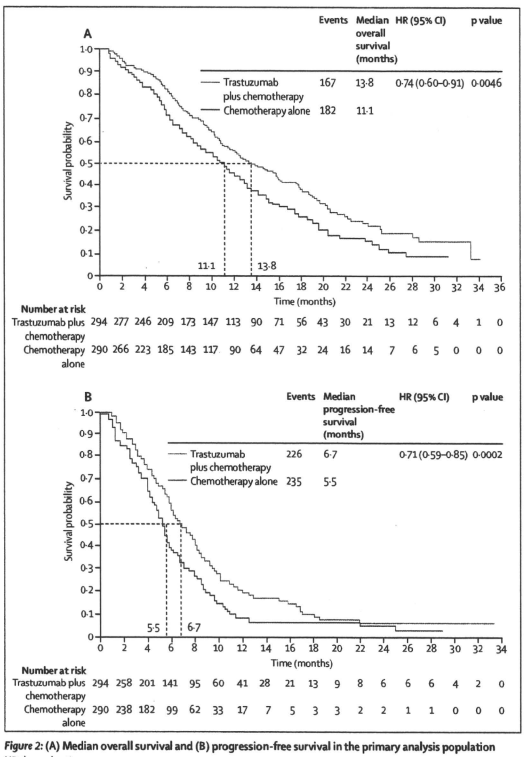

Figure 2: **(A) Median overall survival and (B) progression-free survival in the primary analysis population**
HR=hazard ratio.

Procedures

Chemotherapy was given every 3 weeks for six cycles. Capecitabine 1000 mg/m² was given orally twice a day for 14 days followed by a 1-week rest, or fluorouracil 800 mg/m² per day was given by continuous intravenous infusion on days 1–5 of each cycle. Cisplatin 80 mg/m² on day 1 was given by intravenous infusion. Trastuzumab was given by intravenous infusion at a dose of 8 mg/kg on day 1 of the first cycle, followed by 6 mg/kg every 3 weeks until disease progression, unacceptable toxicity, or withdrawal of consent. Chemotherapy dose adjustments were allowed. Trastuzumab toxicity was managed by treatment interruptions. Crossover to trastuzumab at the time of disease progression was not allowed.

The primary endpoint was overall survival, defined as time from randomisation until death from any cause. Secondary endpoints included progression-free survival, time to progression, overall tumour response rate, duration of response, and safety. LVEF assessments were done at baseline and at least every 12 weeks. Patients were followed up until death, loss to follow-up, or end of study. Efficacy and safety data were monitored by an independent data monitoring committee. Adverse events were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 3.0 and serious adverse events according to International Conference on Harmonisation guidelines. Two interim efficacy analyses were planned, at 50% and 75% of events; the latter was deemed final by the independent data monitoring committee.

After the start of the trial, time to progression was added as a secondary endpoint and the safety follow-up was extended from 4 weeks to 6 months because of the long half-life of trastuzumab.

Statistical analysis

The planned sample size was 584 patients, allowing for a 10% dropout rate and assuming an improvement in overall survival from 10 months to 13 months by the addition of trastuzumab to chemotherapy, following an exponential distribution of survival. The initial median overall survival in this trial was estimated on the basis of data from previous comparative studies and HER2 postitivity being a negative prognostic factor for outcome of patients. Therefore, median overall survival in the comparator group was estimated to be 7 months. Through discussions at several advisory board meetings, an improvement of 3 months to a median of 10 months by the addition of trastuzumab was considered clinically significant. During the study, the independent data monitoring committee noted a lower than expected event rate. On the basis of a longer than projected median survival of patients treated with capecitabine and cisplatin (10·4 months) in the pivotal ML17032 trial,[8] the independent data monitoring committee recommended revision of the original assumptions. To detect an intended difference of 3 months between the two groups and taking into account that the overall survival in the control group could be as long as 10 months, the calculated hazard ratio (HR) increased from 0·7 to 0·77. If the data were to be analysed once (fixed sample study), 460 events were necessary to ensure a power of 80% for a two-sided log-rank test at an α level of 0·05 to show a significant difference in the primary endpoint.

Two efficacy interim analyses were done at approximately 50% and 75% of the total 460 targeted events; the statistical significance level was determined by applying the O'Brien-Fleming boundary with the Lan-DeMets spending function with the actual number of events at the time of the interim efficacy analysis. With approximately 75% of the information (349 events)

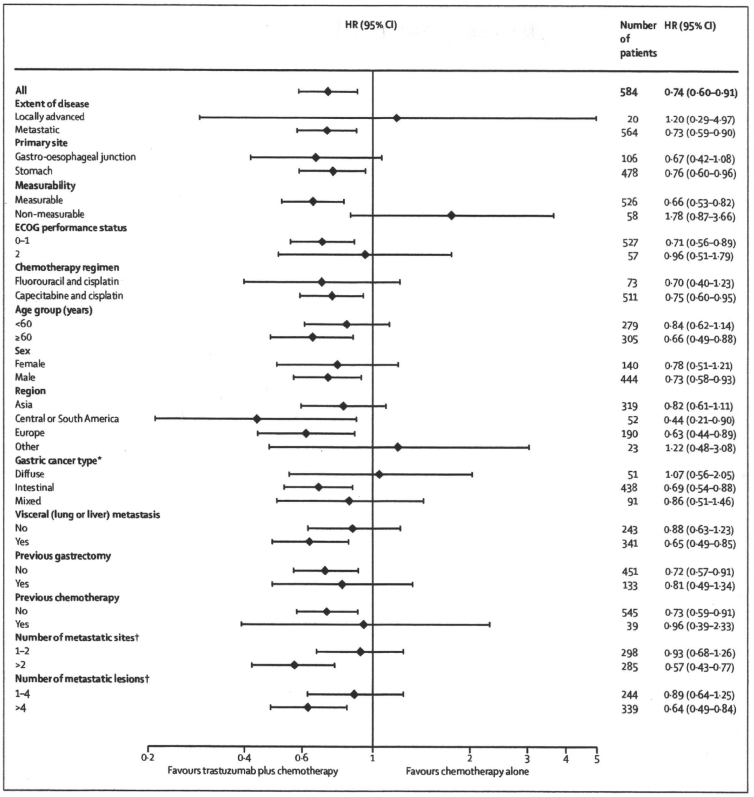

Figure 3: **Hazard ratios and 95% CIs for overall survival in prespecified subgroups**
HR=hazard ratio. ECOG=Eastern Cooperative Oncology Group. *Four patients could not be assessed for gastric cancer type (one patient in the trastuzumab plus chemotherapy group and three patients in the chemotherapy alone group). †One patient did not receive an assessment for target and non-target lesions at baseline in the trastuzumab plus chemotherapy group.

reported, the boundary for statistical significance for overall surivival was crossed.

All randomised patients who received study medication at least once were included in the analysis of the primary endpoint. Patients without an event (death) were censored at the date that they were last known to be alive. Time-to-event endpoints were compared by use of the non-stratified log-rank test. Tumour response rates were analysed with a χ^2 test. All reported p values are two-sided. Kaplan-Meier estimates and Cox regression analyses of overall survival and progression-free survival were done. The log-rank test was used to compare the distribution between the two treatment groups. Both stratified and unstratified analyses were undertaken. Subgroup analyses were undertaken to investigate the consistency of the treatment effect for multiple baseline characteristics by use of a Cox regression model. For the subgroup analysis of overall survival, the HR and 95% CI within each subgroup were summarised and displayed in the forest plot. The log-likelihood ratio test for interactions was used to assess to heterogeneity of treatment effects for levels of baseline characteristics. All

	Trastuzumab plus chemotherapy (n=294)	Chemotherapy alone (n=290)	Non-stratified effect size		Stratified effect size*		Odds ratio	p value
			Hazard ratio (95% CI)	p value	Hazard ratio (95% CI)	p value		
Progression-free survival (months)	6·7 (6–8)	5·5 (5–6)	0·71 (0·59–0·85)	0·0002	0·71 (0·59–0·86)	0·0004	..	..
Time to progression (months)	7·1 (6–8)	5·6 (5–6)	0·70 (0·58–0·85)	0·0003	0·69 (0·57–0·84)	0·0003	..	..
Duration of response (months)	6·9 (6–8)†	4·8 (4–6)‡	0·54 (0·40–0·73)	<0·0001	0·53 (0·39–0·73)	<0·0001	..	..
Tumour response								
Overall tumour response rate	139 (47%)	100 (35%)	..	..	..	..	1·70 (1·22–2·38)	0·0017§
Complete response	16 (5%)	7 (2%)	..	..	..	..	2·33 (0·94–5·74)	0·0599§
Partial response	123 (42%)	93 (32%)	..		..	..	1·52 (1·09–2·14)	0·0145§
Stable disease	93 (32%)	101 (35%)	..	..	..	..	..	..
Progressive disease	35 (12%)	53 (18%)	..	..	..	..	..	..
Missing	27 (9%)	36 (12%)	..	..	..	..	..	..

Data are median (95% CI) or number (%). *Stratified by extent of disease (local vs metastatic), primary tumour site (stomach vs gastro-oesophageal junction), measurability (measurable vs non-measurable), Eastern Cooperative Oncology Group performance status (0–1 vs 2), and fluoropyrimidine regimen (fluorouracil vs capecitabine). †n=139. ‡n=100. §χ^2 test.

Table 3: **Secondary efficacy endpoints**

15 prespecified subgroup analyses are reported. One post-hoc subgroup analysis was done (in patients with immunohistochemistry 2+ and FISH-positive tumours or immunohistochemistry 3+ tumours) and is reported. No adjustment was made for multiple tests between treatment and baseline characteristics; since 15 were tested, one might be significant by chance. Analyses were done with SAS version 8.2.

See Online for webappendix

This study is registered with ClinicalTrials.gov, number NCT01041404 (CenterWatch study number 147440).

Role of the funding source

The sponsor of the study was involved in study design, data interpretation, and the decision to submit the report for publication in conjunction with the authors. Employees of the sponsor collected and managed the data, and undertook data analysis. The two principal investigators (Y-JB and EVC) had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Figure 1 shows the trial profile. Between September, 2005, and December, 2008, 594 patients were randomly assigned to study treatment at 122 centres in 24 countries. 584 randomised patients who received study treatment at least once were included in the analysis.

Table 2 shows demographics and baseline disease characteristics of patients included in the analysis. Most patients received a chemotherapy regimen that included capecitabine (88%). High expression of HER2 protein (ie, immunohistochemistry 2+ and FISH positive or immunohistochemistry 3+) was recorded in 446 (76%) of 584 tumours. More patients assigned to trastuzumab plus chemotherapy had received previous chemotherapy than had patients assigned to chemotherapy alone.

Median follow-up was 18·6 months (IQR 11–25) in the trastuzumab plus chemotherapy group and 17·1 months (9–25) in the chemotherapy alone group. The median number of cycles of trastuzumab therapy was eight (range 1–49). The cumulative dose of chemotherapy agents, treatment duration, and dose intensity did not differ between groups (webappendix). Second-line therapy after disease progression was given to 122 (42%) patients in the trastuzumab plus chemotherapy group (113 [38%] received chemotherapy) compared with 131 (45%) patients in the chemotherapy alone group (124 [43%] received chemotherapy; webappendix).

Median overall survival was 13·8 months (95% CI 12–16) in patients assigned to trastuzumab plus chemotherapy compared with 11·1 months (10–13) in those assigned to chemotherapy alone (HR 0·74; 95% CI 0·60–0·91; p=0·0046; figure 2), corresponding to a 26% reduction in the death rate. Consistent results were provided by a confirmatory analysis that included all 594 randomised patients (data not shown).

A treatment effect could not be excluded in any of the predefined subgroups (figure 3); the overall HR of 0·74 included the 95% CI for all subgroups, apart from the non-measurable disease subgroup. These results must be interpreted with caution because of the small numbers of events within some subgroups. Median progression-free survival was 6·7 months (95% CI 6–8) in the trastuzumab plus chemotherapy group compared with 5·5 months (5–6) in the chemotherapy alone group (HR 0·71, 95% CI 0·59–0·85; p=0·0002; figure 2 and table 3). Overall tumour response rate, time to progression, and duration of response were significantly improved in the trastuzumab plus chemotherapy group compared with the chemotherapy alone group (table 3).

A pre-planned exploratory analysis according to HER2 status suggested that overall survival was longer in patients with high expression of HER2 protein than in patients with low expression (figure 4). To further explore this finding, a post-hoc analysis divided patients into two large subgroups, with either high (immunohistochemistry 2+

and FISH positive or immunohistochemistry 3+; n=446) or low (immunohistochemistry 0 and FISH positive or immunohistochemistry 1+ and FISH positive; n=131) levels of HER2 protein in their tumours (figure 4). The HR for patients whose tumours had high HER2 expression was 0·65 (95% CI 0·51–0·83) and median overall survival was 16·0 months (95% CI 15–19) in those assigned to trastuzumab plus chemotherapy compared with 11·8 months (10–13) in those assigned to chemotherapy alone. There was evidence of a significant interaction test (p=0·036) between treatment and the two HER2 subgroups (high HER2 expression *vs* low HER2 expression).

The adverse event profile was similar between the groups, with no difference in the overall rate of adverse events (all grades or grade 3 or 4; table 4). Nausea, neutropenia, vomiting, and anorexia were the most frequently reported adverse events. Patients assigned to trastuzumab plus chemotherapy had slightly higher rates of diarrhoea, stomatitis, anaemia, thrombocytopenia, fatigue, chills, weight loss, pyrexia, mucosal inflammation, and nasopharyngitis than did patients assigned to chemotherapy alone.

There was no difference between groups in frequency of grade 3 or 4 adverse events apart from diarrhoea (table 4). Serious adverse events were reported in 95 (32%) patients in the trastuzumab plus chemotherapy group and 81 (28%) patients in the chemotherapy alone group. The proportion of patients reporting an adverse event that led to dose modifications or interruptions did not differ between groups (trastuzumab plus chemotherapy, 246 [84%] *vs* chemotherapy alone, 237 [82%]) nor did 60-day mortality (15 deaths [5%] *vs* 20 deaths [7%], respectively); treatment-related mortality was 3% (ten deaths) in the trastuzumab plus chemotherapy group versus 1% (three deaths) in the chemotherapy alone group. Severe (grade ≥3) symptoms typical of an infusion-related reaction (eg, allergic reaction or hypersensitivity, chills, arthralgia, and dyspnoea) were reported infrequently, in 17 (6%) patients in the trastuzumab plus chemotherapy group: none of these reactions were fatal.

Cardiac adverse events were rare with no difference between the trastuzumab plus chemotherapy and chemotherapy alone groups (17 [6%] *vs* 18 [6%]). Frequency of cardiac failure was low, occurring in less than 1% of patients (one patient *vs* two patients, respectively). Rates of grade 3 or 4 cardiac adverse events did not differ between groups. Four (1%) patients in the trastuzumab plus chemotherapy group had a total of five events (cardiac failure [two events in one patient], myocardial infarction, unstable angina, and myocardial ischaemia with tachycardia) compared with nine (3%) patients in the chemotherapy alone group, who had nine events (cardiac failure [two events], myocardial infarction [two events], coronary arteriospasm, atrial flutter, cardiac arrest, cardiorespiratory arrest, and Prinzmetal angina). The number of patients with cardiac dysfunction (defined as a ≥10% drop in LVEF to an absolute value <50%) was low in both treatment groups (trastuzumab plus chemotherapy, 11 [5%] of 237 *vs* chemotherapy alone, two [1%] of 187).

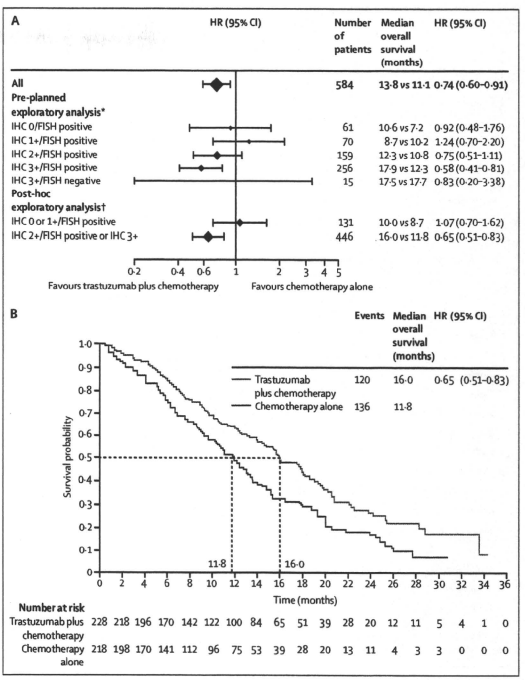

Figure 4: **Exploratory analyses**
HR=hazard ratio. (A) Pre-planned exploratory and post-hoc exploratory analyses of patients stratified by human epidermal growth factor receptor 2 (HER2) status. *n=561; patients with no immunohistochemistry (IHC) data (n=7) or IHC 3+ tumours with no fluorescence in-situ hybridisation (FISH) data (n=16) were excluded from this analysis. †n=577; patients with no IHC data (n=7) were excluded from this analysis. (B) Overall survival according to the post-hoc exploratory analysis (FISH and IHC) in patients with IHC 2+ and FISH-positive tumours or IHC 3+ tumours.

Discussion

In patients with advanced gastric or gastro-oesophageal junction cancer, addition of trastuzumab to chemotherapy significantly improved overall survival compared with chemotherapy alone. Furthermore, an exploratory, post-hoc analysis showed that trastuzumab plus chemotherapy substantially improved overall survival in patients with high expression of HER2 protein (immunohistochemistry 2+ and FISH positive or immunohistochemistry 3+) compared with patients with low expression of HER2 protein (immunohistochemistry 0 or 1+ and FISH positive).

	Trastuzumab plus chemotherapy (n=294)		Chemotherapy alone (n=290)	
	All grades	Grade 3 or 4	All grades	Grade 3 or 4
Any adverse event	292 (99%)	201 (68%)	284 (98%)	198 (68%)
Gastrointestinal disorders				
Nausea	197 (67%)	22 (7%)	184 (63%)	21 (7%)
Vomiting	147 (50%)	18 (6%)	134 (46%)	22 (8%)
Diarrhoea	109 (37%)	27 (9%)	80 (28%)	11 (4%)
Constipation	75 (26%)	2 (1%)	93 (32%)	5 (2%)
Stomatitis	72 (24%)	2 (1%)	43 (15%)	6 (2%)
Abdominal pain	66 (22%)	7 (2%)	56 (19%)	5 (2%)
Dysphagia	19 (6%)	7 (2%)	10 (3%)	1 (<1%)
Blood and lymphatic system disorders				
Neutropenia	157 (53%)	79 (27%)	165 (57%)	88 (30%)
Anaemia	81 (28%)	36 (12%)	61 (21%)	30 (10%)
Thrombocytopenia	47 (16%)	14 (5%)	33 (11%)	8 (3%)
Febrile neutropenia	15 (5%)	15 (5%)	8 (3%)	8 (3%)
General, metabolic, and other disorders				
Anorexia	135 (46%)	19 (6%)	133 (46%)	18 (6%)
Fatigue	102 (35%)	12 (4%)	82 (28%)	7 (2%)
Hand-foot syndrome	75 (26%)	4 (1%)	64 (22%)	5 (2%)
Weight decreased	69 (23%)	6 (2%)	40 (14%)	7 (2%)
Asthenia	55 (19%)	14 (5%)	53 (18%)	10 (3%)
Pyrexia	54 (18%)	3 (1%)	36 (12%)	0
Renal impairment	47 (16%)	2 (1%)	39 (13%)	3 (1%)
Mucosal inflammation	37 (13%)	6 (2%)	18 (6%)	2 (1%)
Nasopharyngitis	37 (13%)	0	17 (6%)	0
Chills	23 (8%)	1 (<1%)	0	0
Hypokalaemia	22 (7%)	13 (4%)	13 (4%)	7 (2%)
Dehydration	18 (6%)	7 (2%)	16 (6%)	5 (2%)
Dyspnoea	9 (3%)	1 (<1%)	16 (6%)	5 (2%)

Data show adverse events of all grades (>5%) and grade 3 or 4 adverse events (≥1%) plus adverse events of any grade with more than 5% difference between groups.

Table 4: **Adverse events**

In recent years, the development of new treatments and combination chemotherapies for advanced gastric cancer has led to a steady increase in overall survival beyond the 3–5 months seen with best supportive care alone.[21,22] Single-agent chemotherapies provided an incremental benefit,[23] but the biggest advances have been seen with two-drug and three-drug combinations, as demonstrated by a meta-analysis showing a 17% reduction in the risk of death with combination regimens (HR 0·83, 95% CI 0·74–0·93).[6]

Van Cutsem and colleagues[24] reported a median overall survival of 9·2 months in patients with advanced gastric cancer who received combination therapy consisting of docetaxel, cisplatin, and fluorouracil, but this finding was associated with fairly high rates of grade 3 or 4 neutropenia, possibly because of the inclusion of a taxane. Recent trials of capecitabine plus cisplatin and of fluorouracil, leucovorin (rINN calcium folinate), and oxaliplatin have resulted in median overall survival of 10·5 months and 10·7 months, respectively.[8,25]

A meta-analysis of two randomised trials, including 1318 patients, showed that capecitabine was non-inferior to fluorouracil in terms of progression-free survival and overall survival in patients with advanced gastric cancer.[26] The addition of an anthracycline to a regimen containing fluorouracil or cisplatin has also been shown to improve overall survival,[27] but before this study, the most promising results had been seen in the randomised, phase 3 REAL-2 (Randomized ECF for Advanced and Locally Advanced Esophagogastric Cancer 2) trial.[7] REAL-2 assessed four different three-drug combination regimens for advanced gastric cancer, and showed median overall survival of 9·9 months with epirubicin, cisplatin, and fluorouracil, 9·9 months with epirubicin, cisplatin, and capecitabine, 9·3 months with epirubicin, oxaliplatin, and fluorouracil, and 11·2 months with epirubicin, oxaliplatin, and capecitabine.[7] In this context, the current median overall survival of 13·8 months in the trastuzumab plus chemotherapy group in all patients with HER2 overexpression or amplification, and 16·0 months in patients with immunohistochemistry 2+ and FISH-positive tumours or immunohistochemistry 3+ tumours represents a clinically significant improvement.

The median overall survival of 11·1 months seen in patients assigned to chemotherapy alone was longer than expected but was in line with other studies of capecitabine-containing regimens in this population of patients.[7,8] One possible explanation is the increased use of second-line therapy in this study compared with other phase 3 studies of combination therapies.[7] Another possible explanation is that HER2 overexpression might already be conferring a better prognosis across both groups of this population of patients. However, HER2 expression leading to a better prognosis is by contrast with recent studies that showed an association between HER2-positive tumours and poor outcomes and aggressive disease;[9,31] further studies are needed to address the issue of whether HER2 has an effect on prognosis in gastric cancer, and whether it confers a good or poor prognosis. A third explanation might have been the higher percentage of patients in the control group of this study who had intestinal-type tumours compared with other phase 3 studies.[24] HER2 expression is more common in intestinal-type tumours[11] and such patients have a better outcome than do those with diffuse-type tumours,[28–30] an effect that might have led to the fairly high overall survival seen in the control group. A significant difference in progression-free survival was seen between treatment groups, but it is worth noting that progression-free survival in both groups in this trial was not substantially longer than that in other phase 3 studies of chemotherapy regimens consisting of three drugs.[7,24]

A potential weakness of this trial was the open-label nature of treatment; however, the study design was deemed acceptable for ethical reasons in seriously ill patients such as these and of low impact on the primary

endpoint of overall survival. Additionally, an independent response review was not done, which might have increased the robustness of the analyses of response rate and progression-free survival, but again this would have had no effect on the primary endpoint, overall survival. Another potential weakness is that the study was not stratified according to patients' region of origin, since other stratification factors were deemed more clinically relevant at the time of study design. At the time this study was initiated, the best possible HER2 testing modality for gastric cancer samples had not been established and the immunohistochemistry scoring criteria proposed by Hofmann and colleagues[10] were further refined during the centralised testing used in the screening phase (table 1).

In metastatic breast cancer, levels of HER2-protein expression predict the response to trastuzumab,[11] and the pre-planned analyses of overall survival according to HER2 expression in this study suggest that this association might occur in gastric cancer. On the basis of these findings, a detailed exploratory, post-hoc analysis of overall survival by subgroups defined by level of HER2-protein expression supported increased efficacy (median overall survival 16·0 months) of trastuzumab associated with high expression of the protein. Therefore, patients with advanced gastric or gastro-oesophageal junction cancer with these tumour characteristics should be offered trastuzumab plus chemotherapy as a treatment option.

The addition of trastuzumab to chemotherapy did not increase toxic effects associated with standard fluoropyrimidine-based and platinum-based chemotherapy. The most common grade 3 and 4 adverse events reported with trastuzumab plus chemotherapy were neutropenia, diarrhoea, and nausea—which occurred at similar rates to those previously described with three-drug combinations containing capecitabine or fluorouracil[2]—and anaemia. The fairly high rates of grade 3 or 4 neutropenia, stomatitis, diarrhoea, and lethargy previously reported in patients treated with the combination of docetaxel, cisplatin, and fluorouracil[24] were not seen with trastuzumab plus chemotherapy. These data show that trastuzumab can be combined with standard chemotherapy without affecting the overall safety profile. Additionally, rates of cardiac adverse events were low in this trial in patients with advanced gastric cancer (including low rates of cardiac failure and decreases in LVEF). The negligible number of patients previously exposed to anthracyclines, which are known to be toxic to myocytes,[12] might have affected these results. Few severe infusion-related reactions were reported in either group.

Thus, addition of trastuzumab to chemotherapy improved survival in patients with advanced gastric or gastro-oesophageal junction cancer compared with chemotherapy alone; this improvement was mainly the result of the survival advantage conferred to patients with high expression of HER2 protein. On the basis of these findings, trastuzumab can be considered as a new standard option for patients with HER2-positive advanced gastric or gastro-oesophageal junction cancer when combined with a chemotherapy regimen consisting of capecitabine plus cisplatin or fluorouracil plus cisplatin.

Contributors

All authors had full access to the original data, reviewed the data analyses, contributed to data interpretation and to the writing of the report, made final decisions on all parts of the report, and approved the final version of the submitted report. EVC, AO, Y-KK, Y-JB, and AF participated in study design. Y-JB, EVC, HCC, LS, AS, FL, AO, YO, TS, GA, EK, and Y-KK enrolled patients. JH undertook statistical analysis. JR undertook HER2 screening. AF and ML contributed to data collation and generation of tables and figures. The academic authors vouch for the completeness and veracity of the data and data analyses.

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Conflicts of interest

Y-JB and JR are advisory board members and Y-JB, EVC, FL, and Y-KK are consultants for F Hoffmann-La Roche. TS is a consultant for Chugai-Roche, Chugai Pharmaceutical, Merck-Serono, Sanofi-Aventis, Bristol-Myers, Yakult Pharmaceutical, Taiho Pharmaceutical, and Daiichi–Sankyo. Y-JB, EVC, FL, JR and Y-KK have received honoraria and Y-JB, EVC, HCC, LS, FL, AO, and GA or their institutions have received grants from F Hoffmann-La Roche. FL, GA, and JR have given educational presentations and Y-JB, JR, and Y-KK have received travel or accommodation fees from F Hoffmann-La Roche. Travel or accommodation fees have been received by AO from Chugai-Roche, and by TS from Chugai Pharmaceutical. AF, JH, and ML are employees of F Hoffmann-La Roche. AS, YO, and EK declare that they have no conflicts of interest.

Acknowledgments

This study was sponsored by F Hoffmann-La Roche. We thank Jeni Fagan (Health Interactions, UK) who provided editorial support on behalf of F Hoffmann-La Roche.

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Can EUS-guided FNA distinguish between gallbladder cancer and xanthogranulomatous cholecystitis?

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Background: EUS-guided FNA (EUS-FNA) is a useful modality for sampling various targets, but its applicability to gallbladder (GB) mass lesions is limited.

Objective: To determine the usefulness of EUS-FNA for diagnosing GB mass lesions.

Design: Single-center, retrospective, case-series study.

Setting: Tertiary-care referral center.

Patients: This study involved 15 consecutive patients who underwent EUS-FNA of GB mass lesions. We punctured GB masses in patients with suspected xanthogranulomatous cholecystitis to distinguish them from malignancy, and in patients with unresectable GB carcinoma for pathological confirmation. The final diagnosis was based on surgical histopathological results or follow-up outcome.

Interventions: EUS-FNA.

Main Outcome Measurements: Evaluation of EUS-FNA sampling adequacy rate and diagnostic yield.

Results: Xanthogranulomatous cholecystitis was suspected in 6 of the 15 patients. EUS-FNA revealed foam cells (n = 3), inflammatory cells (n = 1, proven by cholecystectomy), and GB carcinoma (n = 1), and the amount of the aspirate was insufficient in one case (xanthogranulomatous cholecystitis was later proven by extended hepatectomy). The mean follow-up period of the patients with xanthogranulomatous cholecystitis was 1177 days. Adenocarcinoma was confirmed by EUS-FNA in 8 of the 9 patients with suspected unresectable GB carcinoma, and the FNA was inconclusive in one. All 10 patients with GB carcinoma underwent chemotherapy. The overall sampling adequacy was 86.6%. The accuracy of EUS-FNA for detecting malignancy and for the final diagnosis was 93.3% (95% CI, 62.4%-99.9%) and 80% (95% CI, 54%-93.7%), respectively.

Limitations: A small patient cohort and a retrospective design with potential selection bias.

Conclusions: Malignant GB mass lesions can be safely and accurately differentiated by EUS-FNA. Thus, patients with xanthogranulomatous cholecystitis can avoid undue extensive surgery.

Tissue samples from gallbladder (GB) mass lesions can be obtained through percutaneous image-guided (eg, transabdominal US and CT scanning) FNA and occasionally by open surgery.[1-5] The reported sensitivity and specificity of these modalities are >88% and nearly 100%, respectively. However, the performance characteristics of

Abbreviations: EUS-FNA, EUS-guided FNA; GB, gallbladder; LN, lymph node; XGC, xanthogranulomatous cholecystitis.

DISCLOSURE: *All authors disclosed no financial relationships relevant to this publication.*

0016-5107/$36.00
doi:10.1016/j.gie.2010.05.022

Received February 14, 2010. Accepted May 10, 2010.

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TABLE 1. Detailed summary of 15 GB mass lesions

No.	Sex/age	Symptom	Clinical diagnosis	TNM	FNA aim	Location/EUS findings	Target‡	FNA passes	FNA result	Final	Confirmation	Clinical course
1	F/54	RUQ pain	XGC		Discrimination*	Fundus/homogeneous	GB	3	Ins	XCG	Operation	Alive at 1426 days
2	M/85	RUQ pain	XGC		Discrimination	Body-fundus/homogeneous high echo	GB	2	XCG	XCG	Observation	Alive at 562 days
3	F/77	Appetite loss	XGC		Discrimination	Neck/homogeneous	GB	2	XCG	XCG	Observation	Alive at 1465 days
4	F/57	Appetite loss	XGC		Discrimination	Neck-body/heterogeneous	GB	2	XCG	XCG	Observation	Alive at 951 days
5	M/57	RUQ pain	XGC		Discrimination	Body-fundus/homogeneous	GB	3	IC	XCG	Operation	Alive at 1481 days
6	F/73	Appetite loss	XGC		Discrimination	Body-fundus/heterogeneous	GB	1	GBC	GBC	Chemotherapy	Died at 177 days
7	F/59	Jaundice	GBC	T4N1M0	Evidence†	Neck-body/homogeneous	LN GB	3	GBC	GBC	Chemotherapy	Died at 1109 days
8	M/82	Jaundice	GBC	T4N1M1	Evidence	Neck-body/heterogeneous	LN GB	2	GBC	GBC	Chemotherapy	Died at 602 days
9	M/68	Appetite loss	GBC	T3N0M1	Evidence	Body/homogeneous	GB	3	Ins	GBC	Chemotherapy	Died at 706 days
10	M/51	RUQ pain	GBC	T4N2M0	Evidence	Fundus/heterogeneous	LN GB	3	GBC	GBC	Chemotherapy	Died at 60 days
11	F/81	RUQ pain	GBC	T4N2M1	Evidence	Body-fundus/heterogeneous	GB	1	GBC	GBC	Chemotherapy	Died at 82 days
12	F/67	RUQ pain	GBC	T4N2M1	Evidence	Body/heterogeneous	GB	1	GBC	GBC	Chemotherapy	Died at 1239 days
13	F/55	RUQ pain	GBC	T4N1M0	Evidence	Neck-body/homogeneous	GB	1	GBC	GBC	Chemotherapy	Alive at 126 days
14	M/82	Appetite loss	GBC	T3N0M1	Evidence	Neck/homogeneous	GB	1	GBC	GBC	Chemotherapy	Alive at 92 days
15	M/49	Jaundice	GBC	T4N1M0	Evidence	Neck-body/homogeneous	LN GB	2	GBC	GBC	Chemotherapy	Alive at 67 days

GB, Gallbladder; *TNM*, tumor/node/metastasis tumor staging; *F*, female; *RUQ*, right upper quadrant; *XGC*, xanthogranulomatous cholecystitis; *Ins*, insufficient; *M*, male; *IC*, inflammatory cells; *GBC*, gallbladder carcinoma; *LN*, lymph node.
*Discrimination between benign and malignant lesions.
†Evidence of malignancy before chemotherapy.
‡LN initially targeted; if failed, insufficient, or negative for carcinoma, GB mass lesion was then punctured.

percutaneous aspiration might be suboptimal for smaller GB lesions.[3,5,6-8] Moreover, percutaneous aspiration is associated with risks of abdominal pain (4.5%), bile peritonitis (1%-6%), and needle tract seeding.[7,8] Despite the established role of EUS-guided FNA (EUS-FNA) as a highly accurate tissue sampling modality for various lesions, with a very low complication rate,[9-12] its role in the context of suspected GB malignancies has not been elucidated.[1,2] EUS-FNA could potentially avoid these shortcomings of percutaneous aspiration of GB lesions. Therefore, we studied whether EUS-FNA could be used to differentiate GB mass lesions and diagnose clinically suspected GB malignancies.

PATIENTS AND METHODS

Between March 1997 and October 2009, 1850 EUS-FNA procedures were carried out at Aichi Cancer Center Hospital, Nagoya, Japan. Among these procedures, 51 (2.7%) were done for patients with GB mass lesions either by puncturing the GB mass itself or by targeting regional lymph nodes (LNs). The present study retrospectively included a subset of 15 consecutive patients (mean age 66.4 ± 12.7 years, 8 female) in whom EUS-FNA targeted the GB mass (Table 1).

Our rationale for using EUS-FNA in these patients was to obtain histological evidence of malignancy in clinically suspected, unresectable GB carcinoma and to distinguish between benign and malignant masses when xanthogranulomatous cholecystitis (XGC) was suspected. This clinical diagnosis was suspected after investigation by CT scanning and abdominal US. However, to definitively reach a diagnosis based on imaging features alone was difficult. When a GB mass with an enlarged, regional, intra-abdominal LN was found, we punctured the LN first. If enlarged LNs were not evident, were difficult to puncture (eg, para-aortic location), or the FNA yield from the LN was negative, we punctured the GB mass lesion itself (Fig. 1).

Study procedure

All patients underwent EUS-FNA with a convex array echoendoscope (GF-UCT240; Olympus Optical Corp Ltd, Tokyo, Japan) connected to an US scanning system (SSD 5500; Aloka, Tokyo, Japan). All FNA procedures were performed by using 22-gauge needles (NA-10J-1, NA-10J-KB, NA-11J-KB, or NA-200H-8022; Olympus Medical System Corp Ltd, Tokyo, Japan). Patients were followed-up for 48 hours after the procedure for any procedure-related complications. Cytological samples were processed and analyzed according to established methods of EUS-FNA aspirate processing.[10,13,14] All samples were interpreted through on-site cytological evaluation and by the same experienced cytopathologists (W.H., Y.Y.). Our institu-

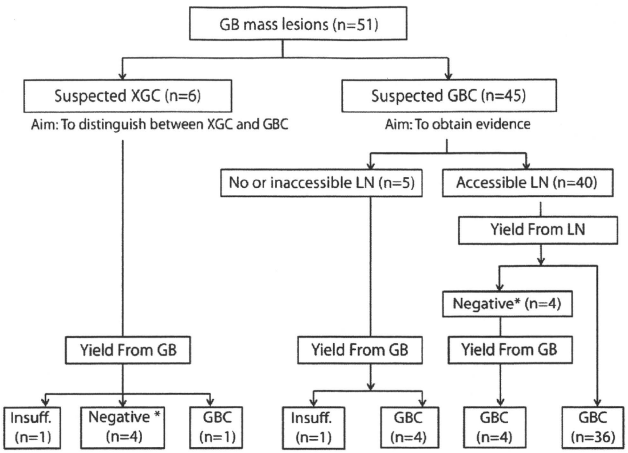

Figure 1. Schematic diagram of a gallbladder mass lesion and EUS-guided FNA yield. *GB*, gallbladder; *XGC*, xanthogranulomatous cholecystitis; *GBC*, gallbladder carcinoma; *LN*, lymph node; *insuff*, insufficient aspirate.

tional review board approved this study. Our main outcome measures were (1) the sampling adequacy rate and (2) the diagnostic yield of EUS-FNA.

Statistical analysis

We used frequencies, proportions (%), and means for descriptive analyses where appropriate. The χ^2 test (with Yates correction) was used as a univariate analysis for comparative statistics. The results of EUS-FNA were compared with the clinical follow-up or with histopathological results obtained after surgical resection. Lesions defined as malignant by EUS-FNA and finally diagnosed as malignant were considered true positive, and lesions defined as malignant by EUS-FNA and finally diagnosed as benign were considered false positive. Likewise, lesions initially categorized and finally diagnosed by EUS-FNA as benign were considered true negative, and lesions initially categorized as benign by EUS-FNA and finally diagnosed as malignant were considered false negative.

RESULTS

The main cause of referral was right upper quadrant pain in 7 patients (46.7%), loss of appetite in 5 patients (33.3%), and obstructive jaundice in 3 patients (20%). Detailed clinical features of these 15 patients are listed in Table 1.

Evaluation of the EUS findings from 51 GB masses (XGC, n = 5; GB carcinoma, n = 46) revealed that only the presence of regional LNs and a disrupted mucosal lining favored a diagnosis of GB carcinoma (Table 2).

TABLE 2. EUS features of XGC and GBC

EUS findings	XGC (n = 5) no. patients (%)	GBC (n = 46) no. patients (%)	*P* value*
Gallbladder			
Focal thickening	1 (20)	7 (15.2)	.7 (NS)
Diffuse thickening	4 (80)	39 (84.8)	
Mucosal line			
Continuous	3 (60)	5 (10.8)	.02
Disrupted	2 (40)	41 (89.1)	
Intramural hypoechoic nodule	3 (60)	12 (26.1)	.2 (NS)
Gallstone	4 (80)	16 (34.8)	.1 (NS)
Lymph node swelling	0 (0)	40 (87)	.0001

XGC, Xanthogranulomatous cholecystitis; *GBC*, gallbladder carcinoma; *NS*, not significant.
*χ^2 test with Yates correction.

A total of 19 punctures (GB masses, n = 15; regional lymphadenopathy, n = 4) were performed in 15 patients with GB masses. The sample adequacy rate for cytological evaluation was 13 of 15 (86.6%; 95% CI, 60.8%-97.5%).

Clinically suspected XGC could not be differentiated from malignancy, and regional lymphadenopathy was un-

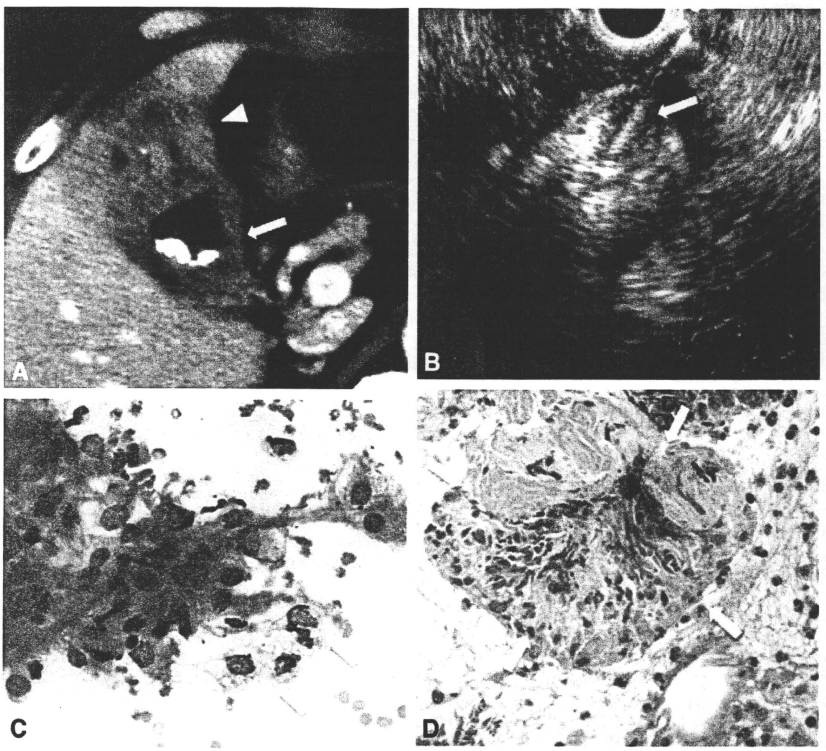

Figure 2. Findings of CT scans and EUS-guided FNA of xanthogranulomatous cholecystitis from patient 5. **A,** Abdominal CT scan shows the diffuse, irregular wall of a thickened gallbladder (*arrow*) with gall stones and irregular, low-density areas in the liver (*arrowhead*). **B,** EUS-guided FNA for a gallbladder mass lesion. The arrow shows the FNA needle inside the lesion. **C,** Foam cells in an FNA-cytology specimen (*arrow*) (Diff-Quik, orig. mag. ×400). **D,** Aggregates of foamy macrophages in cell block sections (*arrow*) (H&E, orig. mag. ×400). These were diagnosed as xanthogranulomatous cholecystitis.

detectable in 6 lesions. Among these, EUS-FNA sampling was inconclusive in 1 patient who underwent surgery (extended right lobe hepatectomy with bile duct resection, transverse colectomy, and partial duodenal resection) because of concerns about GB carcinoma, but XGC was confirmed in the resected specimen. The presence of typical foam cells in 3 patients and inflammatory cells in 1 led to a presumptive EUS-FNA diagnosis of XGC. The presumptive diagnosis was confirmed at follow-up in the 3 patients with foam cells and by simple cholecystectomy for coexistent GB stones in the other with inflammatory cells (Fig. 2). Although XGC with a liver abscess was clinically suspected in the remaining patient, EUS-FNA revealed GB carcinoma. Unresectable GB carcinoma was suspected in another 9 patients, and the aim of puncture was to obtain pathological evidence of malignancy before chemotherapy. Intra-abdominal regional lymphadenopathy was detected in 7 of these patients (4 LNs were punctured, and 3 were too small for puncture). These 4 LN punctures were negative for malignancy, and, hence, the GB mass itself was punctured (Fig. 3). Sampling was sufficient in 8 patients in whom GB carcinoma was diagnosed and yielded only atypical cells from 1 patient that were insufficient to establish a conclusive diagnosis (considered as false negative). This patient was further treated, based on the overall clinical and imaging profile, as having GB carcinoma. All patients with GB carcinoma (n = 10) received chemotherapy. No serious procedure-related com-

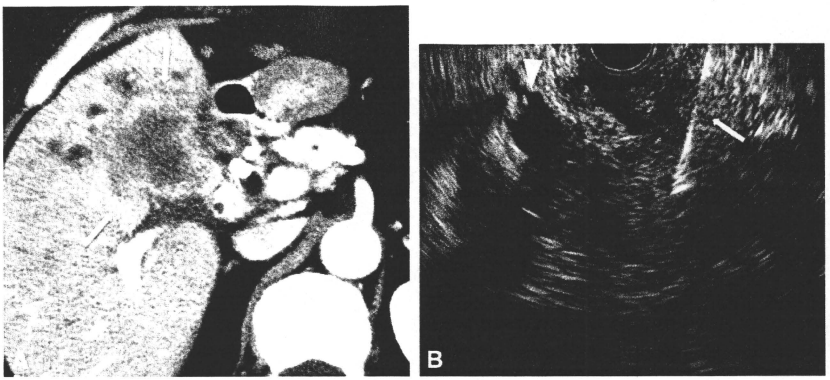

Figure 3. Findings of CT and EUS-guided FNA of gallbladder carcinoma from Patient 13. **A,** A CT image shows diffuse, irregular wall thickening and the disrupted mucosal line of the gallbladder (*arrow*). **B,** EUS-guided FNA for a gallbladder mass lesion (*arrow*, FNA needle inside the lesion; *arrowhead*, the gallbladder lumen).

plications developed in any of the 15 patients within the initial 48 hours. With regard to delayed complications, patients with XGC were followed-up as outpatients at regular intervals, and all those with GB carcinoma were followed-up as outpatients every 1 to 2 weeks for chemotherapy. No serious procedure-related delayed complications developed. The diagnostic accuracy of EUS-FNA to correctly distinguish between benign and malignant masses was 93.3% (14/15; 95% CI, 62.4%-99.9%), with a sensitivity and specificity of 90% (95% CI, 57.4%-99.9%) and 100% (95% CI, 51.9%-100%), respectively. Only one EUS-FNA result was a false negative because of insufficient sampling from unresectable GB carcinoma, and none of the results were false positive (Table 3).

The overall EUS-FNA diagnosis was concordant with the final diagnoses in 12 lesions (accuracy 80%; 95% CI, 54%-93.7%). Among the 3 discordant false-negative results, the EUS-FNA yield was insufficient, and only atypical cells or only inflammatory cells were found in one sample each.

TABLE 3. Diagnostic yield of EUS-guided FNA: benign versus malignant

	Final diagnosis	
	Benign	**Malignant**
EUS-guided FNA diagnosis		
Benign	5 (TN)	1* (FN)
Malignant	0 (FP)	9 (TP)
Total	5	10

TN, True negative; *FN*, false negative; *FP*, false positive; *TP*, true positive; *XGC*, xanthogranulomatous cholecystitis.
Five TNs comprised XGC (n = 4) and insufficient aspirate (n = 1).
*Atypical EUS-guided FNA diagnosis of one FN.

DISCUSSION

EUS-FNA is an established diagnostic tool for obtaining tissue samples from diverse types of lesions. Although EUS has the potential for staging GB masses, it has not been adequately evaluated in the context of GB mass lesions. Only 3 reports have been published. Jacobson et al[1] reviewed 6 patients (GB carcinoma, n = 5; XGC, n = 1) with an 83% accuracy rate. Varadarajulu and Eloubeidi[2] also studied 6 patients (GB carcinoma, n = 5) and achieved 100% sampling adequacy. Meara et al[15] punctured 7 GB mass lesions and obtained 100% specificity and 80% sensitivity. Our sampling adequacy was 86.6% with only 2 insufficient samples. Our approach was to distinguish between benign and malignant masses, especially those that were XGC, because the malignant potential of this lesion cannot be conclusively ruled out based on imaging alone.[16,17,18-20] One of 6 XGC punctures yielded an insufficient aspirate, and the patient underwent extended right hepatectomy, transverse colectomy, and partial duodenal resection. The other 5 patients avoided surgery or at least major resections after EUS-FNA, which justifies the use of EUS-FNA when XGC is suspected. Another point is that coexisting carcinomas cannot be ruled out, because they have been identified in 2% to 15% of patients with XGC.[21-23] Notably, most GB carcinoma associated with XGC occurs in the GB neck region,[20-22] which is thought to be due to increased pressure within the GB. Therefore, we

recommend careful observation of the GB neck region and of the cystic duct by EUS in such circumstances, and adequate sampling from such regions will greatly reduce the incidence of false-negative findings for coexisting carcinomas. We reported sensitivity, specificity, and accuracy of 90%, 100%, and 93.3%, respectively. This level of accuracy might be attributable to immediate cytological analysis and repeated needle passage to acquire adequate samples. We were also concerned about the possibility of puncturing cystic structures like the GB, and, in turn, spilling its contents. Thus, our operators attempted to avoid puncturing the GB mass through any intervening layer of fluid or potential space and targeted the mass by enfacing the probe simply through changing the position of the echoendoscope. The strength of the present study is that relatively more patients with GB mass lesions were recruited than in published reports. We also selected patients who had undergone EUS-FNA for a GB mass itself, regardless of whether an LN had been punctured beforehand. However, the retrospective design and the patient selection algorithm might have led to selection bias. In conclusion, we believe that EUS-FNA is a safe, feasible, and accurate method of detecting malignancies among GB mass lesions, and we recommend its incorporation into diagnostic work-up algorithms.

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original article

Annals of Oncology 21: 2403–2409, 2010
doi:10.1093/annonc/mdq248
Published online 21 May 2010

Neutropenia as a prognostic factor in advanced gastric cancer patients undergoing second-line chemotherapy with weekly paclitaxel

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Received 15 January 2010; revised 26 February 2010; accepted 30 March 2010

Background: Neutropenia during chemotherapy has been reported to be a predictor of better survival in patients with several types of cancers, although there are no reports in pretreated patients.

Methods: We retrospectively analyzed 242 patients with advanced gastric cancer (AGC) who received weekly paclitaxel (Taxol) as second-line chemotherapy. Background characteristics and neutropenia as time-varying covariates (TVCs) were analyzed as prognostic factors.

Results: Of the 242 patients, mild neutropenia (grades 1–2) occurred in 101 patients (41.7%) and severe neutropenia (grades 3–4) occurred in 63 patients (26.0%). The other 78 patients (32.2%) did not experience neutropenia. According to a multivariate Cox model with neutropenia as a TVC, hazard ratios of death were 0.61 [95% confidence interval (CI) 0.43–0.85; $P = 0.004$] for patients with mild neutropenia and 0.61 (95% CI 0.41–0.88; $P = 0.009$) for those with severe neutropenia. Among the patients in landmark analysis (landmark of 2.5 months; median time to treatment failure of paclitaxel), mild and severe neutropenia remained significant prognostic factors.

Conclusions: Our results indicate that neutropenia during chemotherapy is associated with improved survival in patients with AGC who received weekly paclitaxel as second-line chemotherapy. Prospective trials are required to assess whether dosing adjustments based on neutropenia may improve chemotherapy efficacy.

Key words: chemotherapy, gastric cancer, neutropenia, paclitaxel, prognostic factor

introduction

Neutropenia due to cytotoxic chemotherapy is a common adverse event. In general, the recommended doses of cytotoxic agents are determined in dose-finding studies. However, sample sizes in this study phase are not large enough to examine individual differences in drug metabolism; therefore, toxicity profiles are likely to be highly variable [1]. In other words, a standard dose may be insufficient to achieve efficacy for some patients with faster drug elimination times [1]. In support of this hypothesis, toxicity such as neutropenia during chemotherapy has been reported to be associated with favorable clinical outcome in several types of cancer [2–9]. Recently, we analyzed the long-term effects of neutropenia occurring during first-line chemotherapy with FOLFOX (infusional 5-fluorouracil and oxaliplatin) in patients with advanced colorectal cancer, using time-varying covariate (TVC) analysis [9]. Our results in addition to these other reports consistently show that patients experiencing neutropenia during chemotherapy had better prognoses compared with those without neutropenia [2–9]. However, all these studies involved chemo-naive patients, and the clinical impact of neutropenia on pretreated patients who are undergoing second-line chemotherapy has not yet been reported.

Paclitaxel is most commonly used as second-line chemotherapy for advanced gastric cancer (AGC) in Japan [10]. Currently, there is an ongoing phase III study comparing weekly paclitaxel with irinotecan for second-line AGC chemotherapy. Although triweekly administration is the conventional schedule used for paclitaxel, weekly administration (dose dense) was reported to be more effective and feasible in several types of cancer, with neutropenia as the most common schedule-limiting toxicity [11, 12]. Therefore, we conducted this retrospective analysis of patients with AGC who were treated with second-line chemotherapy of weekly paclitaxel after they had progressed on prior chemotherapy in order to evaluate any possible association between neutropenia occurring during chemotherapy and survival.

patients and methods

patients

This was a retrospective cohort study of AGC patients who received weekly paclitaxel (Taxol, Bristol-Myers Squibb, Tokyo, Japan) as second-line chemotherapy. Other principal inclusion criteria were as follows: the

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presence of histologically proven inoperable gastric cancer, Eastern Cooperative Oncology Group performance status (ECOG PS) of zero or two, sufficient bone marrow function (neutrophil count ≥2.0 × 10^9/l, platelet count ≥100 × 10^9/l, hemoglobin ≥8.0 g/dl), and normal liver and renal function.

Between April 2001 and September 2009, 506 patients with AGC were treated with second-line chemotherapy in our institution. Among them, 248 patients met the inclusion criteria and received weekly paclitaxel. Since 6 patients did not undergo any blood test after administration of weekly paclitaxel, a total of 242 patients were analyzed in this study. Written informed consent was obtained from all patients.

treatment delivery

All patients were premedicated i.v. 30 min before therapy with dexamethasone 8–16 mg, cimetidine 300 mg (or a comparable H2 blocker), and diphenhydramine hydrochloride 50 mg. Paclitaxel was administered at a starting dose of 80 mg/m^2 i.v. over 1 h weekly on days 1, 8, and 15 for each 4-week period. In general, chemotherapy was delayed until recovery for a neutrophil count <1.0 × 10^9/l, platelet count <75 × 10^9/l, or any significant persisting nonhematologic toxicity. In case of grade 4 neutropenia, febrile neutropenia, grade 3/4 thrombocytopenia, or grade 2 neuropathy, the paclitaxel dose was reduced by 20%. Paclitaxel was discontinued at the occurrence of grade 3 neuropathy. Other dose

Table 1. Patient characteristics according to highest grade of neutropenia

Characteristics	All (n = 242), n (%)	Absent (n = 78), n (%)	Mild (n = 101), n (%)	Severe (n = 63), n (%)
Age (years)				
Median (range)	62 (29–85)	60 (33–79)	62 (29–85)	64 (32–84)
Gender				
Male	145 (60)	46 (59)	59 (58)	40 (63)
Female	97 (40)	32 (41)	42 (42)	23 (37)
ECOG PS				
0–1	167 (69)	45 (71)	64 (69)	38 (70)
2	75 (31)	27 (29)	29 (31)	19 (30)
Histological type				
Diffuse	168 (70)	55 (71)	73 (72)	40 (64)
Intestinal	74 (30)	23 (29)	28 (28)	23 (36)
Disease status				
Advanced	138 (57)	42 (54)	54 (53)	42 (66)
Recurrent	104 (43)	36 (46)	47 (47)	21 (34)
Prior gastrectomy				
No	110 (45)	40 (51)	41 (41)	29 (46)
Yes	132 (55)	38 (49)	60 (59)	34 (54)
Adjuvant chemotherapy				
No	188 (78)	56 (72)	80 (79)	52 (83)
Yes	54 (22)	22 (28)	21 (21)	11 (17)
Peritoneal metastasis				
Yes	165 (68)	54 (69)	71 (70)	40 (63)
No	77 (32)	24 (31)	30 (30)	23 (36)
Liver metastasis				
Yes	60 (25)	22 (28)	21 (21)	17 (27)
No	182 (75)	56 (72)	80 (79)	46 (73)
Metastatic site				
1	116 (48)	37 (47)	51 (50)	28 (40)
2 or more	126 (52)	41 (53)	50 (50)	35 (60)
First-line chemotherapy				
Monotherapy	165 (68)	54 (69)	73 (73)	38 (60)
Combination	77 (32)	24 (31)	28 (27)	25 (40)
TTF of first line				
Median (months)	5.0	5.6	4.7	5.0
Pretreatment neutrophil				
Median (×10^9/l)	3.98	4.282	3.64	3.53
No. of paclitaxel administrations				
Median times (range)	8 (1–66)	6 (1–51)	9 (1–57)	10 (1–66)
Relative dose intensity				
Median	0.89	0.94	0.89	0.78
Third-line chemotherapy				
Yes	115 (48)	34 (43)	53 (52)	28 (44)

ECOG PS, Eastern Cooperative Oncology Group performance status; TTF, time to treatment failure.

adjustments were made on an individual basis. Treatment was discontinued if the tumor progressed, severe toxicity occurred, or at the patient's request.

The actual dose intensity was defined as the total dose of drug delivered per unit of body surface area per unit of time (mg/m^2/week). The relative dose intensity of paclitaxel was calculated as the ratio between the actual dose intensity and the scheduled dose intensity.

evaluation of neutropenia and supportive therapy

A complete blood cell count was carried out weekly before each chemotherapy. Patients with treatment delay due to toxicity were followed up with weekly or more frequent blood counts. The most severe grade of neutropenia was based on the lowest recorded neutrophil count for a given patient between the first day of paclitaxel administration and 1 week after the last paclitaxel dose was administered and was graded according to the National Cancer Institute Common Toxicity Criteria version 3.0. To evaluate neutropenia during chemotherapy, patients were divided into three categories: neutropenia absent (grade 0), mild (grades 1–2), and severe (grades 3–4).

Indications for using granulocyte colony-stimulating factor (G-CSF) were not specified, but G-CSF was generally used in grade 4 or febrile neutropenia, and its use for prophylaxis was not allowed.

statistical methods

The primary objective of this study was to evaluate the association between neutropenia during weekly paclitaxel and overall survival, which was defined as the interval between the date of beginning weekly paclitaxel and the date of death or last follow-up. To evaluate the impact of neutropenia on overall survival, univariate and multivariate analyses using the Cox proportional hazards model were carried out. Therefore, a measure of association in this study was the hazard ratio (HR) along with the 95% confidence interval (95% CI). As neutropenia varies over time, analyses could be compromised by possible lead-time bias, which results in a false-positive association between chemotherapy-induced neutropenia and longer survival. Therefore, neutropenia was analyzed as a simple prognostic factor or as a TVC in all cohorts and landmark analysis cohorts to avoid this bias as much as possible. Landmark cohorts included patients who survived more than median time to treatment failure of weekly paclitaxel, which was defined as the interval between the date of the start of weekly paclitaxel administration and the last dose. Forward and backward stepwise methods were used for model building. Threshold P values for inclusion or exclusion in the model were defined as 0.10 and 0.20, respectively. Confounding variables considered in the univariate and multivariate analyses were age (<65 years versus 65 years or older), gender (male: 0 versus female: 1), ECOG PS (0–1 versus 2), histological subtype (diffuse: 0 versus intestinal: 1), disease status (advanced: 0 versus recurrent: 1), prior gastrectomy (no: 0 versus yes: 1), prior adjuvant chemotherapy (no: 0 versus yes: 1), presence of peritoneal metastasis (no: 0 versus yes: 1), presence of liver metastasis (no: 0 versus yes: 1), number of metastatic sites (1 versus 2 or more), regimen of prior chemotherapy (monotherapy: 0 versus combination: 1), duration of prior chemotherapy (<median: 0 versus ≥median: 1), prior neutrophil count (<median: 0 versus ≥median: 1), and highest grade of neutropenia during weekly paclitaxel (absent: 0 versus mild: 2 versus severe: 3). The distribution of subject characteristics was assessed by the chi-square test or the Fisher's exact test, as appropriate. Statistical analyses were carried out using STATA ver. 10 (StataCorp LP, College Station, TX). All tests were two-sided, and P values <0.05 were considered statistically significant.

results

patient characteristics

The median follow-up time at the time of this analysis was 39 months (range 5–96 months). Most patients received 5-fluorouracil- or fluoropyrimidine-based first-line chemotherapy (monotherapy in 165, combination in 77), with a median time to treatment failure of 5.0 months.

During treatment with weekly paclitaxel, mild neutropenia (grades 1–2) occurred in 101 patients (41.7%) and severe neutropenia (grades 3–4) occurred in 63 patients (26.0%). The other 78 patients (32.2%) did not experience neutropenia. Among the 164 patients experiencing neutropenia, the highest grade was seen during the second week (day 8) in 27, during the third week (day 15) in 75, during the fourth week (day 22) in 20, and thereafter in 42, indicating that 74.4% of patients with neutropenia experienced their highest grade within 4 weeks. In contrast, only six patients without neutropenia occurring within 4 weeks experienced late-onset neutropenia (all with mild neutropenia).

Patient characteristics categorized according to the highest grade of neutropenia experienced by each patient are shown in Table 1. The pretreatment neutrophil count tended to be higher in patients with neutropenia. No other significant differences

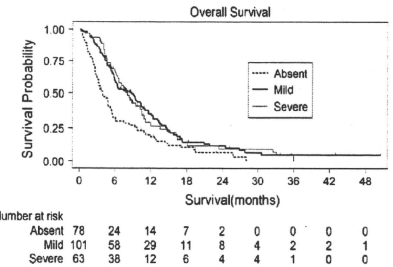

Figure 1. Kaplan–Meier survival curves according to highest grade of neutropenia. The median overall survival times in the absent group, mild group, and severe group were 3.9 months [95% confidence interval (CI) 3.0–5.2], 8.8 months (95% CI 5.9–10.4), and 8.1 months (95% CI 6.3–10.5), respectively.

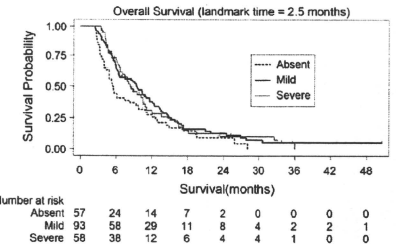

Figure 2. Kaplan–Meier survival curves according to highest grade of neutropenia (landmark analysis). The median overall survival times in the absent group (n = 57), mild group (n = 93), and severe group (n = 58) were 5.4 months [95% confidence interval (CI) 3.9–8.4], 9.4 months (95% CI 6.4–11.6), and 8.4 months (95% CI 6.3–10.5), respectively.

doi:10.1093/annonc/mdq248 |

were seen between the three neutropenia groups. The median time to treatment failure of weekly paclitaxel was 2.5 months, with a median number of paclitaxel administration of 8 (range 1–66). The median number of paclitaxel administrations was lower in patients with absent neutropenia (6, range 1–51) than in patients with mild (9, range 1–57) or severe neutropenia (10, range 1–66). In contrast, the relative dose intensity tended to be higher in the absent group. Third-line chemotherapy after weekly paclitaxel was used in a total of 115 patients (48%), with no significant differences seen between groups (Table 1). The median overall survival time of all patients was 6.3 months (95% CI 5.5–7.8). Kaplan–Meier survival curves according to chemotherapy-induced neutropenia are shown in Figure 1. The median overall survival times in the absent group, mild group, and severe group were 3.9 months (95% CI 2.7–5.2), 8.8 months (95% CI 5.9–10.4), and 8.1 months (95% CI 6.3–10.5), respectively. Kaplan–Meier survival curves of the landmark cohort groups ($n = 208$)

Table 2. Univariate and multivariate analyses with or without TVCs

Baseline and clinical features	Univariate analysis without TVC			Multivariate analysis without TVC			Multivariate analysis with TVC		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Neutropenia									
Absent	1.00	–	–	1.00	–	–	1.00	–	–
Mild	0.60	0.48–0.83	*0.002*	0.50	0.36–0.70	*<0.001*	0.61	0.43–0.85	*0.004*
Severe	0.61	0.42–0.87	*0.008*	0.51	0.35–0.73	*<0.001*	0.61	0.41–0.88	*0.009*
Age (years)									
<65	1.00	–	–	1.00	–	–	1.00	–	–
≥65	1.01	0.77–1.34	0.91	–*	–*	–*	–*	–*	–*
Gender									
Male	1.00	–	–	1.00	–	–	1.00	–	–
Female	1.03	0.78–1.36	0.82	–*	–*	–*	–*	–*	–*
ECOG PS									
0–1	1.00	–	–	1.00	–	–	1.00	–	–
2	2.75	2.02–3.57	*<0.001*	2.92	2.11–4.07	*<0.001*	2.85	2.02–4.03	*<0.001*
Histological type									
Diffuse	1.00	–	–	1.00	–	–	1.00	–	–
Intestinal	0.90	0.67–1.21	0.51	–*	–*	–*	–*	–*	–*
Disease status									
Advanced	1.00	–	–	1.00	–	–	1.00	–	–
Recurrent	0.78	0.57–1.06	0.09	0.77	0.56–1.02	0.08	–*	–*	–*
Prior gastrectomy									
No	1.00	–	–	1.00	–	–	1.00	–	–
Yes	0.80	0.60–1.05	0.11	0.78	0.55–1.10	0.12	0.77	0.56–1.08	0.13
Adjuvant chemotherapy									
No	1.00	–	–	1.00	–	–	1.00	–	–
Yes	0.72	0.51–1.06	0.07	0.70	0.44–1.09	0.10	0.69	0.44–1.08	0.10
Peritoneal metastasis									
No	1.00	–	–	1.00	–	–	1.00	–	–
Yes	1.35	1.01–1.83	*0.045*	–*	–*	–*	1.38	0.90–2.11	0.14
Liver metastasis									
No	1.00	–	–	1.00	–	–	1.00	–	–
Yes	1.02	0.74–1.41	0.88	0.74	0.46–1.08	0.09	0.75	0.50–1.10	0.14
No. of metastatic sites									
1	1.00	–	–	1.00	–	–	1.00	–	–
2 or more	1.96	1.48–2.63	*<0.001*	2.05	1.51–2.82	*<0.001*	2.06	1.50–2.83	*<0.001*
First-line chemotherapy									
Monotherapy	1.00	–	–	1.00	–	–	1.00	–	–
Combination	1.11	0.81–1.51	0.45	–*	–*	–*	–*	–*	–*
TTF of first line									
<Median	1.00	–	–	1.00	–	–	1.00	–	–
≥Median	0.74	0.56–0.98	*0.032*	–*	–*	–*	–*	–*	–*
Pretreatment neutrophil count									
<Median	1.00	–	–	1.00	–	–	1.00	–	–
≥Median	1.31	0.99–1.71	0.057	1.25	0.94–1.66	0.12	1.23	0.92–1.66	0.17

TVC, time-varying covariate; HR, hazard ratio; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; TTF, time to treatment failure. –* indicates variable excluded from the model by stepwise method. Statistically significant values are in italics.

are shown in Figure 2. The median overall survival times in the absent group ($n = 57$), mild group ($n = 93$), and severe group ($n = 58$) were 5.4 months (95% CI 3.9–8.4), 9.4 months (95% CI 6.4–11.6), and 8.4 months (95% CI 6.3–10.5), respectively.

Febrile neutropenia was seen in 10 patients (4.1%), which improved with G-CSF and antibiotics. G-CSF was also used in other 11 patients with grade 4 neutropenia. Grade 3 anemia was seen in eight patients, and one patient developed grade 3 thrombocytopenia. Regarding nonhematologic toxic effects, grade 2 sensory neuropathy was seen in 23 patients, and 4 patients developed grade 3 neuropathy. The incidence of other nonhematologic grades 3–4 toxic effects was low (fatigue in four patients and diarrhea in two patients).

survival analyses including neutropenia

Table 2 shows the results of univariate and multivariate analyses of baseline and clinical characteristics as prognostic factors, including neutropenia. Neutropenia and other two factors (PS and number of metastatic sites) remained significant in multivariate analyses. The HR for mild neutropenia in comparison to absent neutropenia

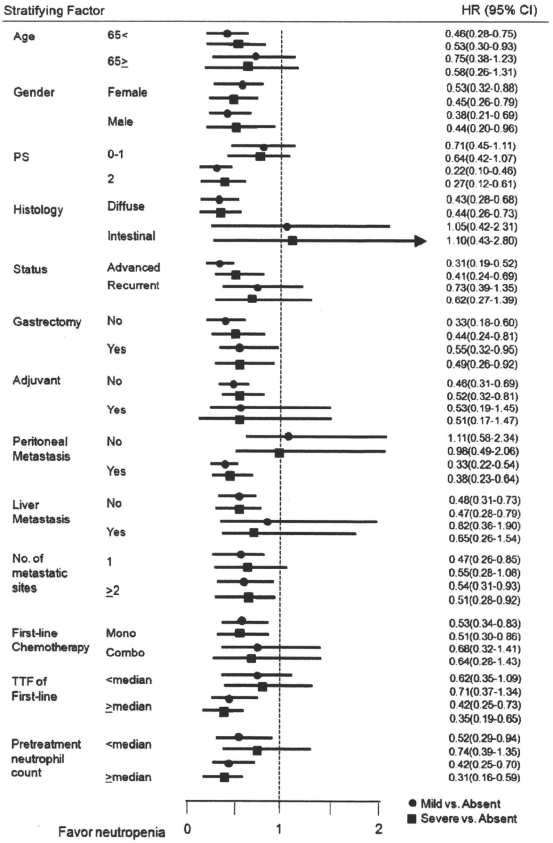

Figure 3. Hazard ratios (HRs) for death and 95% confidence intervals (CIs). In subgroup analyses, both mild and severe neutropenia tended to be associated with improved prognosis in most subgroups.

doi:10.1093/annonc/mdq248 |

was 0.50 (95% CI 0.36–0.70; $P<0.001$), and the HR for severe neutropenia in comparison to absent neutropenia was 0.51 (95% CI 0.35–0.73; $P < 0.001$). The rightmost column of Table 2 also shows the results of multivariate regression analyses with neutropenia as a TVC. Neutropenia was still a highly statistically significant prognostic factor. The HR for mild neutropenia in comparison to absent neutropenia was 0.61 (95% CI 0.43–0.85; $P = 0.004$), and the HR for severe neutropenia in comparison to absent neutropenia was 0.61 (95% CI 0.41–0.88; $P = 0.009$). In subgroup analyses, both mild and severe neutropenia tended to be associated with improved prognosis in most subgroups (Figure 3). Among the patients in landmark cohorts, mild and severe neutropenia remained significant prognostic factors according to survival analyses (Table 3).

discussion

In this study, we found significantly improved survival in patients who experienced neutropenia during weekly paclitaxel administration as second-line chemotherapy for AGC. The

Table 3. Univariate and multivariate analysis with or without TVCs (landmark cohort)

Baseline and clinical features	Univariate analysis without TVC			Multivariate analysis without TVC			Multivariate analysis with TVC		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Neutropenia									
Absent	1.00	–	–	1.00	–	–	1.00	–	–
Mild	0.68	0.49–0.98	*0.039*	0.54	0.36–0.82	*0.004*	0.60	0.41–0.88	*0.009*
Severe	0.71	0.46–1.06	0.13	0.61	0.39–0.95	*0.032*	0.65	0.44–0.98	*0.048*
Age (years)									
<65	1.00	–	–	1.00	–	–	1.00	–	–
≥65	1.15	0.84–1.58	0.39	–*	–*	–*	–*	–*	–*
Gender									
Male	1.00	–	–	1.00	–	–	1.00	–	–
Female	1.11	0.81–1.54	0.52	–*	–*	–*	–*	–*	–*
ECOG PS									
0–1	1.00	–	–	1.00	–	–	1.00	–	–
2	2.21	1.53–3.19	*<0.001*	2.25	1.51–3.38	*<0.001*	2.27	1.57–3.28	*<0.001*
Histological type									
Diffuse	1.00	–	–	1.00	–	–	1.00	–	–
Intestinal	0.90	0.63–1.29	0.54	0.72	0.49–1.09	0.12	0.74	0.50–1.11	0.13
Disease status									
Advanced	1.00	–	–	1.00	–	–	1.00	–	–
Recurrent	0.75	0.54–1.01	0.06	–*	–*	–*	–*	–*	–*
Prior gastrectomy									
No	1.00	–	–	1.00	–	–	1.00	–	–
Yes	0.85	0.62–1.16	0.31	–*	–*	–*	–*	–*	–*
Adjuvant chemotherapy									
No	1.00	–	–	1.00	–	–	1.00	–	–
Yes	0.67	0.46–0.98	*0.043*	0.60	0.38–0.91	*0.03*	0.66	0.45–0.97	*0.035*
Peritoneal metastasis									
No	1.00	–	–	1.00	–	–	1.00	–	–
Yes	1.36	0.97–1.89	0.07	–*	–*	–*	1.29	0.91–1.82	0.14
Liver metastasis									
No	1.00	–	–	1.00	–	–	1.00	–	–
Yes	1.08	0.76–1.53	0.67	–*	–*	–*	–*	–*	–*
No. of metastatic sites									
1	1.00	–	–	1.00	–	–	1.00	–	–
2 or more	1.90	1.38–2.61	*<0.001*	2.09	1.42–3.09	*<0.001*	1.91	1.39–2.62	*<0.001*
First-line chemotherapy									
Monotherapy	1.00	–	–	1.00	–	–	1.00	–	–
Combination	1.12	0.79–1.57	0.52	–*	–*	–*	–*	–*	–*
TTF of first line									
<Median	1.00	–	–	1.00	–	–	1.00	–	–
≥Median	0.83	0.60–1.12	0.21	1.28	0.90–1.81	0.16	1.21	0.89–1.83	0.19
Pretreatment neutrophil count									
<Median	1.00	–	–	1.00	–	–	1.00	–	–
≥Median	1.28	0.94–1.74	0.11	1.29	0.92–1.80	0.14	1.25	0.90–1.75	0.18

TVC, time-varying covariate; HR, hazard ratio; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; TTF, time to treatment failure. –* indicates variable excluded from the model by stepwise method. Statistically significant values are in italics.