

Recurrence in node-negative advanced gastric cancer with novel clinical and pathologic findings

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Research article

Keywords: Gastric cancer, Recurrence, Node-negative, Prognosis, Ki67, P53

Posted Date: July 1st, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-38675/v1>

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Abstract

Background

Node-negative gastric cancer patients carry a better prognosis than node-positive. However, a subset of these patients eventually died due to the high recurrence rate of recurrence. This study investigated the clinic-pathologic factors for recurrent patterns and prognosis.

Methods

The detailed medical records of 947 gastric cancer patients who underwent gastrectomy from the prospectively collected database of the Gastrointestinal Surgery Department of the First Affiliated Hospital of Zhejiang Chinese Medical University From January 2012 to December 2014 were analyzed retrospectively.

Results

Tumor size, tumor invasion, histological grading were the predictive factors for locoregional recurrence. Tumor invasion, histological grading, Lauren type and lymphatic vessel invasion resulted significantly in predicting peritoneal recurrence. Two parameters, tumor invasion and lymphatic vessel invasion, were significantly associated with hematogenous spread. The combinatorial biomarker of positive for both Ki67 and P53 was significantly associated with recurrence-free survival (RFS) ($P = 0.037$). Histological grading ($P = 0.020$), tumor invasion ($P = 0.007$) and lymphatic vessel invasion ($P = 0.012$) were independent factors related to overall survival (OS) in node-negative advanced gastric cancer patients.

Conclusion

The reported clinicopathologic factors for different recurrence patterns and the prognostic factors of RFS and OS should be considered to guide clinicians choose appropriate postoperative treatment strategy and construct individual follow-up schedule to improve prognosis of node-negative gastric cancer.

1 Introduction

Gastric cancer (GC) remains one of the most common malignancies in the world and accounts for the third leading cause of cancer-related deaths^[1]. To date, the standard approach of radical gastrectomy is D2 lymphadenectomy for resectable advanced gastric cancer (AGC)^[2]. Moreover, novel chemotherapeutic agents of improved therapeutic regimens have been used to treat this disease^[3]. However, patients with AGC usually have fatal outcomes because of the high rate of recurrence^[4].

Lymph node (LN) metastasis is the most significant prognostic parameter for recurrence in GC of curative resection (R0)^[5]. Although patients with node-negative GC show an importantly better overall survival than node-positive patients, a number of these patients also experience recurrence and metastasis^[6]. Previous reports consistently found that the depth of invasion was an independent predictor in node-negative GC that included early gastric cancer (EGC)^[7]. Furthermore, there is no consensus about other clinicopathologic factors of the prognostic significance, including

patient gender, age, tumor location, tumor size, histological grading, Lauren type and Lymphatic vessel invasion^[8, 9]. In addition, little is known about the recurrent patterns of node-negative AGC after D2 curative resection.

Nowadays, staging classification of GC has been implemented by TNM staging system. Because of the multifactorial nature of GC, TNM classification system can not guide the clinic treatment or predict the prognostic value adequately, so we need to determine the best therapeutic schedule by more instruments such as cancer biomarkers that can associate with patient survival^[10]. It will be more appropriate that using a combination of dual cancer biomarkers to substitute for single cancer biomarker which is found to allow quantification to predict the state of GC. Several studies using combinatorial biomarkers in cervical cancer or breast cancer, but little is published in GC^[11, 12].

On the basis of these considerations, the goals of our study are to investigate the clinic-pathologic factors of recurrence and recurrent patterns in node-negative AGC after R0 resection, and to evaluate the correlation between the outcome of these patients and predictive factors such as combinatorial cancer biomarkers.

2 Methods

2.1 Patients

We analyzed retrospectively the detailed medical records of 947 GC patients who underwent gastrectomy (R0) from the prospectively collected database of the Gastrointestinal Surgery Department of the First Affiliated Hospital of Zhejiang Chinese Medical University From January 2012 to December 2014. The inclusion criteria were as follows: (1) gastric adenocarcinoma; (2) primary AGC patients with T2, T3 or T4a stage according to the 3rd Japanese classification of gastric carcinoma^[13]; (3) patients with more than 15 retrieved nodes after D2 gastrectomy and (4) patients with negative for metastases by routine hematoxylin-eosin staining. The exclusion criteria were as follows: (1) patients with EGC; (2) patients underwent neoadjuvant chemotherapy or post-operative radiotherapy; (3) patients with severe basic diseases such as the system problem of heart or (4) cases of operative mortality. In conformity to the eligibility criteria described above, 307 patients were enrolled in this study ultimately, including 47 patients who developed cancer recurrence (Fig. 1).

The following clinicopathologic data were obtained: age, gender, tumor location, tumor size, depth of tumor invasion(T), histological grading(G), Lauren type, lymphatic vessel invasion, adjuvant chemotherapy, recurrence patterns (locoregional recurrence/ peritoneal seeding/ hematogenous spread) and the oncological outcomes of recurrence-free survival (RFS) and overall survival (OS).

Postoperative recurrence was diagnosed by the pieces of evidence of clinical, imaging and pathologic findings. Recurrence patterns included locoregional recurrence, peritoneal seeding and hematogenous spread. Locoregional recurrence was defined as GC tumors were detected in regional gastric nodes, gastric bed, anastomotic stoma or gastric stump. Peritoneal seeding was regarded as tumor metastasis to adjacent to the peritoneum of stomach, the region of ovary or the distant peritoneum. Hematogenous spread included cancer metastasis in viscera such as liver, brain and lung, or detected in distant lymph node.

This study, in accordance with the Declaration of Helsinki, was granted by the ethics committee of the First Affiliated Hospital of Zhejiang Chinese Medical University.

2.2 Immunohistochemical Scoring

The sections of surgical specimens were formalin-fixed and paraffin-embedded, then immunohistochemical (IHC) pathological cancer markers included P53, Ki67, HER2 were evaluated independently by two pathologists who were

blinded to the information of patients. The positive immunostaining of P53 and Ki67 was expressed in nuclear staining by brown or yellow granules. P53 and Ki67 expression were graded as below: no staining observed in tumor cells or stained tumor cells < 10% in specimens were defined as negative for immunostaining and specimens in which $\geq 10\%$ of stained tumor cells were defined as positive. The presence of HER2 was expressed by membrane staining with brownish-yellow granules. HER2 expression was scored as follows: no staining or staining in $\leq 10\%$ of GC cells (score 0); faint/barely perceptible partial staining in > 10% (score 1+); weak-to-moderate complete staining in > 10% (score 2+) and intensely complete staining in > 10%(score 3+). Score 0 and score 1 + were graded as negative HER2 status, and score 3 + was graded as the positive status of HER2. Score 2 + had to be evaluated further by fluorescence in situ hybridization (FISH).

2.3 Follow-up

After gastrectomy, patients were followed every 3 months in the first 2 years and then every 6 months until death or, December 2018, the cut-off date of follow-up. Physical examination and hematological examination such as serum tumor marker including CEA and CA199 were performed in every visit. Chest X-rays, abdominal ultrasonography, computed tomography scans of the abdomen and gastroscopy were performed annually.

2.4 Statistical analysis

We analyzed categorical variables by the Pearson's Chi-square test or Fisher's exact test as appropriate. Patient survival data, RFS and OS, were assessed using the Kaplan–Meier method and calculated by the log-rank test to perform univariate analyses. independent prognostic factors were obtained by multivariate analyses with the Cox proportional hazards model. In this test, all P values were two-sided and P values < 0.05 were considered statistically significant. Statistical analysis was performed with IBM SPSS Statistics for Windows, version 21.0 (IBM Corp., Armonk, NY, USA).

3 Results

3.1 Features of node-negative AGC in terms of recurrence

The clinicopathologic factors of the 307 patients with node-negative AGC are summarized in Table 1. During follow-up, 47 patients (15.3%) developed recurrence. Tumor size (P = 0.002), tumor invasion (P = 0.001), Lauren type (P = 0.038), Lymphatic vessel invasion (P = 0.015) and Ki67 expression (P = 0.034) were risk factors for recurrence of node-negative AGC, While age, gender, tumor location, histological grading, adjuvant chemotherapy, P53 expression and HER2 expression were no significant correlation observed between patients with tumor recurrence or not.

Table 1
Features of node-negative advanced GC in terms of recurrence

Parameter	Recurrence			P
	Total(307)	No(n = 260)	Yes(n = 47)	
Age(years)				0.227
≤ 65	145	119	26	
> 65	162	141	21	
Gender				0.662
Male	234	197	37	
Female	73	63	10	
Tumor location				0.117
Upper	42	36	6	
Middle	108	87	21	
Lower	148	131	17	
Entire	9	6	3	
Tumor size				0.002
≤ 5 cm	139	126	13	
> 5 cm	168	134	34	
Tumor invasion(T)				0.001
T2	92	87	5	
T3	40	36	4	
T4a	175	137	38	
Histological Grading(G)				0.080
G1	36	33	3	
G2	157	137	20	
G3	114	90	24	
Lauren type				0.038
Intestinal type	122	110	12	
Diffuse type	138	109	29	
Mixed type	47	41	6	
Lymphatic vessel invasion				0.015
Positive	34	24	10	
Negative	273	236	37	

Recurrence			
Adjuvant chemotherapy			0.240
Yes	230	198	32
No	77	62	15
P53 expression			0.060
Positive	89	70	19
Negative	218	190	28
Ki67 expression			0.034
Positive	200	163	37
Negative	107	97	10
HER2 expression			0.189
Positive	46	36	10
Negative	261	224	37

3.2 Pattern and incidence of recurrence

33 (70.2%) patients recurred at a single site: locoregional recurrence (n = 15, 31.9%), peritoneal seeding (n = 8, 17.0%), hematogenous metastasis (n = 10, 21.3%). 14 (29.8%) patients had recurrence at multiple sites including 1 (2.1%) patient with locoregional and peritoneal recurrence, 5 (10.6%) patients with locoregional and hematogenous recurrence, 6 (12.8%) patients with peritoneal and hematogenous recurrence, 2 (4.3%) patients with locoregional, peritoneal and hematogenous recurrence.

3.3 Clinicopathologic factors for different recurrence patterns

Table 2 demonstrates the correlation between the different recurrence patterns and clinicopathologic factors. Tumor size (P = 0.018), tumor invasion (P = 0.007), histological grading (P = 0.013) were the predictive factors for locoregional recurrence. Tumor invasion (P = 0.049), histological grading (P = 0.017), Lauren type (P = 0.029) and lymphatic vessel invasion (P = 0.029) resulted significantly in predicting peritoneal recurrence. Two parameters, tumor invasion (P = 0.037) and lymphatic vessel invasion (P = 0.030), were significantly associated with hematogenous spread.

Table 2
Clinicopathologic factors for different recurrence patterns in patients with node-negative AGC

Parameter	Locoregional recurrence			Peritoneal seeding			Hematogenous spread		
	Negative (n = 284)	Positive(n = 23)	P value	Negative (n = 290)	Positive (n = 17)	P value	Negative (n = 284)	Positive (n = 23)	P value
Age(years)			0.418			0.607			0.173
≤ 65	136	9		138	7		131	14	
> 65	148	14		152	10		153	9	
Gender			0.209			0.366			0.811
Male	214	20		219	15		216	18	
Female	70	3		71	2		68	5	
Tumor location			0.100			0.073			0.400
Upper	41	1		39	3		38	4	
Middle	95	13		103	5		103	5	
Lower	140	8		141	7		135	13	
Entire	8	1		7	2		8	1	
Tumor size			0.018			0.064			0.137
≤ 5 cm	134	5		135	4		132	7	
> 5 cm	150	18		155	13		152	16	
Tumor invasion(T)			0.007			0.049			0.037
T2	89	3		91	1		90	2	
T3	40	0		38	2		37	3	
T4a	155	20		161	14		157	18	
Histological grading(G)			0.013			0.017			0.821
G1	34	2		35	1		34	2	
G2	151	6		153	4		146	11	
G3	99	15		102	12		104	10	
Lauren type			0.263			0.029			0.111
Intestinal type	116	6		120	2		115	7	
Diffuse type	124	14		127	11		123	15	
Mixed type	44	3		43	4		46	1	

Parameter	Locoregional recurrence			Peritoneal seeding			Hematogenous spread		
	Negative (n = 284)	Positive(n = 23)	P value	Negative (n = 290)	Positive (n = 17)	P value	Negative (n = 284)	Positive (n = 23)	P value
Lymphatic vessel invasion			0.302			0.029			0.030
Positive	30	4		29	5		28	6	
Negative	254	19		261	12		256	17	
Adjuvant chemotherapy			0.701			0.147			0.264
Yes	212	18		220	10		215	15	
No	72	5		70	7		69	8	

3.4 Prognostic factors of RFS and OS

In regard to RFS, Kaplan-Meier survival curves and the log-rank test showed that histological grading (P = 0.029), tumor invasion (P = 0.011) had significantly worse prognosis. Then the significant prognostic Parameters (p < 0.1) were enrolled in the Cox proportional hazards model. Only tumor invasion (P = 0.009) as an independent factor for RFS in node-negative AGC patients. In terms of OS, there were significant relationship with tumor size (P = 0.045), histological grading (P = 0.031), tumor invasion (P = 0.008) and lymphatic vessel invasion (P = 0.024) by the univariate analysis. The multivariate analysis indicated that histological grading (P = 0.020), tumor invasion (P = 0.007) and lymphatic vessel invasion (P = 0.012) were independent factors related to OS.

According to the expression of Ki67 and P53, we divided the 307 patients into three groups: Group1 (negative for both Ki67 and P53, n = 94), Group2 (positive for either Ki67 or P53, n = 153), Group3 (positive for both Ki67 and P53, n = 60). Group3 had shorter RFS (P = 0.006) by univariate analysis and shorter RFS (HR,1.516; 95%CI, 1.251–1.837; P = 0.037) by multivariate analysis in comparison with Group1 and Group2. However, the Group3 was no an independent parameter for OS. (Table 3)

Table 3
Prognostic factors of recurrence-free survival and overall survival (univariate and multivariate analyses)

Prognostic factor	RFS univariate analysis	RFS multivariate analysis			OS univariate analysis	OS multivariate analysis		
	P value	HR	95% CI	P value	P value	HR	95% CI	P value
Ki67/P53 expression								
(Group1/Group2 vs. Grpup3)	0.006	1.516	1.251–1.837	0.037	0.083	2.007	0.929–4.336	0.153
Tumor size								
(≤ 5 cm vs. > 5 cm)	0.132				0.045	1.013	0.775–1.324	0.082
Histological grading								
(G1/G2 vs. G3)	0.029	1.143	0.823–1.587	0.068	0.031	1.579	1.217–2.049	0.020
Tumor invasion(T)								
(T2/3 vs. T4a)	0.011	1.994	1.533–2.593	0.009	0.008	2.162	1.340–3.488	0.007
Lymphatic vessel invasion								
(Negative vs. Positive)	0.095	1.129	0.750–1.700	0.190	0.024	1.717	1.302–2.264	0.012
Adjuvant chemotherapy								
(Negative vs. Positive)	0.830				0.504			

Figure 2 demonstrated that the RFS (P = 0.002) and the OS (P = 0.030) of Group3 had significantly worse to Group1 respectively.

4 Discussion

Although the incidence of gastric cancer was gradually decreased year by year, gastric cancer is still the third leading cause of cancer-related deaths all over the world because of poor biological behavior. Node-negative GC patients carry a significantly better prognosis than node-positive GC. However, a subset of these patients eventually died due to recurrence. Chou et al. reported the cumulative RFS rate and OS rate were 83.2% and 84.3%, respectively, in node-negative AGC patients underwent gastrectomy (R0) at five-year^[14]. Correspondingly, Zhao et al. investigated the cumulative RFS rate and OS rate were 71.1% and 63.5%, respectively^[15]. In the present study, With the median follow-up period of 57.5 months, 47 (15.3%) of 307 patients had tumor recurrences. The entire 307 patients with node-negative AGC who underwent D2 lymphadenectomy with the cumulative RFS rate and OS rate were 84.9% and 77.2% at five-year, respectively. In consideration of the high recurrence rate for node-negative AGC and the situation that these patients lacking independent prognosis factors except for the depth of invasion, it is necessary to find predictive

outcome indicators that may complement TNM staging system and guide clinicians not only to choose appropriate postoperative treatment strategy but also to construct individual follow-up schedule to improve prognosis of GC^[6, 16].

In this study, we excluded patients with T1 and T4b stage, on account of early gastric cancer (T1N0) associated with favorable prognosis who had low lymph node metastatic rate. On the contrary, T4b patients who had lower 5-year survival rate usually received postoperative chemotherapy by reason of the malignant biological behavior of gastric cancer^[17]. To some extent, it can reduce their confounding effect in the research.

Implementing appropriate adjuvant therapy is vital for gastric cancer patients after complete tumor resection to prevent recurrence and improve overall survival. Sasako et al. reported the adjuvant chemotherapy of S-1 can prolong curatively resected GC patients' overall survival. Combination of fluoropyrimidines and platinum is remaining the first line treatment of AGC^[18]. Sakamoto et al. put forward capecitabine plus cisplatin were effective to patients with early relapse^[19]. The 1-year RFS rates of stage II patients (n = 41) and stage III patients (n = 59) were 87% and 84% respectively found in JCLASSIC-II. In conclusion, capecitabine plus oxaliplatin was beneficial following resection of GC in Japanese patients^[20]. However, we didn't find that a statistically significant association between improved prognosis and postoperative chemotherapy. Although 230 patients (74.9%) were received chemotherapy, only 148 patients (64.3%) patients finished > 80% of the scheduled cycles, the other patients owing to the side effects of the chemotherapy and economic reasons didn't receive chemotherapy or complete it, which might affect the conflicting results.

Of note, different patterns of recurrence correspond to different postoperative adjuvant therapy regimens. Hence, it is significant to predict the recurrence pattern to scheme personalized therapy^[21]. Previously, Huang et al. reviewed the medical records of 317 node-negative GC patients and the results showed that 51 patients had recurred, the population patients with locoregional recurrence, peritoneal seeding accounted for 51.0% separately and 21 (41.1%) patients had hematogenous metastasis^[15]. Chou et al. indicated that the main pattern of recurrence in node-negative patients was locoregional disease^[14]. This study is not in accordance with previous results that the main pattern were locoregional (n = 28, 48.9%) and hematogenous (n = 28, 48.9%) recurrence. This difference might be out of with/without receiving chemotherapy after surgery, regional difference and the variability of tumor biology. Tumor size, the depth of tumor invasion (T stage), Lauren type and the presence of lymphatic vessel invasion have been identified as risk factors for recurrence in this series^[22]. Furthermore, we demonstrated that tumor size, T stage and histological differentiation were significant risk indicators for locoregional recurrence in node-negative GAC. T stage, tumor differentiation, Lauren type and lymphatic vessel invasion were associated with higher risks of peritoneal seeding. Of these factors, the depth of tumor invasion and the presence of lymphatic vessel invasion were relevant to hematogenous spread. Actually, in regard to the predictors of recurrence patterns in GC have been explored previously, but few in node-negative^[23-25]. Interestingly, pT stage had been confirmed as a risk predictor in any of recurrence patterns in relevant research fields^[26, 27]. The depth of tumor invasion was also the most unanimous significant prognostic parameter of node-negative GC patients, which was consistent with the multivariate analysis (P = 0.007) of independent factors related to OS in this study. In the aspect of tumor size, it is no consistency for the prognostic significance in node-negative AGC, unlike breast and lung cancer^[28, 29]. Chou et al. demonstrated that bigger tumor was correlated with hematogenous metastasis, but we only observed tumor size was related to Locoregional recurrence^[14]. The discrepancy may be explained by enrolling T4b patients who had dismal prognosis in their research that was not included ours. Although several studies found tumor size increased the hazard for recurrence and survival in advanced node-negative GC underwent R0 resection, this result was not repeated in other researches^[30-32]. Univariate analysis revealed tumor size was significantly associated with 5-year OS (P = 0.045) in our series but it lost any significance in the multivariate analysis with the Cox proportional hazards model (P = 0.082). In terms of the presence of lymphatic vessel invasion, it

was a predictive marker both for peritoneal seeding and hematogenous spread. On the other hand, it was an important poor prognostic indicator to shorten the time of OS. Noteworthily, The above results of lymphatic vessel invasion were consistent with this study. Zhao et al. hypothesized lymphatic vessel invasion was the startup phase based on it was associated with lymph node micrometastasis^[15]. Unfortunately, we have not observed it had prognostic value in recurrence-free survival.

In general, the combined use of the pathological cancer markers, has more complex and more heterogeneous, might has more advantages than that of single markers for GC patients in the prognostic value. p53 is a tumor-suppressor gene that located on chromosome 17p and encoded a 53-kDa protein^[33]. The p53 gene plays an important role in the cell cycle by G1 arrest checkpoint cell cycle to modulate cell proliferation and suppress the development of tumor^[34]. However, during the development of GC, the functions of p53 protein are often blocked because it has undergone mutation. Compare to normal p53 protein, mutated p53 protein prolong its half-life on account of changing configuration and increasing stability. Therefore, it can be detected by immunohistochemical staining. Several studies have reported that overexpression of p53 were independent risk factors for EGC patients^[35,36]. Ki67 is a nuclear proliferation-associated antigen, which encodes two protein isoforms with molecular weights of 345 and 395 kDa. In addition, it is universally expressed throughout the cell cycle in proliferating (late G1, S, G2 and M-phases), but is absent in quiescent cells^[37]. The prognostic value of pKi67 has been investigated as a reliable marker having been shown in several types of cancer, such as colorectal and breast cancer, according to activating the trigger of downregulation for the gene of apoptosis^[38,39]. Whereas, some analyses found the expression of Ki67 was correlated with survival in gastric cancer, others found no significant difference between Ki67 expression and oncological outcomes^[40,41]. In a word, p53 and Ki67 are important biomarkers for cancer progression. However, little research reported on whether the combination of p53 and Ki67 is an independent prognosis factor in node-negative AGC after D2 curative resection. The whole patients were divided into 3 groups: Group1 (negative for both Ki67 and P53), Group2 (positive for either biomarker), Group3 (positive for both biomarkers). We found Group3 was a significant prognostic parameter in terms of RFS and OS compared with Group1, $P = 0.002$ and $P = 0.030$ respectively. Meanwhile, Group 3 had shorter recurrence-free survival compared with Group1 and Group2 in the multivariate analysis with the Cox proportional hazards model ($P = 0.037$), but do not find the significant difference between Group 3 and OS.

Several limitations of the present study should be acknowledged. Firstly, on account of the retrospective nature of this study, all the result may be influenced. Secondly, it was single institutional research with the limitation of the number of patients. On the side, our observations are necessary to be confirmed by more large-scale prospective studies with more longer follow-up time.

5 Discussion

The data presented in this report demonstrate that tumor size, tumor invasion and histological grading resulted significantly in predicting locoregional recurrence with node-negative AGC after D2 curative resection. Tumor invasion, histological grading, Lauren type and lymphatic vessel invasion were the predictive factors for peritoneal recurrence. Tumor invasion and lymphatic vessel invasion were significantly associated with hematogenous spread. The combinatorial biomarker of positive for both Ki67 and P53, histological grading, tumor invasion and lymphatic vessel invasion were independent factors related to prognosis.

Abbreviations

GC

Gastric cancer; AGC:Advanced gastric cancer; LN:Lymph node; R0:Curative resection; EGC:Early gastric cancer; T:Depth of tumor invasion; G:Histological grading; RFS:Recurrence-free survival; OS:Overall survival; IHC:Immunohistochemical; FISH:Fluorescence in situ hybridization

Declarations

Availability of data and materials

The clinical datasets supporting the results of this article are available from the corresponding author.

Ethics approval and consent to participate

The study was conducted with strict adherence to the Declaration of Helsinki. The study protocol was reviewed and approved by the ethics committee of the First Affiliated Hospital of Zhejiang Chinese Medical University. Informed consent to participate this study was obtained from all patients in writing or orally. The procedure for oral consent was approved by the ethics committee.

Consent for publication

Not applicable.

Competing interests

The Authors declare no conflict of Interest exists in regard to this study.

Funding

This work was supported by the Major Science and Technology Project of Zhejiang Province of China (Grant No. 2019C03049); Natural Science Foundation of Zhejiang Province (Grants no. LY18H290006); Traditional Chinese Medicine Scientific Research Foundation of Zhejiang Province (Grant no. 2019ZZ010, 2018ZY006); National Natural Science Foundation of China (Grant No. 81573953); Zhejiang Provincial Medical and Healthy Science and Technology Projects (Grants No. 2016KYB220)

Authors' contributions

CSQ, HC, XZY, and CXD designed the study. CSQ, XZY, HC, YJF, and WXF performed data acquisition and analysis. CSQ, XZY, HC, LH, WSY, and SCW performed statistical analysis and data interpretation. CSQ prepared the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

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Figures

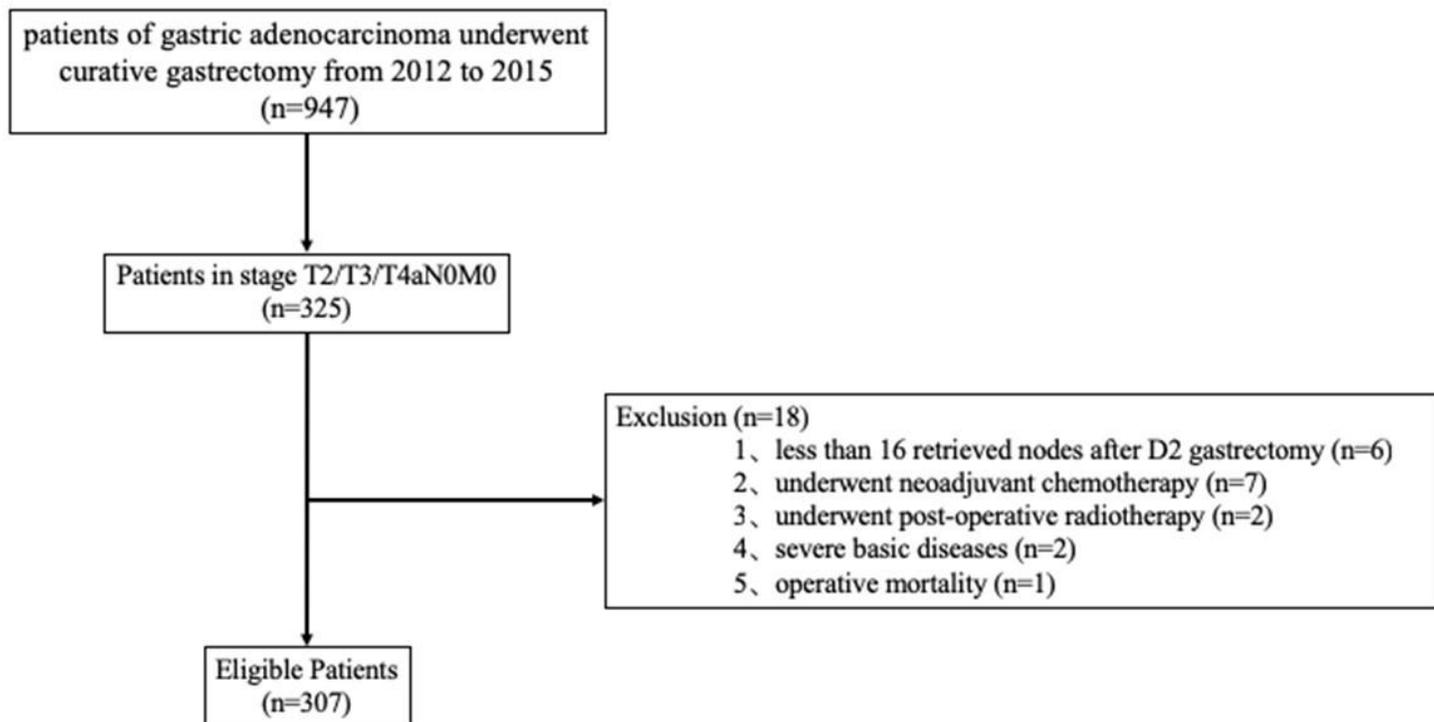


Figure 1

Flow chart of the inclusion process for eligible patients in this study.

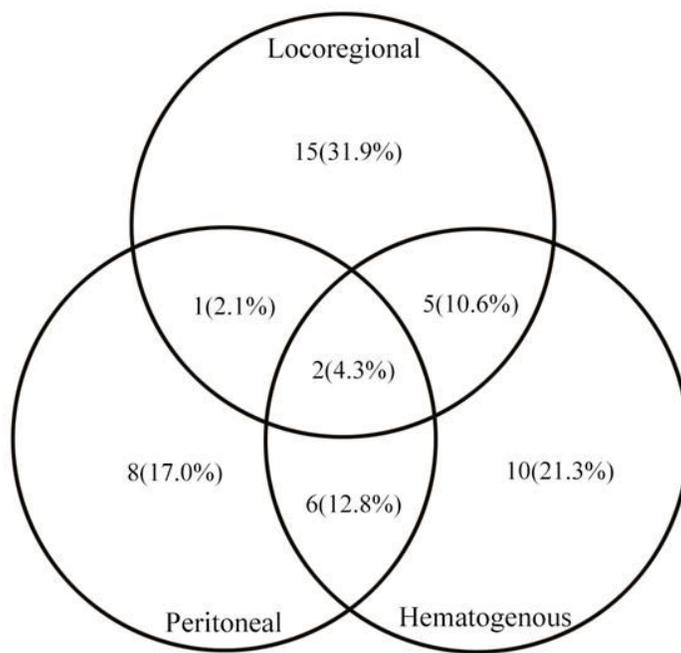


Figure 2

Recurrence-free survival curves (a) and overall survival curves (b) for node-negative AGC patients in combination with p53 and Ki67 expression (Group1-3).

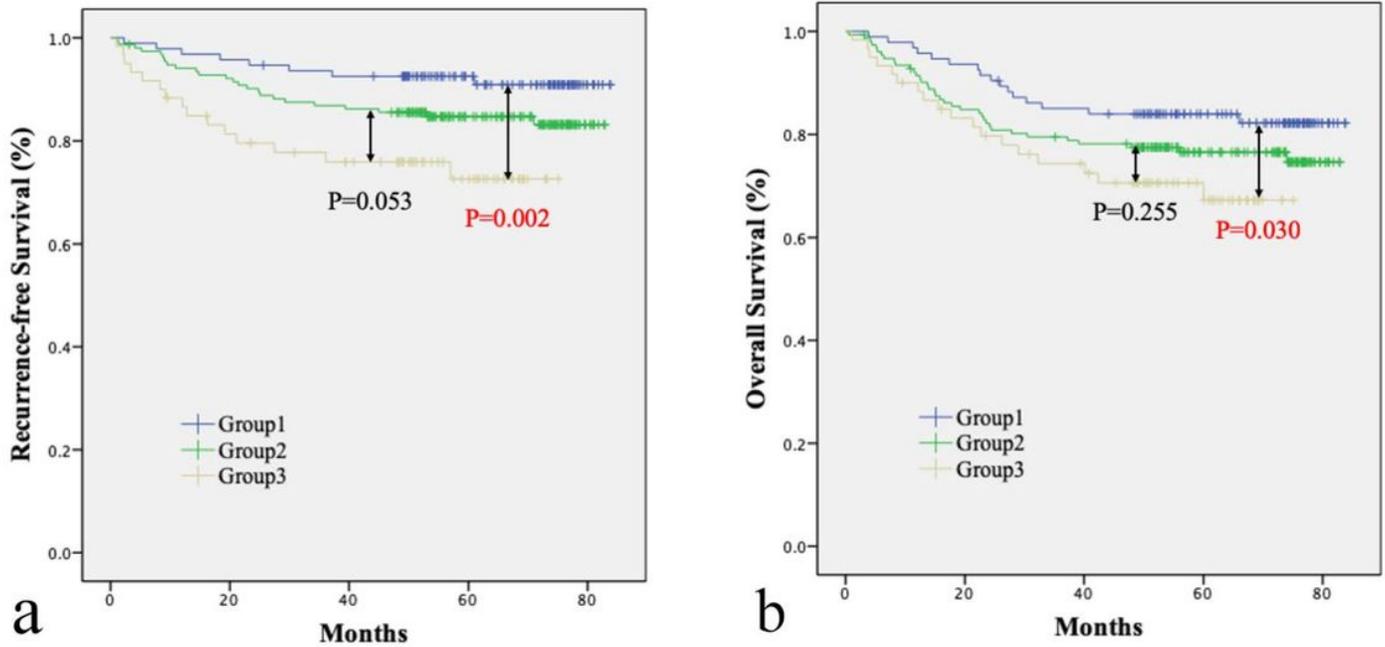


Figure 3

Figure 3

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