Title: **Extra-Perigastric Extranodal Metastasis is a Significant Prognostic Factor in Node-Positive Gastric Cancer**

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Abstract (223 words)

Background: Extranodal metastasis is an isolated tumor nodule without a residual lymph node structure and has been reported as a poor prognostic factor in gastric cancer. The aim of this study is to assess the prognostic value of extranodal metastasis, especially from the viewpoint of its anatomical distribution.

Methods: A total of 139 consecutive gastric cancer patients who underwent curative surgery with lymph node metastasis between 2008 and 2009 were included. Clinicopathological features and patient survival outcomes were retrospectively assessed. Patients with extranodal metastasis were sub-divided into two groups: perigastric extranodal metastasis, located near the perigastric area (#1-#7 according to the Japanese classification of gastric carcinoma 15th edition), and extra-perigastric extranodal metastasis, located alongside the major vessels (#8-#12).

Results: Extranodal metastasis was found in 51 patients (37%), and it was more frequent in those with bulky, ≥pT3, and pStage III tumors. All patients with extra-perigastric extranodal metastasis had recurrence, resulting in a 0% 5-year overall survival rate, which was significantly worse than that of patients with perigastric extranodal metastasis (59%), or those without extranodal metastasis (84%; $P<0.001$). Multivariable analysis identified the presence of extra-perigastric extranodal metastasis as an independent poor prognostic factor.

Conclusions: Extranodal metastasis, especially extra-perigastric extranodal metastasis, was a pivotal poor prognostic factor in node-positive gastric cancer. Recognizing extra-perigastric

extranodal metastasis would help provide optimal therapeutic options to these high-risk patients.

Introduction

Gastric cancer is one of the most common cancers in Eastern countries, including Japan, and is one of the leading causes of cancer-related death worldwide [1]. The tumor node metastasis (TNM) classification system, developed by the American Joint Committee on Cancer (AJCC) and Union for International Cancer Control (UICC), is the most widely accepted system to predict the prognosis of gastric cancer and plays an important role in selecting the optimal treatment strategy [2-4]. However, risk stratification is still needed to precisely predict gastric cancer patient outcomes to provide more appropriate clinical management.

Extranodal metastasis (ENM) is a histological term that indicates the presence of an isolated tumor nodule without a residual lymph node structure and was first recognized by W.B. Gabriel et al. in 1935. The clinical significance of ENM had been controversial; however, this "tumor deposit," a synonym of ENM, was recognized to be a significant predictor of a worse prognosis for colorectal cancer and is now included in the TNM staging system [4-6].

In the TNM classification criteria for gastric cancer, extranodal metastasis is described as the presence of tumor nodules in the perigastric fat adjacent to a gastric carcinoma, without any evidence of identifiable lymph node tissue, or vascular or neural structures. Although there have been several reports suggesting that the presence of ENM is an independent predictor of poor prognosis in gastric cancer [7-12], no consensus on ENM management in clinical practice has been reached. Additionally, it remains unclear whether all ENMs have the same clinical impact because ENM can be a result of heterogeneous malignant biological behaviors of gastric cancer, such as the extramural spread of a primary tumor and peritoneal metastasis.

The aim of this study was to assess the prognostic value of ENM, especially from the viewpoint of its anatomical distribution. Here, we report the results of our extensive review of the clinicopathological features of node-positive gastric cancer with ENM using a simple definition.

Patients and Methods

Patients

A total of 452 patients underwent curative gastrectomy for gastric cancer between January 2008 and December 2009 at the National Cancer Center Hospital East. Of these 139 patients had pathologically positive lymph nodes, and were included in this study. Patients with carcinoma in the gastric stump, those with another malignancy, and those with a previous history of chemotherapy were excluded. All clinicopathological data were reviewed retrospectively and analyzed based on the 7th AJCC TNM classification of malignant tumors. The follow-up duration was defined as the time between the operation and the time of the patient's last outpatient clinic visit or death. During the study period, adjuvant chemotherapy with S-1 was administered to patients with pathological stage II/III tumors. This study complied with the standards of the Declaration of Helsinki and current ethical guidelines, and it was approved by the institutional ethics board. The requirement of written informed consent for participation was waived, because this study did not report the findings of a clinical trial, and the study was retrospective in nature, with data analyzed anonymously.

Pathological examination of ENM and the definition of extra-perigastric ENM

All surgically resected gastric carcinoma specimens from each patient were fixed in formalin and macroscopically examined in detail. The tumors were cut at a thickness of 5 mm, embedded in paraffin, and thinly sliced at 4-μm intervals from the paraffin-embedded block. The slices of the primary lesion and one maximum cross section of each lymph node were then stained with hematoxylin and eosin for routine microscopic pathologic examination. Each specimen of the primary lesion and retrieved lymph nodes was reviewed by M.S. and A.T. ENM was defined as microscopic depositions of carcinoma observed around the lymphadenectomy specimens and/or perigastric connective tissue without any residual lymph node structures (**Fig. 1**). The positive/negative status of ENM was recorded, and patients were divided into ENM-positive or ENM-negative groups. In addition, the ENM-positive group was sub-divided into two groups based on the anatomical location of ENM according to the Japanese classification of gastric carcinoma (JCGC) 15th edition. ENM located at perigastric area (#1-#7 according to the JCGC 15th edition) was defined as perigastric ENM (P-ENM). That located alongside the major vessels (#8-#12 according to the JCGC 15th edition) was defined as extra-perigastric ENM (EP-ENM). The morphologic pattern of ENM was sub-classified into a separate nodular pattern, perivascular pattern, lymphatic and endovascular pattern (suggestive of derivation from lymphovascular invasion), or a perineural pattern, based on a previous study.

Statistical analysis

All continuous variables are presented as the median (range), and comparisons between groups were performed using the Mann-Whitney *U* test for continuous and ordinal variables and the Fisher's exact test for categorical variables. The overall survival (OS) rates were calculated from the date of the operation to death from any cause. Cumulative OS curves were estimated using the Kaplan-Meier method, and the survival curves were compared with the log-rank test. To assess the potential prognostic factors, both univariate and multivariable analyses for OS were performed using the Cox proportional hazards model. P values less than 0.05 were considered statistically significant. All statistical analyses were conducted using the JMP Statistics software program version 11 (SAS Institute Inc, Cary, NC).

Results

Patients and tumor characteristics

The clinicopathological characteristics of patients with (n=51) and without (n=88) ENM are listed in **Table 1**. The median number of retrieved lymph nodes and metastatic lymph nodes was 44 (range 15-107) and 4 (range 1-61), respectively. The positive lymph node ratio, which is the ratio of the metastatic lymph node number to the retrieved lymph node number, ranged from 0.01 to 0.77 (median, 0.10). Histological analysis revealed that the proportion of deeper tumor invasion and bulky tumors >80 mm was significantly higher in the ENM-positive

group. The prevalence of pathological N (pN) stage (based on the 7th UICC), lymphatic invasion, and venous invasion did not differ significantly between the two groups.

Of the 51 patients (36.7%) with ENM, 40 patients (28.8%) had P-ENM and 11 patients (8.0%) had EP-ENM; clinicopathological differences between the groups were also assessed (**Table 2**). No significant differences were noted in tumor size, depth of invasion, or the presence of lymphovascular invasion. In contrast, pN stage and the positive lymph node ratio were significantly higher in the EP-ENM group. The prevalence of the morphologic patterns of ENM was similar between these two groups.

Survival analysis

The results of survival analysis are shown in **Figure 2**. The median follow-up duration was 60 months (range, 1-108) for all patients. The 3-year and 5-year overall survival (OS) rates were 54.2% and 45.5%, respectively, in the ENM-positive group, and lower than those (91.8% and 84.4%, respectively) in the ENM-negative group (P<0.001) (**Fig. 2a**).

Survival curves of the ENM-positive group are stratified by the location of ENM (P-ENM or EP-ENM) in (**Fig. 2b)**. The 5y-OS was significantly worse in the EP-ENM group (0%) than in the P-ENM group (59%) and ENM-negative group (84%).

Postoperative recurrence rate was also significantly higher in the P-ENM group (45%) and EP-ENM group (100%) than in the ENM-negative group (16%). The site of recurrence did not differ among the groups (**Electronic supplementary material 1**).

Prognostic factors in univariate and multivariable analysis

The results of univariate and multivariable analyses for OS with all patients (n=139) are shown in **Table 3a.** The univariate analyses revealed that advanced pathological T stage, N stage, bulky tumors, and the presence of ENM were predictors of poor prognosis. Multivariable analyses revealed that ENM (HR, 3.71; 95% CI, 1.89- 7.67; P=0.0002) was independently associated with OS. For the ENM-positive patients (n=51), multivariable analysis demonstrated that EP-ENM (HR, 5.98; 95% CI, 2.71-15.7; P<0.0001) was independently associated with poorer OS **(Table 3b)**.

Discussion

In this study, we not only confirmed that the presence of ENM is a poor prognostic factor in our node-positive gastric cancer cohort, but also revealed that the presence of ENM beyond the perigastric region, a condition we have named extra-perigastric ENM (EP-ENM), is an independent and significant risk factor for poor clinical outcome.

ENM is reportedly related to positive surgical margin or lymph node metastasis in patients with colorectal cancer, contributing to poor survival outcomes [5,6]. Poor survival outcomes following gastrectomy in patients with ENM have also been reported [7-13], but the contributing factors might be different from those for colorectal cancer. ENM in gastric

cancer may include peritoneal metastasis, which may be one reason that the presence of ENM leads to poor prognosis [8].

Gastric cancer with EP-ENM had a critical negative impact on OS, compared to that with P-ENM. Initially, we thought that the etiology might differ between EP- and P-ENM and considered that EP-ENMs were peritoneal metastases. However, unexpectedly, the incidence of peritoneal recurrence did not differ (**Electronic supplementary material 1**), and the results of histological analysis of the morphological pattern were similar, between the EP-ENM and P-ENM groups (**Table 2**), indicating a low possibility that EP-ENM was associated with peritoneal metastasis. However, there was a significant correlation between the presence of EP-ENM and higher pN stage (UICC 7th); therefore, the presence of EP-ENM may reflect the aggressive lymphatic tumor spread.

Our new finding with regard to EP-ENM is important as it clearly demonstrates the insidious heterogeneity of the risk of ENM. Previously, Etoh et al. stratified patients with ENM according to the number of ENM, and demonstrated that it affects survival outcomes. In our study, survival outcome was also worse in patients with multiple ENM (5y OS; 33.5%) than in those with single ENM (5y OS, 68.8%; p=0.0128) (**Electronic supplementary material 2**). However, the number of ENM was affected by the number of retrieved lymph nodes. In addition, ENM sometimes infiltrates into adjacent fat tissue and could merge with another ENM, which makes it difficult to count the number exactly. Therefore, we focused on the location of ENM rather than the number of ENM, and the anatomical location of ENM was

adopted for the risk stratification model. This approach revealed that EP-ENM affects survival outcomes to a greater degree than P-ENM. Moreover, we should also emphasize that the recognition of this critical prognostic factor requires no additional processes beyond routine microscopic examination.

In our multivariable analyses, pT stage and pN stages that are generally common prognostic factors, were not revealed as the independent poor prognostic factors. In this limited patient group with positive lymph node metastases, this would imply that the presence of ENM has greater impact on the poor prognosis than pT or pN stage. Similarly, in the ENM positive patient group, pT or pN stage was not found to be the prognostic indicator, especially compared to the EP-ENM group. Taking it into consideration that the 5-year OS was 0%, the presence of ENM, especially EP-ENM, would be useful for identifying patients with quite unfavorable prognosis.

Gastrectomy followed by adjuvant chemotherapy has been a standard treatment for locally advanced gastric cancer in Japan, and therefore, post-operative risk stratification based on pathological diagnosis is indispensable to enable the selection of optimal treatment strategies [13-17]. As we have demonstrated here, the prognosis of patients with EP-ENM, regardless of their pathological state, was identical to that of those with N3b or even close to that those with of M1 disease [18]. Therefore, EP-ENM could be integrated into future TNM classification systems, and patients with EP-ENM should be classified to have N3b irrespective of actual number of positive lymph nodes. The novel prognostic factor reported here can help stratify

the currently underestimated risk of these patients and can contribute to improving their outcomes by providing appropriate multidisciplinary therapeutic options that address their high risk levels.

There are several limitations to this study. First, these data were retrospectively analyzed in a single institution, and the number of the targeted patients was relatively small. The result of this study should be validated in the future, preferably via a multicenter prospective study. Second, the definition of ENM has not been precisely determined with regard to various morphologic patterns; therefore, the definition used for the present study included all morphologic types of ENM. However, simple definitions could avoid interobserver difference among pathologists, and we therefore believe this can lead to high reproducibility.

In conclusion, we have confirmed ENM to be a poor prognostic factor in node-positive gastric cancer and have identified a subpopulation of ENM, EP-ENM, which is associated with critically worse clinical outcomes. To the best of our knowledge, this is the first study to have successfully demonstrate that the location of ENM has a strong effect on prognosis. As EP-ENM is easily recognized in routine pathological practice, we hope these findings will be widely used to stratify the risk of the patients and consequently improve the prognosis of these highly advanced diseases.

Human rights statement and informed consent:

This study complied with the standards of the Declaration of Helsinki and current ethical guidelines and was approved by the institutional ethics board. Written informed consent for

participation in this study was not obtained from the patients, because this study did not report on a clinical trial and the data were retrospective in nature and analyzed anonymously.

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Figure legends

Figure 1. Histology of ENMs. Tumor cell aggregate without lymph node structure of a) differentiated and b) undifferentiated cancer cells, respectively (x40). Scale bar 1mm

Figure 2. Kaplan-Meier estimates of overall survival after curative gastrectomy in patients a) with ENM vs without ENM, and b) with perigastric ENM vs with extra-perigastric ENM (P<0.001, log-rank test).

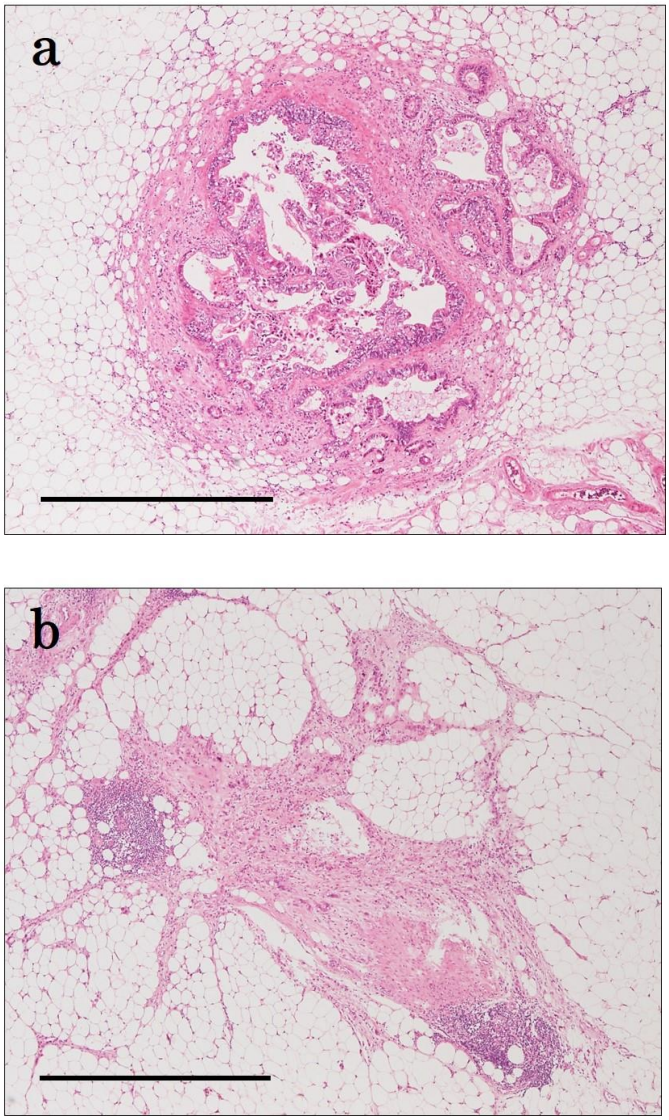
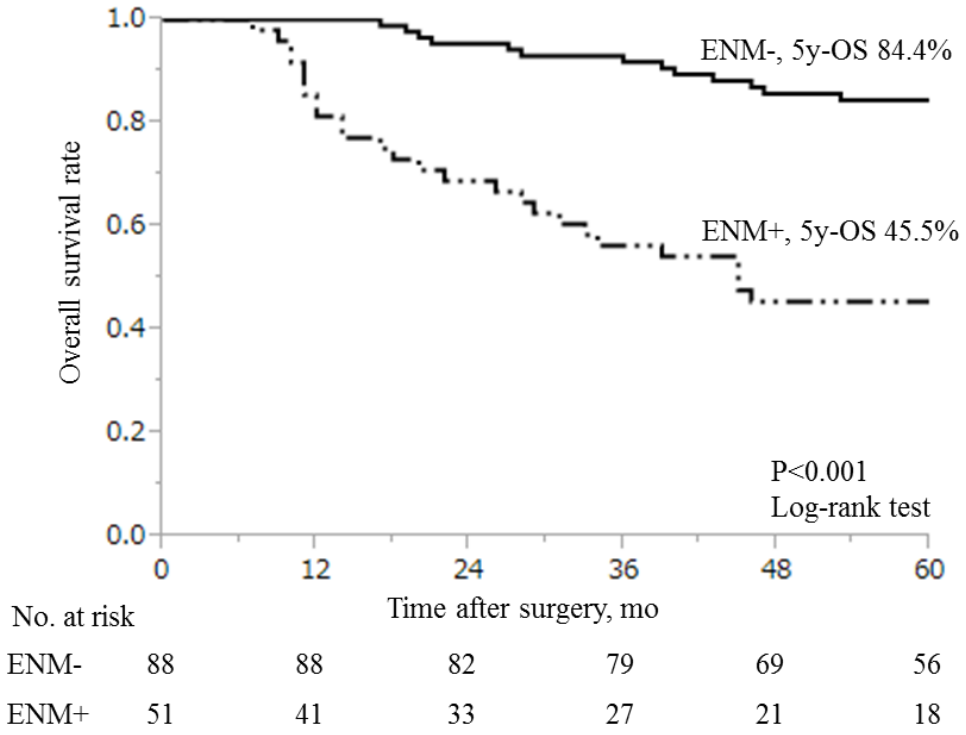
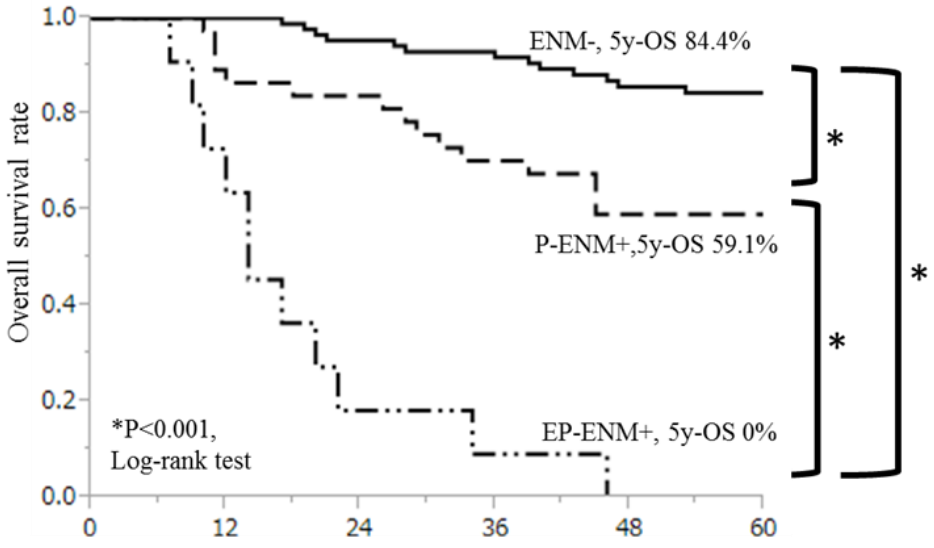


Figure 1.



No. at risk						
ENM-	88	88	82	79	69	56
ENM+	51	41	33	27	21	18

Figure 2a



No. at risk						
ENM -	88	88	82	79	69	56
P-ENM	40	33	31	26	21	18
EP-ENM	11	8	2	1	0	0

Figure 2b

Table 1. Clinicopathological characteristics of patientswith and without ENM

Variables	ENM-positive (n=51)	ENM-negative (n=88)	P value
Age (years) median (range)	65 (36-86)	68 (39-86)	0.57
Sex Male Female	31 20	57 31	0.64
Tumor size < 80mm ≥ 80mm	31 20	72 16	0.006
Macroscopic type Type 0-2 Type 3, 4	18 33	46 42	0.053
Depth of invasion pT1 pT2 pT3 pT4a/T4b	2 3 12 31/3	19 16 29 21/3	<0.001
LN metastasis (UICC 7th) pN1 pN2 pN3a pN3b	7 12 19 13	42 27 17 2	0.23
Histology Differentiated type Undifferentiated type Mucinous type	20 27 4	42 42 4	0.52
Location Upper third Middle third Lower third Entire	15 22 13 1	20 31 36 1	0.33
Lymphatic invasion Absent Present	5 46	13 75	0.40
Venous invasion Absent Present	7 44	21 67	0.15
Lymph node ratio median (range)	0.19 (0.03-0.73)	0.06 (0.01-0.77)	<0.001
Morphological features Separate nodular pattern Perivascular pattern Perineural pattern Lymphatic pattern Endovascular pattern	36 22 1 9 13	- - - - -	

Table 2. Clinicopathological characteristics of patients with P-ENM and EP-ENM

Variables	P-ENM (n=40)	EP-ENM (n=11)	P value
Tumor size			0.06
< 80mm	27	4	
≥ 80mm	13	7	
Macroscopic type			0.53
Type 0-2	15	3	
Type 3, 4	25	8	
Depth of invasion			0.22
pT1	2	0	
pT2	3	0	
pT3	9	3	
pT4a/T4b	24/2	7/1	
LN metastasis (UICC 7th)			0.029
pN1	7	0	
pN2	11	1	
pN3a/3b	16/6	3/7	
Histology			0.062
Differentiated type	19	1	
Undifferentiated type	18	8	
Mucinous type	3	2	
Lymphatic invasion			0.22
Absent	5	0	
Present	35	11	
Venous invasion			0.61
Absent	6	1	
Present	34	10	
Lymph node ratio			0.018
median (range)	0.15 (0.03-0.54)	0.31 (0.11-0.73)	
Morphological features			0.65
Separate nodular pattern	26	10	
Perivascular pattern	14	8	
Perineural pattern	1	0	
Lymphatic pattern	8	1	
Endovascular pattern	9	4	
Number of ENM			0.008
Single	17	0	
Multiple	23	11	

Table 3. Cox regression analysis of prognostic factors for overall survival in all patients (n=139, Table 3a) and in ENM-positive patients (n=51; Table 3b).

Table 3a.

Parameters	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Age (years) ≥ 65 vs < 65	0.82 (0.45-1.53)	0.54		
Sex Male vs Female	1.15 (0.62-2.26)	0.66		
Tumor size ≥ 80mm vs <80mm	**2.35 (1.26-4.31)**	**0.008**	1.39 (0.72-2.66)	0.33
Macroscopic type Type 3,4 vs Type 0-2	1.30 (0.70-2.44)	0.41		
pT stage T4 vs T1-3	**2.57 (1.39-4.84)**	**0.024**	1.28 (0.66-2.55)	0.47
pN stage (UICC 7th) N2-3 vs N1	**4.08 (1.85-10.8)**	**<0.01**	2.18 (0.92-6.03)	0.078
ENM-positive vs ENM-negative	**5.06 (2.71-9.92)**	**<0.01**	**3.71 (1.89-7.67)**	**0.0002**

Table 3b.

Parameters	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Age (years) ≥ 65 vs < 65	1.05 (0.49-2.22)	0.90		
Sex Male vs Female	1.08 (0.51-2.44)	0.84		
Tumor size ≥ 80mm vs <80mm	1.43 (0.67-3.01)	0.35		
Macroscopic type Type 3,4 vs Type 0-2	1.30 (0.70-2.44)	0.57		
pT stage T4 vs T1-3	1.47 (0.67-3.56)	0.34	1.47 (0.67-3.55)	0.35
pN stage (UICC 7th) N2-3 vs N1	1.10 (0.43-3.76)	0.85	1.08 (0.41-3.69)	0.88
EP-ENM vs P-ENM	**6.01 (2.58-13.6)**	**<0.01**	**5.98 (2.71-15.7)**	**<0.0001**

Electronic supplementary material 1. Recurrence patterns in the ENM-negative, P-ENM, and EP-ENM groups.

	ENM- (n=88)	P-ENM+ (n=40)	EP-ENM+ (n=11)
No. of patients with recurrence*	14 (16%)	18 (45%)	11 (100%)
Peritoneal dissemination	5	6	6
Hematogenous	6	10	6
Lymphatic	6	4	1
Local	0	1	0
Others, unknown	0	1	1

*with duplication

Follow-up period (median, 60 month; range: 1-108 months)

Electronic supplementary material 2. Kaplan-Meier estimates of overall survival comparing patients with single ENM vs multiple ENM, (P=0.0128, log-rank test).