Application of serum anti-ENO1 and anti-SSNA1 antibody biomarkers in predicting the prognosis of gastric cancer

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Abstract. Given the high malignancy of advanced gastric cancer, the identification of biomarkers for the diagnosis and prognosis prediction of advanced gastric cancer is of importance. The present study conducted the serological identification of antigens by recombinant cDNA expression cloning and determined enolase 1 (ENO1) and Sjögren syndrome nuclear autoantigen 1 (SSNA1) as tumor antigens recognized by serum immunoglobulin G (IgG) antibodies in patients with gastric cancer. The clinicopathological significance of preoperative autoantibodies were assessed, namely serum anti-ENO1 antibodies (s-ENO1-Abs) and serum anti-SSNA1 antibodies (s-SSNA-Abs), in the sera of 166 patients with gastric cancer who underwent radical surgery and 96 healthy donors. The s-ENO1-Ab and s-SSNA-Ab titer levels were significantly increased in patients with gastric cancer compared with that in healthy donors (P<0.01). Areas under the receiver operating characteristic curves of s-ENO1-Ab and s-SSNA1-Ab were 0.656 and 0.607, respectively. None of the clinicopathological factors, such as sex, age, histological type, tumor size, tumor depth, nodal status, cytology, peritoneal dissemination and stage demonstrated association with the s-ENO1-Ab or s-SSNA1-Ab titer levels. High s-ENO1-Ab and s-SSNA1-Ab titer levels were associated with improved overall survival, but the differences were not statistically significant. According to the Human Protein Atlas dataset, high mRNA expression levels of ENO1 and SSNA1 showed a trend towards shorter overall survival, while low expression levels showed a trend towards longer overall survival (ENO1: P=0.07, SSNA1: P<0.05). Combination analysis indicated that the s-ENO1-Ab-positive (+)/carcinoembryonic antigen (CEA)-negative (-) group demonstrated a significantly improved prognosis compared with that of the s-ENO1-Ab(-)/CEA(+) group (P<0.01), while a comparison of the s-SSNA1-Ab(+)/CEA(-) group with the s-SSNA1-Ab(-)/CEA(+) group also demonstrated a significant improvement in prognosis (P<0.01). Thus, s-ENO1-Abs and s-SSNA-Abs may be useful biomarkers for predicting gastric cancer prognosis, providing future research directions for novel approaches to target and treat gastric cancer.

Introduction

Gastric cancer is a major global health challenge due to its high mortality and morbidity rates, especially in East Asia, Eastern Europe and South America. Despite an overall decreasing trend in incidence and mortality in different countries over the past decades, gastric cancer is the fifth most common malignant tumor and the fourth leading cause of cancer-related deaths worldwide (1-3). To improve early diagnosis and prognosis, research into various biomarkers and immune-related therapies is ongoing (4-6).

Autoantibodies against tumor-associated antigens (TAAs) are antibodies produced in response to self-proteins that are abnormally expressed or altered by cancer cells. These are being researched for their potential as biomarkers in early cancer detection, diagnosis, prognosis prediction and monitoring of treatment efficacy (7,8). It has been reported that autoantibodies against tumor-associated antigens can be detected with increased sensitivity compared with the tumor-associated antigens themselves (9,10). However, only a few autoantibodies have been applied as biomarkers in clinical practice (11,12).

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Abbreviations: ENO1, enolase 1; SSNA1, Sjögren syndrome nuclear autoantigen 1; s-ENO1-Abs, serum anti-ENO1 antibodies; s-SSNA1-Abs, serum anti-SSNA1 antibodies

Key words: antibody biomarker, gastric cancer, overall survival, ENO1, SSNA1

A large-scale screening for esophageal cancer antigens through the serological identification of antigens was performed in our previous studies using the recombinant cDNA expression cloning (serological analysis of recombinant tumor cDNA expression libraries; SEREX) method, which demonstrated that autoantibody levels against these antigens (TROP2, SURF1, LOC146223 and HOOK2) were increased in the sera of patients with esophageal cancer compared with those in healthy donors (13,14). Cancer antigens not only for esophageal cancer are currently being investigated, but also for other types of cancer, such as lung cancer and colorectal cancer (15,16). The p53 antibody, as a tumor marker using an autoantibody that targets tumor antigens, has been applied for esophageal, colorectal and breast cancer, but not gastric cancer in Japan (17,18). Conversely, some of these autoantibodies, such as Anti-FIRΔexon2, sorting nexin 15, and spermatogenesis and oogenesis specific basic helix-loop-helix 1 antibodies, were increased in the sera of patients with gastric cancer (14,19-21). Therefore, the association of the esophageal cancer autoantibody markers with gastric cancer were investigated.

Materials and methods

Collection of serum samples

Patients and sera. Blood samples were prospectively recruited from patients with gastric cancer at the Toho University Omori Medical Center (Tokyo, Japan) to search for new tumor markers. The present study included 166 patients with gastric cancer who underwent radical surgery, endoscopic submucosal dissection or probe laparotomy at Toho University Omori Medical Center from May 2010 to May 2013. Patients with other types of cancer and those aged ≤18 years were excluded. The control group comprised 96 healthy donors from a health screening clinic, Port Square Kashiwado Clinic (Chiba, Japan). Healthy donor blood samples were obtained from consecutive patients who had undergone brain checkups between April 2013 and March 2014. According to the inclusion criteria for healthy donors, those with a medication history and lifestyle-related diseases were excluded. The patients with gastric cancer comprised 118 male and 48 female patients (mean age, 64.9 years; range, 33-90 years). The number of patients in each pathological tumor stage were: I, n=90; II, n=26; III, n=28; and IV, n=22. The serum samples were analyzed at Chiba University (Chiba, Japan). Patient hospital records were first accessed in September 2019.

Ethical approval. The study was conducted under the guidelines of the Declaration of Helsinki. The Ethics Committee of Faculty of Medicine, Toho University (approval nos. A18103_A17052_A16035_A16001_26095_25024_24038_22047 and 25131_23005; Tokyo, Japan), Toho University Omori Medical Center (approval no. 26-255), Chiba University Graduate School of Medicine (approval nos. 2018-320, 2020-1129, 2022-623 and 2023-836), and Port Square Kashiwado Clinic, Kashiwado Memorial Foundation (approval no. 2012-001) approved the collection of serum samples. All patients signed written informed consent. The Ethics Committee of Faculty of Medicine, Toho University (approval no. A22038_A21089_A19030) and Toho University Omori Medical Center (approval no. M22211) approved the retrospective analysis of patients' medical records. Participants were allowed to decline to be further enrolled in the present study (opt-out). Before treatment, 5 ml blood samples were collected, centrifuged at 3,000 x g for 10 min, and serum was collected and stored at -80˚C until use.

Measurement of s-ENO1-Abs, s-SSNA-Abs and conventional serum markers. Glutathione S-transferase (GST)-ENO1, GST-SSNA1 and control GST were expressed in *Escherichia coli* and purified using affinity-chromatography with glutathione-Sepharose (Cytiva) as previously described (20). The s-ENO1-Ab and s-SSNA1-Ab levels were measured using amplified luminescence proximity homogeneous assay-linked immunosorbent assay [AlphaLISA™; Revvity, Inc.; AlphaLISA Anti-Human HA Acceptor Beads, cat. no. AL170M; AlphaScreen GSH Donor Beads, cat. no. 6765301; AlphaLISA buffer, cat. no. AL000F; 384-well microtiter plates (white opaque OptiPlate™), cat. no. 6008280]. AlphaLISA was conducted using 384-well microtiter plates (Revvity, Inc.) containing 2.5 μl 1/100-diluted sera and 2.5 μl GST, GST-ENO1 and GST-SSNA1 proteins (10 μg/ml) in AlphaLISA buffer (25 mM HEPES, pH 7.4, 0.1% casein, 0.5% Triton X-100, 1 mg/ml dextran-500 and 0.05% Proclin-300) following the manufacturer's instructions (Revvity, Inc.). The reaction mixture was incubated at room temperature for 6-8 h, and 2.5 μl anti-human IgG-conjugated acceptor beads (40 μg/ml) and 2.5 μl glutathione-conjugated donor beads (40 μg/ml) were then added and incubated further for 7-21 days at room temperature in the dark. The chemical emission at 607-623 nm (Alpha photon count) which indicates the antigen-antibody binding level was read using an EnSpire™ Alpha microplate reader (Revvity, Inc.) as previously described (6,11,20,21-24). Specific reactions were estimated by subtracting the Alpha values of GST control from the GST-fusion protein values. Additionally, the carcinoembryonic antigen (CEA) levels were assessed as previously described (25). The cut-off value for CEA was set at 5.0 ng/ml following the manufacturer's instruction.

Comparison of the overall survival rates according to the expression levels of the mRNA. Comparison of overall survival rates according to mRNA expression levels were conducted, using reference data from The Human Protein Atlas (https://www.proteinatlas.org/), from gastric cancer tissue obtained during diagnosis.

Statistical analyses. Differences between the two variables were analyzed using Fisher's exact test. Corresponding differences between the two variables were identified using the Mann-Whitney U-test. Receiver operating characteristic curve (ROC) analysis was utilized to identify the predictive qualities of putative disease markers and cut-off values were determined to maximize the total sensitivity and specificity (Youden index). Optimal cut-off values for serum antibody levels that affect overall survival were identified using the X-tile software (version 3.6.1; Yale University) as previously described (26). The Kaplan-Meier method was utilized to analyze survival and survival curves were drawn. Additionally, the survival distributions of the two groups were compared

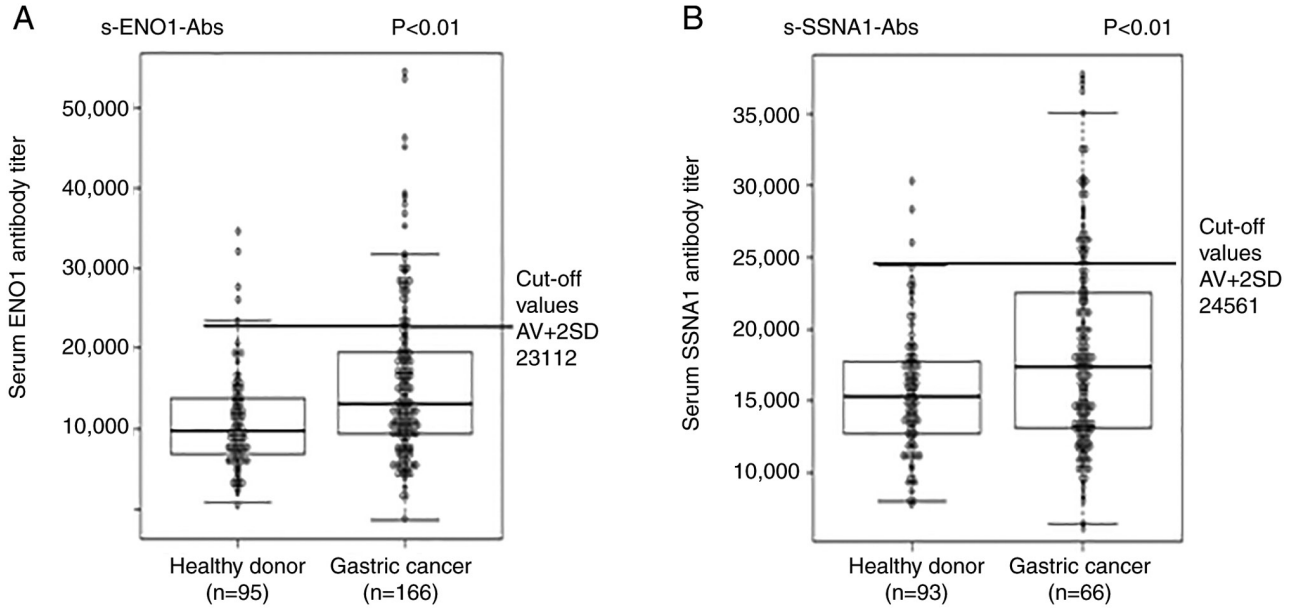

Figure 1. Comparison of s-ENO1-Ab and s-SSNA1-Ab levels between healthy donors and surgically treated 166 patients with gastric cancer. The levels of (A) s-ENO1-Abs and (B) s-SSNA1-Abs in patients with gastric cancer and healthy donors were examined using an AlphaLISA assay. Data shown in box-whisker plots. Box plots represent the 25, 50 and 75th percentiles. The upper and lower horizontal lines represent the 90th percentile. Data evaluated using the Mann Whitney U test. P<0.01. ENO1, enolase 1; SSNA1, Sjögren syndrome nuclear autoantigen 1; s-ENO1-Abs, serum anti-ENO1 antibodies; s-SSNA1-Abs, serum anti-SSNA1 antibodies; AV+2SD, mean + 2 standard deviations.

using the log-rank test. Clinicopathological variables related to overall survival were assessed by univariate analysis followed by multivariate analysis using the Cox proportional hazards model. Statistical analyses were conducted using EZR software (version 1.55; Jichi Medical University; https://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmed.html) (27). P<0.05 was considered to indicate a statistically significant difference.

Results

Comparison of s-ENO1-Abs and s-SSNA1-Abs levels between patients with gastric cancer and healthy donors. The s-ENO1-Abs and s-SSNA1-Abs titer levels were measured using the AlphaLISA assay with GST-ENO1 and GST-SSNA1 as antigens. s-ENO1-Ab and s-SSNA1-Ab titer levels were significantly increased in patients with gastric cancer compared with that in healthy donors (Fig. 1A and B; P<0.01). The ROC curve analysis demonstrated that the areas under the ROC curve of s-ENO1-Abs and s-SSNA1-Abs were 0.656 and 0.607, respectively, for gastric cancer (Fig. 2A and B). The sensitivity and specificity were 39.1 and 87.3% for s-ENO1-Abs, and 37.3 and 84.9% for s-SSNA1-Abs respectively, using cut-off values that were determined as per the Youden index to maximize the sum of sensitivity and specificity.

The positive rate of s-ENO1-Abs was 18.7% (31/166) and the false positive rate of healthy donors was 5.3% (5/95; one sample measurement failed); the positive rate of s-SSNA1-Abs was 19.9% (33/166) and the false positive rate of healthy donors was 3.2% (3/93; three sample measurements failed), when the cut-off values were set at the mean value + 2 x SD value of healthy donors (Fig. 1). Patients with positive s-ENO1-Abs and s-SSNA1-Abs demonstrated little overlap with patients in the CEA-positive (+) group and exhibited different positive patterns (Fig. 3).

The positive rates of s-ENO1-Abs, s-SSNA1-Abs and CEA among various tumor stages (I-IV) were compared. The positive rates of CEA elevated as the stage progressed, whereas those of s-ENO1-Abs and s-SSNA1-Abs were independent of the stage. The positive rates in stages I and II increased significantly in the combination group positive for all three markers compared with that in CEA alone (Fig. 4; P<0.01 and P<0.05, respectively).

Correlation of s-ENO1-Ab and s-SSNA1-Ab levels with clinicopathological parameters. The examination of s-ENO1-Abs, s-SSNA1-Abs and CEA in terms of clinicopathological factors indicated the s-ENO1-Ab and s-SSNA1-Ab levels were stratified based on the cut-off values identified using X-tile analysis (26). Fisher's exact test demonstrated a significant association between patients with CEA(+) and tumor depth, lymph node metastasis and disease stage (Table I). However, other clinicopathological variables and patients with positive s-ENO1-Ab or s-SSNA1-Ab levels exhibited no significant association.

Combined analysis of s-ENO1-Ab and s-SSNA1-Ab titer levels in relation to patient survival. The prognostic significance of s-ENO1-Ab and s-SSNA1-Ab titer levels was evaluated by generating survival curves with the Kaplan-Meier method (Fig. 5). The s-ENO1-Ab and s-SSNA1-Ab levels were categorized into positive and negative groups according to the cut-off level from the X-tile analysis (26). No statistically significant difference was observed in the patients' overall survival rates between the s-ENO1-Ab(+) and s-ENO1-Ab-negative (-) groups; however, the s-ENO1-Ab(+) group demonstrated a notable trend toward an improved prognosis (P=0.28; Fig. 5A).

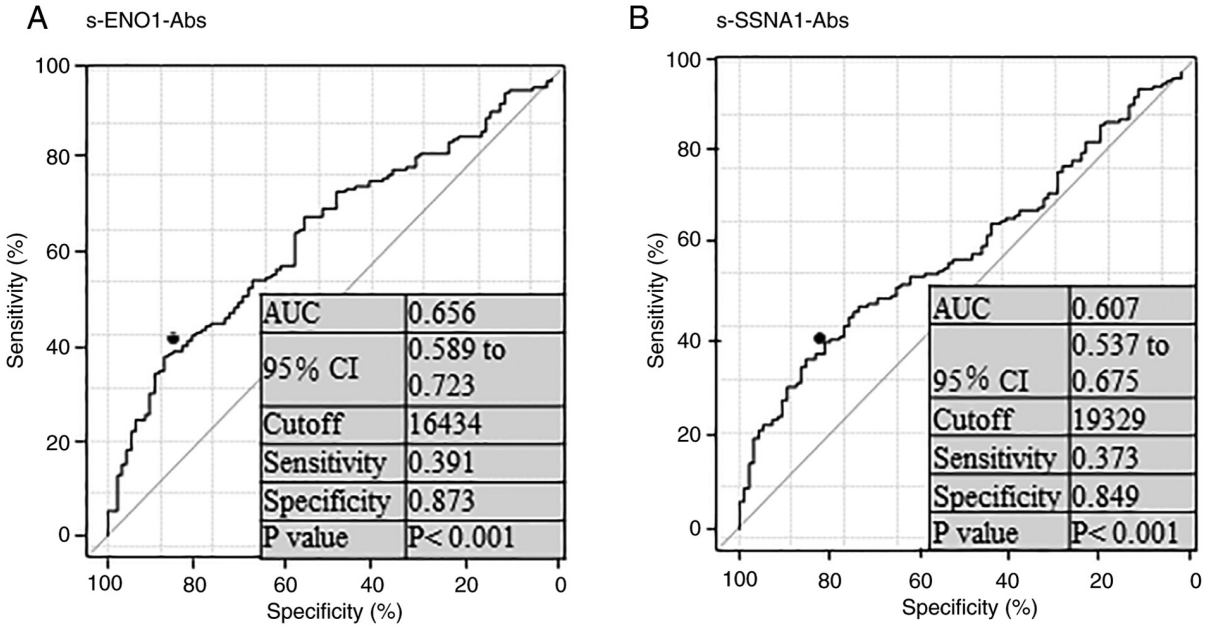

Figure 2. Receiver operating characteristic curve analysis for s-ENO1-Abs and s-ENO1-Abs. Data in (A) s-ENO1-Abs and (B) s-ENO1-Abs analysis represents the AUC, 95% CI, cut-off level, sensitivity, specificity and P-values. ENO1, enolase 1; SSNA1, Sjögren syndrome nuclear autoantigen 1; s-ENO1-Abs, serum anti-ENO1 antibodies; s-SSNA1-Abs, serum anti-SSNA1 antibodies; CI, confidence interval; AUC, area under curve.

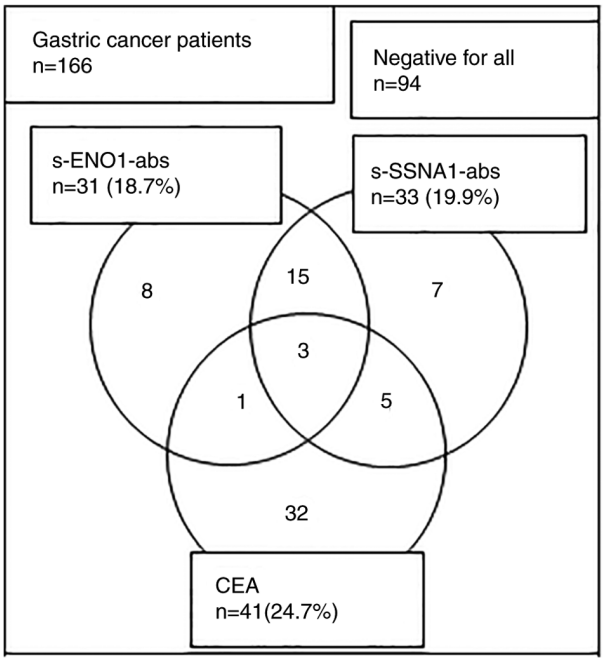

Figure 3. Relationship among positive sample numbers of s-ENO1-Abs, s-SSNA1-Abs and CEA in patients with gastric cancer. Total positive numbers of s-ENO1-Abs, s-SSNA1-Abs, and CEA were 31, 33 and 41, respectively. The numbers in the figure represent single, double- and triple-positive sample numbers. ENO1, enolase 1; SSNA1, Sjögren syndrome nuclear autoantigen 1; s-ENO1-Abs, serum anti-ENO1 antibodies; s-SSNA1-Abs, serum anti-SSNA1 antibodies; CEA, carcinoembryonic antigen.

Similarly, no statistically significant difference was observed in the patients' overall survival rates between the s-SSNA1-Ab(+) and s-SSNA1-Ab(-) groups; however, the s-SSNA1-Ab(+) group demonstrated a trend toward a more favorable prognosis (P=0.30; Fig. 5B). By contrast, a significant difference was found in the improvement of overall survival rate between the CEA(+) and CEA(-) groups (P<0.05; Fig. 5C).

The s-ENO1-Ab(+)/s-SSNA1-Ab(+) group demonstrated a notable trend towards an improved prognosis compared with that of the s-ENO1-Ab(-)/s-SSNA1-Ab(-) group when considering the combination of two types of markers as prognostic factors (P=0.13; Fig. 5D). Moreover, the s-ENO1-Ab(+)/CEA(-) group demonstrated a significantly improved prognosis compared with that of the s-ENO1-Ab(-)/CEA(+) group (P<0.01; Fig. 5E). Similarly, the s-SSNA1-Ab(+)/CEA(-) group demonstrated a significantly improved prognosis compared with that of the s-SSNA1-Ab(-)/CEA(+) group (P<0.01; Fig. 5F).

Univariate and multivariate analysis of prognostic impact of clinicopathological variables including autoantibody status. Univariate and multivariate analyses included the overall survival and patient characteristics, including sex, age, histological type, tumor size, tumor depth, lymph node metastasis, laparotomy lavage cytology, peritoneal dissemination, stage, s-ENO1-Abs, s-SSNA1-Abs and CEA levels. Both univariate and multivariate analyses identified tumor size, tumor depth, lymph node metastasis, laparotomy lavage cytology, peritoneal dissemination, stage and CEA levels as significant prognostic variables; however, s-ENO1-Abs or s-SSNA1-Abs were not significant prognostic indicators (Table II).

Comparison of the overall survival rates according to the expression levels of the mRNA. Cut-off values identified by yields maximal difference regarding survival between the two groups at the lowest log-rank P-value were utilized to divide the participants into high- and low-expression groups. The high ENO1 expression group demonstrated a marked increase in overall survival rates compared with that of the

Table I. Clinicopathological significance of positive status of three biomarkers, s-ENO1-Ab, s-SSNA-Ab and CEA.

Characteristics	No. of patients	s-ENO1-Ab >9,358[b], n	P-value[a]	s-SSNA-Ab >11,711[b], n	P-value[a]	CEA >5.0 ng/ml, n	P-value[a]
Sex (n=166)							
Female	48	34	0.56	42	0.64	11	0.84
Male	118	90		99		30	
Age, years (n=166)							
≤65	67	54	0.20	55	0.51	15	0.59
>65	99	70		86		26	
Histological type (n=163)							
Differentiated	88	64	0.59	73	0.66	24	0.27
Undifferentiated	75	58		65		14	
Tumor size, mm (n=163)							
≤40	95	71	0.85	84	0.16	21	0.84
>40	57	44		45		14	
Tumor depth (n=144)							
T1-2	78	59	>0.99	67	>0.99	11	0.04
T3-4	66	50		56		19	
Nodal status (n=124)							
Negative	75	51	0.31	62	0.61	7	<0.01
Positive	49	38		43		19	
Cytology (n=118)							
Negative	110	87	0.68	94	0.35	23	0.37
Positive	8	6		6		3	
Peritoneal dissemination (n=108)							
Negative	101	79	0.65	87	0.28	15	0.30
Positive	7	5		5		2	
Stage (n=166)							
I/II	116	87	>0.99	98	>0.99	17	<0.01
III/IV	50	37		43		24	

[a]Fisher's exact probability test. [b]Optimal cut-off values for serum antibody levels that affect overall survival were identified with X tile software (version 3.6.1; Yale University). ENO1, enolase 1; SSNA1, Sjögren syndrome nuclear autoantigen 1; s-ENO1-Abs, serum anti-ENO1 antibodies; s-SSNA1-Abs, serum anti-SSNA1 antibodies; CEA, carcinoembryonic antigen.

low ENO1 expression groups, but the difference was not significant (P=0.07; Fig. 6A). Conversely, the high SSNA1 expression group demonstrated a significantly improved prognosis compared with that of the low SSNA1 expression group (P<0.05; Fig. 6B). No discernible difference was observed in prognosis between high and low CEA expression (Fig. 6C).

The combined analysis demonstrated that the high ENO1/high SSNA1 expression group demonstrated significantly improved overall survival rates compared with that of the low ENO1/low SSNA1 expression group (P<0.05; Fig. 6D). Furthermore, the high ENO1/low CEA expression group exhibited significantly improved overall survival rates compared with that of the low ENO1/high CEA expression group (P<0.05; Fig. 6E). No significant difference was observed in the overall survival rates between the high SSNA1/low CEA expression and the low SSNA1/high CEA expression groups (P=0.06; Fig. 6F).

Discussion

Our previous studies identified ENO1 and SSNA1, based on SEREX screening, as antigens recognized by serum IgG antibodies in patients with esophageal cancer (14,20). The present study demonstrated that patients with gastric cancer exhibited significantly increased s-ENO1-Ab and s-SSNA1-Ab levels compared with that of the healthy donors.

Autoantibody development frequently accompanies the high antigen expression in tumor tissue (18,20,23,24). The comparison of overall survival rates according to mRNA expression levels mirrors the results obtained for autoantibodies. These are consistent with the suggestion that autoantibodies increase against antigen leakage due to high expression in cancer tissues and tissue destruction accompanying cancer progression (28).

The present study demonstrated that risk factors for overall survival in 166 cases were associated with tumor size, tumor

Table II. Univariate and multivariate analysis of the risk factors associated with the overall survival of 166 patients with surgically treated for gastric cancer.

	Univariate analysis		Multivariate analysis	
Variables	P-value[a]	Hazard ratio	95% CI	P-value[b]
Sex, male vs. female	0.47			0.47
Age, >65 vs. ≤65 years	0.15	1.721	0.829-3.573	0.15
Histological type, differentiated vs. undifferentiated	0.06	2.106	0.985-4.503	0.06
Tumor size, ≤40 vs. >40 mm	0.03	2.531	1.095-5.850	0.03
Tumor depth, T1-2 vs. T3-4	<0.01	30.360	4.087-225.500	<0.01
Nodal status, negative vs. positive	<0.01	3.633	1.536-8.594	<0.01
Cytology, negative vs. positive	<0.01	5.850	2.105-16.260	<0.01
Peritoneal dissemination, negative vs. positive	<0.01	18.370	5.859-57.580	<0.01
Stage, I and II vs. III and IV	<0.01	6.978	3.286-14.820	<0.01
s-ENO1-Abs, negative vs. positive	0.28			0.28
s-SSNA-Abs, negative vs. positive	0.30			0.30
CEA, negative vs. positive	0.01	2.438	1.189-4.997	0.01

[a]Log-rank test. [b]Cox proportional hazard model. P<0.05 was considered to indicate a statistically significant difference. ENO1, enolase 1; SSNA1, Sjögren syndrome nuclear autoantigen 1; s-ENO1-Abs, serum anti-ENO1 antibodies; s-SSNA1-Abs, serum anti-SSNA1 antibodies; CEA, carcinoembryonic antigen.

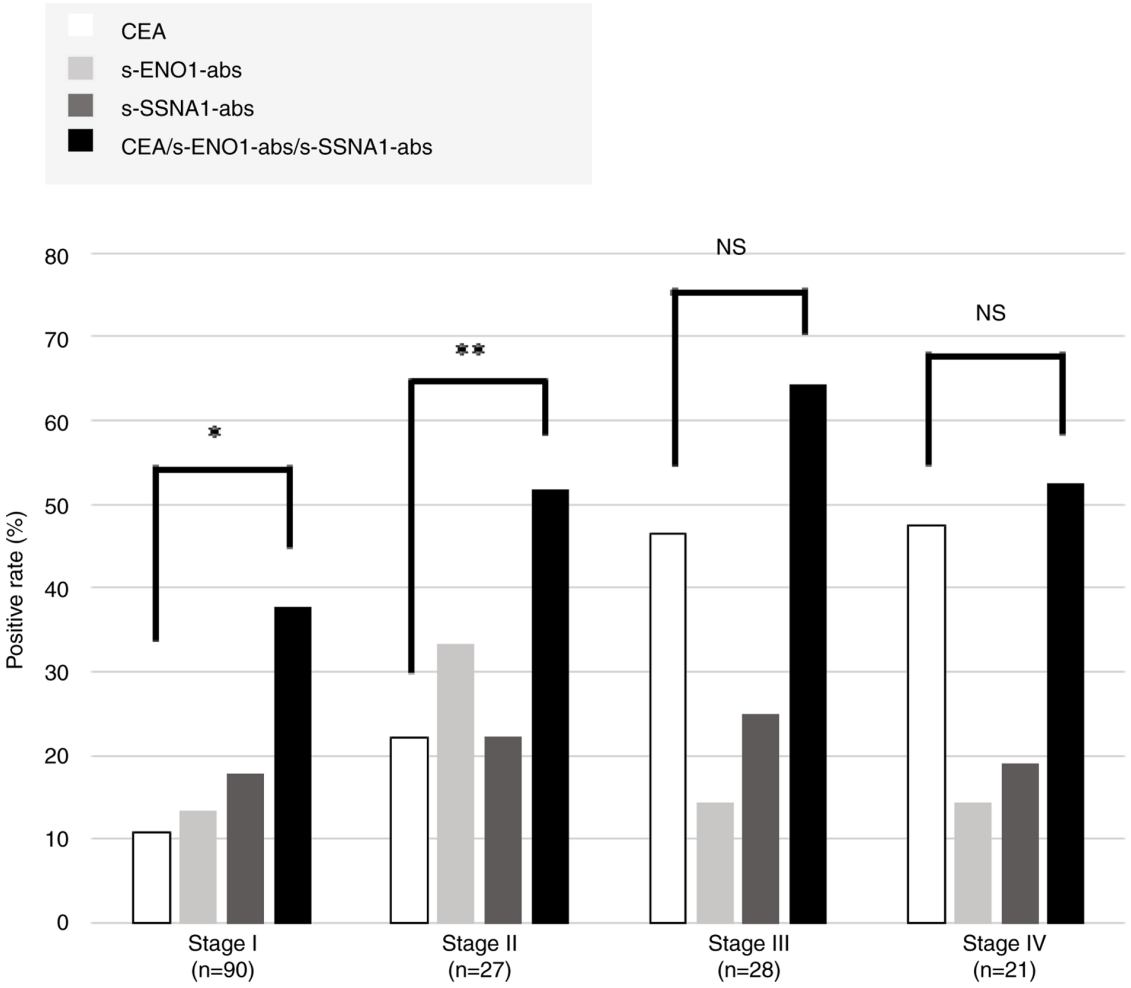

Figure 4. Comparison of positivity rates for CEA, s-ENO1-Abs, s-SSNA1-Abs and combination of all three markers in patients with gastric cancer according to the tumor stage. The P-values were calculated using Fisher's exact probability test. *P<0.01, **P<0.05. NS; not significant; ENO1, enolase 1; SSNA1, Sjögren syndrome nuclear autoantigen 1; s-ENO1-Abs, serum anti-ENO1 antibodies; s-SSNA1-Abs, serum anti-SSNA1 antibodies; CEA, carcinoembryonic antigen.

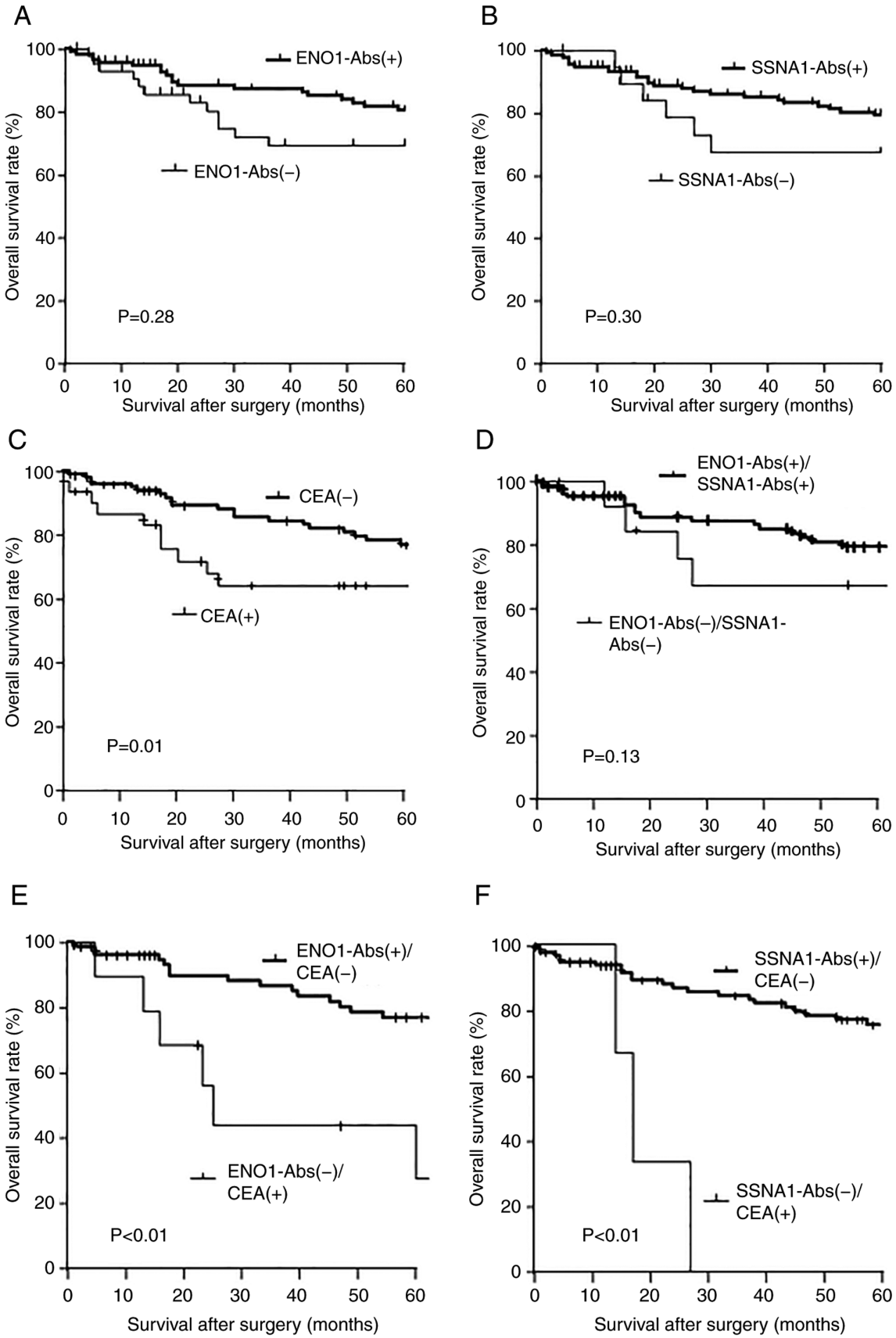

Figure 5. Comparison of the overall survival between the positive and negative groups of s-ENO1-Abs, s-SSNA1-Abs and CEA alone or their combination. The overall survival of surgically treated gastric cancer shown in Kaplan-Meyer plots of (A) s-ENO1-Abs and (B) s-SSNA1-Abs. The levels of s-ENO1-Abs and s-SSNA1-Abs were used to dived patients into positive and negative groups. The s-ENO1-Abs and s-SSNA1-Abs levels were categorized into positive and negative groups according to the cut-off level from the X-tile analysis (s-ENO1-Abs cut-off, 9,358; s-SSNA1-Abs cut-off, 11,711). (C) Comparison of groups with CEA >5.0 ng/ml.) Comparison of prognosis between the group (D) s-ENO1-Abs+/s-SSNA1-Abs+ and s-ENO1-Abs-/s-SSNA1-Abs-, (E) s-ENO1-Abs+/CEA- and s-ENO1-Abs-/CEA+ and (F) s-SSNA1-Abs+/CEA- and s-SSNA1-Abs-/CEA+. ENO1, enolase 1; SSNA1, Sjögren syndrome nuclear autoantigen 1; s-ENO1-Abs, serum anti-ENO1 antibodies; s-SSNA1-Abs, serum anti-SSNA1 antibodies; CEA, carcinoembryonic antigen.

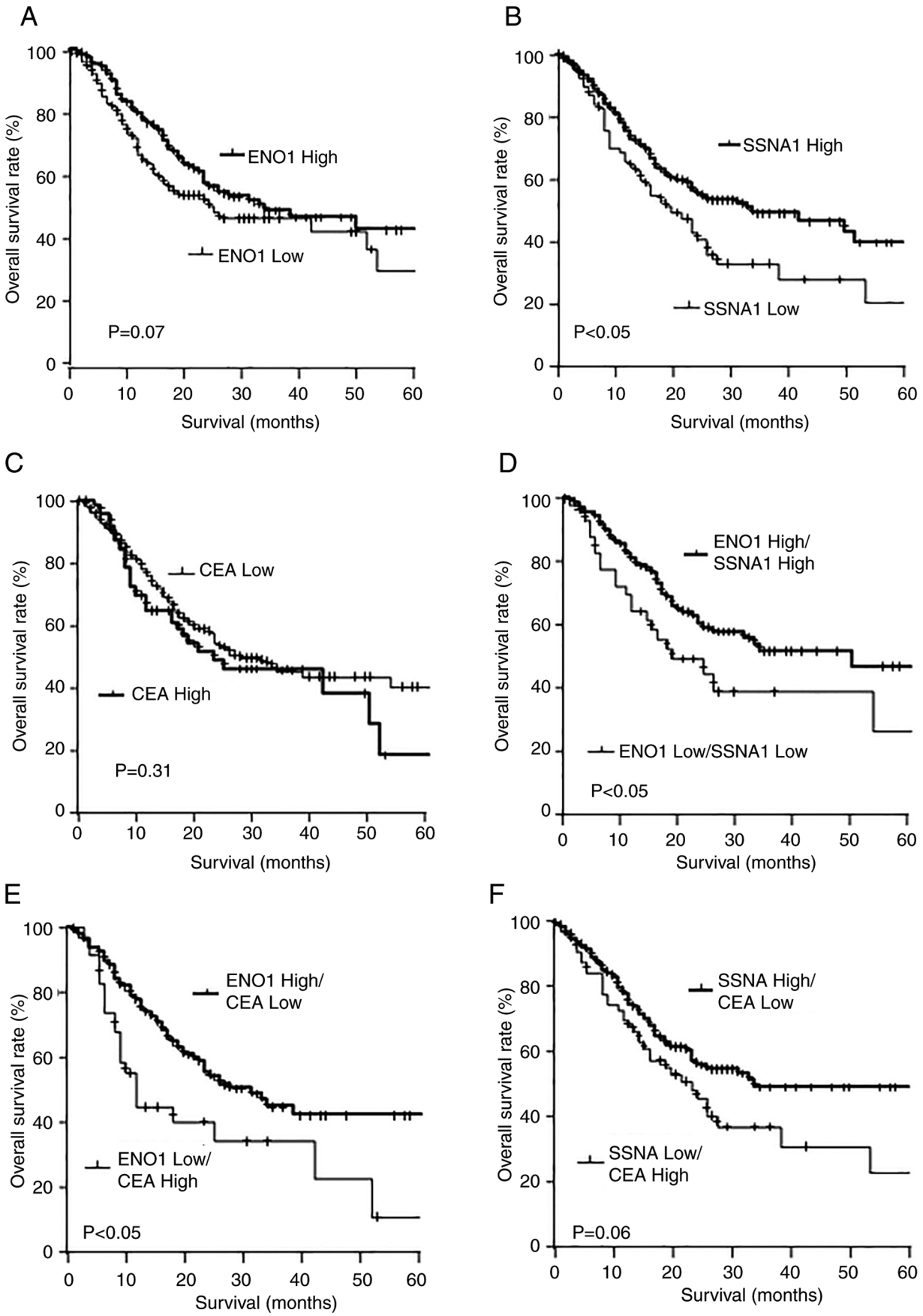

Figure 6. Comparison of the overall survival rates according to the mRNA expression levels. The levels of mRNA of ENO1, SSNA1 and CEA were obtained from the public database, the Human Protein Atlas. Overall survival of gastric cancer was depicted with Kaplan-Meier plots of (A) ENO1, (B) SSNA1 and (C) CEA, (D) ENO1 high/SSNA1 high and ENO1 low/SSNA1 low, (E) ENO1 high/CEA low and ENO1 low/CEA high, (F) SSNA1 high/CEA low and SSNA1 low/CEA high. ENO1, enolase 1; SSNA1, Sjögren syndrome nuclear autoantigen 1; s-ENO1-Abs, serum anti-ENO1 antibodies; s-SSNA1-Abs, serum anti-SSNA1 antibodies; CEA, carcinoembryonic antigen.

depth, lymph node status, cytology and peritoneal dissemination but not with s-ENO1-Abs or s-SSNA1-Abs (Table II). CEA levels were associated with tumor depth, lymph node status and stages (Table I) as previously reported (29,30), whereas s-ENO1-Abs and s-SSNA1-Abs were not. Patients that were CEA(+) demonstrated a poorer prognosis compared with those that were CEA(-). The positivity and negativity of s-ENO1-Abs or s-SSNA1-Abs demonstrated no significant difference in the overall survival rates, but the combination of CEA(+) and s-ENO1-Ab(+) or s-SSNA1-Ab(+) exhibited more significant associations compared with CEA alone. Thus, s-ENO1-Abs and s-SSNA1-Abs may be involved in the overall survival by presenting some prognosis-associated aspects other than tumor status, lymph node status or metastasis.

ENO1 protein is known to localize not only in the mitochondria membrane but also in the cell surface to mediate intracellular signaling such as PI3K/AKT, AMPK/mTOR and Wnt/β-catenin pathways (31,32). Serum anti-ENO1 autoantibodies block the signaling via direct binding to cell surface ENO1 if these signaling molecules are involved in gastric cancer proliferation/progression (33,34). Hence, the autoantibodies actively affect the cancer development. Treatment using anti-ENO1 monoclonal antibodies has been proposed for lung cancer (32). These are consistent with the present findings that s-ENO1-Ab(+) patients demonstrated more favorable prognoses compared with s-ENO1-Ab(-) patients in combination with CEA positivity. CEA levels, a major tumor marker for gastric cancer, have been reported to correlate with TNM stage (35).

By contrast, autoantibodies measure IgG antibodies against mutant protein antigens, and may not correlate with tumor mass. The antibody markers may decrease in the late stages of cancer (36). Although s-p53-Abs, which is used clinically to measure this IgG antibody, has been reported to not correlate with survival rates in gastric cancer (18), although a large-scale multi-center cohort study is necessary to verify this as the sample size was small. ENO1 protein binds to HGFR and activates its signal transduction. By contrast, HGFR serves an important role in the prognosis of gastric cancer (37). ENO1 is highly expressed as a glycolytic enzyme, and as a result, ENO1 antibodies increase through the destruction of cancer tissue. As the stage progresses, HGFR signals are further required, but ENO1 antibodies bind to ENO1 protein on the cell surface and block the signals. Therefore, this may explain the slightly improved prognosis in the s-ENO1-Ab(+) group demonstrated in the present study. ENO1 antibodies do not increase further in advanced cancer. ENO1 is upregulated in gastric cancer cells to obtain energy, which results in the production of antibodies due to tissue destruction (38,39). The autoantibodies produced inhibit the HGFR signaling of gastric cancer cells, which may improve prognosis. This feedback phenomenon is important; since the HGFR signaling promotes the proliferation of cancer cells themselves rather than invasion and metastasis (40,41), therefore, suppressing this signaling could ultimately lead to improved prognosis.

SSNA1, which is localized in the centrosome and presumed to be involved in cell division by regulating microtubule polymerization, currently has limited studies on its association with gastrointestinal cancer (42,43). SSNA1 is reported to promote metastasis in hepatocellular carcinoma (44). Additionally, SSNA1 mRNA expression levels demonstrate a more favorable positive trend toward overall survival in liver cancer (44). The functional relationship between s-SSNA1-Abs and SSNA1 protein was not confirmed in the present study, although s-SSNA1-Ab positivity exhibited a similar tendency for an improved prognosis as s-ENO1-Ab positivity.

The present study had several limitations. First, the association between protein expression levels in the resected specimens and the two serum antibody levels in the same patients was not assessed. Second, to the best of our knowledge, this was the first study to investigate the association between the two serum antibodies levels and gastric cancer; thus, a cut-off value for the two serum antibodies was identified using a test cohort of all patients. Due to the small sample size and single-center nature of the study, a large multi-institutional cohort is required for assessment.

Serum antibodies are less expensive than tissue mRNA assays (45,46), making them a useful marker. Additionally, s-ENO1-Abs and s-SSNA1-Abs may be utilized as diagnostic and prognostic biomarkers for gastric cancer and may indicate future research directions for innovative approaches to target and treat gastric cancer.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

SY, MI, TH and HS conceived and designed the present study. TS, YO, MS and FS analyzed the data. TH developed the AlphaLISA™ system. HT, SY, YO and FS acquired serum samples. SYL, BSZ, YY and TM analyzed patient data and drafted the manuscript. HS and SY confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics

Committee of Toho University, Graduate School of Medicine (approval nos. A18103_A17052_A16035_A16001_26095_25024_24038_22047_22047; Tokyo, Japan) and the retrospective analysis of patients' collected blood samples and medical records was approved by the Ethics Committee of Faculty of Medicine, Toho University (approval no. A22038_A21089_A19030) and Toho University Omori Medical Center (approval nos. M22211 and M23174 21320 21039 20200 20196 19056 18002; Tokyo, Japan). Ethics Committee of Chiba University Graduate School of Medicine (approval nos. 2018-320, 2020-1129, 2022-623, 2023-836; Chiba, Japan) and Port Square Kashiwado Clinic, Kashiwado Memorial Foundation (approval no. 2012-001) were also approved. Sera was collected from patients who had provided written informed consent.

Patient consent for publication

Not applicable.

Competing interest

The authors declare that they have no competing interests.

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