

Lymph Node Metastasis Density (ND)-factor Association with Malignant Degree and ND40 as “Non-curative Factor” in Gastric Cancer

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Abstract. *Advanced gastric cancer is not controlled by surgery alone, although early gastric cancer can be cured by curative gastrectomy. The TNM factor, representing physical tumor spread, accurately predicts patient prognosis in advanced gastric cancer, however, more elaborate prognostic factors reflecting the malignant degree are necessary in order to determine the therapeutic target. The purpose of this study was to select a significant prognostic indicator of gastric cancer with curative intent, independent of TNM stage among daily-feasible clinical parameters. The current study included 382 patients with stage IB to III gastric cancer after curative surgery, in which the robust univariate prognostic predictors were age ($p < 0.0001$), lymph node metastasis density of 40% or over (ND40) ($p < 0.0001$), and preoperative high value of carcinoembryonic antigen (CEA) (preCEA) ($p = 0.0012$), as well as TNM stage, preoperative high value of CA19-9 (preCA19-9), a glyco-chain antigen recognized by monoclonal antibody NS19-9, and vascular invasion. In multivariate prognostic analysis, age ($p = 0.0002$), ND40 ($p = 0.02$), and preCEA ($p = 0.03$) remained independent prognostic factors. The ND-factor provided excellent prognostic stratification within the same stage, and stage III ND40 predicted a similar prognosis to stage IV and could be considered a non-curative factor. Interestingly, ND40 relevance was validated in the prospective and independent set of gastric cancer patients ($p = 0.002$). Conclusion: The ND factor reflects the malignant degree of gastric cancer, and ND40 is a novel “non-curative factor” in gastric cancer patients. A search for the molecules*

associated with higher ND value within an identical stage could lead to objective determination of the degree of malignancy and development of novel strategies against advanced gastric cancer.

The incidence and mortality of gastric cancer have fallen dramatically even outside Japan over the past several decades (1). Nonetheless, gastric cancer remains a major public health issue as the fourth most common cancer and the second leading cause of cancer death worldwide because of the defective strategy against advanced gastric cancer (2). Surgeons and oncologists must accurately assess the chance of operative success (3), and more sophisticated classification of the prognosis than the classical TNM stage by the JCGC (Japanese Classification of Gastric Cancer) and the UICC (Union International Contre Le Cancer) is required for the development of a clinical nanogram (4, 5). Such prognostic prediction by using parameters other than the TNM factor could lead to the development of a more efficient treatment, and the identification of such a clinical factor could lead to the determination of target molecules linked to the prognostic phenotype.

Stage IV disease is an excellent model for analyzing the biological character of gastric cancer, because no accepted treatment has provided notable improvement on patient survival of stage IV disease and only a modest prognosis is expected. We recently determined a striking prognostic predictor, the lymph node metastasis density (ND)-factor in stage IV gastric cancer (6) and esophageal squamous cell carcinoma (7). In this current study, the ND-factor was adopted as a clinical parameter in advanced gastric cancer treated with curative intent, and its clinical significance was evaluated with other known prognostic factors.

Patients and Methods

Retrospective registration of patients. A total of 1,039 patients who had undergone gastrectomy at the Kitasato University Hospital between January 1, 1990 and January 31, 2000, and had been followed-up over 5 years were registered into a retrospective

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Key Words: Gastric cancer, ND-factor, prognosis, non-curative factor.

database. From these, 451 patients with pathological stage IB to III gastric cancer who had undergone radical gastrectomy with D2 or D3 lymphadenectomy were identified. The 35 cases with double carcinomas (gastric and colorectal) and the 15 cases with operative death or death from other diseases were excluded from this analysis. Out of the remaining 401 cases, 382 gastric carcinomas with stage IB (n=169), II (n=106), and III (n=107; A 66 cases, B 41 cases) had data for preoperative carcinoembryonic antigen (CEA) value (preCEA), preoperative CA19-9, a glyco-chain antigen recognized by monoclonal antibody NS19-9, value (preCA19-9) and lymph node metastasis density (ND)-factor, as well as other basic clinical parameters. The ND-factor was registered, if the extracted number of lymph nodes was beyond 7, as in the previous study (6). The number of resected lymph nodes ranged from 9 to 127. The patient demographics, tumor characteristics, and postoperative course were recorded and analyzed. The tumor stage was classified according to the 13th JCGC staging system, because it was more suitable for evaluating the prognosis of Japanese patients with gastric cancer than the UICC classification (8-10).

Prospective registration and characteristics of recent patients with stage III gastric cancer. Between January 1, 2001 and March 31, 2004, 110 sporadic gastric cancer patients were enrolled and were eligible for this prospective study at Kitasato University Hospital. Patients were eligible if they had undergone curative surgery of histologically proven gastric adenocarcinoma with pathological stage III in the JCGC. Other eligibility criteria included age of 20 to 75 years; life expectancy >3 months, the absence of prior chemo-immunotherapy or radiotherapy and the absence of severe liver dysfunction, heart failure, renal dysfunction or other severe systemic complications. If adjuvant therapy (ADT) was performed, it had to start within 7 weeks after surgery. Most of the ADT regimens consisted of TS1-based (54 cases: 73%) or UFT-based regimens (10 cases: 14%) of at least 1 month or 1 cycle, both of which are 5-FU-based anticancer drugs administered orally. TS-1 standard protocol was repeated in cycles of 4-week administration and 2-week discontinuation, while UFT was taken repeated cycles of 5-day administration and 2-day discontinuation. Patients not fitting the above ADT criteria were excluded from the group with ADT, which, as a result, consisted of 74 patients (67%). All the patients were followed-up for at least 2 years (2 years to 5 years) or until death, and patients alive at 5 years were censored at 5 years after surgery.

Statistical analysis. Statistical computations were performed using SAS software package (SAS Institute, Cary, NC, USA), StatView version 5.0. A result was considered statistically significant when the *p*-value was <5% (*p*<0.05). The time of follow-up was calculated from the date of the first operation. Disease-specific survival (DSS) was estimated according to the Kaplan-Meier method and compared using the log-rank test (11, 12). A multivariate logistic model was built using the variables that had prognostic potential suggested by the univariate analysis (*p*<0.1).

Results

Univariate prognostic factors from the retrospective data of stage IB to III gastric cancer. The characteristics of the 382 patients included in this study are displayed in Table I. The average age of the patients was 59 years (range, 21 to 86

Table I. Distribution of clinical and pathological factors and univariate analysis of prognostic factors in 382 patients undergoing radical gastrectomy of stage IB to III gastric cancer.

Variable	No. of patients	%	DSS	
			Average survival (months)	<i>P</i> *
Gender				
Male	263	69	46	NS
Female	119	31	45	
Age, years				
<60	174	46	51	<0.0001
> or =60	208	54	41	
Tumor position				
Upper	175	46	44	NS
Middle	110	29	48	
Lower	97	25	45	
Differentiation				
Poor	224	59	44	NS
Other	158	41	47	
Lymphatic invasion				
Present	371	97	45	NS
Absent	11	3	43	
Vascular invasion				
Present	344	90	44	0.015
Absent	38	10	52	
ND (%)				
> or = 40	21	5	17	<0.0001
<40	361	95	47	
Stage				
IB	171	45	54	<0.0001
II	104	27	48	
III (A, B)	107	28	29	
Preoperative CEA				
> or = 2.5	51	13	36	0.0012
< 2.5	331	87	47	
Preoperative CA19-9				
> or = 37	39	10	37	0.0027
<37	343	90	46	

DSS, disease-specific survival; NS, not significant; ND, lymph node metastasis density; CEA, carcinoembryonic antigen; CA19-9, glyco-antigen recognized by monoclonal antibody NS19-9. *Log-rank test.

years). The average follow-up for all patients was 45 months, with a range of 0 to 60 months. Stage, age, ND40, preCEA, preCA19-9, and vascular invasion were significantly associated with a poor outcome by the univariate analysis (Table I and Figure 1). The average number of resected lymph nodes is shown in Figure 2. In the current analysis, the T factor and N factor were not included, because the JCGC TNM stage was determined by these two factors, and our aim was to reveal prognostic factors independent of the TNM stage. Excellent stratification of prognosis according to ND-factor was found in stage II (Supplemental Figure 1A) and stage III (Figure 3A). ND40 (lymph node metastasis

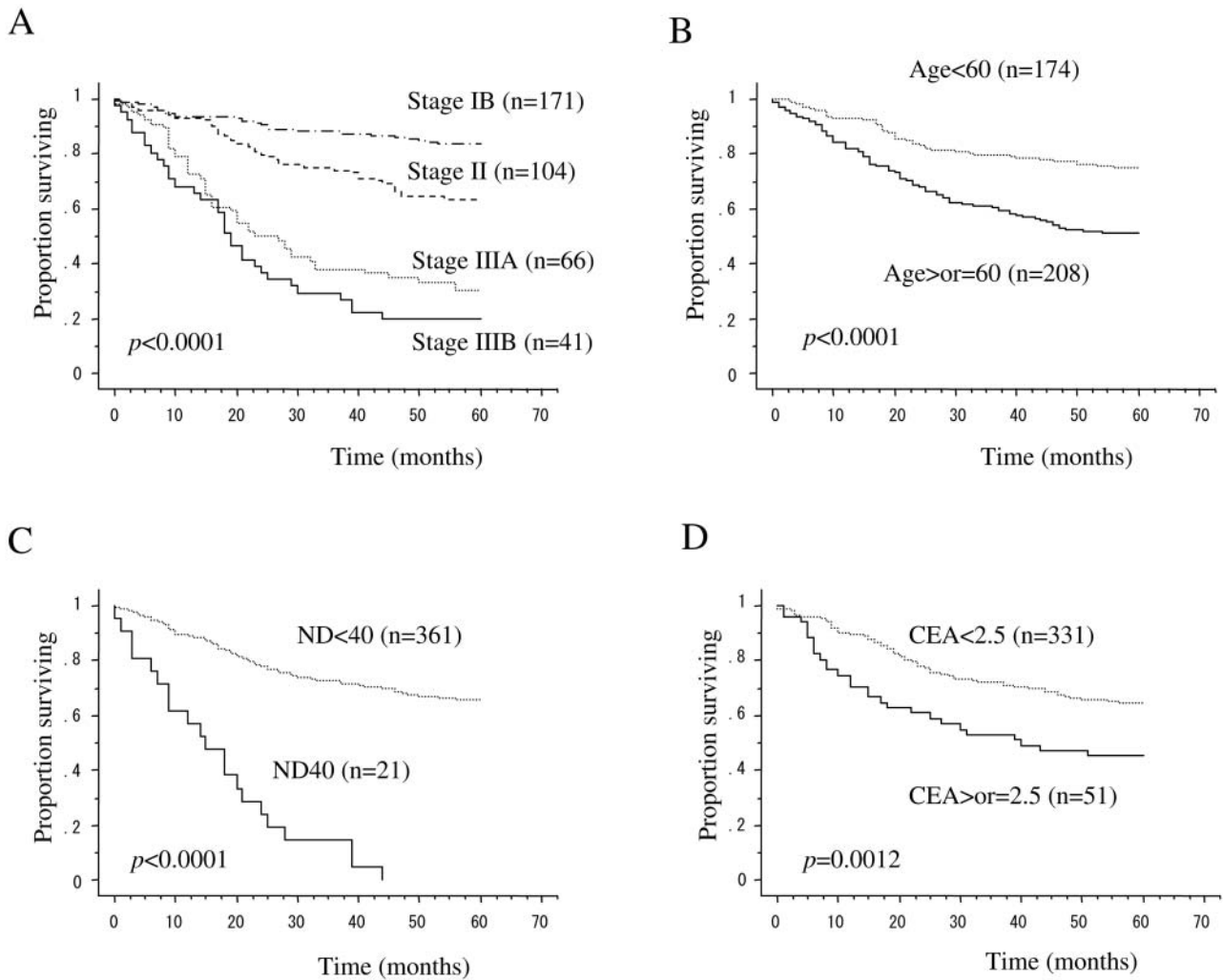


Figure 1. Disease-specific survival of gastric cancer patients after gastrectomy by Kaplan-Meier analysis. (A) JCGC staging system; (B) age, (C) lymph node metastasis density (ND) factor, and (D) preoperative CEA value.

density of 40% or over) could predict non-curability in stage III patients (Figure 3A). Note, patients with ND40 showed a similar prognosis to those with ND30, however, patients with ND40 all died within 5 years after surgery. The patients with stage III ND40 showed a similar prognosis to stage IV patients (Figure 3B). There was no case with ND40 in either stage IB or II, and the ND-factor was shown to be clearly proportional to stage (Figure 4).

Independent prognostic factors from the retrospective data by multivariate analysis. All factors that had prognostic potential as suggested by univariate analysis ($p < 0.1$) were included. The multivariate analysis defined TNM stage, age, preCEA and ND40 as independent factors in curable stage IB to stage III gastric cancer (Table II). Both preCA19-9 and vascular invasion were eliminated after multivariate analysis.

Prognostic significance of ND40 in stage III gastric cancer, and association of ND-factor and stage. As stated earlier, ND40 was not found in either stage IB or stage II. The clinical significance of ND40 in stage III gastric cancer after radical gastrectomy with lymphadenectomy was investigated. Univariate prognostic analysis revealed that ND40 was the striking prognostic factor in addition to age in stage III gastric cancer (Table III). Multivariate analysis revealed that age, ND40, and preCEA were stronger independent prognostic factors than classical AB staging in stage III gastric cancer (Table IV).

Validation of the independent prognostic factors in stage III gastric cancer. The 5-year DSS rate was 61.5 % in 110 prospective follow-up cases with stage III gastric cancer (Figure 5A). ND40 reproducibly showed significantly worse

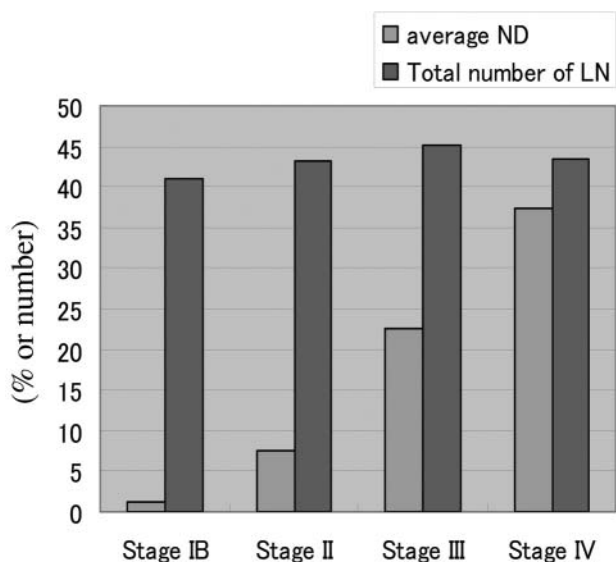


Figure 2. Distribution of average lymph node metastasis density (ND) (%) (blue boxes) and total number of resected lymph nodes (LN) (red boxes) according to each stage.

DSS ($p=0.002$, Figure 5C), although preCEA and age did not show a significant association with prognosis in this setting. The elderly patients showed a marginal association with poor outcome ($p=0.06$), however preCEA showed a rather inverse association with prognosis (while not significant).

Discussion

The ND-factor has been demonstrated to be useful as a prognostic factor in gastric cancer (6, 13-16), but this was the first multivariate validation of its independency among other clinically relevant parameters in gastric cancer treated with curative intent. Interestingly, the ND-factor was closely proportional to stage (Figure 4), and even within the same stage, it could accurately predict the prognosis (stage III, Figure 3A and stage II, Supplemental Figure 1C).

ND40 was used as the optimal cut-off, since ND50, which could also be a non-curative factor, was so stringent in stage III gastric cancer that it resulted in a relatively small number of patients in the group (11/107: 10.3%) as compared with those with ND40 (20%) (Figure 4). On the other hand, ND30 was used as the cut-off, it could be recognized in a portion of stage IB and stage II patients (Figure 3), and would no longer be a non-curative factor (Figure 2A). As a prognostic factor, ND20 resulted in excellent stratification of stage III gastric cancer ($p=0.0001$, Supplemental Figure 1B) and stage II gastric cancer ($p=0.015$, Supplemental Figure 1C). Interestingly, stage II ND20 patients showed a similar prognosis to stage III patients with ND<20 (Supplemental Figure 1D) and as did

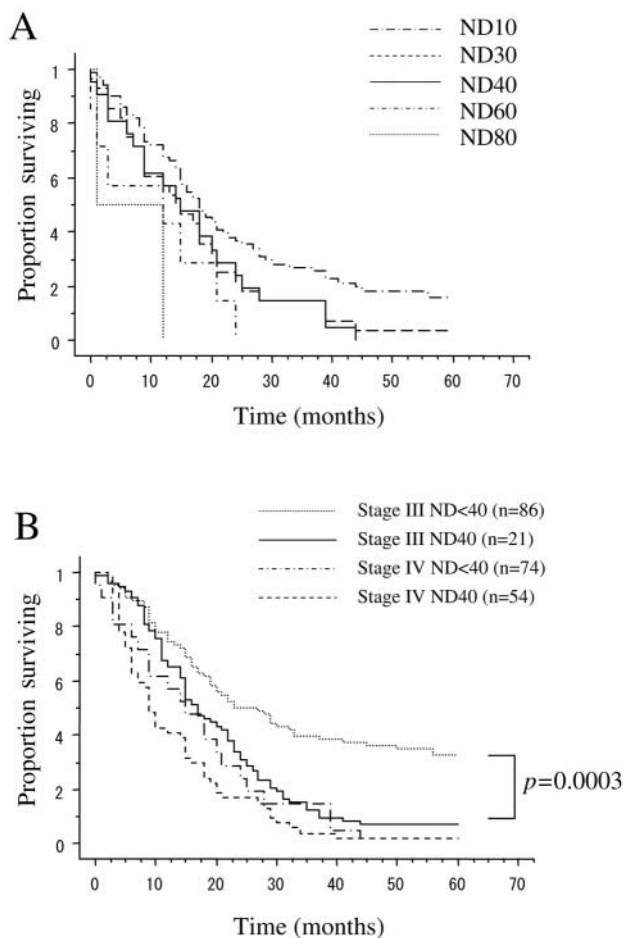


Figure 3. Prognostic analysis of stage III gastric cancer patients after curative surgery. (A) Prognostic stratification according to ND values. (B) Comparison with Stage IV patients by ND40 status.

stage III ND40 to stage IV. Thus a higher ND value represented a higher malignant degree even in gastric carcinomas with identical stages.

In this study, we identified ND40 as a novel non-curative factor in gastric cancer, and this finding was validated in our independent prospective analysis (Figure 5). Curability is the supreme criterion for surgery, and a non-curable factor is a critical determinant for the selection of operative patients. However, the ND-factor is a pathological factor, and cannot be determined preoperatively. There is thus a need to develop prediction methods for higher ND value in order to apply this result to the actual selection of patients for surgery. Either an imaging system such as positron emission tomography (PET) (17, 18) or molecular biology (19) may reach such a goal in the near future.

Another interesting finding of our current study was age as the most potent independent prognostic factor in gastric cancer patients after radical surgery. This has also

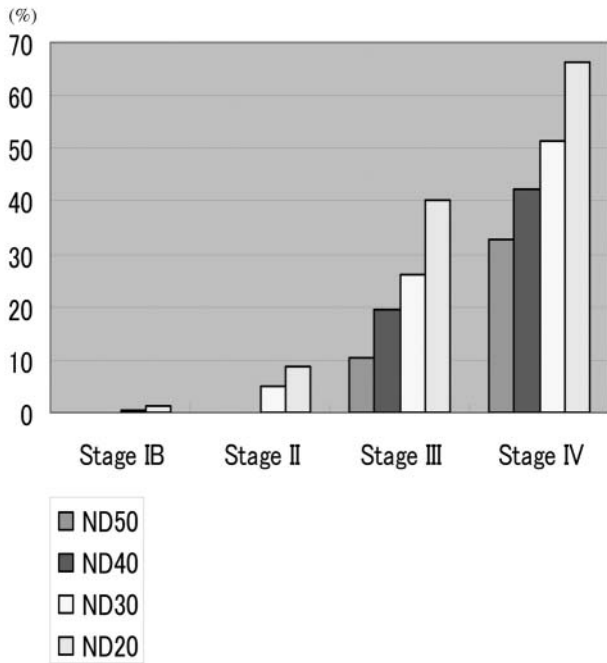


Figure 4. Relation of ND factor to JCGC stage.

Table II. Multivariate analysis of factors associated with disease-specific survival in curable stage IB to III gastric cancer.

Factor	Multivariate analysis		
	RR	95% CI	P-value
TNM stage IB	7.27	4.00-13.20	<0.0001
TNM stage II	3.38	1.92-5.95	<0.0001
Age	2.02	1.40-2.90	0.0002
Preoperative CEA	1.72	1.09-2.70	0.02
ND40	2.05	1.09-3.83	0.03
Preoperative CA19-9	1.18	0.72-1.95	NS
Vascular invasion	1.1	0.54-2.23	NS

RR, relative risk; CI, confidence interval.

recently been noticed by other groups (20-24). Among them, Saito *et al.* demonstrated that age was closely associated with limited lymph node dissection (D0, D1 vs. D2, D3), limited chemotherapy, more differentiated histology and vascular permeation, nevertheless it could be a potent independent prognostic factor in gastric cancer after curative surgery (24). The patients in the present study had all undergone by at least D2 dissection. Hence, worse prognosis in the elderly persons was unlikely to have been affected by the surgical treatment.

Table III. Distribution of clinical and pathological factors and univariate analysis of prognostic factors in 107 patients who had undergone radical gastrectomy of stage III gastric cancer.

Variable	No. of patients	%	DSS	
			Average survival (months)	P*
Gender				
Male	65	61	29	NS
Female	42	39	29	
Age, years				
<60	42	39	38	<0.0001
> or =60	65	61	23	
Tumor position				
Upper	57	53	31	NS
Middle	18	17	25	
Lower	32	30	29	
Differentiation				
Poor	224	59	44	NS
Other	158	41	47	
Lymphatic invasion				
Present	107	100	29	-
Absent	0	0	-	
Vascular invasion				
Present	104	97	29	NS
Absent	3	3	43	
T factor				
pT2	39	36	29	NS
pT3/pT4	68	64	29	
N factor				
n0	5	5	40	NS
n1	31	29	31	
n2	71	66	27	
ND (%)				
> or = 40	21	20	17	0.0003
<40	86	80	32	
Stage				
IIIA	66	62	32	NS (0.1)
IIIB	41	38	25	
CEA				
> or = 2.5	19	18	21	NS (0.07)
<2.5	88	82	31	
CA19-9				
> or = 37	39	10	29	NS
<37	343	90	29	

DSS, disease-specific survival; NS, not significant; ND, lymph node metastasis density; CEA, carcinoembryonic antigen; CA19-9, glyco-antigen recognized by monoclonal antibody NS19-9. *Log-rank test.

Recently, the hypothesis that innate and adaptive immune function decline with age has become well accepted, and it is presumed to be associated with increased susceptibility to cancer (25, 26). Such a decline in immunity might be involved in the worse prognosis in the elderly patients even after curative surgery.

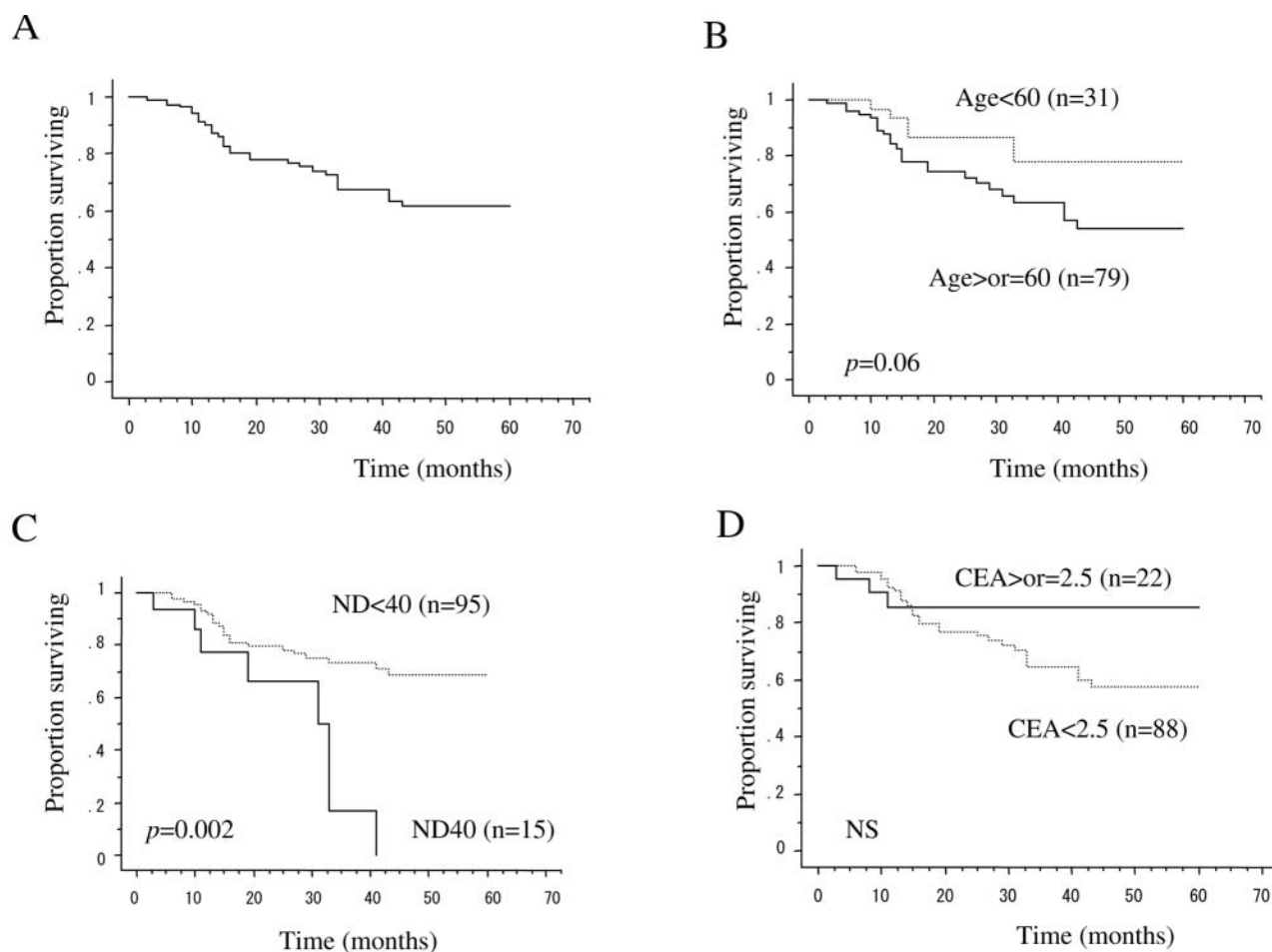


Figure 5. Disease-specific survival of gastric cancer after gastrectomy by Kaplan-Meier analysis. (A) 110 cases with stage III gastric cancer, (B) age, (C) ND-factor and (D) preCEA.

Table IV. Multivariate analysis of factors associated with disease-specific survival in curable stage III gastric cancer.

	Multivariate analysis		
	RR	95% CI	P-value
Age	2.05	1.31-3.19	0.0016
ND40	1.95	1.15-3.31	0.013
CEA	2.02	1.14-3.56	0.015
Stage (A or B)	1.23	0.78-1.94	NS

RR, relative risk; CI, confidence interval; ND, lymph node metastasis density; CEA, carcinoembryonic antigen.

In conclusion, ND40 is as a novel non-curative factor in stage IB to III gastric cancer after radical surgery, and more importantly the ND-factor could represent the malignant degree even within identical stages of gastric cancer. Application of the ND-factor in addition to TNM stage

would enable more precise stratification of gastric cancer patients' prognosis, and could have potential as a clinical tool for patient counseling, in follow-up scheduling and in clinical trial eligibility. The search for molecules involved in higher ND values of the identical stage disease may lead to the objective determination of the degree of lymph node metastasis and aid the development of new approaches for treating advanced gastric cancer.

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Received August 23, 2007
Revised November 1, 2007
Accepted November 29, 2007