

Improved Sampling Method to Enhance the Positive Rate of HER2 Detection in Gastric Cancer

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Background: To analyze the efficacy of the improved sampling method in enhancing the accuracy of Human Epidermal Growth Factor Receptor 2 (HER2) detection within gastric cancer surgical specimens, in order to decrease the false negative rate of HER2 detection results.

Methods: In this study, the participants were gastric cancer patients recruited from the Shandong Cancer Hospital Affiliated with Shandong First Medical University. Between December 2018 to August 2020, 310 surgical specimens of gastric cancer were procured and processed using traditional sampling methods. Biopsy specimens ($n = 235$), and surgical specimens ($n = 400$) indicated for processing by improved sampling methods, were obtained during the period from September 2020 to July 2021. Clinical data including sex, age, stage, degree of differentiation, tumor site, operation duration and blood loss was collected. Sex, operation duration and blood loss among three groups were compared by One-Way analysis of variance (ANOVA), and sex, stage, degree of differentiation and tumor site comparisons were performed by Chi-squared test (χ^2). HER2 detection was carried out for all specimens by means of immunohistochemical (IHC) assay. The results of HER2 detection among three groups were compared by Chi-squared test (χ^2) using SPSS software.

Results: There was no significant correlation between HER2 expression and pathological stage ($p = 0.468$). HER2 expression was related to tumor differentiation and tumor site: the HER2 expression in moderately differentiated tumors was higher than that in poorly differentiated tumors ($p < 0.001$); and it was significantly higher in gastric fundus than that in the gastric body and antrum ($p = 0.031$). The positive expression of HER2 in gastric cancer was 59.3% (0), 20.0% (1+), 13.4% (2+) and 7.3% (3+). Among them, a 2+ HER2 expression rate of 11.3%, 11.1%, and 16.5% were detected in specimens processed by the traditional sampling method, biopsy method, and tissues processed by improved sampling method, respectively. Meanwhile, a 3+ HER2 expression rate was found to be 4.8%, 8.1%, and 8.8%, respectively, for the specimens in the same order. Evidently, 1+ HER2 expression rate in specimens obtained by the improved sampling method and biopsy method was higher than in those by traditional sampling method ($p < 0.001$ and $p = 0.007$, respectively).

Conclusions: The improved sampling method significantly improves the positive HER2 expression rate (1+) in gastric cancer specimens, thereby reducing false-negative HER2 detection rates by immunohistochemistry. This method warrants further clinical implementation, particularly in resource-limited settings.

Keywords: HER2; IHC; improved sampling method; gastric cancer

Introduction

Despite comprising one-fifth of the global population, China bears a disproportionate burden of gastric cancer, accounting for 44% of cases worldwide. The latest statistical report of the International Cancer Research Agency (ICRA) in 2022 showed that the incidence rate of gastric cancer was about 12.1 per 100,000 individuals, with a mortality rate of 4.9%, which ranks the fifth among all malignant tumors. It is worthy to mention that the mortality rate attributable to

gastric cancer in China was much higher than the world's average [1,2].

In China, about 30% of patients found that their condition had progressed to advanced stage when they were first diagnosed with gastric cancer [3]. For this group of patients, they have missed the golden opportunity to receive radical surgery, and their 5-year survival rate following receiving palliative treatment would drop to below 5% [4–7]. A glimmer of hope happened to the advanced-stage patients in 2010 when the Trastuzumab for Gastric Cancer (ToGA)

study, published on the *Lancet*, introduced a targeted treatment for gastric cancer, bringing new hope to these patients [8]. Nevertheless, trastuzumab combined with systemic chemotherapy remains the preferred treatment for Human Epidermal Growth Factor Receptor 2 (HER2)-positive gastric cancer [9]. Besides, the evaluation of HER2 expression and gene amplification in patients with gastric cancer is key to evaluating whether patients could benefit from the trastuzumab treatment.

Adopting a standardized sampling method is a premise for accurate HER2 expression detection. The standards and requirements of sampling surgical specimens of gastric cancer have been clearly stipulated in The China's Guidelines for HER2 detection (2016 Edition) [10]. However, due to certain limitations in clinical practice that pave the way for objective and subjective conditions, such as the shortage of operating room personnel and pathologists, most medical institutions—especially the grassroots hospitals—are unable to follow the standard process in sampling appropriate gastric cancer specimens for clinical and laboratory examination. The current study aimed to assess the efficacy of an improved version of the sampling method for gastric cancer surgical specimens, modified based on the guidelines.

Materials and Methods

General Information

This study included patients with gastric cancer recruited at Shandong Cancer Hospital Affiliated with Shandong First Medical University from December 2018 to July 2021. Between December 2018 and August 2020, 310 surgical specimens were procured and processed by traditional sampling method for the detection of HER2 expression. Biopsy specimens (n = 235), as well as surgical specimens (n = 400) indicated for processing by improved sampling method, were obtained from patients from September 2020 to July 2021. All participants had given informed consent prior to participation in this study. The study was conducted in strict compliance with the Declaration of Helsinki and approved by the Ethics Committee of Shandong Cancer Hospital (NO. SDZLEC2020-104-01).

Sampling Methods

To obtain biopsy specimens, biopsy forceps were used to retrieve 4–6 mucosal specimens of the lesion with the aid of a gastroscope. After confirmation, the samples were immersed in 10% neutral formalin solution (G2161, Solarbio, Beijing, China) for fixation, prior to HER2 expression detection in the pathology department.

Surgical specimens indicated for processing by the traditional sampling methods were handled post-surgery. Following a thorough check by surgeon, the specimens were stored specimen bags containing the fixation solution (BK6090, Seroat, Escondido, CA, USA) (Fig. 1A,B).

While implementing the improved sampling method, surgical specimens were sampled in less than 5 minutes and handled post-surgery. Surgeons practicing this method are encouraged to replace gloves after retrieving the specimens before continuing with the operation. The gastric wall of the specimens was trimmed away from the center of the tumor in order to fully expose the lesion, while retaining at least the depth of the muscle layer. Two parallel incisions along the center of the longitudinal axis of the ulcer were made, with an interval of about 3 mm. A strip-shaped tissue of 2–3 cm in length, including normal mucosa and tumor tissue, was cut out from each specimen. The trimmed specimens were then immersed in about 90 mL of 10% neutral formalin solution (G2161, Solarbio, Beijing, China) for fixation (Fig. 1C,D).

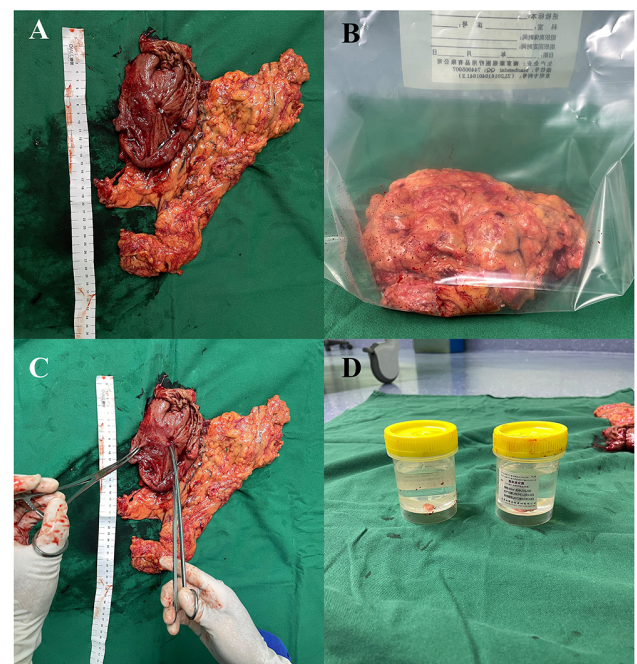


Fig. 1. Specimen sampling and fixation. (A) The surgical specimen from gastric cancer. (B) The surgical specimen was immersed in specimen bag containing fixation solution. (C) The tumor was cut from a surgical specimen. (D) The tumor was immersed in 10% neutral formalin solution.

Immunohistochemical Detection

The formalin-fixed and paraffin-embedded (FFPE) sections were obtained from specimens of gastric cancer. The FFPE specimens were dewaxed and rehydrated. Subsequently, they were immersed in H₂O₂ solution to block endogenous peroxidase, in sodium citrate buffer (0.01 M, pH 6.0) for antigen retrieval, in anti-HER2 antibody solution (1:2000 dilution; ab314070, Abcam, Cambridge, UK), and in the secondary antibody solution (1:5000 dilution; ab7068, Abcam, Cambridge, UK). The FFPE sections were

Table 1. Summary of HER2 expression status of all patients.

Variable		Negative HER2 expression		1+ HER2 expression		2+ HER2 expression		3+ HER2 expression		
		Number	Proportion	Number	Proportion	Number	Proportion	Number	Proportion	
Total (n = 945)		Negative	560	59.3%	189	20.0%	127	13.4%	69	7.3%
Degree of differentiation	Poorly differentiated gastric cancer (n = 656)	Negative	413	63%	139	21.2%	71	10.8%	33	5%
	Moderately differentiated gastric cancer (n = 241)	Negative	124	51.5%	34	14.1%	51	21.2%	32	13.2%
	Highly differentiated gastric cancer (n = 48)	Negative	23	47.9%	16	33.3%	5	10.4%	4	8.3%
		χ^2/p	-		17.792 (<0.001*)					
Tumor stage	Stage I (n = 192)	Negative	112	58.3%	38	19.8%	23	12%	19	9.9%
	Stage II (n = 213)	Negative	120	56.3%	51	23.9%	28	13.1%	14	6.6%
	Stage III (n = 462)	Negative	283	61.3%	80	17.3%	69	14.9%	30	6.5%
	Stage IV (n = 78)	Negative	45	57.7%	20	25.6%	7	9%	6	7.7%
		χ^2/p	-		2.540 (0.468)					
Tumor site	Gastric fundus (n = 281)	Negative	166	59.1%	51	18.1%	35	12.5%	29	10.3%
	Gastric body (n = 233)	Negative	148	63.5%	49	21%	26	11.2%	10	4.3%
	Antrum of stomach (n = 431)	Negative	246	57.1%	89	20.6%	66	15.3%	30	7%
		χ^2/p	-		6.976 (0.031*)					

Notes: 1+ indicates weak staining; 2+ indicates moderate staining; and 3+ indicates strong staining in IHC assay. * $p < 0.05$. IHC, immunohistochemical; χ^2 , Chi-squared test; HER2, Human Epidermal Growth Factor Receptor 2.

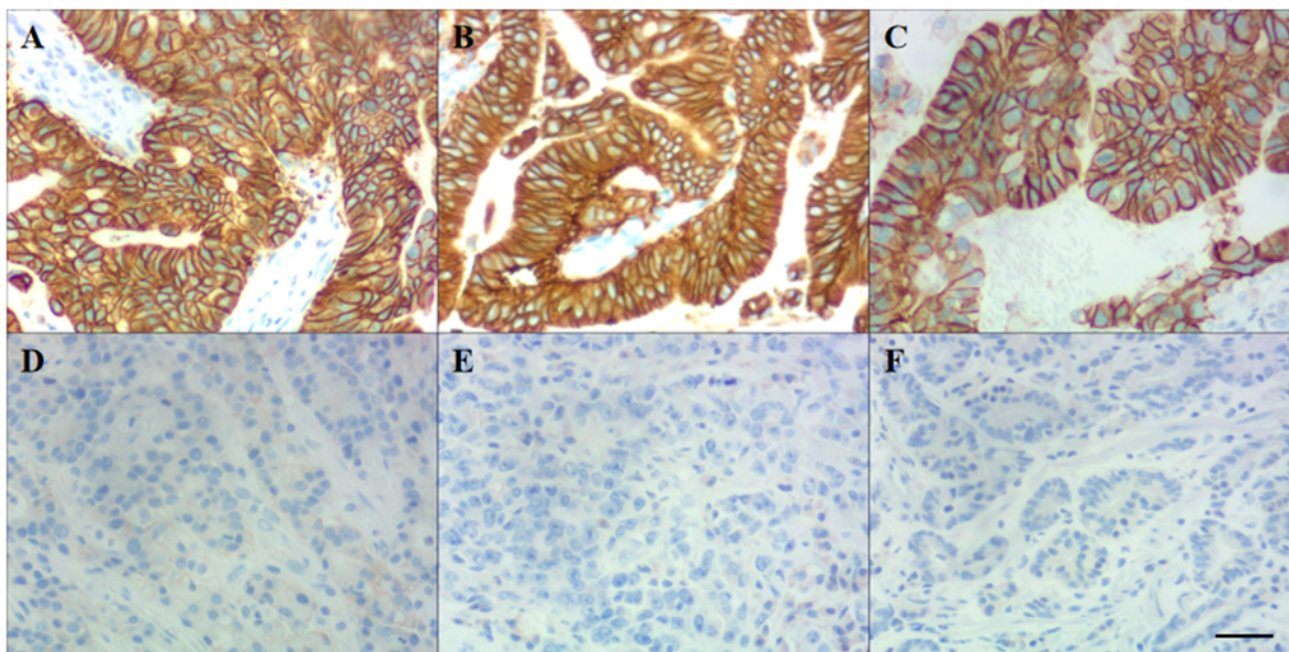


Fig. 2. Representative microscopic images of IHC detection results of HER2 expression in gastric cancer specimens. The images show samples with positive and negative HER2 expression following the implementation of the traditional sampling method (A,D), biopsy method (B,E), and improved sampling method (C,F). Scale bar, 50 μ m.

stained using diaminobenzidine (DAB) Peroxidase Substrate Kit for immunohistochemical (IHC) (36302ES, Yeason, Shanghai, China). The IHC results were observed under a microscope. The criteria for determining HER2 expression refer to *Guideline for HER2 testing in breast cancer (2024 version)* [11].

Statistical Analysis

SPSS 19.0 software (25.0, Armonk, NY, USA) was used to perform statistical analysis. Data normality was tested using Shapiro-Wilk. The difference among three groups for continuous variables was evaluated by One-Way analysis of variance (ANOVA). If ANOVA showed a statistically significant difference, the Tukey's test was then used for pairwise difference comparison. For categorical variables, the difference was compared using Chi-squared (χ^2) test. Results with $p < 0.05$ were considered statistically significant.

Results

Baseline Data

The HER2 expression of a total of 945 patients with gastric cancer is shown in Table 1. HER2 expression in gastric cancer was not significantly associated with post-operative pathological stage ($p = 0.468$). There was significant association between the expression of HER2 and the primary site of gastric cancer ($p = 0.031$). The positive rate of HER2 expression (3+) in patients with primary tumor in the gastric fundus was significantly higher than

those with tumor in the gastric body or gastric antrum. We found that the HER2 expression varied significantly across highly, moderately and poorly differentiated adenocarcinomas of gastric cancer. The 2+ and 3+ HER2 expression in moderately differentiated gastric cancer was the highest, while that in poorly differentiated gastric cancer was the lowest ($p < 0.001$).

Comparison of Positive HER2 Expression Rate

General Information

Three groups of specimens were investigated in this study to compare the efficacy of the traditional sampling method and improved sampling method, namely surgical specimens processed by traditional sampling method; biopsy specimens. As shown in Table 2, there was no significant difference in the clinico-pathological characteristics among the three groups, such as gender ($p = 0.812$), age ($p = 0.252$), tumor stage ($p = 0.059$), tumor differentiation ($p = 0.12$) and tumor site ($p = 0.133$), operation duration ($p = 0.408$) and blood loss ($p = 0.312$).

Comparison of the Positive HER2 Expression Rates Among the Three Specimen Groups Processed by Different Sampling Methods

The positive rates of HER2 expression detected by means of immunohistochemistry among the three specimen groups processed by different sampling methods were compared. The HER2 expression rate results are shown in Table 3, whereas representative microscopic images of immunohistochemistry-stained sections are depicted in

Table 2. General information comparison among the three groups.

Variable	Traditional sampling method (n = 310)	Biopsy method (n = 235)	Improved sampling method (n = 400)	χ^2/F	<i>p</i>
Sex					
Male	241 (77.7%)	184 (78.3%)	305 (76.2%)	0.417	0.812
Female	69 (22.3%)	51 (21.7%)	95 (23.8%)		
Age	59.96 ± 10.77	61.02 ± 11.213	61.29 ± 10.76	1.380	0.252
Stage					
I	70 (22.6%)	48 (20.4%)	74 (18.5%)	5.652	0.059
II	43 (13.9%)	46 (19.6%)	124 (31%)		
III	174 (56.1%)	113 (48.1%)	175 (43.8%)		
IV	23 (7.4%)	28 (11.9%)	27 (6.8%)		
Degree of differentiation					
Low	204 (65.8%)	168 (71.5%)	284 (71%)	4.234	0.120
Moderate	80 (8.4%)	60 (3%)	101 (3.8%)		
High	26 (25.8%)	7 (25.5%)	15 (25.2%)		
Tumor site					
Gastric fundus	101 (32.6%)	61 (26%)	119 (29.8%)	7.046	0.133
Gastric body	65 (21%)	57 (24.3%)	111 (27.8%)		
Antrum of stomach	144 (46.4%)	117 (49.7%)	170 (42.4%)		
Perioperative indexes					
Operation duration	3.23 ± 1.04	3.10 ± 1.14	3.20 ± 1.20	0.897	0.408
Blood loss	155.82 ± 33.33	156.53 ± 31.73	159.70 ± 39.95	1.168	0.312

Table 3. The comparison of the positive rate of HER2 expression among three groups.

Expression	Traditional sampling method (n = 310)	Biopsy method (n = 235)	Improved sampling method (n = 400)	χ^2	<i>p</i>
1+	36 (11.6%)	47 (20%)**	106 (26.5%)***	24.19	0.000
2+	35 (11.3%)	26 (11.1%)	66 (16.5%)	5.592	0.061
3+	15 (4.8%)	19 (8.1%)	35 (8.8%)	4.231	0.121

*** $p < 0.001$, ** $p < 0.01$, compared with traditional sampling method (36/310).

Fig. 2. The positive rates of HER2 expression rated as 1+, 2+ and 3+ in specimens processed by improved sampling method were 26.5%, 16.5% and 8.8%, respectively, with a total positive rate of 51.75% as detected by IHC, which was almost similar to those processed by the biopsy method but higher than those by the traditional sampling method. Only 1+ HER2 expression rate in specimens obtained by the improved sampling method and biopsy method was higher than in those by the traditional sampling method ($p < 0.001$ and $p = 0.007$, respectively).

Discussion

The proportion of patients with advanced gastric cancer was much higher in China than in Japan and South Korea. The proportion of early gastric cancer in China was 20%, with advanced gastric cancer accounting for as high as 80% [12,13]. The incidence rate of gastric cancer has also been rising in recent years. At present, trastuzumab combined with chemotherapy remains the primary treatment for patients with HER2-positive advanced gastric cancer and those with advanced gastric cancer patients after treatment. Therefore, accurate HER2 detection results are

of great clinical significance for the identification of gastric cancer patients whose conditions are amenable to the standard treatment regimens.

The positive rate of HER2 expression in gastric cancer reported in different geographical regions ranged from 3.7% to 20.2% [14–17], of which the positive rate of HER2 in Europe and America was about 14% [18], and the data provided by four large medical centers in China was 13% [19]. Based on the global HER2 research project on gastric cancer, the positive HER2 expression rate in China was 12% [20], which was largely based on the data provided by the large class III hospitals; therefore, we speculate that the cumulative positive rate would be much lower given that the data from a large number of grassroots hospitals in China were not included in the statistical calculations. The data in China were generally lower than those in Europe and the United States. It should also be highlighted that even the data reported in various parts of China presented inconsistencies, which were possibly related to a variety of factors. Thus, adopting the right specimen sampling and fixation step is highly imperative to address the inconsistencies.

The accurate sampling and fixation of surgical specimens represents the key step to ensuring high-quality HER2

detection, which has a direct and important impact on the results of HER2 detection. In Europe and the United States, there have been clear technical guidelines for performing HER2 detection. On this basis, guidelines for HER2 detection of gastric cancer tailored to the demographic realities in China have been formulated in 2011 by pathology experts, and revised in 2016 [10]. However, in most hospitals, the sampling and fixation of specimens were not regularly carried out in strict accordance with the guidelines; for instance, the specimens were not subject to fixation within a stipulated period of time following specimen isolation, the fixed specimens were not cut open, and the fixation solution was insufficient. According to the standard guidelines, the specimens should be sampled, cut and fixed by the staff in the operating room or pathology technician within 20–30 minutes after isolation. But in practice, these steps are often completed by the surgeons. Gastric cancer specimens *in vitro* also need digestive tract reconstruction and other surgical operations. The time was often more than 1 hour, so it was difficult to fix the surgical specimen in 20–30 minutes. To ensure that the tissue was fully immersed in the fixative, the guidelines recommended that the volume of the fixative should be at least 10 times that of the tissue. However, in most cases, the isolated specimens of gastric cancer were very large in size, which is unfeasible environmentally and consumable-wise, since using a large amount of formalin solution is impractical in real-life settings. Therefore, the primary focus of the surgeons should be to isolate small-sized specimens. It should be highlighted that due to tissue dehydration, deformation and other reasons, the histopathologic results of the fixed samples may become inaccurate.

In this study, the improved sampling method necessitates immediate processing of the specimens isolated by the surgeon. The specimens are then fixed and subjected to histopathological analyses. This modified method boasts several advantages: (i) instant fixation of surgical specimens following isolation to prevent significant protein degradation; (ii) complete immersion of small-sized specimens in fixative; (iii) reduced sampling errors as the surgeon isolated fresh surgical specimens to ensure the accuracy and reliability of all samples submitted for pathological examination; and (iv) sampling site including tissue regions from normal gastric mucosa tissue to gastric cancer tissue. Sampling along the maximum diameter of the lesion could reduce the result deviation caused by different levels of HER2 expression due to tumor heterogeneity.

Our experimental data showed that the positive HER2 expression rate in specimens processed by the improved sampling method was significantly higher than in those by the traditional sampling method, and also higher than the results reported by multiple medical centers in China. The positive rates of 2+ and 3+ HER2 expression in specimens processed by the improved sampling method were significantly higher than in those by the traditional method—a finding that unequivocally underlines the advantages of the

improved sampling method. In this study, we did not incorporate the FISH results since the previous FISH inspection rate had been low and the improved method was carried out. Through the improved sampling method, the positive rate of 2+ HER2 expression, as detected by FISH, was 34.8% (23/66), which was significantly lower than that reported in ToGA study (54.65%) [21]. Of note, the majority of the samples used in the ToGA study were European and North American. The proportion of intestinal gastric cancer cases in Europe and North America was much higher than that in East Asia, of which the positive rate of HER2 expression for intestinal gastric cancer was significantly higher than that for diffuse and mixed gastric cancer. Our research findings resonate with other studies, in which the positive HER2 expression rate ranges from 7% to 27% [14,22]. According to the existing research in China, the positive rate of HER2 expression, as detected by FISH, in patients with gastric cancer was about 30%, but the rate was found to be in the range of 8.8–12% in gastric cancer cases enrolled from multiple centers in China [20].

The positive HER2 expression rate in biopsy specimens was higher than that in specimens processed by traditional methods. One of the prime reasons is the lower standard utilized to interpret the parameter in biopsy specimens than that for the surgical specimens processed by traditional approach. Short fixation time and inadequate fixative are also cited as other plausible reasons. Both the biopsy specimens and the specimens processed by the improved method share almost comparable rates of positive HER2 expression. This is probably attributed to the rather standard sampling duration and fixation method used in the biopsy specimens. In terms of reducing the risk of false-negative results caused by tumor heterogeneity, the improved sampling method outperformed the biopsy method. However, it should be noted that HER2 expression is only detectable by IHC methods, whereas FISH is not capable of identifying 2+ HER2 expression in samples. Such limitations in these detection methods may lead to inconsistent interpretations of the histopathological results, mostly due to the 2+ HER2 expression that is hard to capture. Thus, more versatile and efficient detection method must be explored and developed with the hope to reduce interpretation inconsistencies and ease the detection process.

Conclusions

In conclusion, relative to the traditional sampling method, the improved method effectively enhances the positive rate of HER2 expression (2+ and 3+) in gastric cancer, and is less time-consuming and more efficient. Hence, this method is worthy of promotion in the majority of medical institutions, especially in grassroots hospitals.

Availability of Data and Materials

The datasets generated and/or analyzed during the current study are not publicly available due to the need for follow-up research but are available from the corresponding author on reasonable request.

Author Contributions

KL designed the research program and led the work of sample collection. HS and LQL were responsible for the overall execution and guidance of the experiment. Immunohistochemical detection was performed by LQL, XWW, and XLS, data induction and sorting by YHQ and LGW, statistical analysis and difference comparison by BL, XS, JC, and SJW. HS wrote the article. All authors contributed significantly to editorial changes of important content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study was conducted in strict compliance with the Declaration of Helsinki and approved by the Ethics Committee of Shandong Cancer Hospital (NO. SDZLEC2020-104-01). All participants had given informed consent prior to participation in this study.

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Conflict of Interest

The authors declare no conflict of interest.

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