B747). Regarding gender, the depth of invasion, and histological differentiation, patients either with EGC or presenting well-differentiated tumor

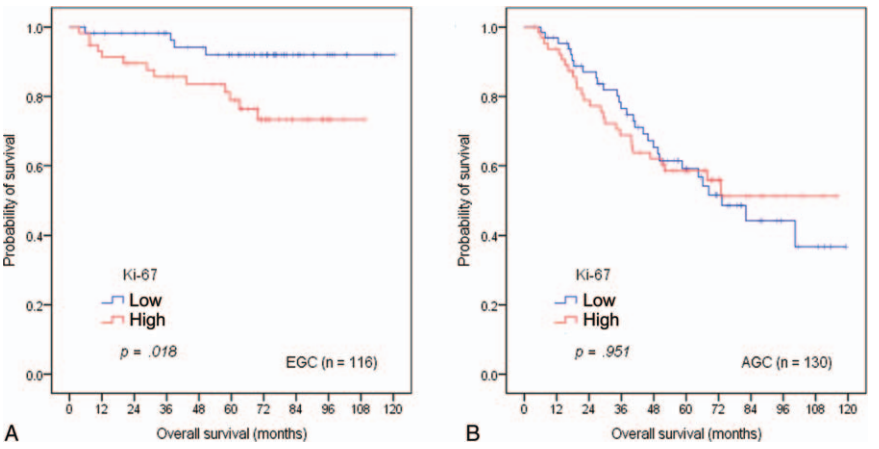

Figure 3. Kaplan–Meier curves for OS according to Ki-67 expression in patients with (A) EGC and (B) AGC. AGC = advanced gastric cancer, EGC = early gastric cancer, OS = overall survival.

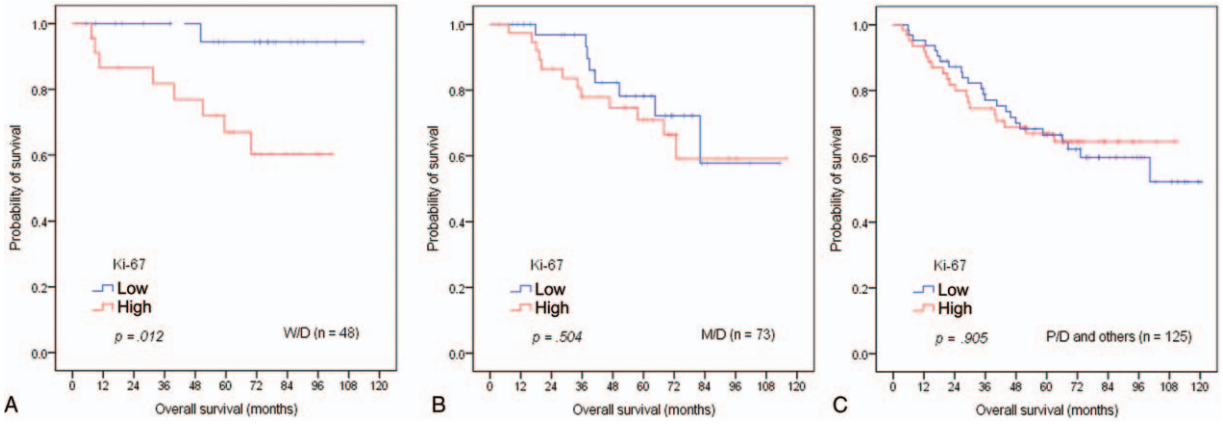

Figure 4. Kaplan–Meier curves for OS according to Ki-67 expression in patients with (A) W/D, (B) M/D, and (C) P/D and others. M/D = moderately-differentiated, OS = overall survival, Others = undifferentiated and mucinous adenocarcinoma, and signet ring cell carcinoma, P/D = poorly-differentiated, W/D = well-differentiated.

histology in the Elevated Ki-67 group were likely to have a worse prognosis than corresponding patients in the Ki-67-low group regardless of the gender, although the difference did not reach statistical significance among female patients (Supplementary Fig. S2, http://links.lww.com/MD/B747). In contrast to patients with either EGC or well-differentiated histology, the Ki-67 status did not affect the prognosis of those with AGC or with moderately to poorly differentiated histology irrespective of the tumor location and gender (data not shown). These results suggest that elevated Ki-67 expression is associated with poor prognosis of patients with EGC but not AGC whose primary tumors are located in the lower third of the stomach and that

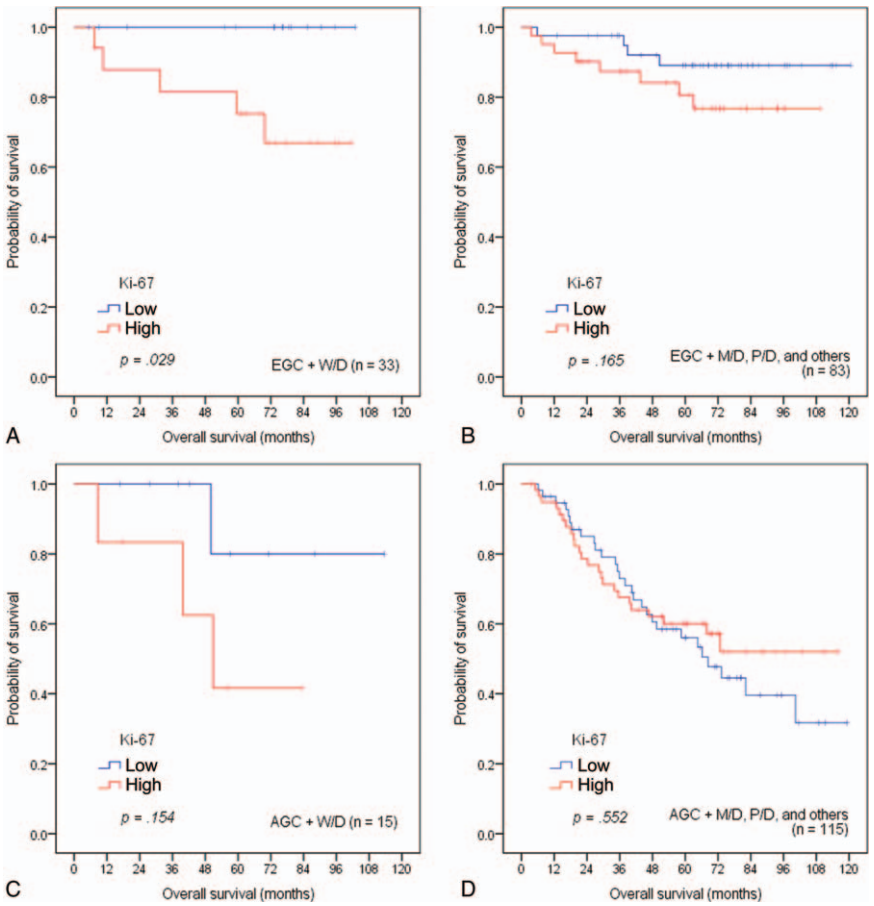

Figure 5. Kaplan–Meier curves for OS according to Ki-67 expression in patients with (A) EGC + W/D, (B) EGC + M/D, P/D, and others, (C) AGC + W/D, and (D) AGC + M/D, P/D, and others. AGC = advanced gastric cancer, EGC = early gastric cancer, M/D = moderately-differentiated, OS = overall survival, Others = undifferentiated and mucinous adenocarcinoma, and signet ring cell carcinoma, P/D = poorly-differentiated, W/D = well-differentiated.

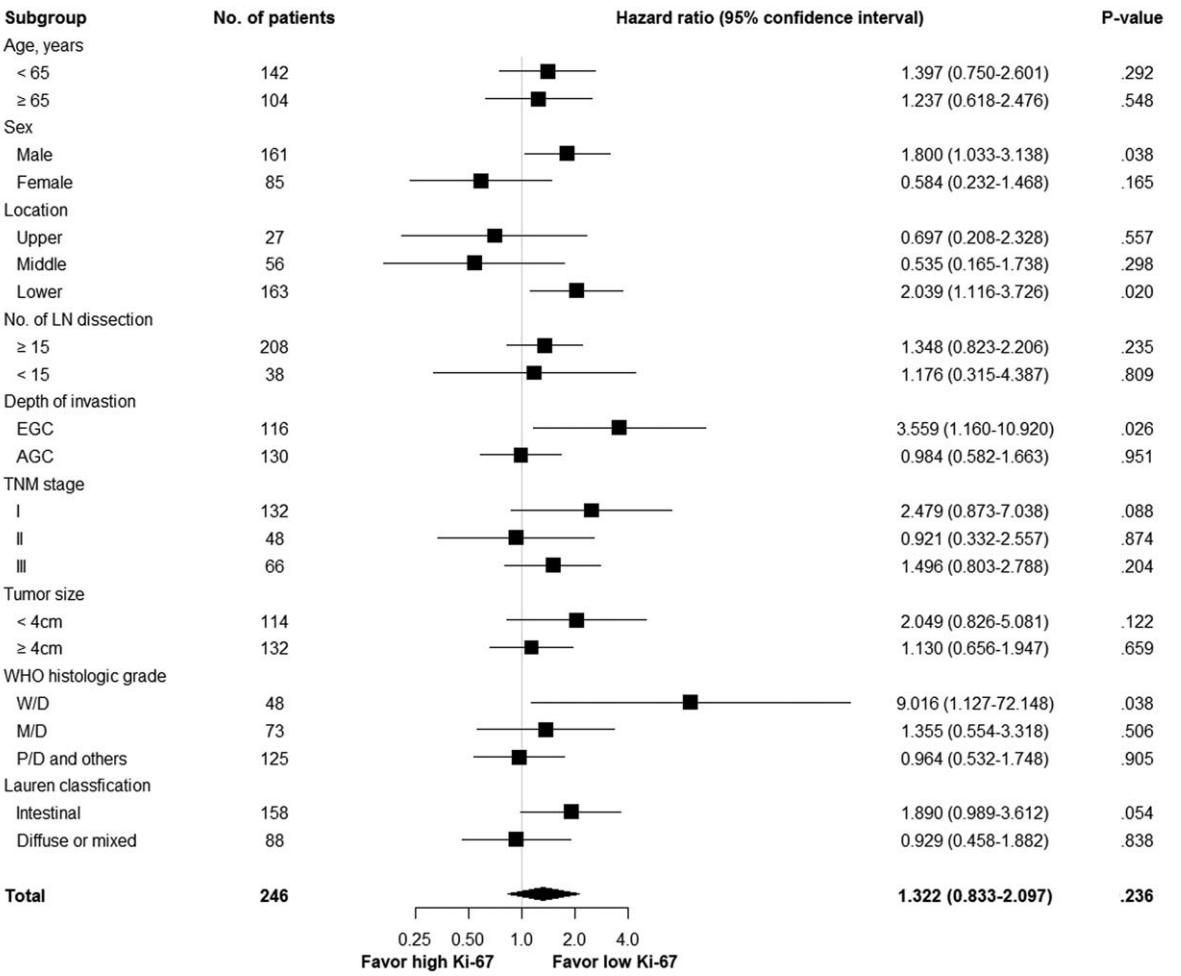

Figure 6. Forest plot of subgroup analysis for OS. OS = overall survival.

gender does not have a significant influence on the prognostic role of Ki-67 expression in this population.

The multivariate analysis revealed that the Ki-67 status was not a statistically significant prognostic factor in the entire cohort, but when the same analysis was performed in elderly patients with EGC, the Ki-67 status (HR 4.346, 95% CI 1.397–13.515, $P = .011$) was the only independent prognostic factor. No other clinical factors were predictive for the prognosis of patients with EGC. Using bootstrap resampling, we validated these results (Table 2).

4. Discussion

The current study was designed to determine whether Ki-67 could serve as a prognostic biomarker for EGC. To accomplish this, we compared the role of elevated Ki-67 expression as a prognostic biomarker in both EGC and AGC patients. The results indicated that the Ki-67 LI can serve as a biomarker to predict the prognosis in EGC.

Here, we first found that elevated Ki-67 expression was significantly associated with worse prognosis in EGC but did not serve as a marker of poor prognosis in AGC. Then, we also noticed significant differences in the positive rate of Ki-67 between elderly and non-elderly populations as well as between the diffuse-type and intestinal-type in the Lauren classification. Therefore, we performed propensity score-matched analysis for age and the Lauren classification to reduce the selection bias and remove confounding factors. The results also showed that elevated Ki-67 expression serves as a poor prognostic biomarker in EGC. To confirm this finding, we also performed internal validation via the bootstrap method for overall survival analysis in EGC patients.

Previous studies showed that the Ki-67 LI did not serve as a prognostic biomarker.[18] This finding can be partially explained by our previous study suggesting that finding a single prognostic biomarker for various stages of gastric cancer is much harder to find for AGC than for EGC because AGC includes more heterogeneous cells, especially in mixed types,[13] and contains more complicated genetic abnormalities than EGC,[14] suggesting that a single prognostic biomarker might not serve well in individuals with highly heterogeneous gastric cancer cells. The increased heterogeneity of AGC can be easily explained by the following findings: the major histologic type of EGC among the elderly is the well-differentiated type, but that of AGC among the elderly is mixed type, which comprises well-differentiated adenocarcinoma in the superficial area and poorly differentiated adenocarcinoma in the deeper area.[19,20] This finding suggests that majority of elderly AGCs are principally well-differentiated adenocarcinomas that have progressed to poorly differentiated adenocarcinomas and are different from the initial histological

Table 2

Cox proportional hazard model for overall survival.

	HR	95% CI	*P*	*P* (bootstrap)
Total (n = 246)				
Depth of invasion (AGC vs EGC)	2.827	1.561–5.119	.001	.002
Tumor size (≥ 4 cm vs < 4 cm)	1.600	0.919–2.786	.097	.084
WHO classification (P/D and others* vs W/D and M/D)	1.017	0.580–1.783	.953	.949
Lauren classification (diffuse or mixed vs intestinal)	1.545	0.886–2.696	.125	.106
Ki-67 (high vs low)	1.375	0.863–2.191	.180	.161
EGC (n = 116)				
Age, y (≥ 65 vs < 65)	3.100	1.163–8.262	.024	.006
Ki-67 (high vs low)	4.346	1.397–13.515	.011	.011

AGC = advanced gastric cancer, CI = confidence interval, EGC = early gastric cancer, HR = hazard ratio, M/D = moderately-differentiated, P/D = poorly-differentiated, W/D = well-differentiated.
* Undifferentiated and mucinous adenocarcinoma and signet-ring cell carcinoma were included.

type as they progress over time. During the period when EGCs are progressing and changing into AGCs, ECGs would gain various additional genetic abnormalities, which make AGCs more heterogeneous. Another supporting finding that a molecular biomarker may fare better in more homogeneous subtypes is that elevated Ki-67 expression was statistically significant factor of poor prognosis in EGC patients with well-differentiated adenocarcinoma even though there was a small sample size (n = 33). Recent comprehensive molecular characterization[14] by The Cancer Genome Atlas (TCGA) network revealed that gastric cancer can be subclassified into 4 genomic subtypes. These 4 genomic subtypes have anatomic distinctions.[14] The most prevalent type, chromosomal instability (CIN) type usually develops in the G-E junctional area; the next 2 prevalent subtypes, microsatellite instable (MSI) and genomically stable (GS) type, usually develop in the lower third, and the least frequent type, Epstein–Barr Virus (EBV), is often positive throughout the entire body. The proposed prognostic biomarker would be different among each genomic subtype. In this context, our finding indicates that elevated Ki-67 expression serves as a marker of poor prognosis in patients with EGC with well-differentiated histology who develop tumors in the lower third but neither in EGC patients with moderately to poorly differentiated histology nor in individuals with disease at the body and fundus area. From these findings, we assumed that Ki-67 is a biomarker for poor prognosis in patients with EGC with differentiated histology who developed tumors in the lower third area. We also evaluated the role of the Ki-67 LI in this group. The results showed that the patients with elevated Ki-67 expression clearly showed a poor prognosis, but the differences did not reach statistical significance (5-YSR, elevated Ki-67 vs low, 73% vs 100%, $P = .062$; Supplementary Fig. S3A, http://links.lww.com/MD/B747), which would be derived from small sample numbers. Therefore, a validation study is warranted to confirm this finding in a larger patient group.

The Ki-67 LI has been shown to be an independent biomarker of poor prognosis in some patients with gastric cancer,[21–25] but the final conclusions of these studies were inconsistent; some reports evaluated the clinical significance of Ki-67 with other candidate biomarkers by estimating the clinical value of coexpression of Ki-67 and other biomarkers,[21,23,24] whereas other studies evaluated the correlation between elevated Ki-67 expression and other biomarkers or clinical variables such as tumor size and lymph node metastasis, but the enrolled gastric cancer patients were at various stages and presented multiple histologic differentiation states, which have highly different genetic abnormalities.[14,25,26] Here, we solve the complicated problems using propensity score-matched analysis and subgroup analysis. In addition, to further validate the role of Ki-67 expression in EGC, we used the bootstrapping method. We also investigated the correlation between the expression of Ki-67 and CD44v9, a marker characteristic of cancer stem-like cells that has been shown to be a prognostic biomarker for EGC (data not shown).[13] We found a correlation between the expression levels of Ki-67 and CD44v9. This finding suggests that rapid tumor cell proliferation is a critical feature for tumor aggressiveness even in tumors within the same TNM stage.

For the evaluation of Ki-67 expression, we used TMA technology, but some authors doubt the utility of the TMA approach for Ki-67 and recommend assessing the Ki-67 LI on multiple cores of the TMA because it is recognized that the Ki-67 expression pattern shows considerable heterogeneity among different tumor areas. A previous study showed that all expected associations between the Ki-67 LI and clinicopathological parameters in cancer TMAs were independent of the number of analyzed cores. To avoid the considerable heterogeneity, we obtained TMA tissue cores from the area near the invasive front (which contains more cancer tissue) because the number of stained cancer cell nuclei were scored out of 400 cancer cells, and the ratio of stained cells to total cells expressed as the percentage was defined as the Ki-67 LI. In addition, there are reports strongly suggesting that TMAs are highly useful to study cancer cell proliferation.

There are several limitations in this study. First, this study is retrospective in nature. To overcome this weakness, we validated the findings using bootstrap resampling. Second, for the positive criteria for Ki-67 expression, we scored 2 or 3 when more than 20% cancer cells were stained. We used a positive cutoff value of 20% while most other studies used a cutoff of 5 or 10%[21–25] to obtain similar populations of patients with elevated and low levels of Ki-67, which enable the increase in the statistical power.

5. Conclusions

In conclusion, these results suggest that Ki-67 expression can be a good prognostic biomarker for EGC but not for AGC. Furthermore, Ki-67 expression is a better prognostic marker in gastric cancers with well-differentiated histology, although well-differentiated histology was associated with EGC; therefore, further large-scale studies are warranted to validate role of Ki-67 expression in AGC with well-differentiated histology. These findings support that Ki-67 expression is closely related to the prognosis of EGC and gastric cancers with well-differentiated histology.

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