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ORIGINAL ARTICLE

Hyperfibrinogenemia is associated with inflammatory mediators and poor prognosis in patients with gastric cancer

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Abstract

Purpose Hyperfibrinogenemia is associated with poor prognosis in various cancers; however, its clinical relevance in gastric cancer has not been well analyzed. We conducted this study to assess the clinicopathological significance and prognostic value of hyperfibrinogenemia in patients with gastric cancer.

Methods Plasma fibrinogen levels were measured preoperatively in 315 patients undergoing surgery for gastric cancer. We then evaluated the clinicopathological significance of hyperfibrinogenemia and its relationship with several biomarkers, including white blood cell (WBC), C-reactive protein (CRP), platelet count, prothrombin time (PT) and activated partial thromboplastin time (APTT). Postoperative plasma levels were compared with preoperative levels. The multivariate prognostic value of hyperfibrinogenemia was calculated using the Cox proportional hazards model.

Results Tumor progression was significantly associated with hyperfibrinogenemia, as were the CRP level and platelet counts. Plasma fibrinogen levels decreased significantly after radical surgery. Adjusting for TNM factors, multivariate analysis indicated that hyperfibrinogenemia was an independent prognostic factor for poor survival (hazard ratio = 2.607, 95 % confidence interval = 1.180–5.761, $P = 0.018$).

Conclusion Preoperative hyperfibrinogenemia was associated with tumor progression, inflammatory mediators, and poor overall survival in patients with gastric cancer.

Keywords Gastric cancer · Plasma fibrinogen · Hyperfibrinogenemia

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Introduction

Fibrin, or fibrinogen, was identified as one of the major components of the tumor stroma enveloping tumor cells [1]. Hyperfibrinogenemia has been associated with various malignancies [2–7]. A consequence of hyperfibrinogenemia is that several plasma components, including fibrinogen, accumulate in the tumor stroma, where fibrinogen can be converted to cross-linked fibrin by the coagulant/procoagulant activities of malignant cells [8] and/or by tumor-infiltrating macrophages [9]. Several studies have reported that hyperfibrinogenemia is associated with poor prognosis and tumor progression [2–5, 7]. Although there is evidence that tumor cells secrete humoral factors that may eventually lead to hyperfibrinogenemia, the pathogenesis of hyperfibrinogenemia in gastric cancer remains unclear [10, 11]. Although increasing evidence suggests that fibrinogen expression and inflammation in various cancers are associated with poor prognosis [2–4], the association between the plasma fibrinogen concentrations and gastric cancer prognosis has only been examined by univariate analysis [10, 11]. Therefore, we analyzed inflammatory mediators in 315 patients with primary gastric adenocarcinoma before surgery, to identify correlations between inflammatory mediators and hyperfibrinogenemia. Possible correlations between fibrinogen levels and several biomarkers, namely, white blood cell (WBC) count, C-reactive protein (CRP)

level, prothrombin time (PT), activated partial thromboplastin time (APTT), carcinoembryonic antigen (CEA), CA19-9, CA125, and D-dimer, were also analyzed. Finally, we assessed the prognostic significance of hyperfibrinogenemia after adjusting for the selected clinicopathological variables by multivariate analysis.

Methods

Patients

We reviewed, retrospectively, the laboratory data and clinicopathological parameters of 315 patients who underwent surgery for histologically confirmed primary gastric adenocarcinoma, at Toho University Hospital (Tokyo, Japan) between 2008 and 2013. The patient population comprised 212 men (67.3 %) and 103 women (32.7 %), with a median age of 69 years (range 33–93 years). The study protocol was approved by the institutional review board of Toho University (IRB #26–256).

After surgery, all patients were classified according to the 14th edition (3rd English edition) of the Japanese Classification of Gastric Carcinoma [12]. The distribution of patients according to their cancer stages was as follows: stage I, 146; stage II, 71; stage III, 65; and stage IV, 33. All patients with disease classified as stage IV had distant lymph node metastasis, but no organ metastasis. All patients underwent either total or subtotal gastrectomy with standard lymphadenectomy. All patients were followed up regularly until July 2015 or death. The follow-up period ranged from 0 months to 88 months (median 28 months).

Blood sample analysis for fibrinogen levels and other biomarkers

Venous blood samples were collected before the initiation of treatment. Serum CEA was analyzed in 293 patients, serum CA19-9 in 290, serum CA125 in 165, APTT in 314, D-dimer in 231, and fibrin/fibrinogen degradation products in 235. Hyperfibrinogenemia status was re-analyzed in some patients after surgery. Repeated freezing and thawing of samples was avoided. Each sample was centrifuged at $3000g$ for 5 min and then frozen at -80 °C until the time of the assay. Plasma fibrinogen levels were measured using a previously established solid phase human fibrinogen immunoassay enzyme-linked immunosorbent assay kit (reagent: Coagpia®Fbg; analyzer: Automated Coagulation Analyzer CP3000®, Sekisui Medical Co., Tokyo, Japan) [13]. Patients were divided into groups of high and low plasma fibrinogen levels using three cutoff values (300, 350, and 400 mg/dl), according to institutional standards [14] and previous publications [2, 3, 10, 11]. We also used cutoff

values for the WBC count (9000 cells/mm^3) and CRP levels (0.2 mg/ml), in accordance with institutional standards.

Statistical analyses

Serum biomarker levels are expressed as means ± standard deviations. Comparisons between unpaired groups for these variables were conducted using the Mann–Whitney U test. Overall survival was calculated using the Kaplan–Meier product limit estimate. Survival differences between groups were determined using the log-rank test. Significant predictors identified by univariate analysis were assessed by multivariate analysis using the Cox proportional hazards model. All statistical analyses were performed using EZR statistical software [15]. Two-sided P values of <0.05 were considered significant.

Results

Clinicopathological factors in the high vs. low plasma fibrinogen level groups

Plasma fibrinogen levels were significantly associated with age, stage, tumor size, tumor depth, nodal involvement, and liver metastasis, but not with gender or histology (Table 1). We also compared the frequency of hyperfibrinogenemia in relation to clinicopathological factors, based on the three cutoff plasma fibrinogen levels (Table 2). Hyperfibrinogenemia was consistently associated with nodal involvement, tumor depth, and large tumor size using any of the three cutoff levels (Table 2).

A receiver operating characteristic curve was constructed to assess plasma fibrinogen cutoff levels to differentiate advanced tumors from T1N0 or T2N0 tumors. The best cutoff value was found to be 347 mg/dl, similar to 350 mg/dl (Fig. 1). Based on Table 2, we assessed the differences in frequencies of hyperfibrinogenemia between patients with stage I/II and those with stage III/IV disease. We observed the biggest difference when 350 mg/dl was used as the cutoff value. Based on Fig. 2, we then assessed differences in the prognostic impact of hyperfibrinogenemia. We observed the biggest difference when 50 mg/dl was used as the cutoff value. Therefore, we used 350 mg/dl as the cutoff value for plasma fibrinogen levels for further analyses.

Association between hyperfibrinogenemia, pathological factors, and biomarkers

Various inflammatory mediators were significantly associated with hyperfibrinogenemia (Table 3). The frequencies of hyperfibrinogenemia were significantly associated with the following parameters: large tumors, deep tumors,

Table 1 Comparison of plasma fibrinogen levels according to clinicopathological factors

Variables	Number of patients (total = 315)	Mean ± SD	P value
Gender			
Female	103	332 ± 81	0.279
Male	212	343 ± 89	
Age			
≤65	114	326 ± 88	0.019
>65	201	347 ± 85	
Histology[a]			
Differentiated	163	342 ± 90	0.883
Poorly differentiated	148	336 ± 82	
Tumor[a] size			
≤50 mm	197	321 ± 77	<0.001
>50 mm	103	369 ± 94	
Tumor depth			
T1–2	161	315 ± 75	<0.001
T3–4	154	364 ± 91	
Nodal status			
Negative	174	321 ± 81	<0.001
Positive	141	362 ± 87	
Liver metastasis			
Negative	310	338 ± 86	0.033
Positive	5	423 ± 74	
Peritoneal metastasis			
Negative	303	336 ± 83	0.054
Positive	12	411 ± 126	
Residual tumor			
R0	273	333 ± 83	0.005
R1	42	378 ± 100	
Stage			
Stage I/II	217	327 ± 82	<0.001
Stage III/IV	98	366 ± 91	

P values were calculated by Mann–Whitney *U* test

[a] Unknown cases were not included

positive lymph nodes, high WBC, high CRP, high platelet count, and high APTT (Tables 2, 3). Therefore, we evaluated the contribution of all these seven parameters to hyperfibrinogenemia by multivariate analysis (Table 4). Multivariate analysis revealed that the CRP levels ($P < 0.001$) and platelet count ($P = 0.044$) were independent risk factors for hyperfibrinogenemia.

Prognostic impact of plasma fibrinogen levels

Fifty-nine (90.8 %) of the 65 patients who died by the end of July 2015 succumbed to gastric cancer. Hyperfibrinogenemia, as defined by any of the three cutoff values, was consistently associated with poor patient survival (Fig. 2). Based on the survival curves obtained after dividing the patients into low-risk groups, a plasma fibrinogen level of 350 mg/dl seemed to be the best cutoff value to identify high-risk patients with a poor prognosis (Fig. 2). Univariate analysis revealed that survival was significantly worse in the high fibrinogen level group than in the low fibrinogen level group ($P < 0.001$), and that large tumors (>50 mm), deep tumors (>T3), and lymph node metastasis or peritoneal metastasis were significant prognostic factors for poor overall survival (Table 5). According to the multivariate analysis, not only tumor depth and nodal status, but also hyperfibrinogenemia, were selected as independent poor prognostic factors for patients with gastric adenocarcinoma (Table 5).

Postoperative plasma fibrinogen levels in patients with preoperative hyperfibrinogenemia

Plasma fibrinogen levels were re-examined in 32 of the 122 patients with hyperfibrinogenemia, 6–12 months after surgery. The postoperative plasma fibrinogen levels were significantly lower than the preoperative levels (Fig. 3a; $P < 0.001$). Recurrence developed in 7 of these 32 patients. Figure 3b shows the changing plasma fibrinogen levels between radical surgery and when tumor recurrence was evident in a representative case from among these seven patients. Plasma fibrinogen levels decreased after surgery, but then began increasing after lymph node recurrence was found.

Discussion

The present study found that an increase in plasma fibrinogen levels was associated with tumor progression, high CRP levels, and poor prognosis. Although previous reports have defined hyperfibrinogenemia as a plasma fibrinogen level of 300–400 mg/dl, we defined it as >350 mg/dl. When we used a cutoff level of >350 mg/dl, hyperfibrinogenemia was present in 122 (38.7 %) of the 315 patients. The frequency of hyperfibrinogenemia in the present study was relatively lower than that reported for other solid tumors [2–7], being 55 % for esophageal cancer [2], 50 % for pancreatic cancer [3], 42 % for hepatocellular carcinoma [4], 43 % for breast cancer [5], and 77 % for colon cancer [6]. However, the frequency of hyperfibrinogenemia in gastric cancer was relatively higher than that for prostate cancer (12 %) [7]. This can be partly explained by the lower frequency of advanced tumors in the present series of patients with gastric cancer.

The results of this study identified hyperfibrinogenemia as an independent prognostic indicator of poor patient

Table 2 Frequency of hyperfibrinogenemia according to clinicopathological factors based on three cutoff levels

Variables	Number of patients (total = 315)	Number of patients with hyperfi-brinogenemia (>400 mg/dl) (%)	P value[a]	Number of patients with hyperfi-brinogenemia (>350 mg/dl) (%)	P value[a]	Number of patients with hyperfi-brinogenemia (>300 mg/dl) (%)	P value[a]
Gender							
Female	103	19 (18.4)	0.384	33 (32.0)	0.109	64 (62.1)	0.804
Male	212	50 (23.6)		89 (42.0)		135 (63.7)	
Age							
≤65	114	21 (18.4)	0.321	38 (33.3)	0.150	61 (53.5)	0.011
>65	201	48 (23.9)		84 (41.8)		138 (68.7)	
Histology[b]							
Differentiated	163	38 (23.3)	0.490	64 (39.3)	0.816	101 (62)	0.725
Poorly differenti-ated	148	29 (19.6)		56 (37.8)		95 (64.2)	
Tumor[b] size							
≤50 mm	197	29 (14.7)	<0.001	55 (27.9)	<0.001	111 (56.3)	0.004
>50 mm	103	34 (33)		59 (57.3)		76 (73.8)	
Tumor depth							
T1–2	161	22 (13.7)	<0.001	39 (24.2)	<0.001	85 (52.8)	<0.001
T3–4	154	47 (30.5)		83 (53.9)		114 (74)	
Nodal status							
Negative	174	28 (16.1)	0.006	46 (26.4)	<0.001	95 (54.6)	<0.001
Positive	141	41 (29.1)		76 (53.9)		104 (73.8)	
Liver metastasis							
Negative	310	66 (21.3)	0.072	118 (38.1)	0.076	194 (62.6)	0.162
Positive	5	3 (60)		4 (80)		5 (100)	
P factor							
Negative	302	64 (21.1)	0.145	113 (37.3)	0.066	189 (62.6)	0.546
Positive	12	5 (41.7)		8 (66.7)		9 (75)	
Residual tumor							
R0	273	53 (19.4)	0.009	96 (35.2)	0.001	167 (61.2)	0.085
R1	42	7 (31.8)		12 (54.5)		15 (68.2)	
Stage							
Stage I/II	217	38 (17.5)	0.008	68 (31.3)	<0.001	127 (58.5)	0.012
Stage III/IV	98	31 (31.6)		54 (55.1)		72 (73.5)	

[a] P values were calculated by Fisher's exact probability test

[b] Unknown cases were not included

Fig. 1 Receiver operating characteristic curve of plasma fibrinogen levels to differentiate stage I (T1N0 and T2N0) disease
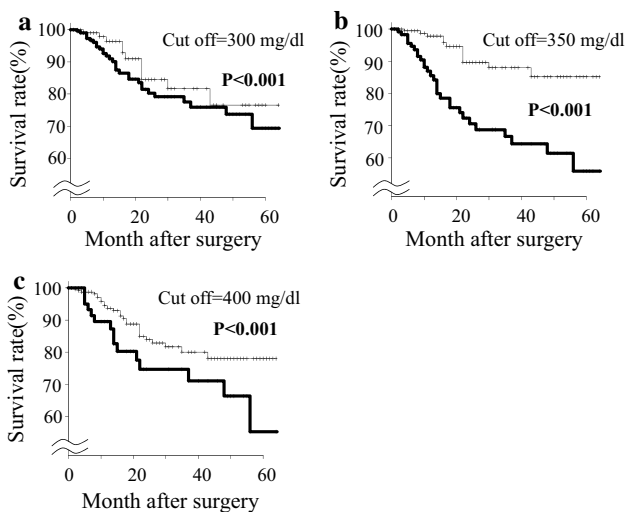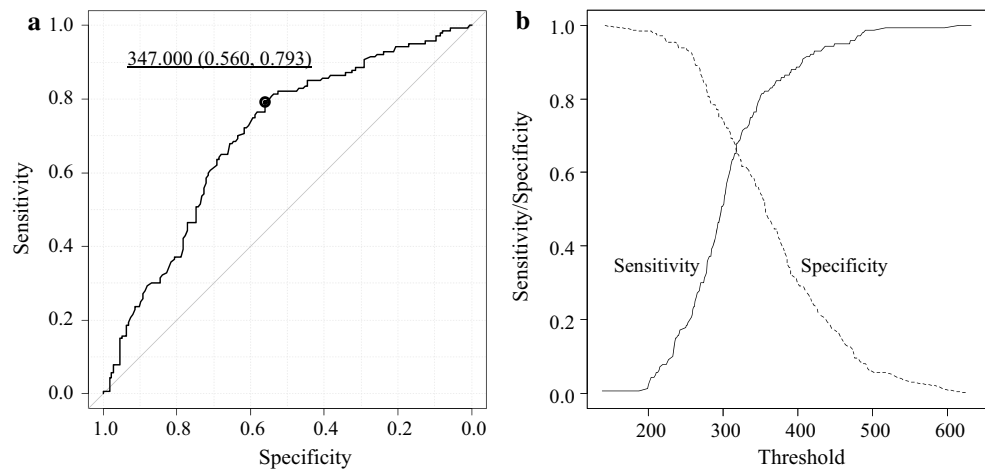

Fig. 2 Survival curves according to various plasma fibrinogen cutoff levels. *Thick lines* represent the survival curves of patients with high plasma fibrinogen levels. *Thin lines* represent the survival curves of patients with low plasma fibrinogen levels

survival when adjusted for tumor stage. Other cutoff values (300 and 400 mg/dl) were not selected as independent prognostic factors (data not shown). Based on the interactions between IL-6, platelets, and cancer cells [16], hyperfibrinogenemia could trigger lymph node metastasis [17].

The pathophysiological mechanism of hyperfibrinogenemia may be secondary to tumor-associated elevations of circulating inflammatory mediators and/or intra-abdominal infectious disease [18, 19]. We also analyzed several other biomarkers such as FDP, D-Dimer, CEA, CA19-9, and CA125 in those parameters lacking many data. FDP and D-Dimer were significantly associated with hyperfibrinogenemia, whereas CEA, CA19-9, and CA125 were not (supplemental Table 1). Because hyperfibrinogenemia is an independent prognostic factor predictive of worse survival, patients with hyperfibrinogenemia may be candidates for neoadjuvant chemotherapy to inhibit tumor recurrence. Of the 122 patients with hyperfibrinogenemia, 76 (62 %) had lymph node metastases. For such patients, D2 lymph node dissection should be done, even if tumors are clinically negative for lymph node metastases. As inflammatory mediators are also associated with prognosis, combinatory assessment could be helpful to identify patients at risk of recurrence even after curative surgery.

Postoperative plasma fibrinogen levels should decrease if cancer cells are a cause of hyperfibrinogenemia. Although plasma fibrinogen levels were monitored only in some patients, we found that the levels decreased significantly after surgery. Although plasma fibrinogen levels after recurrence were monitored only in some patients, we found that the levels increased at the time of diagnosis of recurrent disease. Because postoperative plasma fibrinogen

Table 3 Associations between hyperfibrinogenemia and various biomarkers

Variables	Number of patients (total = 315)	Mean + SD	P value[a]	Number of patients with hyperfibrinogenemia (%)	P value[b]
WBC					
≤9000/μl	298	336 ± 83	0.005	110 (36.9)	0.009
>9000/μl	17	397 ± 116		12 (70.6)	
CRP[c]					
≤0.2 mg/dl	215	308 ± 64	<0.001	49 (22.8)	<0.001
>0.2 mg/dl	98	405 ± 88		72 (73.5)	
Pletelet					
≤400 × 10⁹/l	306	337 ± 86	0.002	114 (37.3)	0.003
>400 × 10⁹/l	9	413 ± 58		8 (88.9)	
PT					
≤14.0 s	312	338 ± 86	0.029	119 (38.1)	0.057
>14.0 s	3	468 ± 68		3 (100)	
APTT[c]					
≤40.0 s	298	336 ± 84	0.005	110 (36.9)	0.006
>40.0 s	15	394 ± 116		11 (73.3)	

[a] P values were calculated by the Mann–Whitney U test

[b] P values were calculated by Fisher's exact probability test

[c] Unknown cases were not included

Table 4 Logistic regression analysis of relationships between hyperfibrinogenemia and various biomarkers

Variables	Odds ratio	95 % CI[a]	Multivariate P value[b]
Tumor size			
≤50 mm	1.580	0.801–3.110	0.188
>50 mm			
Tumor depth			
T1–2	1.480	0.668–3.260	0.335
T3–4			
Nodal status			
Negative	1.940	0.918–4.110	0.083
Positive			
WBC			
≤9000/μl	2.450	0.619–9.680	0.202
>9000/μl			
CRP			
≤0.2 mg/dl	8.350	4.490–15.50	<0.001
>0.2 mg/dl			
Platelet			
≤400 × 10⁹/l	11.200	1.070–117.0	0.044
>400 × 10⁹/l			
APTT			
≤40.0 s	1.650	0.357–7.630	0.521
>40.0 s			

[a] Adjusted 95% confidence interval

[b] P values were calculated by Logistic regression analysis

Table 5 Univariate and multivariate analyses of risk factors for survival

Variables	Univariate P value[a]	H.R.[b]	95 % CI[c]	Multivariate P value[d]
Gender				
Female	0.155	0.627	0.309–1.274	0.197
Male				
Age				
≤65	0.936	0.904	0.434–1.882	0.788
>65				
Tumor size				
≤50 mm	<0.001	0.895	0.426–1.884	0.771
>50 mm				
Tumor depth				
T1–2	<0.001	4.178	1.141–15.30	0.031
T3–4				
Nodal status				
Negative	<0.001	5.183	1.479–18.16	0.01
Positive				
Peritoneal status				
Negative	<0.001	2.502	0.924–6.778	0.071
Positive				
Fibrinogen				
≤350 mg/dl	<0.001	2.607	1.180–5.761	0.018
>350 mg/dl				

[a] Log-rank test

[b] Adjusted hazards ratio

[c] Adjusted 95 % confidence interval

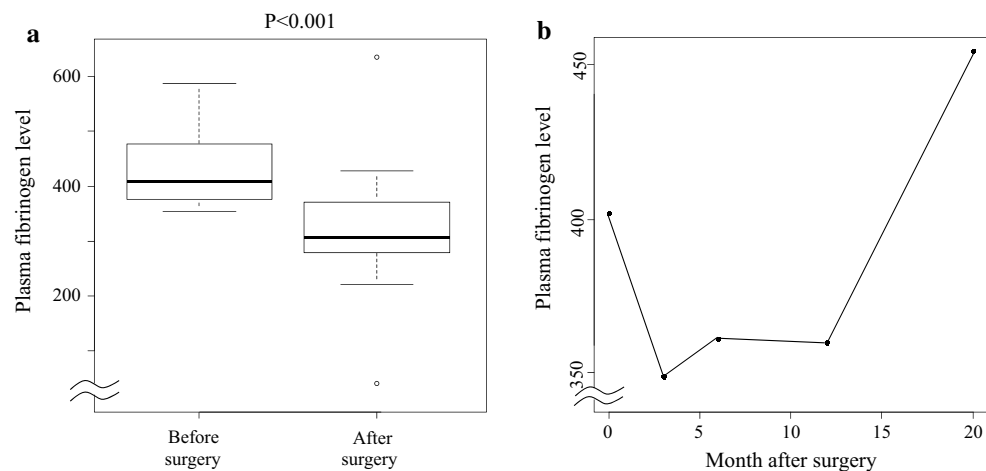[d] Cox proportional hazards model

Fig. 3 Postoperative plasma fibrinogen levels of patients with preoperative hyperfibrinogenemia. **a** Median and standard deviation of plasma fibrinogen levels before and after surgery. **b** Representative pattern of changing plasma fibrinogen levels between surgery and recurrence

levels were available for only some patients to assess this interaction, further analyses are needed to identify underlying mechanisms.

In conclusion, hyperfibrinogenemia was significantly associated with tumor load and overall survival, and our results demonstrate its usefulness as a clinical biomarker. Thus, preoperative assessment of plasma fibrinogen levels may assist in the prediction of tumor stage and overall survival.

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