

A pilot clinical study to evaluate feasibility of using single patient classifier as a prognostic test in stage II–III gastric cancer patients

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Abstract

Objective: Precision medicine approaches emphasize the importance of reliable prognostic tools for guiding individualized therapy decisions. In this study, we evaluated the clinical feasibility of the single patient classifier (SPC) test, a new clinical-grade prognostic assay, in stage II–III gastric cancer patients.

Methods: A prospective multicenter study was conducted, involving 237 patients who underwent gastrectomy between September 2019 and August 2020 across nine hospitals. The SPC test was employed to stratify patients into risk groups, and its feasibility and performance were evaluated. The primary endpoint was the proportion of the cases in which the test results were timely delivered before selecting postoperative treatment. Furthermore, 3-year disease-free survivals of risk groups were analyzed.

Results: The SPC test met the primary endpoint criteria. The 99.5% of SPC tests were timely delivered to hospitals before the postoperative treatment started. In a clinical setting, the median time from the specimen transfer to laboratory to the result delivery to hospital was 4 d. Furthermore, 3-year disease-free survivals were significantly different between risk groups classified with SPC tests.

Conclusions: This study highlights the SPC test's feasibility in offering crucial information timely delivered for making informed decisions regarding postoperative treatment strategies. It also provides evidence to support the implementation of a future prospective clinical trial aimed at evaluating the clinical utility of the SPC test in guiding personalized treatment decisions for gastric cancer patients.

Keywords: Prognostic test; advanced gastric cancer; adjuvant chemotherapy; feasibility; gastrectomy

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Introduction

Adjuvant chemotherapy is the recommended standard treatment for stage II–III advanced gastric cancer (AGC) in Korea and Japan (1-5). However, despite the TNM stage being used to determine the progression of AGC (1,5,6), it is evident that the prognosis can significantly differ among patients within the same stage. Recognizing this variability, there is a pressing need for additional prognostic markers that can contribute to improved patient outcomes, enabling tailored treatment approaches (7,8). By categorizing patients into these risk groups, medical care can employ less toxic chemotherapy regimens for those in the low-risk group, thus minimizing unnecessary side effects, while administering more aggressive chemotherapy with closer monitoring for those in the high-risk group (9,10).

In recent years, significant advancements have been witnessed in the field of precision medicine. One of the recent notable developments is a clinical-grade prognostic

assay for patients with AGC following surgery (11). This assay, known as the single patient classifier (SPC) (or nProfiler® 1 Stomach Cancer Assay by tradename, Novomics, Seoul, Korea), uses formalin-fixed, paraffin-embedded (FFPE) tissue and employs a real-time polymerase chain reaction (PCR) approach. SPC categorizes AGC patients into immune/stem cell/epithelial subtypes, effectively differentiating patients into three distinct risk groups (low, intermediate, and high; *Figure 1A*) with varying survival rates. This finding has been validated through independent retrospective cohorts of AGC patients (12); however, further prospective validation is still warranted to solidify its clinical applicability.

Prior to initiating prospective clinical trials, it is essential to assess the feasibility of implementing the test in a clinical environment (8,13-17). To achieve this, a feasibility study was prospectively designed. The primary objective of this observational study was to assess the practicality of various steps, starting from the point of requesting the test after

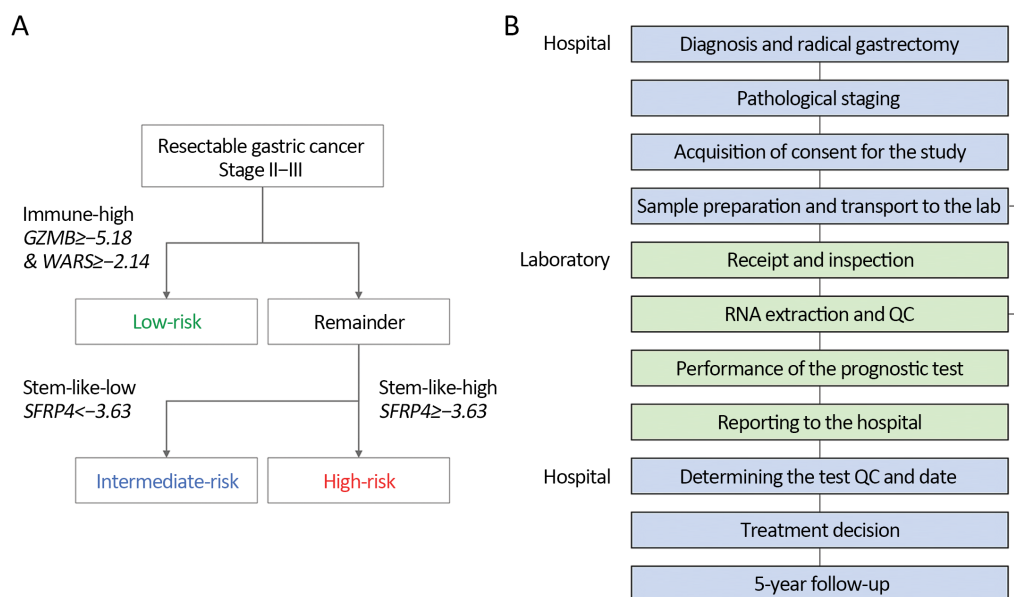


Figure 1 SPC algorithm and a flowchart of the study. (A) SPC categorizes stage II–III gastric cancer patients into immune/stem cell/epithelial subtypes, effectively differentiating patients into three distinct risk groups (low, intermediate, and high) with varying survival rates. To be more specific, the low-risk group was identified by the high expression of immune genes, *GZMB* and *WARS*, with cut-off values of -5.18 and -2.14 , respectively. Meanwhile, the intermediate and high-risk groups were differentiated by the expression of the stem-like gene *SFRP4*, where the high-risk group exhibited a high level of *SFRP4* with a cut-off value of -3.63 ; (B) This study evaluated the feasibility of SPC test in a clinical setting. When the FFPE specimen slides of the enrolled patients were prepared, they were transported to the laboratory for analysis with nProfiler® 1 Stomach Cancer Assay. The results were reported back to the test referred hospital. We defined the cases with “timely delivery”, when the SPC test results were delivered back to the test referred hospitals before the treatment decision is made to patients following gastrectomy. The primary endpoint of this study is the proportion of cases with “timely delivery” of test results. The secondary endpoint is 3-year DFS of the enrolled patients. SPC, single patient classifier; FFPE, formalin-fixed, paraffin-embedded; DFS, disease-free survival; QC, quality control.

postoperative pathological examination, including sample preparation and delivery, assay testing and analysis, and the timely return of results. Given that the prognostic test results provide considerable information in determining the appropriate postoperative treatment plan, it is crucial that the test results are delivered to the physician in a timely manner, before the treatment course is decided upon. Therefore, the primary endpoint was set to be the timely delivery rate, which refers to whether the test results are provided to the physician before the decision-making stage for postoperative treatment. Furthermore, a secondary endpoint was established as the evaluation of significant differences in the survival outcomes between patients in different prognostic risk groups.

In this report, we present a pre-trial feasibility study, which encompass the timely delivery rate and the 3-year disease-free survival (DFS) rate. These results are expected to contribute additional clinical evidence, providing valuable insights for the design and implementation of prospective clinical trials in the future.

Materials and methods

Study design and participants

The current study was a prospective pilot clinical study conducted across nine hospitals in South Korea (Samsung Medical Center, National Cancer Center, Severance Hospital, Chonnam National University Hwasun Hospital, Gangnam Severance Hospital, Yeungnam University Medical Center, Soonchunhyang University Bucheon, Cheonan Hospital, and Chung-Ang University Hospital). The patients were enrolled between September 2019 and August 2020. Eligible participants met specific criteria, including 1) being ≥ 19 years of age; 2) having a histologically confirmed diagnosis of stage II or III AGC according to the American Joint Committee on Cancer (AJCC) staging system (sixth edition); 3) underwent radical gastrectomy without prior neo-adjuvant chemoradiotherapy and did not receive adjuvant chemotherapy; and 4) having no visually or microscopically detectable residual tumors. In addition, participants were required to provide written informed consent for the study and the use of human-derived materials. Those with insufficient quantity or quality of RNA for analysis were excluded from the study. The study protocol was reviewed and approved by the institutional review board in accordance with applicable regulations at each participating site. The study was

registered in ClinicalTrials.gov (NCT06521528). We adhered to the Reporting Recommendations for Tumor Marker Prognostic Studies Guideline (16,18).

Primary and secondary endpoints

The primary endpoint of this study was the proportion of cases with “timely delivery” of test results using the nProfiler® 1 Stomach Cancer Assay. The definition of “timely delivery” is that the SPC test results which met the required quality control criteria approved by The Korean Ministry of Food and Drug Safety, were delivered back to the test referred hospitals before the treatment guidelines were provided to patients following surgery. To evaluate the median turnaround time, we measured the period between the date of transferring the FFPE specimen slides to the test laboratory and the date when the test results were delivered to the hospital that requested the test. Because this study was designed to evaluate only the feasibility of timely delivery, the test results were not used for patient treatment purposes during the study period. Therefore, the results were not disclosed to patients so as to maintain patients’ safety during the standard treatment period.

The secondary endpoint was 3-year DFS rates for each prognostic group classified with SPC tests in full analysis set. A follow-up period of 5 years was scheduled after completion of the enrollment phase to collect diagnostic and treatment information from the patients’ medical records.

nProfiler® 1 Stomach Cancer Assay

The nProfiler® 1 Stomach Cancer Assay is an *in vitro* diagnostic quantitative reverse transcription polymerase chain reaction (RT-qPCR) assay that provides prognostic information on a patient’s 5-year survival rate. The assay measures the expression levels of nine genes (*GZMB*, *WARS*, *SFRP4*, *CDX1*; reference genes: *ACTB*, *ATP5E*, *HPRT1*, *GPX1*, *UBB*) in FFPE tissue samples obtained from specimens after gastrectomy. The SPC within the assay classifies patients into low-, intermediate-, and high-risk groups using the expression levels of specific target genes (Figure 1A) (11). To be more specific, the low-risk group was identified by the high expression of immune genes, *GZMB* and *WARS*, with cut-off values of -5.18 and -2.14 , respectively. Meanwhile, the intermediate- and high-risk groups were differentiated by the expression of the stem-like gene *SFRP4*, where the high-risk group exhibited a

high level of SFRP4 with a cut-off value of -3.63 .

Procedure

Figure 1B shows a flowchart of this study. Patients who received radical gastrectomy following a diagnosis of AGC through pathological evaluation of tumor histology and grade were provided with information about this study. Participants provided consent for research. FFPE specimen slides containing surgically resected tissues were prepared and sent to the designated testing laboratory (Novomics). At the testing laboratory, total RNA extraction and nProfil[®] 1 Stomach Cancer Assay testing were performed on the samples. If the quality control (QC) assessment failed due to insufficient quantity or inadequate quality of the extracted total RNA or genomic DNA contamination, a request was made to the requesting hospital for a new sample. Upon successful QC assessment, the testing agency provided the requesting hospital with the test results and confirmation of meeting the QC standards. The requesting hospital then determined the timeliness of the delivery of test results based on whether they were received before the patient's adjuvant chemotherapy treatment began.

Sample size estimation

The sample size calculation for this study aimed to ensure achievement of the primary objective of delivering test results in a timely manner before treatment decisions after surgery. The number of samples was determined by considering the desired level of confidence (95%, $\alpha=5\%$) and its associated standard error associated with the probability of timely delivery of test results (margin of error $=3\%$). The calculation employed the Clopper-Pearson Interval method (19,20), which is an exact method for binomial distribution, determining that at least 238 individuals were required for this study. We planned to enroll a total of 265 patients for the study, including an additional 10% of patients considering the possibility of QC standards not being met, re-testing, or dropout.

Trial enrollment termination

This study was initially planned with a total of 265 participants. However, the trial committee agreed that the study enrollment could close earlier due to an observation in June 2020 that one case failing out of consecutive 186 cases met the criteria for timely delivery of test results

during that period. The timely delivery success rate for this period was 99.5% with 95% confidence interval (95% CI) 98.4%–100%, exceeding the primary endpoint requirement, as the lower confidence limit of the timely delivery rate (98.4%) exceeded 95% with a margin of error of 3%. Consequently, the enrollment was closed after screening 237 participants in August 2020.

Statistical analysis

We defined two different population sets for analysis. First, there was per-protocol set for analysis of timely delivery of the SPC test results. Unexpected drop out cases, where timely delivery could not be determined were excluded from per-protocol analysis. In addition, after the trial monitoring program, a set of patients classified as stage II or III GC according to the eighth edition of the AJCC staging system but as stage I ($n=7$) or IV ($n=28$) according to the sixth edition (e.g., T3N0, T1N2, T4bN1+, or T1+N3b cases according to the eighth edition of the AJCC staging system). Patients diagnosed as stage I or IV as of the sixth edition ($n=35$) were excluded from per-protocol analysis. The 3-year DFS of the risk groups was performed using this full analysis set of patients with stage II or III AGC according to the eighth edition of the AJCC staging system (6), which is currently used in clinical practice.

The primary endpoint was evaluated using a per-protocol analysis set. The success rate of timely delivery results was provided with a 95% CI estimated by the simple asymptotic method, and the turnaround time was presented in working days, excluding the weekends and holidays.

The 3-year DFS of full analysis set which is the secondary endpoint, was estimated using the Kaplan-Meier method. The significance of the prognostic test results was determined using the log-rank test and Cox model. The log-rank test was used as the primary test to assess the survival difference in the Kaplan-Meier curves of the risk groups. Multivariate Cox regression analysis was performed to calculate each adjusted hazard ratio (HR) and its corresponding 95% CI.

Continuous variables were expressed as mean and 95% CI values or median and interquartile range (IQR) values, while categorical variables were presented as proportions. Statistical significance was determined using a two-sided P value of <0.05 . Sample size and power estimations were conducted using Power Analysis and Sample Size (PASS), version 15.0.2 (NCSS, LLC, Kaysville, Utah, USA) — specifically, the Confidence Intervals for One Proportion.

Survival analysis was performed using R version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Patients

From September 2019 to August 2020, a total of 237 individuals were assessed for this study (Figure 2). Among them, 35 patients were classified as stage I or IV (31 patients were classified as stage II or III according to the eighth edition of the AJCC staging system). There were five cases where timely delivery could not be determined: in two cases, the test was requested after the initiation of postoperative treatment, while there was one case of patient death before the delivery of test results and one case of the patient being transferred to another hospital after surgery. In addition, one patient was identified as having another solid tumor. These were classified as dropouts. Among the patients in the per-protocol set, 93.9% (185/197) underwent adjuvant chemotherapy (Table 1).

Primary endpoint: feasibility and turnaround time of prognostic test

In the per-protocol set for timely delivery, 197 patients met the quality standards for the test results, and the test results of 196 patients were delivered to the hospital before the postoperative treatment decision. The timely delivery rate was 99.5% (95% CI, 98.5%–100%), surpassing the primary endpoint of 95% with a margin of error of 3%, thereby meeting the requirement. The time interval from surgery

to the treatment decision varied across hospitals (Kruskal-Wallis $P < 0.001$; median IQR, 19.5–23.0 working days), and the median of the turnaround time was 4 (IQR, 3–5) working days.

In this trial, there was one instance of failed timely delivery, due to an omission in the sample preparation process, resulting in the delivery of the test result after the postoperative treatment had already started. In this case, however, the turnaround time was 5 d after the prepared sample was transported to the laboratory. Additionally, there were two cases with requests for additional sample preparation due to QC failure (genomic DNA contamination) that were timely delivered before treatment decision with the total turnaround times of 12 and 14 d, respectively.

Secondary endpoint: 3-year DFS analysis and prognostic performance of risk groups

Three-year DFS in the full analysis set ($n=228$) was conducted. The median follow-up period was 27.1 (IQR, 12.6–33.1) months. The clinical characteristics of this cohort are shown in Table 1. No significant differences were observed between the distribution of clinical variables in the per-protocol set and the full analysis set (Table 1). The 3-year DFS rates were 81.7% (95% CI, 73.1%–91.4%) and 62.6% (95% CI, 54.5%–71.8%) in the stage II and III groups, respectively (log-rank $P=0.003$) (Figure 3A). The prognostic test revealed 30 (13.2%) patients in the low-risk group, 133 (58.3%) in the intermediate-risk group, and 65 (28.5%) in the high-risk group. The Kaplan-Meier curves depicted the survival differences by the stage and the

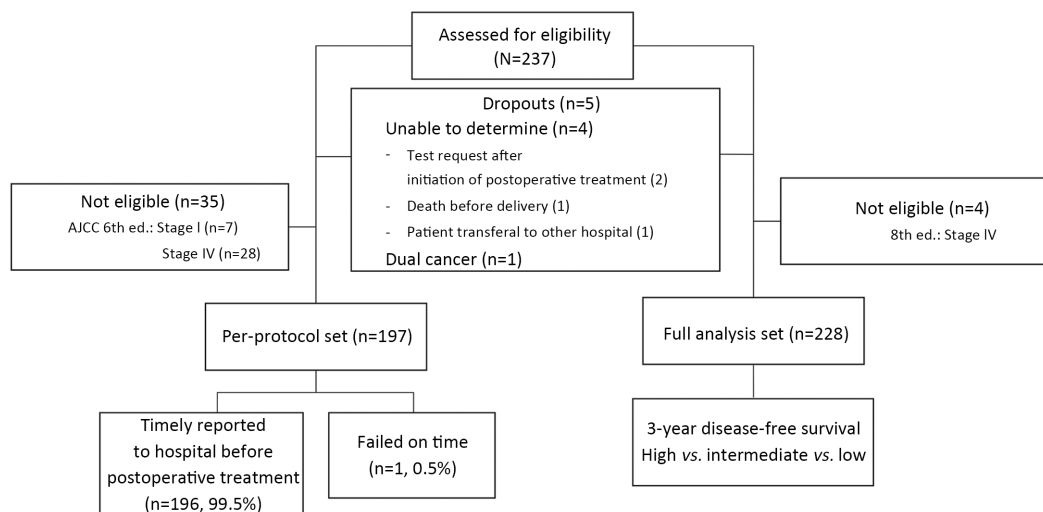


Figure 2 A flow diagram of patient selection through the study. AJCC, American Joint Committee on Cancer.

Table 1 Baseline characteristics of study cohort

Characteristics	n (%)		Chi-squared P
	Per protocol (N=197)	Full analysis (N=228)	
Age (year)			1.00
<70	129 (65.5)	148 (64.9)	
≥70	68 (34.5)	80 (35.1)	
Sex			0.99
Male	130 (66.0)	153 (67.1)	
Female	67 (34.0)	75 (32.9)	
Stage ^a			0.85
II	78 (39.6)	85 (37.3)	
III	119 (60.4)	143 (62.7)	
Chemotherapy			1.00
Yes	185 (93.9)	215 (94.3)	
No	12 (6.1)	13 (5.7)	
Recurrence			0.95
Yes	49 (24.9)	60 (26.3)	
No	148 (75.1)	168 (73.7)	

^a, According to the eighth edition of the American Joint Committee on Cancer Staging system.

prognostic test (Figure 3B). The 3-year DFS rates were 92.7% (95% CI, 83.4%–100%) in the low-risk group, 69.7% (95% CI, 61.7%–78.7%) in the intermediate-risk group, and 59.4% (95% CI, 47.7%–73.9%) in the high-risk group (log-rank $P=0.021$) (estimated power=93%) (Figure 3B). Furthermore, the low-risk group exhibited a higher 3-year DFS rate than that of the stage II group (92.7% vs. 81.7%). It is worth noting that the stage II high-risk subgroup had a 3-year DFS rate of 57.5% ($n=15$), which was lower than the 3-year DFS rate of the stage III group (62.6%, Figure 3C). Conversely, the stage III low-risk subgroup demonstrated a 3-year DFS rate of 90.0% ($n=30$), surpassing the 3-year DFS rate of the stage II group (81.7%) (Figure 3D). Distribution of the risk groups was associated with the TNM stage (χ^2 , $P<0.001$) and recurrence ($P=0.013$), but not with other clinical variables (Table 2). An additional stratified analysis by sex showed that the survival difference trend by the prognostic groups conserved in both male and female groups (Supplementary Figure S1). In the multivariate Cox analysis, the adjusted HR for the risk group, compared to the low-risk group, was

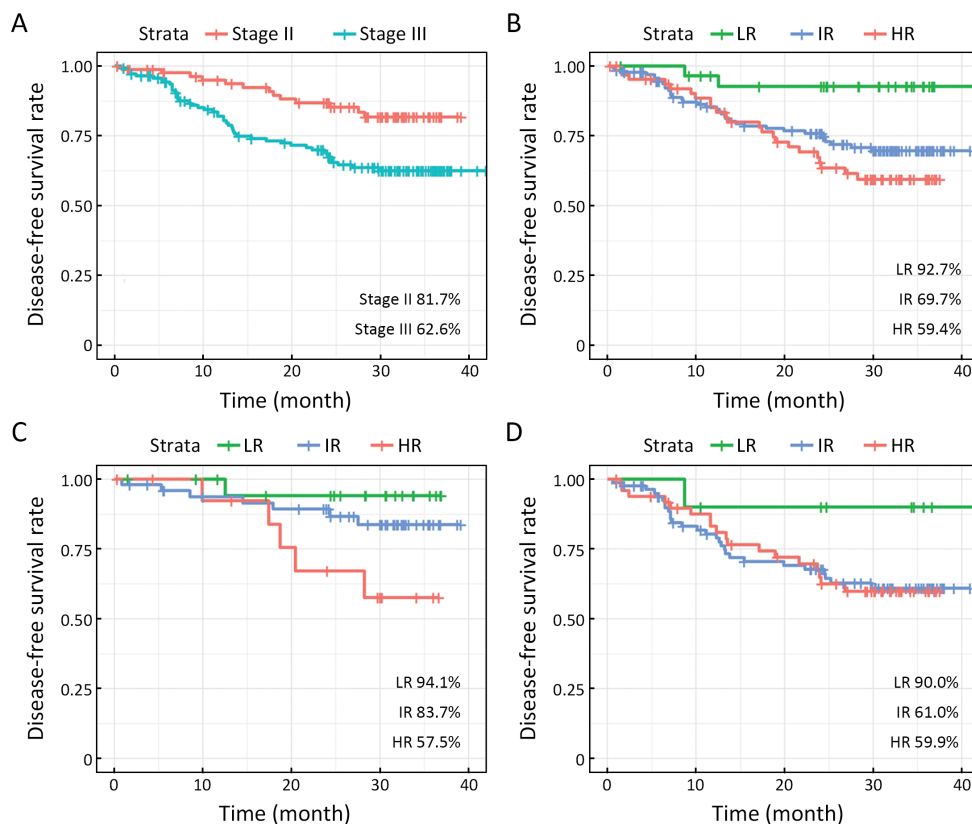


Figure 3 Kaplan-Meier survival curves according to stage ($P=0.003$) (A), prognostic group ($P=0.021$) (B), prognostic group in stage II ($P=0.053$) (C) and stage III ($P=0.310$) (D) subgroups in the full analysis set. LR, low risk; IR, intermediate risk; HR, high risk.

3.60 (95% CI, 0.85–15.20; P=0.08) for the intermediate-risk group and 4.40 (95% CI, 1.00–19.20; P<0.05) for the high-risk group (Table 3). The prognostic information showed independently significant prognostic performance regardless of the tumor stage and the receipt of

chemotherapy.

Discussion

This study represents the pilot clinical study to see the

Table 2 Association of prognostic groups with clinical variables in full analysis set

Characteristic	Prognostic group [n (%)]			P
	Low risk (N=30)	Intermediate risk (N=133)	High risk (N=65)	
Age (year)				0.180
<70	16 (53.3)	85 (63.9)	47 (72.3)	
≥70	14 (46.7)	48 (36.1)	18 (27.7)	
Sex				0.920
Male	21 (70.0)	88 (66.2)	44 (67.7)	
Female	9 (30.0)	45 (33.8)	21 (32.3)	
Stage				<0.001
II	20 (66.7)	50 (37.6)	15 (23.1)	
III	10 (33.3)	83 (62.4)	50 (76.9)	
Chemotherapy				0.350
Yes	30 (100)	124 (93.2)	61 (93.8)	
No	0 (0)	9 (6.8)	4 (6.2)	
Recurrence				0.013
Yes	2 (6.7)	35 (26.3)	23 (35.4)	
No	28 (93.3)	98 (73.7)	42 (64.6)	

Table 3 DFS analyzed by a Cox proportional hazards model in full analysis set

Characteristics	Univariate analysis		Multivariate analysis	
	HR	P	HR	P
Age (year)				
<70	1		1	
≥70	1.46 (0.87–2.44)	0.16	1.42 (0.81–2.49)	0.23
Sex				
Female	1		1	
Male	0.55 (0.33–0.92)	0.02	0.59 (0.35–0.98)	0.04
Stage				
II	1		1	
III	2.46 (1.33–4.55)	<0.01	2.21 (1.18–4.15)	0.01
Chemotherapy				
No	1		1	
Yes	0.38 (0.17–0.90)	0.03	0.54 (0.22–1.36)	0.19
Prognostic group				
Low	1		1	
Intermediate	4.47 (1.08–18.60)	0.04	3.60 (0.85–15.20)	0.08
High	6.10 (1.44–25.90)	0.01	4.40 (1.00–19.20)	<0.05

DFS, disease-free survival; HR, hazard ratio.

clinical feasibility conducted after the development and validation of the SPC prognostic test through retrospective research. The timely delivered rate of the test results was 99.5%, with a median turnaround time of 4 d in a clinical setting. Despite variations in protocols and procedures among the nine hospitals, the test met the primary endpoint criteria for timely delivery before making decisions about postoperative treatment methods. It was deemed feasible for the test to undergo prospective clinical trials to evaluate its clinical validity.

Furthermore, in the prospectively collected cohort, we demonstrated the independent prognostic significance of the risk group, irrespective of the TNM stage and adjuvant chemotherapy. Interestingly, the 3-year DFS was >90.0% in the low-risk group and higher than rates of both stage II (81.7%) and stage III (62.6%) patients, respectively. Even among patients diagnosed with stage II cancer, the 3-year DFS was 57.5% in the high-risk subgroup, and it was lower than the rate of stage III patients (62.6%). Additionally, in the per-protocol set, the high-risk group exhibited a significantly poorer prognosis compared to the low-risk group (log-rank $P=0.03$). This result revealed that the SPC test presented a better-than-conventional TNM staging system in discriminating the individual patient's risk in terms of prognosis. Furthermore, based on this SPC test result, physicians can recommend more intense chemotherapy for stage II patients who were classified as at high risk or omit chemotherapy for stage II or III patients who were classified as at low risk.

Comparison between this study and a previous validation study using archived FFPE tumor samples from patients who underwent gastrectomy between 2006 and 2009 from the CLASSIC trial (adjuvant capecitabine plus oxaliplatin for gastric cancer after D2 gastrectomy) revealed no significant differences in the distribution of the SPC risk groups ($\chi^2 P=0.21$) (11). Although a direct comparison of survival rates between this study and the previous validation study is challenging due to differences in the endpoints (3-year DFS and 5-year overall survival), the observed trends in the survival curves of the risk groups were similar. A comprehensive analysis of the secondary endpoint will be conducted after a 5-year follow-up period.

Though confirmatory clinical trial after this preliminary study should tell later, we assume that the clinical potential of the SPC test as a new clinical-grade prognostic tool lies in its ability to provide more accurate stratification of

patients with stage II–III AGC. Building upon previous research, where the SPC risk group demonstrated independence as a prognostic factor to the traditional TNM staging system, Choi *et al.* proposed a modified TNM staging that incorporates SPC information by a single-grade down-staging for patients classified into the low-risk group, leading to an improvement in the C-index (12). Similarly, in the present study, we observed an improvement in the C-index (from 0.680 for the original TNM to 0.686 for modified TNM staging in the full analysis set). These findings hold significant implications for clinical practice, emphasizing the importance of integrating both TNM staging and risk group stratification. Specifically, it is prudent to consider the use of less toxic treatment regimens (with shorter cycles) for stage II patients classified in the low-risk group, as this approach helps minimize unnecessary side effects. Conversely, stage III patients belonging to the high-risk group may benefit from more intensive chemotherapy protocols accompanied by close monitoring (9,10). By adopting this tailored treatment approach, it is expected that healthcare providers can optimize patient outcomes while minimizing treatment-related complications.

To accomplish this objective, Riley *et al.*, McShane *et al.*, and Altman *et al.* emphasized the importance of prioritizing large, protocol-driven primary investigations and subsequently performed systematic reviews and meta-analyses to consolidate existing evidence (8,18,21). Moreover, the initiation of prospectively planned pooled analyses can further strengthen the reliability and generalizability of prognostic marker research findings. Collaborative efforts among multiple research centers and disciplines will play a pivotal role in assembling sufficient sample sizes and facilitating the exchange of knowledge and expertise, ultimately propelling the field toward evidence-based practices and improved patient outcomes. We believe that the present study serves to contribute to this collective effort.

Conclusions

The SPC prognostic tests in stage II–III gastric cancer patients are feasible in clinics. The test results can be timely delivered to hospitals with adequate quality before the patients start their postoperative treatment. Timely delivered results will guide clinicians to personalized

treatment decisions for those patients. Future research should continue to explore and validate the utility of the SPC test in larger prospective clinical trials and in diverse clinical settings.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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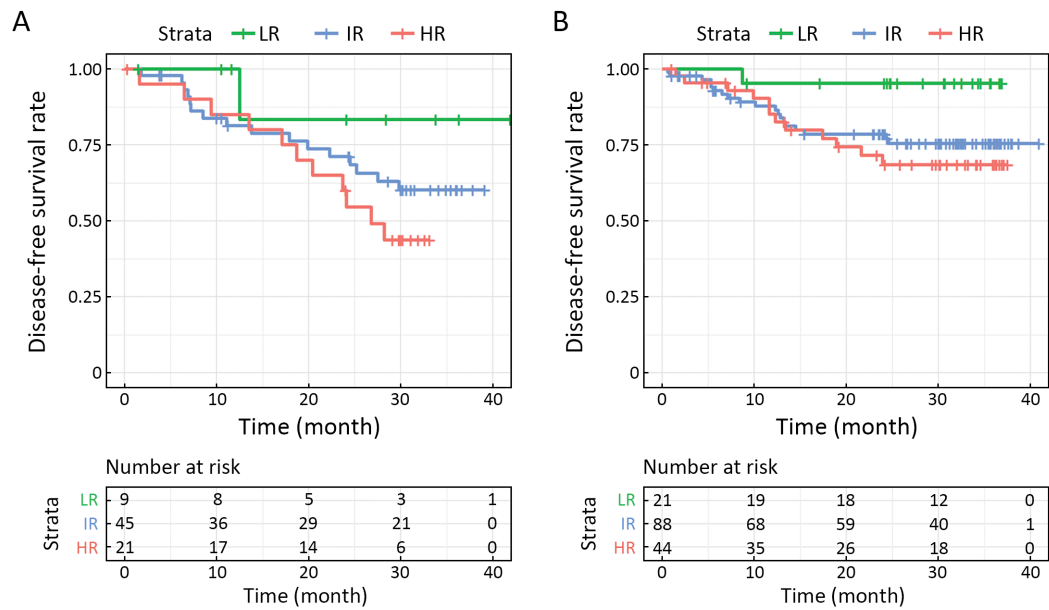


Figure S1 Stratified analysis by sex. (A) Female (n=75) (P=0.24); (B) Male (n=153) (P=0.10).