CA19-9, and CA72-4 may be elevated in patients with gastric cancer at various stages [1]. AFP [2], CA125 [3], and STN [4] can be used to detect liver metastases and/or peritoneal metastases. However, low rates of sensitivity and specificity prevent the use of any of these serum markers in early diagnosis. The National Comprehensive Cancer Network guidelines (http://www.nccn.org) do not recommend serum marker testing for preoperative evaluation and staging of gastric cancer.

In this context, the Task Force of the Japanese Gastric Cancer Association for Research Promotion (directed by Dr. Motoki Ninomiya) planned to reevaluate the clinical impact of serum tumor markers in a systematic review of previous publications, focusing mainly on CEA, CA19-9, and CA72-4. The clinical significance of the three other serum markers, AFP, CA125, and STN, was also addressed. Prospective clinical studies can be planned based on the results of this systematic review to elucidate the clinical utility of serum tumor markers.

Manuscript selection

A computer-aided search of the PubMed website (http://www.ncbi.nlm.nih.gov/sites/entrez) was conducted to retrieve relevant articles on serum tumor markers used for gastric cancer. The keywords "gastric cancer" and "tumor marker" and the serum markers CEA, CA19-9, CA72-4, AFP, CA125, STN, TPA, and IAP were used to search for relevant articles published before the end of November 2012 (Table 1). Studies investigating the clinicopathological impact of preoperative serum tumor markers used for assessing patients with gastric cancer were selected. Furthermore, case reports, review article, non-English articles, articles that included less than 30 patients, and articles that addressed cancers other than gastric cancer were excluded. Four researchers (H.S., T.N., M.O., and Y.T.) reviewed all the articles, and after applying the inclusion and exclusion criteria arrived at a consensus about articles to be selected at a working meeting. A total of 657 articles were selected from the PubMed database using the keyword "CEA." A total of 46 articles were selected as references for the present review article to evaluate the positive rates for CEA (n = 8,104), CA19-9 (n = 5,300), and CA72-4 (n = 2,774) [4–49]. In this review, the number of positive patients reported as positive, based on the definition in each original paper, was used to calculate the combined positive rates of patients with early/advanced gastric cancer. The positive rates for each marker at each stage were calculated. Among these 46 articles, 19 articles [1, 9, 12, 15, 19, 20, 22, 26, 27, 31, 32, 34–37, 39, 42, 43, 50] analyzed all three markers, CEA, CA19-9, and CA72-4, which included 2,774 patients (Table 2). Four discussion points were evaluated for CEA, CA19-9, and CA72-4 from selected articles as follows: (1) positive rates, (2) clinicopathological significance, (3) prognostic impact, and (4) clinical impact during follow-up after surgery and/or during chemotherapy. Finally, we selected 10 other publications that focused only on AFP [51–54], CA125 [3, 55–57], and STN [58, 59] in patients with gastric cancer to analyze the clinical significance of these three serum markers.

Table 1 Key words with "gastric cancer and tumor marker" and number of publications from PUBMED search

Key words	Number of publications
CEA	657
CA19-9	281
CEA + CA19-9	187
AFP	179
CA125	44
CEA + AFP	42
CEA + CA125	28
CA72-4	26
STN	26
CEA + CA72-4	25
CEA + CA19-9 + CA72-4	24
IAP	21
CA50	7

Positive rates for each serum marker according to the TNM stages

In the initial 46 articles, the overall positive rates for each marker were as follows: 24.0 % (1,945/8,104) for CEA, 27.0 % (1,431/5,300) for CA19-9, and 29.9 % (829/2,774) for CA72-4. The positive rates for CEA during each stage were as follows: stage I = 13.7 %, stage II = 23.0 %, stage III = 25.6 %, and stage IV = 39.5 %. The positive rates for CA19-9 during each stage were as follows: stage I = 9.0 %, stage II = 19.9 %, stage III = 32.2 %, and stage IV = 44.7 %. The positive rates for CA72-4 during each stage were as follows: stage I = 12.0 %, stage II = 15.6 %, stage III = 36.7 %, and stage IV = 49.6 % (Fig. 1).

The 19 manuscripts that evaluated all three markers in the same group of patients (n = 2,774) showed a similar trend as shown in Fig. 1 (Table 2). The positive rate for CA72-4 was the highest among the three markers (Fig. 2). Among these 19 articles, 12 articles indicated that the positive rate for CA72-4 was the highest among the three serum markers (Table 2). The combination with the highest positive rate was CA19-9 and CA72-4 [15, 50]. Because the average frequency of these three markers was approximately 10 % at stage I (CEA = 13.7 %, CA19-

Table 2 A total of 19 publications analyzed all three serum markers CEA, CA19-9, and CA72-4 in patients with gastric cancer

Reference	Author	Journal	Year	Number of patients	CEA (%)	CA19-9 (%)	CA72-4 (%)
9	Guadagni F	Cancer Res	1992	94	20	32	43
12	Guadagni F	Anticancer Res	1993	161	42	34	22
15	Filella X	Acta Oncol	1994	79	33	46	47
19	Fernandez-Fernandez L	Int Surg	1996	167	21	26	60
20	Spila A	Anticancer Res	1996	242	22	33	41
22	Pectasides D	Am J Clin Oncol	1997	62	49	65	70
26	Tocchi A	J Cancer Res Clin Oncol	1998	59	58	39	19
28	Marrelli D	Oncology	1999	254	21	35	28
31	Marrelli D	J Surg Oncol	2001	167	16	34	20
32	Marrelli D	Am J Surg	2001	133	16	35	20
34	Gaspar MJ	Tumour Biol	2001	82	16	33	34
35	Mattar R	Rev Hosp Clin Fac Med Sao Paulo	2002	44	25	25	48
36	Lai IR	Hepatogastroenterology	2002	195	32	16	16
37	Aloe S	Int J Biol Markers	2003	166	23	25	37
39	Louhimo J	Int J Cancer	2004	146	18	31	34
42	Goral V	Hepatogastroenterology	2007	47	30.5	30	46.8
43	Ucar E	Adv Ther	2008	95	24.2	41	32.6
1	Kim DH	J Surg Oncol.	2011	312	1	1	5
1	Kim DH	J Surg Oncol.	2011	167	5	13	15
50	Emoto S	Gastric Cancer	2012	102	19	37	44.9

Reference [1] presented data of "early" and "advanced" tumors separately

Positive rates are shown as %

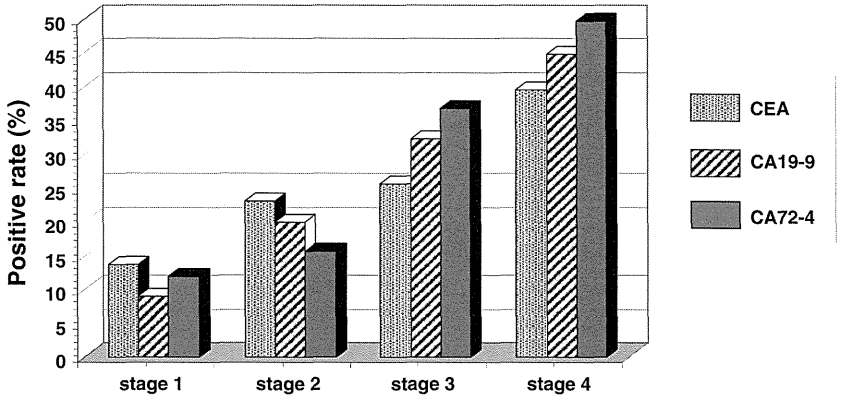

Fig. 1 Positive rate of serum tumor markers in gastric cancer according to stage

9 = 9.0 %, and CA72-4 = 12.0 %), they may not be useful for early cancer screening. Although the positive rates for CEA, CA19-9, and CA72-4 were similar in detecting major tumors, CA72-4 had the highest positive rate in patients with nodal involvement or serosal invasion. Therefore, CA72-4 was the most useful marker for detecting advanced gastric cancer [34, 39, 42, 43].

A recent meta-analysis of Chinese studies also showed that CA72-4 was the best of these three serum markers [60]. The accumulated accuracy rate of CA72-4 was 77 %, which was better than others. CA72-4 was the most highly correlated serum tumor biomarker for gastric cancer in the Chinese population. A combination of CA72-4 + CEA + CA19-9 considerably improved the positive rate without impairing the specificity.

Association of elevated serum markers with clinicopathological factors

CEA

The overall positive rates for CEA were 16–68 %. CEA was strongly associated with the T factor [29, 30, 33, 37],

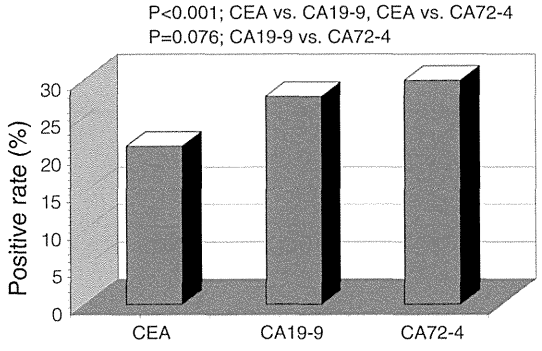

Fig. 2 Positive rate of each serum tumor marker in gastric cancer: In 19 articles, a total of 2,774 patients showed positive rates of all three serum markers

N factor [18, 24, 27, 29, 30], M factor [5, 6, 11, 24, 27, 33, 43], and stage [18, 27, 29, 39, 40, 45, 48]. Ikeda et al. [11] analyzed 68 patients with stage IV gastric cancer using multivariate analysis and concluded that an elevated CEA level was an independent risk factor for predicting liver metastases. A few reports have shown a significant association between elevated CEA and peritoneal metastases [18, 34]. Although several reports showed that elevated CEA was significantly associated with differentiated tumor types [7, 10, 48, 61], a few reports indicated an association with poorly differentiated types of tumors [29]. Maehara et al. analyzed the CEA levels in 221 patients with well-differentiated gastric cancer. The CEA-positive patients had larger tumors, greater serosal invasion, more frequent lymphatic and vascular involvement, less expansive tumor growth, and higher rates of lymph node and hepatic metastases than CEA-negative patients [17].

CA19-9

The overall positive rates for CA19-9 were reported as 14–68 %. Elevated CA19-9 was associated with tumor depth [30, 37], nodal involvement [18, 34, 40, 43, 47, 48], peritoneal metastases [18, 34, 43], and stage [18, 30, 34, 43, 47]. Of these various clinicopathological factors, CA19-9 was frequently reported to be associated with nodal involvement. The positive predictive value for nodal involvement was reported to be 78–96 % [18, 43, 47]. The positive predictive value for peritoneal metastases was reported to be 27 % [18] or 24 % [17]. Kodera et al. [18] reported that elevated CA19-9 levels were strongly associated with liver metastases.

CA72-4

The overall positive rates for CA72-4 were reported to be 16–70 %, which were generally higher than CEA and CA19-9 [1, 12, 37, 39, 42, 50]. Elevated CA72-4 was associated with tumor depth [28, 37, 43], nodal involvement [10, 12, 20, 24, 28, 34, 37, 42, 43], peritoneal metastases [10, 24, 34, 43], distant metastases [34, 43], and stage [9, 20, 28, 34, 35, 39, 42, 43]. Ucar et al. [43] reported that 9 of 11 patients (82 %) with liver metastases were positive for CA72-4. Because the positive rate for CA72-4 in patients with poorly differentiated adenocarcinoma was significantly higher than that for CEA (36 vs. 8 %), the overall positive rate for CA72-4 was higher than that for CEA [25]. In patients with Bormann type 2, 3, and 4, the positive rates for CA72-4 were higher than that for CEA. In particular, the positive rate for CA72-4 was significantly higher than that for CEA in patients with Bormann type 4 (67 vs. 11 %) [10]. The positive rate for CA72-4 was higher than that for CEA in stage III or IV patients. In particular, the positive rate for CA72-4 was significantly higher than that for CEA in patients with peritoneal metastases (69 vs. 23 %) [10].

Association of elevated serum markers with recurrence and patient survival

Because elevated serum markers were generally associated with tumor progression, most previous reports concluded that preoperative elevated serum markers were significantly associated with poor long-term patient survival. The prognostic value of preoperative CEA was confirmed by univariate analysis [18, 27, 28, 30, 34, 43, 44] and multivariate analysis using TNM factors [13, 14, 23, 25, 26, 62]. The prognostic value of preoperative CA19-9 was also confirmed by univariate analysis [25, 27, 28, 30, 31, 34, 39, 40, 43] and multivariate analysis using TNM factors [18, 26, 29, 44, 63]. The prognostic value of preoperative CA72-4 was also confirmed by univariate analysis [23, 24] and multivariate analysis using TNM factors [33, 39, 43, 63]. Although none of the three markers was associated with peritoneal recurrences, preoperative positivity for CEA, CA19-9, or CA72-4 was an independent risk factor for hematogenous recurrences of gastric carcinoma, and this point should be considered when selecting adjuvant chemotherapy after surgery for gastric cancer [1, 24, 32, 37]. Among these three markers, preoperative elevated CA72-4 was an independent risk factor for reduced patient survival in a multivariate analysis when co-analyzed with CEA and CA19-9 [63].

Takahashi et al. [38] reported that the CEA levels and/or CA19-9 levels increased for the first time at recurrence (54.7 and 40.0 %, respectively). Sensitivities for CEA and CA19-9, and combinations of the two markers, for indicating recurrence were 65.8, 55.0, and 85.0 % [38]. More than 90 % of patients with elevated preoperative levels of CEA had increased CEA levels again at the time of

recurrence. Similarly, the CA19-9 level increased again at recurrence in more than 90 % of patients with high preoperative levels of this marker [38]. Kim et al. confirmed these findings based on follow-up data from 1,117 patients. They concluded that the postoperative elevation of CEA and/or CA72-4 were both independent risk factors for recurrence [1]. Liu et al. [64] also reported that CA72-4 was the highest in sensitivity (35 %) and that the combined triple markers had 62 % sensitivity in the diagnosis of recurrence. They also reported false-positive rates of CEA, CA19-9, CA72-4, and the triple markers were 5.6, 7.0, 9.9, and 18.3 %, respectively [64]. Choi et al. [65] reported that the majority (90 %) of cases with recurrence to the liver had an elevated CEA, whereas an elevated CA 19-9 postoperatively was more predictive of a peritoneal recurrence (78.9 %). CA19-9 may be particularly useful as a marker of peritoneal recurrence, whereas CEA could be a useful marker for recurrence in the liver [65].

Therefore, the patients should have a set of markers evaluated once preoperatively. Measurement during the postoperative follow-up would then be particularly important for those who had elevated preoperative values, although one cannot deny the relevance of measuring tumor markers among patients who did not have an elevated preoperative value.

Doubling time and lead time of elevated serum markers

The reported doubling time estimate, based on the serum level of CEA, agreed with the actual tumor doubling time in 112 previously untreated patients with recurrent gastric cancers [66]. The CEA doubling time ranged from 12 to 105 days, with a mean of 37.5 days. The CEA doubling time was significantly shorter in patients with papillary adenocarcinoma compared with those with well- or moderately differentiated tubular adenocarcinoma. The doubling time was also significantly shorter in patients with liver metastasis than those with lymph node metastasis or peritoneal dissemination. There was also a significant correlation between the CEA doubling time and postoperative survival time of patients who received no chemotherapy.

The serum markers were frequently elevated several months before imaging abnormalities. Thus, the lead time before imaging abnormalities was reduced gradually because of improvements in imaging technology. In 1982, Tamada et al. [5] reported that the lead time for CEA was 8.3 months. Because of improvements in imaging modalities, recent studies (after 2000) reported a shorter mean lead time than that reported earlier (3–5 months for CEA and 2–5 months for CA19-9) [31, 38]. The preoperative seropositive group was more likely to have a longer lead time than the preoperative seronegative group. The lead times for recurrences in the liver, peritoneum, and lymph nodes were 1.2, 3.4, and 3.7 months, respectively, for CEA and 2.1, 1.0, and 3.6 months, respectively, for CA19-9. The lead time for CEA for liver recurrence was significantly shorter than those for peritoneum and lymph node metastases [38]. It was concluded that because the lead time depends on the imaging modalities and follow-up interval, a large-scale prospective study is required to clarify the best strategy for follow-up to improve overall patient survival.

Clinical significance of serum marker monitoring during chemotherapy

Yamao et al. [66] monitored changing patterns in CEA, CA19-9, and CA125 levels during systemic chemotherapy to determine the relationship between changes in the serum tumor marker levels and the response assessment in imaging studies throughout the treatment course. The sensitivity and negative predictive value of falling tumor marker levels after chemotherapy for a partial response in imaging was 100 %. On categorizing the patients as responders or nonresponders, a significant correlation was observed between the assessment of response by tumor markers and by imaging studies. The survival time of responders assessed by tumor markers was significantly longer than that of nonresponders [66]. Catalano et al. analyzed the CEA levels in 175 patients with advanced gastric cancer who received second-line chemotherapy. Univariate and multivariate analyses showed that elevated CEA levels >50 ng/ml were significantly associated with poor overall survival. This analysis suggests that readily available clinical factors may help to select patients with advanced gastric cancer who may benefit from second-line chemotherapy [67].

Regarding elevated serum marker levels immediately after chemotherapy, Kim et al. [68] reported a transient increase in the CEA or CA19-9 levels despite the clinical benefits of chemotherapy in patients with metastatic or recurrent gastric cancer. CEA and CA 19-9 surges were defined as >20 % increases in these tumor marker levels from the baseline, followed by >20 % drop in subsequent levels compared with the baseline. Of 51 patients who were evaluated for CEA surges, nine (18 %) patients had CEA surges. The median time to the CEA peak and the duration of the CEA surge were 2.8 and 9.1 weeks, respectively. Of 40 patients who were evaluated for CA19-9 surges, 7 (18 %) had CA19-9 surges. The median time to the peak and the duration of the CA19-9 surge were 2.3 and 7.1 weeks, respectively. All patients with these surge phenomena received clinical benefits from chemotherapy. Although increases in serum tumor markers after chemotherapy were general indicators of tumor progression, an initial rise in the CEA or CA19-9 levels after the initiation

Table 3 Clinical significance of serum tumor markers in gastric cancer

	T	N	M	P	Histology	Prognosis	Recurrence pattern
CEA	Yes	Yes	Yes	No	Yes	Yes	Distant
CA19-9	Yes	Yes	Yes	Yes	No	Yes	Distant
CA72-4	Yes	Yes	Yes	Yes	No	Yes	Distant and/or peritoneal
AFP	NA	NA	Yes	NA	Yes	Yes	Liver
CA125	NA	NA	NA	Yes	No	Yes	Peritoneal
STN	NA	NA	Yes	Yes	No	Yes	Peritoneal

NA not enough evidence to evaluate clinical significance was available

of chemotherapy should not be an indicator of progressive disease in some cases [68].

Other useful serum markers for gastric cancer: alpha-fetoprotein (AFP), CA125, and sialyl Tn antigens (STN)

AFP-producing gastric cancers behave aggressively and have a high potential for metastasis to the liver [2, 52–54]. There was poorer differentiation, a higher incidence of lymph node metastasis, and more marked lymphatic and vascular invasion in the AFP-positive group than in the AFP-negative group [54].

The diagnostic ability of the serum CA125 was more reliable than other imaging modalities including computed tomography, ultrasonography, and the other serum tumor markers for peritoneal metastasis from gastric carcinoma [3]. The predictive values of the serum CA125 levels at a cutoff value of 35 U/ml resulted in a sensitivity of 39.4 %, a specificity of 95.7 %, and a diagnostic accuracy of 90.8 %. Hwang et al. [57] analyzed the utility of diagnostic imaging and CA125 levels in the sera of 768 patients with gastric cancer. The serum CA125 levels had high sensitivity (38.6 %), specificity (98.4 %), and diagnostic accuracy (91.5 %). Emoto et al. [50] also showed the sensitivities of CA125 for peritoneal metastasis at the initial diagnosis was 46 %. The CA125 level was significantly correlated with the degree of peritoneal dissemination and patient survival [50, 55, 57].

Takahashi et al. [4, 58] evaluated the clinical significance of the serum STN level as a tumor marker in 350 patients with gastric cancer. Histologically, the tumors in the high STN group were deeply penetrating and the rates of lymphatic involvement, vascular involvement, and lymph node and hepatic metastases were higher. The 5-year survival rate for patients in the high STN group was significantly less than that of patients in the low STN group (44.8 vs. 75.1 %, $P < 0.05$). Nakagoe et al. [59] confirmed similar conclusions that high serum STN was an independent factor that predicted liver metastasis and a worse outcome in gastric cancer patients.

Because these three markers could classify advanced gastric cancer into a specific category, measurement during the postoperative follow-up would be particularly important for those who had elevated preoperative values.

In conclusion, this systematic review evaluated 657 publications related to serum tumor markers in patients with gastric cancer (Table 3). Although no prospective trial has yet been completed to evaluate the clinical significance of these serum markers, this literature survey suggests that combinations of CEA, CA19-9, and CA72-4 are the most effective ways for staging before surgery and chemotherapy. Monitoring those positive markers after treatment should be important. AFP is useful for detecting and predicting liver metastases. CA125 and STN are useful for detecting peritoneal metastases. Any of these serum markers may be a risk factor for poor patient survival. Final conclusions about the clinical utility of these serum markers for patients with gastric cancer during treatment should be clarified in a phase III prospective randomized trial for certain anticancer agents or radical surgery.

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ORIGINAL ARTICLE

Clathrin heavy chain is a useful immunohistochemical marker for esophageal squamous intraepithelial neoplasia

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Abstract
Background Recent advances in the endoscopic diagnosis and treatment of esophageal cancer have facilitated the detection and treatment of minute tumors, necessitating the accurate histopathological diagnosis of early esophageal cancer or precancerous lesions. This study evaluated the usefulness of immunohistochemical analysis (IHC) of clathrin heavy chain (CHC) as a marker for early esophageal cancer.
Methods The immunoreactivity of CHC was analyzed in 409 esophageal specimens using a tissue array. Immunoreactivities of CHC, p53, and Ki67 were then compared in 44 endoscopically resected specimens.
Results CHC expression was significantly stronger in the cytoplasm of esophageal squamous cell carcinomas compared with non-tumor specimens in the tissue array. CHC expression in endoscopic specimens was significantly stronger in the cytoplasm of high-grade intraepithelial neoplasias and superficial carcinomas than in benign squamous epithelium and low-grade intraepithelial neoplasias. The sensitivity and specificity of CHC for the diagnosis of esophageal lesions were 75 and 96 %, respectively. These accuracies were comparable with those of p53 (43 and 98 %) and Ki67 (68 and 100 %). In addition, the sensitivity was increased by using a combination of markers as follows: 80 %, CHC + p53; 78 %, CHC + Ki67; 90 %, CHC + p53 + Ki67.
Conclusions CHC detected by IHC may be a useful marker for the pathological diagnosis of esophageal squamous intraepithelial neoplasia.

Keywords Clathrin heavy chain · Endoscopy · Esophageal cancer · Immunohistochemistry · Marker

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Introduction

Early esophageal cancer generally lacks symptoms, and many cases are therefore discovered at an advanced stage when the prognosis is poor [1]. Carcinoembryonic antigen, matrix metalloproteinase-9, squamous cell carcinoma antigen, and p53 are commonly used as diagnostic markers for esophageal cancer [2–5], but their sensitivities and specificities are relatively low, and they are therefore inadequate for identifying early stage esophageal cancer. Esophageal cancer can be detected early by endoscopic screening [6], and lesions identified by endoscopy are then biopsied for histopathological diagnosis. However, determining malignancy by histopathological examination of very small samples, such as biopsy specimens, is difficult, and new pathological diagnostic markers are needed to improve this process.

We recently reported strong expression of clathrin heavy chain (CHC) in the cytoplasm and cell membrane of hepatocellular carcinoma cells, detected by proteome analysis of primary hepatocellular carcinoma (HCC). These results suggest that CHC may be useful as a pathological diagnostic marker for early HCC [7]. CHC plays a major role in cellular endocytosis [8] and is involved in the stability of spindle microtubules in the nucleus [9], which is essential for equipartitioning into sister chromatids and the regulation of p53 transcription activity by binding to p53 [10]. CHC is therefore an interesting protein in relation to carcinogenesis and cancer progression, and may serve as a pathological diagnostic marker for cancers other than HCC. We investigated its immunoreactivity in various esophageal cancer types using tissue arrays. The immunoreactivity of CHC in esophageal cancerous regions was significantly increased, and we therefore evaluated the value of CHC immunoreactivity for the diagnosis of early esophageal cancer. In this study, we subjected tissue arrays and endoscopically resected esophageal tissues to immunohistochemical analysis (IHC) to evaluate CHC immunoreactivity and compared CHC immunoreactivity with those of the current auxiliary diagnostic markers, p53 and Ki67.

Materials and methods

Patients

The subjects were patients who underwent resection at the Department of Frontier Surgery, Chiba University Hospital. Written informed consent was obtained from each patient before surgery. Specimens included mucosal regions resected by endoscopic mucosal resection or endoscopic submucosal dissection in 44 cases. Formalin-fixed paraffin-embedded preparations were used. Preparations were diagnosed by pathologists at our hospital according to the WHO Classification of Esophageal Cancer [11]. Of the 44 cases of mucosal lesions, 12 were low-grade intraepithelial neoplasias (LINs) (including 8 cases in which LIN regions were independently present near malignant regions), 10 high-grade intraepithelial neoplasia (HINs), and 30 superficial carcinomas. Benign squamous epithelia from non-tumorous regions (margins of resected regions) from all 44 cases were used as negative controls.

Tissue array

We used a commercial esophageal tissue array to validate CHC immunoreactivity in esophageal tissues. Tissue arrays (ES2001, ES2084, and ES2082; US Biomax, Inc., Rockville, MD, USA) containing 319 tumor (266 squamous cell carcinoma, 5 adenosquamous carcinoma, 26 adenocarcinoma, and 22 small cell undifferentiated carcinoma) and 90 non-tumor (64 cancer-adjacent normal esophageal tissue, 16 chronic inflammation of esophageal mucosa, and 10 hyperplasia of squamous epithelium) esophageal tissue samples were used for IHC.

Immunohistochemistry

IHC was performed as described previously [7]. Briefly, 4-μm sections from paraffin-embedded tissue were fixed on slide glasses. Anti-CHC mouse monoclonal antibody (BD Biosciences Tokyo, Japan) was diluted 1:200 in blocking buffer (Dako Real™ Antibody Diluent; Dako, Kyoto, Japan). The EnVision + system (Dako) was used to visualize tissue antigens. For double staining of esophageal intraepithelial neoplasia, anti-CHC mouse monoclonal antibody was deactivated by microwave irradiation for 5 min in citric buffer, pH 6.0, after CHC staining, followed by staining with anti-p53 mouse monoclonal antibody (Dako) diluted 1:50 or anti-Ki67 mouse monoclonal antibody (Dako) diluted 1:100 in blocking buffer (Dako Real™ Antibody Diluent). The EnVision Gl2 System/AP (Rabbit/Mouse (Permanent Red); Dako) was used to visualize tissue antigens. CHC-positive regions were stained brown in the cytoplasm, and p53- and Ki67-positive regions were stained red with Permanent Red in the nucleus. The staining intensity was classified as negative or positive: For CHC, intense staining more than 30 % was judged as positive (Fig. 1). For p53, intense staining more than 30 % was judged as positive. For Ki67, the mucosa was divided into the upper and lower (basal) layers and staining was judged in each layer. Staining more than 30 % was judged as positive. IHC of the samples was evaluated by our two pathologists (T.T., Y.N.).

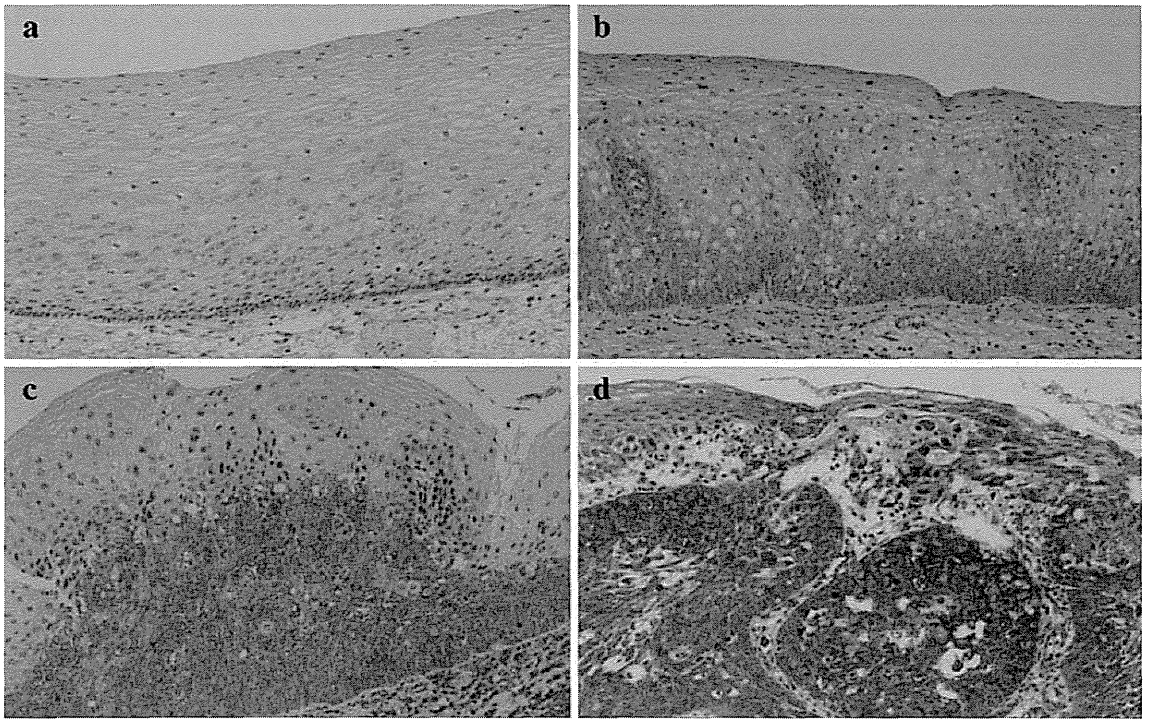

Fig. 1 The staining intensity of clathrin heavy chain (CHC) **a** negative (benign squamous epithelia), **b** negative (low grade dysplasia), **c** positive (superficial carcinoma), and **d** positive (advanced carcinoma)

Table 1 Immunoreactivity of CHC in tissue array

CHC expression level	Non-tumor			Tumor			
	Normal	Chronic inflammation	Hyperplasia	Squamous cell carcinoma	Adenosquamous carcinoma	Adeno-carcinoma	Small cell undifferentiated carcinoma
Negative	63	15	10	119	3	7	15
Positive	1	1	0	147	2	19	7

Results

Immunoreactivity of CHC in the tissue array

CHC expression was positive in many esophagus carcinoma tissue samples (55 %). In contrast, most non-tumor tissues (98 %) were negative (Table 1).

Immunoreactivities of CHC, p53, and Ki67 in endoscopic specimens

IHC double staining of CHC-p53 and CHC-Ki67 is shown in Fig. 2. CHC was positive in 75 % of HINs or superficial carcinomas, and p53 was positive in 43 % of HINs or superficial carcinomas. Ki67 was positive in the lower layer of the esophageal epithelium in 66 % cases of benign squamous epithelium or LIN. However, no expression was noted in the upper layer of benign squamous epithelium or LIN. Positive staining was noted in the lower (93 %) and upper layers (68 %) in many cases of HIN or superficial carcinoma (Table 2).

We set the expression conditions of the markers to differentiate between malignant (HIN and superficial carcinoma) and benign lesions (benign squamous epithelium and LIN). Positivity was judged to be the criterion for differentiating between malignant and benign lesions in terms of CHC, with a sensitivity and specificity of 75 and 96 %, respectively. Similarly, positivity was the optimal p53 criterion, with a sensitivity and specificity of 43 and 98 %, respectively. Positivity in the upper layer was the optimal criterion for Ki67 protein staining, with a sensitivity and specificity of 68 and 100 %, respectively. The sensitivity could be increased by using a combination of these markers (Table 3). There were significant differences in the immunoreactivities of all proteins between squamous epithelium/LIN and HIN/superficial carcinoma ($p < 0.001$).

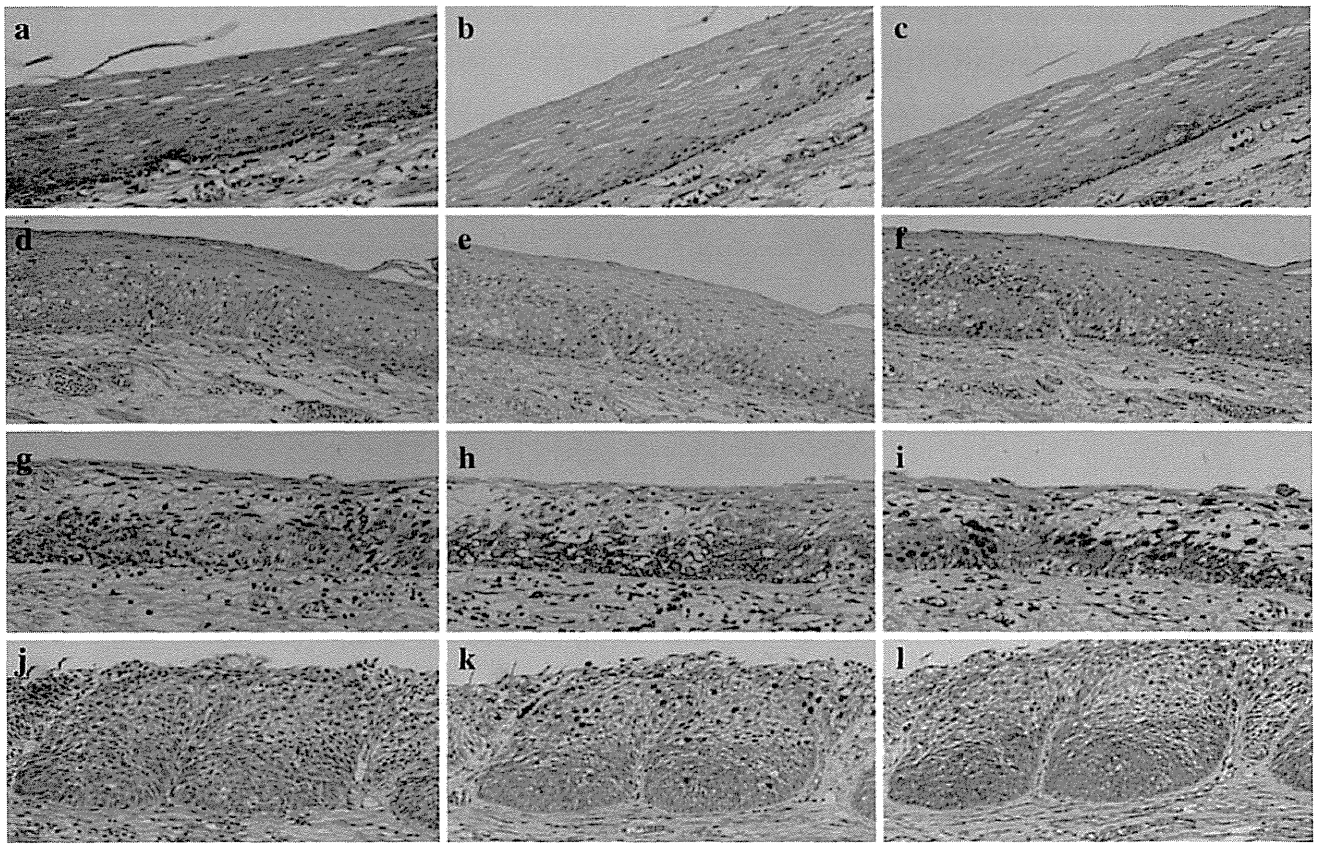

Fig. 2 Immunohistochemical double staining of esophageal epithelium CHC was stained brown in the cytoplasm, and p53 or Ki67 was stained red in the nucleus. **a–c** Benign squamous epithelial region, **d–e** low-grade intraepithelial neoplasia, **g–i** high-grade intraepithelial neoplasia, and **j–l** superficial carcinoma (carcinoma invading the lamina propria). **a**, **d**, **g**, **j** Conventional hematoxylin-eosin staining, **b**, **e**, **h**, **k** immunohistochemical double staining with CHC and p53, and **c**, **f**, **i**, **l** immunohistochemical double staining with CHC and Ki67

Table 2 Immunoreactivity of proteins in mucosal lesions

Marker	Immunoreactivity	Benign lesions		Malignant lesions	
		Benign squamous epithelium	LIN*1	HIN*2	Superficial carcinoma
CHC	Negative	42	12	3	7
	Positive	2	0	7	23
P53	Negative	43	12	7	16
	Positive	1	0	3	14
Ki67 (in lower layer)	Negative	16	3	1	2
	Positive	28	9	9	28
Ki67 (in upper layer)	Negative	44	12	5	8
	Positive	0	0	5	22

* 1, low-grade intraepithelial neoplasia; * 2, high-grade intraepithelial neoplasia

Discussion

The risk of developing invasive cancer in LIN (without basal layer type squamous cell carcinoma in situ) is low, while HIN, in contrast, is likely to progress to invasive cancer and thus requires early treatment [12–16]. Differentiating between LIN and HIN is therefore very important. Unfortunately, however, it is difficult to make an accurate histopathological diagnosis based on small tissue samples. p53 and Ki67 are currently used as immunohistochemical esophageal markers, but there have been very few reports on immunohistochemical markers of esophageal intraepithelial tumors [17], and there is no clear information regarding the differentiation of benign squamous

Table 3 Diagnostic accuracy of immunoreactivity employing each protein alone and in combination

Marker	Sensitivity (%)	Specificity (%)
CHC	75	95
p53	43	98
Ki67	68	100
Combination		
CHC and p53	80	95
CHC and Ki67	78	95
p53 and Ki67	78	98
CHC and p53 and Ki67	90	95

* Decision criterion for differentiating between high-grade intraepithelial neoplasia/superficial carcinoma and benign squamous epithelium/low-grade intraepithelial neoplasia/LIN

epithelium/LIN from malignant lesions (HIN/superficial carcinoma).

We investigated the usefulness of CHC IHC for the histological diagnosis of esophageal cancer in 409 cases, using commercial esophageal tissue arrays. CHC was positive in only 2 % of benign squamous epithelia, but in 55 % of malignant cases. Apparent differences in CHC expression were noted between mucosal preparations of benign squamous epithelium/LIN and HIN/superficial carcinoma. The sensitivity and specificity of CHC for the diagnosis of esophageal lesions were 75 and 96 %, respectively, indicating the usefulness of CHC as an immunohistochemical marker of esophageal epithelial lesions.

IHC double staining of CHC/p53 and CHC/Ki67 was used to compare CHC with p53 and Ki67, which are currently used as auxiliary diagnostic markers of esophageal cancer. p53 is a tumor suppressor protein, but wild-type p53 is rapidly degraded and is normally present in the nucleus at a very low level. In comparison, mutant p53 protein has markedly delayed intracellular degradation and accumulates in the nucleus, allowing its detection by IHC [18–20]. Residual p53 protein is observed in early esophageal squamous cell carcinomas and precancerous lesions. A high incidence of mutant p53 has recently been demonstrated in LIN and HIN tissues by laser capture microdissection [21]. In the current study, p53 staining was slightly positive in the lower (basal) layer in 17 % of LIN cases (data not shown), but no regions showed positivity. In contrast, the lower layer demonstrated positivity in 30 % of HIN cases, suggesting that not only the presence/absence of mutant p53, but also the level of residual p53 protein in the nucleus may be associated with carcinogenesis and cancer progression.

Ki67 protein is expressed in the G1, S, G2, and M phases, but not in the G0 phase during cell division, showing that an increase in Ki67 protein indicates active cell proliferation, and high Ki67 expression levels have been reported in tumor cells [22, 23]. Ki67 was positive in the lower layer of the esophageal epithelium in many cases, reflecting active cell division in the lower layer, while Ki67 was negative in the upper layer of tissue samples in benign squamous epithelium and LIN, but positive or in many cases of HIN, suggesting active cell division in the upper layer of the esophagus in HIN.

We investigated the optimal condition for each marker in terms of differentiating between benign (benign squamous epithelium/LIN) and malignant lesions (HIN/superficial carcinoma). Positivity for p53 and positivity in the upper layer of the mucosa for Ki67 were considered to be optimal conditions, respectively. The sensitivity and specificity of CHC alone were 75 and 96 %, respectively, which were comparable with or superior to those of p53 (43 and 98 %) and Ki67 (68 and 100 %). In addition, the sensitivity could be increased using a combination of these markers, to 80 % for CHC + p53, 78 % for CHC + Ki67, and 90 % for CHC + p53 + Ki67.

These results suggest that CHC IHC could be a useful histological auxiliary diagnostic technique for differentiating between benign squamous epithelium/LIN and HIN/superficial carcinoma in the histopathological diagnosis of esophageal intraepithelial tumors. CHC IHC might contribute to the accurate pathological diagnosis of esophageal cancers that are difficult to diagnose by conventional hematoxylin-eosin staining. The mechanisms by which the expression levels of CHC are enhanced in esophageal squamous cell carcinoma remain to be investigated. Clarification of these mechanisms may lead to further understanding of the tumor biology of esophageal cancers.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standard statement The authors work conformed to the guidelines set forth in the Helsinki Declaration of 1975, as revised in 2000.

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ORIGINAL ARTICLE

Correlation between preoperative systemic inflammation and postoperative infection in patients with gastrointestinal cancer: a multicenter study

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Abstract
Purpose Our aim was to examine the association between postoperative infection and preoperative systemic inflammation in patients undergoing resection of gastrointestinal cancer.
Methods We studied 862 patients who underwent elective gastrointestinal cancer surgery at six institutions. The levels of C-reactive protein and albumin were included as parameters of preoperative systemic inflammation measured using the Glasgow prognostic score. The Glasgow prognostic score was calculated based on the admission data as follows: patients with an elevated level of C-reactive protein (>1.0 mg/dl) and hypoalbuminemia (<3.5 g/dl) were allocated a score of 2, while patients showing one or none of these blood chemistry abnormalities were allocated a score of 1 or 0, respectively. The significance of the Glasgow prognostic score for predicting postoperative infection was analyzed using a multivariate analysis.
Results After surgery, 182 (21 %) patients developed postoperative infections. According to a multivariate analysis, the Glasgow prognostic score ($p < 0.01$) was independently associated with an increased risk of developing a postoperative infection. When the postoperative infections were divided into surgical site infections and remote site infections, the Glasgow prognostic score was significantly associated with an increased risk of developing remote site infections.
Conclusions Preoperative systemic inflammation is associated with postoperative infection in patients undergoing resection of gastrointestinal cancer.

Keywords Systemic inflammation · Postoperative complication · Gastrointestinal cancer

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Introduction

Patients undergoing major oncological resection are at high-risk for developing postoperative infection [1]. Recent therapeutic advances, both medical and surgical, have enabled clinicians to reduce the rate of early postoperative mortality. Despite this progress, some patients continue to have a high-risk of infection and the attendant risk of increased morbidity and mortality. Moreover, there is increasing evidence that postoperative complications due to infection following gastrointestinal cancer surgery are significantly associated with negative short- and long-term outcomes [2–4]. Many studies have reported possible risk factors for postoperative infectious complications, such as the severity of the underlying disease, an advanced age, trauma, loss of skin integrity, and malnutrition [5, 6]. Malnutrition is a common problem in surgical patients, which adversely affects the outcomes [7]. The nutritional status is evaluated based on weight loss, the serum albumin level, and the body mass index. The albumin level is the most commonly used and reliable indicator of a patient's nutritional status and is also an indicator of a negative

acute phase problem [8]. In the past decade, it has been shown that an elevated C-reactive protein (CRP) level is associated with the loss of lean body mass and increased resting energy expenditure [9, 10]. Therefore, both low albumin and high CRP levels are linked to malnutrition in oncology patients.

With regard to the measurement of the systemic inflammatory response [Glasgow prognostic score (GPS)], the combination of CRP and albumin measurement improves the prediction of cancer-specific survival in patients with a variety of common solid tumors, including stomach and colorectal cancer [11–13]. However, the relationship between preoperative systemic inflammation and postoperative infection has not been well studied. It is unknown whether a preoperative systemic inflammatory response is associated with postoperative infection in patients who have undergone gastrointestinal cancer surgery regardless of the tumor site.

The present study was conducted to evaluate preoperative systemic inflammation, as indicated by elevated CRP and low albumin levels, with respect to the prediction of postoperative infectious complications in patients with gastrointestinal cancer.

Patients and methods

A retrospective study of all patients who underwent elective gastrointestinal cancer surgery at six institutions was performed. The patients were identified using a postoperative infection database or hospital records. The data were recorded prospectively for each patient in the database immediately postoperatively by the operating surgeon. The patients were assessed for postoperative infectious complications, including surgical site infection (SSI) and remote site infection (RI), by the surgeon or attending doctor. The presence of SSI was determined according to the Centers for Disease Control and Prevention definition [14]. The definition of RI was derived from Garner and associates [15].

The subjects included in this study were patients who underwent elective surgery for stomach and/or colorectal cancer. The surgical wound class in all patients was classified as clean contaminated. Patients who did not undergo primary resection, those who had been treated with antibiotics in the past 2 weeks and those with an infection at the time of surgery were omitted from this study.

Standard procedures were used in all patients. Antimicrobial prophylaxis was administered 30 min before the procedure. The hair in the operative field was shaved using electric clippers following induction of general anesthesia. The surgical site was wiped with 10 % povidone-iodine solution before surgery and draped with a disposable towel. Absorbable synthetic sutures were used to close the fascia and peritoneum. The intra-abdominal drainage tubes were passed through a stab incision separate from the wound. The skin was closed using stainless steel staples, and the wound was then wiped with normal saline. No local irrigation of tissues with solutions containing antimicrobial agents was used. The site was kept covered with a film dressing until removal of the staples. Patients with colorectal cancer received the same protocol of mechanical bowel preparation. Routine postoperative care was provided to each patient, and each patient was followed-up for a minimum of 30 days.

The study was approved by the Ethics Review Board of each institution in which it was undertaken, and written informed consent was obtained from each patient or legal representative before entering into the study.

The data included age, sex, tumor site, operative approach, and American Society of Anesthesiologists (ASA) grade. Preoperative laboratory measurements of the white cell count and levels of hemoglobin, albumin, and CRP were obtained. The score of systemic inflammation before surgery (GPS) was estimated as previously described [16]. Briefly, patients with both an elevated CRP (>1.0 mg/dl) level and hypoalbuminemia (<3.5 g/dl) were allocated a score of 2. Patients with only one of these biochemical abnormalities were allocated a score of 1. Patients with neither of these abnormalities were allocated a score of 0.

Statistical analysis

The data are presented as the median (interquartile range). Comparisons between groups of patients were made using the Chi-square test for categorical variables and Wilcoxon rank test for continuous variables. A multiple logistic regression analysis was used to examine the effects of the variables on the development of postoperative infection, including SSI and RI. To test the independence of the risk factors, the variables found to be significant ($p < 0.1$) in the univariate analysis were entered into a multivariate logistic regression model. A p value of <0.05 was considered to be statistically significant.

Results

Data were obtained and analyzed for 862 patients who underwent elective gastrointestinal cancer surgery. There were 558 males and 304 females with 406 gastric cancers and 456 colorectal cancers. Table 1 compares the patient demographics and the incidence of postoperative infection. After surgery, 182 (21.1 %) patients developed a postoperative infection: 117 patients developed an SSI, 83

Table 1 Patient demographics

Characteristics	Total *N* (%)	Postoperative infection (−) (%)	Postoperative infection (+) (%)	*p*
Total	862	680 (78.9)	182 (21.1)	
Age, median (IQR) (years)	67 (59–74)	67 (58–74)	69 (61.5–74)	0.0487
Sex				
Male	558 (64.7)	424 (62.4)	134 (73.6)	0.0047
Female	304 (35.3)	256 (37.6)	48 (16.4)	
ASA				
Grade I	508 (59)	409 (60.1)	99 (54.4)	<0.0001
Grade II	320 (37)	255 (37.5)	65 (35.7)	
Grade III	34 (4)	16 (2.4)	18 (9.9)	
Hb, median (IQR) (g/dl)	12.2 (10.5–13.7)	12.3 (10.7–13.7)	12 (10.5–13.5)	0.0666
WBC, median (IQR) ($\times 10^9$/l)	5.9 (4.9–7.1)	5.8 (4.9–7.1)	6.1 (5.0–7.2)	0.2673
CRP, median (IQR) (mg/dl)	0.25 (0.2–0.6)	0.23 (0.2–0.5)	0.27 (0.2–1.2)	<0.0001
Alb, median (IQR) (g/dl)	3.8 (3.5–4.1)	3.8 (3.6–4.2)	3.7 (3.4–4.0)	0.0008
Time, median (IQR) (min)	240 (173–310)	230 (169–300)	272 (195.8–351.3)	<0.0001
Laparoscopic surgery				
Yes	182	5	177	0.0041
No	680	60	620	
Blood loss, median (IQR) (g)	340 (180–693)	314 (167–593.5)	546 (236.8–995.3)	<0.0001
Tumor site				
Stomach	406 (47)	332 (48.8)	74 (40.7)	0.0021
Colon	201 (23)	166 (24.4)	35 (19.2)	
Rectum	255 (30)	182 (26.8)	73 (40.1)	
R				
0	659 (76.5)	526 (77.4)	133 (73.1)	0.4804
1	59 (6.8)	45 (6.6)	14 (7.7)	
2	144 (16.7)	109 (16.0)	35 (19.2)	

ASA American Society of Anesthesiologists grade, *Hb* hemoglobin, *WBC* white blood cell count, *CRP* C-reactive protein, *Alb* albumin, *R* residual tumor classification, *IQR* interquartile range

patients developed an RI and 18 patients developed both an SSI and RI. There were significant differences between the patients with postoperative infection and the patients without postoperative infection in terms of age at the time of surgery, sex (male), a high grade of ASA, tumor site, operative time, operative approach, amount of blood loss, and the albumin and CRP levels.

The relationships between preoperative systemic inflammation (GPS) and the clinicolaboratory characteristics in the patients undergoing resection of gastrointestinal cancer are shown in Table 2. The tumor site, operative time, and amount of intraoperative blood loss did not exhibit any significant relationships with GPS. On the other hand, an increased GPS was significantly associated with an advanced age, a high ASA grade, non-curability, a high WBC count, a low hemoglobin level, and the incidence of postoperative infection and RI.

The results of the univariate analyses of postoperative infection, using the same factors as those shown in Table 1, are presented in Table 3. Sex and the ASA grade, tumor site, laparoscopic surgery, operative time, amount of blood loss, and GPS were associated with postoperative infection. The multivariate analyses revealed that sex (male) and the GPS, ASA grade, tumor site, operative time, and amount of blood loss were independently associated with an increased risk of developing a postoperative infection (Table 3). The GPS was also an independent factor for the development of postoperative infectious complications in each tumor site when the data were analyzed according to the tumor site (gastric cancer and colorectal cancer).

The relationships between SSI and the clinicolaboratory characteristics, including preoperative systemic inflammation, are shown in Table 4. According to the univariate logistic regression analysis, sex (male) and the tumor site (rectum), laparoscopic surgery, operative time, and amount of blood loss were associated with an increased risk of developing an SSI. According to the multivariate analysis, the tumor site and amount of blood loss were independently

Table 2 Relationships between clinicolaboratory characteristics and GPS

	GPS 0 ($N = 616$)	GPS 1 ($N = 160$)	GPS 2 ($N = 86$)	p
Age (years)				
<60	188 (78.3)	33 (13.8)	19 (7.9)	0.0006
60–75	330 (72.5)	80 (17.6)	45 (9.9)	
>75	98 (58.7)	47 (28.1)	22 (13.2)	
Sex				
Male	408 (73.1)	92 (16.5)	58 (10.4)	0.1028
Female	208 (68.4)	68 (22.4)	28 (9.2)	
ASA				
I	382 (75.2)	85 (16.7)	41 (8.1)	0.0014
II	217 (67.8)	67 (20.9)	36 (11.3)	
III	17 (50)	8 (23.5)	9 (26.5)	
Site of tumor				
Gastric cancer	291 (71.7)	74 (18.2)	41 (10.1)	0.9692
Colorectal cancer	325 (71.3)	86 (18.9)	45 (9.9)	
R				
R0	504 (76.5)	112 (17)	43 (6.5)	<0.0001
R1	40 (67.8)	13 (22)	6 (10.2)	
R2	72 (50)	35 (24.3)	37 (25.7)	
WBC				
<5000/mm^3	187 (78.9)	42 (17.7)	8 (3.4)	<0.0001
5000–7000/mm^3	296 (74.4)	72 (18.9)	30 (7.5)	
>7000/mm^3	133 (58.6)	46 (20.3)	48 (21.2)	
Hb				
<10 g/dl	65 (38.7)	49 (29.2)	54 (32.1)	<0.0001
10–14 g/dl	400 (75.1)	105 (19.6)	28 (5.3)	
>14 g/dl	151 (93.8)	6 (3.7)	4 (2.5)	
Operation time				
<170 min	150 (71.1)	39 (18.5)	22 (10.4)	0.9341
170–300 min	304 (72.8)	75 (17.9)	39 (9.3)	
>300 min	162 (69.5)	46 (19.7)	25 (10.7)	
Blood loss				
<180 ml	161 (73.9)	37 (17)	20 (9.2)	0.1874
180–700 ml	317 (73)	80 (18.4)	37 (8.5)	
>700 ml	138 (65.7)	43 (20.5)	29 (13.8)	
Postoperative infection				
Yes	112 (61.5)	42 (23.1)	28 (15.4)	0.0020
No	504 (74.1)	118 (17.4)	58 (8.5)	
Surgical site infection				
Yes	77 (65.8)	25 (21.4)	15 (12.8)	0.3204
No	539 (72.4)	135 (15.7)	71 (9.5)	
Remote site infection				
Yes	44 (53)	22 (26.5)	17 (20.5)	0.0001
No	572 (73.4)	138 (17.7)	69 (8.9)	

ASA American Society of Anesthesiologists grade, *R* residual tumor classification, *WBC* white blood cell count, *Hb* hemoglobin

associated with an increased risk of developing an SSI. When the data were analyzed according to the two tumor sites, the extent of gastrectomy (total gastrectomy) was found to be the only independent factor for the development of SSI in the patients undergoing gastric cancer surgery. In contrast, a male gender and the amount of blood loss were found to be independent factors for the development of SSI in the patients undergoing colorectal cancer surgery.

The relationships between RI and the clinicolaboratory characteristics are shown in Table 5. According to the

Table 3 Relationships between the studied variables and postoperative infection in patients treated with gastrointestinal cancer surgery

	Overall				Gastric cancer				Colorectal cancer			
	Univariate	Multivariate			Univariate	Multivariate			Univariate	Multivariate		
	p	RR	95 % CI	*p*	*p*	RR	95 % CI	*p*	*p*	RR	95 % CI	*p*
Age (<60/60–75/ >75 years)	0.10				0.31				0.18			
Sex (female/male)	0.004	1.64	1.11–2.45	0.01	0.69				0.0002	2.09	1.27–3.50	0.003
ASA (I/II/III)	0.006	2.27	1.26–4.07	0.006	0.003	4.03	1.62–10.24	0.003	0.25			
GPS (0/1/2)	0.001	1.89	1.11–3.21	0.02	0.007	2.43	1.10–5.33	0.03	0.03	2.25	1.14–2.10	0.02
Hb (>14/10–14/ <10 g/dl)	0.07	1.32	0.70–2.48	0.39	0.03	0.90	0.57–2.45	0.83	0.77			
WBC (<5.0/5.0–7.0/ >7.0 × 10^3/mm^3)	0.36				0.99				0.26			
Tumor site (stomach/colon/ rectum)	0.003	1.92	1.29–2.88	0.001	–	–	–	–	–	–	–	–
R (R0/R1/R2)	0.24				0.04	1.05	0.50–2.12	0.90	0.90			
Laparoscopic surgery (yes/no)	0.002	0.50	0.17–1.20	0.13	0.047	0.74	1.36–2.10	0.59	0.03	0.42	0.02–2.25	0.36
Operation time (<170/170–300/ >300 min)	<0.001	2.13	1.13–4.04	0.02	0.005	1.58	0.59–4.31	0.36	<0.0001	1.60	0.67–3.87	0.29
Blood loss (<180/ 180–700/ >700 ml)	<0.001	2.30	1.20–4.45	0.01	0.002	1.05	0.37–2.98	0.93	<0.0001	2.97	1.22–2.23	0.02
Total gastrectomy	–	–	–	–	<0.0001	3.41	1.85–5.33	<0.001	–	–	–	–
Rectal surgery	–	–	–	–	–	–	–	–	0.005	1.31	0.77–2.23	0.32

ASA American Society of Anesthesiologists grade, *GPS* Glasgow prognostic score, *Hb* hemoglobin, *WBC* white blood cell count, *R* residual tumor classification, *RR* risk ratio, *95 % CI* 95 % confidence interval

univariate logistic regression analysis, an advanced age, sex (male), the ASA grade, a low hemoglobin level, the amount of blood loss, and the GPS were associated with an increased risk of developing an RI. According to the multivariate analysis, an advanced age, sex (male) and the GPS were independently associated with an increased risk of developing an RI. Among the patients undergoing gastric cancer surgery, the ASA grade, GPS, and extent of gastrectomy were independent factors for the development of RI. Among the patients undergoing colorectal cancer surgery, an advanced age and the amount of blood loss, but not the GPS, were independent factors for the development of RI.

Discussion

Our study based on individual data for 862 patients undergoing resection for gastrointestinal cancer demonstrated that preoperative systemic inflammation (GPS) is independently associated with an increased risk of postoperative infection. Given that postoperative infections are relatively common in patients undergoing gastrointestinal surgery and are associated with increased hospital stays and treatment costs, it is possible that the simple routine preoperative measurement of the GPS is clinically useful in identifying patients at high-risk of developing infections. To the best of our knowledge, few studies have evaluated common risk factors for postoperative infections in patients undergoing various gastrointestinal cancer surgeries. The primary advantage of this study is that a significant number of patients had data for prospective postoperative infection surveillance. Furthermore, this was a multicenter study that included private medical centers, institutional hospitals, and university hospitals. Therefore, our results are more representative of oncological surgical practice, avoiding the bias of single center recruitment.

Resection of gastrointestinal cancer is associated with a high rate of postoperative infections, ranging from 20 to 40 % [1]. Recent advances in both surgical techniques and perioperative care have led to a reduction in morbidity and mortality following gastrointestinal cancer surgery. Despite these advances, infectious complications still pose a major clinical problem, particularly in high-risk patient populations. Previous studies have shown that the clinical diagnosis of a complicated postoperative course may be

Table 4 Relationships between the studied variables and surgical site infection in patients treated with gastrointestinal cancer surgery ($n = 862$)

	Overall				Gastric cancer				Colorectal cancer			
	Univariate	Multivariate			Univariate	Multivariate			Univariate	Multivariate		
	p	RR	95 % CI	*p*	*p*	RR	95 % CI	*p*	*p*	RR	95 % CI	*p*
Age (<60/60–75/ >75 years)	0.7602				0.99				0.73			
Sex (female/male)	0.0274	1.437	0.922–2.285	0.1102	0.99				0.002	1.90	1.07–3.52	0.03
ASA (I/II/III)	0.2069				0.08	1.59	0.93–2.70	0.09	0.74			
GPS (0/1/2)	0.1441				0.90				0.052	2.12	0.97–4.60	0.06
Hb (>14/10–14/ <10 g/dl)	0.5144				0.77				0.74			
WBC (<5.0/ 5.0–7.0/ >7.0 × 10^3/ mm^3)	0.2044				0.49				0.04	1.56	0.73–3.41	0.25
Tumor site (stomach/colon/ rectum)	0.0005	2.038	1.138–1.796	0.0021	–	–	–	–	–	–	–	–
R (R0/R1/R2)	0.8851				0.99				0.91			
Laparoscopic surgery	0.0134	0.475	0.112–1.368	0.1843	0.096	0.53	0.08–2.00	0.39	0.16			
Operation time (<170/170–300/ >300 min)	<0.0001	2.097	0.996–4.493	0.0527	0.002	2.67	0.79–9.50	0.11	<0.0001	1.30	0.47–3.64	0.61
Blood loss (<180/ 180–700/ >700 ml)	<0.0001	2.828	1.303–6.215	0.0084	0.003	1.39	0.39–5.06	0.61	<0.0001	3.73	1.33–10.76	0.01
Total gastrectomy (yes/no)	–	–	–	–	0.0005	2.35	1.15–4.89	0.02	–	–	–	–
Rectal surgery (yes/no)	–	–	–	–	–	–	–	–	0.003	1.59	0.86–3.03	0.14

ASA American Society of Anesthesiologists grade, *GPS* Glasgow prognostic score, *Hb* hemoglobin, *WBC* white blood cell count, *R* residual tumor classification, *RR* risk ratio, *95 % CI* 95 % confidence interval

significantly delayed, with the median time to diagnosis occurring on day 8 [17]. Preoperatively identifying patients at risk of postoperative infections may affect surgical management modifications, and thus reduce mortality associated with infectious morbidities.

Previous research has indicated that an advanced patient age, comorbidities, longer operative times, malnutrition, and the use of blood transfusions are associated with a high rate of postoperative infection. Malnutrition is common among oncology patients and leads to a reduction in the performance status and quality of life [5, 6]. The presence of systemic inflammation, as indicated by an increased CRP level and a decreased albumin level, is associated with weight loss in patients with various solid tumors [18–20]. Therefore, both high CRP and low albumin levels are linked to malnutrition. To date, there are few data in the literature regarding the effects of preoperative systemic inflammation on the development of postoperative infection. Recently, Moyes et al. [21] reported an independent association between systemic inflammation and postoperative infection following curative resection of colorectal cancer. However, the precise role of preoperative systemic inflammation in the development of postoperative infection is unclear. The current study showed that the GPS can be used to identify patients at an increased risk for infection following resection of gastrointestinal tumors, regardless of the tumor site. We also investigated the impact of the GPS on the development of postoperative infection according to the tumor site. A significant association between the GPS and the development of postoperative infection was observed with respect to both tumor sites. Therefore, we confirmed that preoperative systemic inflammation, as evidenced by the GPS, is an independent factor for the development of postoperative infection, regardless of the tumor site.

Postoperative infections are usually classified as SSIs or RIs. SSIs and RIs may have a distinct pathogenesis, being specific to different underlying mechanisms, and may

Table 5 Relationships between the studied variables and remote site infection in patients treated with gastrointestinal cancer surgery ($n = 862$)

	Overall				Gastric cancer				Colorectal cancer			
	Univariate	Multivariate			Univariate	Multivariate			Univariate	Multivariate		
	p	RR	95 % CI	*p*	*p*	RR	95 % CI	*p*	*p*	RR	95 % CI	*p*
Age (<60/60–75/ >75 years)	0.0007	2.805	1.255–6.491	0.0116	0.053	1.92	0.79–2.46	0.26	0.005	2.27	1.38–3.83	0.001
Sex (female/male)	0.0200	1.757	1.025–3.122	0.0402	0.25				0.04	1.91	0.95–4.12	0.07
ASA (I/II/III)	0.0022	1.675	0.692–3.900	0.2477	0.005	1.89	1.00–3.51	0.049	0.12			
GPS (0/1/2)	<0.0001	2.305	1.143–4.597	0.0199	<0.0001	2.17	1.63–3.69	0.004	0.14			
Hb (>14/10–14/ <10 g/dl)	0.0209	1.378	0.579–3.289	0.4690	0.01	0.79	0.40–3.69	0.50	0.37			
WBC (<5.0/ 5.0–7.0/ >7.0 × 10^3/ mm^3)	0.5328				0.71				0.60			
Tumor site (stomach/colon/ rectum)	0.9421				–	–	–	–	–	–	–	–
R (R0/R1/R2)	0.0884	1.252	0.683–2.225	0.4584	0.003	1.32	0.85–4.02	0.21	0.57			
Laparoscopic surgery	0.2954				0.37				0.54			
Operation time (<170/170–300/ >300 min)	0.0550	1.764	0.747–4.337	0.1957	0.28				0.11			
Blood loss (<180/ 180–700/ >700 ml)	0.0237	1.500	0.631–3.594	0.3589	0.20				0.06	2.71	1.11–6.80	0.03
Total gastrectomy (yes/no)	–	–	–	–	0.003	2.90	1.39–6.21	0.005	–	–	–	–
Rectal surgery (yes/no)	–	–	–	–	–	–	–	–	0.53			

ASA American Society of Anesthesiologists grade, *GPS* Glasgow prognostic score, *Hb* hemoglobin, *WBC* white blood cell count, *R* residual tumor classification, *RR* risk ratio, *95 % CI* 95 % confidence interval

differ from each other with respect to risk factors. It is important to identify which clinical and laboratory factors can predict site-specific patterns of postoperative infection in patients with SSIs and RIs, as this information may assist surgeons in developing specific strategies to prevent infection according to an oncology patient's risk factors. Previous studies have not evaluated whether the presence of preoperative systemic inflammation can be used to predict site-specific patterns of postoperative infection [21]. In the present study, we examined site-specific risk factors for SSIs and RIs in gastrointestinal cancer patients. We found that the tumor site (rectum) and amount of blood loss were independently associated with the development of SSI, whereas the GPS and sex (male) were independently associated with the development of RI. Similar to the findings of previous reports [4, 22], our results showed that the risk factors for SSI and RI are different. With respect to the development of RI, the GPS was found to be an independent risk factor in the patients undergoing gastric cancer surgery, but not in those undergoing colorectal cancer surgery. Although the association between the GPS and RI observed in the patients undergoing colorectal cancer surgery was not significant, there was a higher incidence of RI among the patients with an elevated GPS (GPS0 8 %, GPS1 12.8 %, and GPS2 13.3 %). An evaluation of a larger cohort is needed to determine whether preoperative systemic inflammation is an indicator of site-specific infection in each tumor site.

The basis of the independent relationship between an increased GPS before surgery and postoperative infection in patients with gastrointestinal cancer is unclear. The increased CRP levels observed in oncology patients may be due to the increased production of proinflammatory cytokines by tumors or tissue necrosis. Indeed, the serum IL-6 levels have been shown to be closely correlated with the CRP levels in patients with gastrointestinal cancer [23–25]. On the other hand, a decrease in the albumin level from 4.5 to 2.1 g/dl is associated with an increase in morbidity from