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Pretreatment neutrophil count as an independent prognostic factor in advanced non-small-cell lung cancer: An analysis of Japan Multinational Trial Organisation LC00-03

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ABSTRACT

We examined the impact of pretreatment neutrophil count on survival in patients with advanced non-small-cell lung cancer (NSCLC). A total of 388 chemo-naïve patients with stage IIIB or IV NSCLC from a randomized controlled trial were evaluated. The effects of pretreatment peripheral blood neutrophil, lymphocyte and monocyte counts and neutrophil-lymphocyte ratio on survival were examined using the proportional hazards regression model to estimate hazard ratios after adjustment for covariates. The optimal cut-off value was determined by proportional hazards regression analysis with the minimum P-value approach and shrinkage procedure. After adjustment for prognostic factors, the pretreatment elevated neutrophil count was statistically significantly associated with short overall (P=0.0008) and progression-free survival (P=0.024), whereas no association was found between prognosis and lymphocyte or monocyte count. The cut-off value selected for neutrophil count was 4500/mm^3 (corrected hazard ratio: 1.67, 95% confidence interval (CI), 1.09-2.54). The median survival time was 19.3 months (95%CI, 16.5-21.4) for the low-neutrophil group (<4500/mm^3, n=204) and 10.2 months (95%CI, 8.0-12.3) for the high-neutrophil group ($\geq$4500/mm^3, n=184). We confirmed that pretreatment elevated neutrophil count is an independent prognostic factor in patients with advanced NSCLC receiving modern chemotherapy. Neutrophil count is easily measured at low

cost, and it may be a useful indicator of patient prognosis.

Key words: prognostic factors, neutrophil count, non-small-cell lung cancer

1. Introduction

The prognosis for patients with advanced non-small-cell lung cancer (NSCLC) (TNM stage IIIB with a positive pleural effusion, or stage IV) has improved with recent advances in systemic chemotherapy, but still remains poor, with a median overall survival time between 4 and 15 months.[1] Prognostic factors identified in previous studies include tumour stage, performance status (PS), weight loss, sex, plasma lactate dehydrogenase (LDH) level and the presence of bone, liver or skin metastases.[2] Although novel immunological and histological biomarkers have been identified, these are often time-consuming to measure, and this is not part of standard practice.

It is now evident that inflammatory cells in the tumour microenvironment have significant effects on tumour development.[3-6] Elevation in the pretreatment neutrophil count has been proposed as a prognostic factor for poor survival in patients with metastatic renal cell carcinoma,[7-9] and elevated neutrophil, monocyte or leukocyte count has been associated with poor survival in patients with metastatic melanoma.[10,11] A high neutrophil-lymphocyte ratio may be related to poor prognosis in patients with colorectal cancer[12] and in those with advanced gastric cancer.[13] The European Lung Cancer Working Group found that the high-neutrophil count was an independent prognostic factor for poor survival in patients with unresectable advanced NSCLC[14] and in those with

small-cell lung cancer.[15] A retrospective study found that neutrophil count was of prognostic value in patients with lung cancer.[16]

The aim of this study was to examine and confirm the impact of pretreatment peripheral blood neutrophil, monocyte and lymphocyte counts on overall and progression-free survival in a well-defined population of patients with advanced NSCLC being treated with regimens using newer chemotherapeutic agents in a randomized controlled clinical trial.

2. Patients and methods

2.1 Study population

A total of 401 chemo-naïve NSCLC patients with stage IIIB with pleural effusion or stage IV without brain metastasis, who had Eastern Cooperative Oncology Group (ECOG) PS of 0 or 1, were enrolled from 45 institutions in Japan between March 2001 and April 2005 into Japan Multinational Trial Organisation LC00-03[17] (registered with ClinicalTrials.gov identifier NCT00079287). Patients underwent one of two treatment regimens: intravenous vinorelbine (25 mg/m^2) plus gemcitabine (1000 mg/m^2) on days 1 and 8 every 21 d for three cycles, followed by intravenous docetaxel (60 mg/m^2) on day 1 every 21 d for three cycles [VGD arm, n=196] versus intravenous paclitaxel (225 mg/m^2) and carboplatin (area under the curve=6) for 3 h on day1, every 21 d for six cycles

[PC arm, n=197]). As there were no significant differences between treatment groups in terms of either overall (hazard ratio: 0.996, P=0.974) or progression-free survival (hazard ratio: 0.966, P=0.742), the combined data from the two arms were analysed in this study. Of 393 eligible patients, information regarding pretreatment neutrophils in peripheral blood was not available for five patients. Thus, data from 388 patients were included in the present study.

2.2 Statistical analysis

Overall survival was defined as the time from randomisation until death from any cause, and progression-free survival was defined as the time from randomisation until objective tumour progression or death. Survival curves were estimated with the Kaplan-Meier method. Associations between the factors and the prognosis were examined with the log-rank test in univariate analyses. The prognostic impact of pretreatment peripheral blood neutrophil, lymphocyte and monocyte counts, and neutrophil-lymphocyte ratio were examined using the proportional hazards regression model to estimate hazard ratios after adjustment for covariates without variable selection. Optimal cut-off points for continuous variables were selected using the minimum P-value approach with correction of the P-value.[18] The corrected hazard ratio and its 95% confidence

interval (CI) were estimated using a shrinkage procedure with bootstrap resampling.[19] All statistical analyses were done using SAS version 9.1 (SAS Institute, Cary, NC).

3. Results

3.1 Patient characteristics

Of 388 patients, 276 patients had died, and the median follow-up time for 112 surviving patients was 567 d (range: 70-1711 d). The characteristics of the 388 patients (276 men [71%], 112 women [29%], median age 65 years [range, 33-81 years], included in the present study are shown in Table 1. Median pretreatment counts of neutrophils, lymphocytes and monocytes were 4304/mm^3, 1386/mm^3, and 404.2/mm^3, respectively. Spearman's rank correlations were 0.351 for neutrophils and monocytes, −0.034 for neutrophils and lymphocytes and 0.352 for monocytes and lymphocytes.

3.2 Relationship between pretreatment neutrophil, lymphocyte and monocytes counts and survival

In univariate analyses, pretreatment elevated counts of neutrophils were statistically significantly associated with short overall (Fig. 1A, P<0.0001) and progression-free survival (Fig. 1B, P=0.0001). Although lymphocyte count did

not correlate with survival, there were significant relationships between high-neutrophil-lymphocyte ratio and short overall (P<0.0001) and progression-free survival (P=0.005). The elevated monocyte count was also significantly associated with short overall survival (P=0.004), and moderately related to short progression-free survival (P=0.052). We selected sex, smoking history, stage, ECOG PS, weight loss, plasma LDH and presence of bone, liver or skin metastases as the known pretreatment prognostic factors.[2,14] Adjusted hazard ratios for the relationship between pretreatment neutrophil, lymphocyte and monocyte counts and neutrophil-lymphocyte ratio and overall and progression-free survival after adjustment for the known prognostic factors are shown in Table 2. There was a statistically significant association between elevated neutrophil count and short overall (P=0.0008) and progression-free survival (P=0.024), and between high-neutrophil-lymphocyte ratio and short overall (P=0.011) and progression-free survival (P=0.040), whereas no association was found between lymphocyte or monocyte count and prognosis. The relationship between neutrophil count and both overall and progression-free survival was linear, whereas the relationship between neutrophil-lymphocyte ratio and overall survival was to some degree non-linear.

3.3 Optimal cut-off value for pretreatment neutrophil count

In selecting optimal cut-off values for the effect of neutrophil count on overall survival, the range between the 5th percentile (2205/mm^3) and the 95th percentile (9657/mm^3) for distribution of neutrophils was selected, and the possible cut-off points at intervals of 500/mm^3 from 2500/mm^3 to 9500/mm^3 were considered (giving 15 candidate cut-off points). Using the minimum P-value approach, the selected cut-off value for neutrophil count was 4500/mm^3 (corrected P=0.0009) and the corrected shrunk hazard ratio was 1.67 (95%CI, 1.09-2.54, from 100 bootstrap samples; Table 3). The selected optimal cut-off value did not change even if we used the stratified proportional hazards model, stratified by the combination of all covariates. The median survival time was 19.3 months (95%CI, 16.5-21.4) for the low-neutrophil group (<4500/mm^3, n=204) and 10.2 months (95%CI, 8.0-12.3) for the high-neutrophil group ($\geq$4500/mm^3, n=184) (Fig. 2). The results of prognostic factor analysis for overall survival are shown in Table 4. In terms of the relative order of significance, neutrophil count was one of the most important prognostic factors along with ECOG PS (p<0.0001), LDH (p=0.001) and smoking history (p=0.002). The adjusted hazard ratios for the relationship between neutrophil count (<4500/mm^3 versus $\geq$4500/mm^3) and survival according to the treatment groups were 1.62 (95%CI, 1.14-2.30) in the PC arm (n=195) and 1.74 (95%CI, 1.22-2.48) in the VGD arm (n=193). There was no interaction between the

neutrophil count and the treatment arms (P for interaction = 0.437).

3.4 Relationship between pretreatment neutrophil count and intensity of chemotherapy

In order to evaluate the effect of neutrophil count on administration of chemotherapy and toxicity, we analysed the dose intensity of chemotherapeutic agents and the incidence of toxicity in each arm. In the VGD arm, there was no significant difference in the relative dose intensity of vinorelbine or gemcitabine between the low-neutrophil group (<4500/mm^3) and the high-neutrophil group (≥4500/mm^3). However the relative dose intensity of docetaxel was significantly lower in the high-neutrophils group (median, 33%) than in the low-neutrophils group (median, 87%) (P=0.040, Wilcoxon test).

The toxicity due to treatment was also analysed. In the VGD arm, the incidence of grade 3 or 4 non-hematological toxicity within the first three cycles of treatment was significantly higher in the high-neutrophil group than in the low-neutrophil group (26.5% versus 8.5%; P=0.002, Fisher's exact test). Significantly fewer cycles were administered in the high-neutrophil group than in the low-neutrophil group (mean, 2.9 cycles versus 4.7 cycles; P<0.0001, Wilcoxon test). None of the patients in the high-neutrophil group who experienced grade 3 or 4 non-hematological toxicity within the first three cycles

completed the planned six cycles. The proportion of patients requiring reductions in the doses of vinorelbine or gemcitabine within the first two cycles of treatment was significantly higher in the low-neutrophil group (45.2%) than in the high-neutrophil group (26.4%) (P=0.007, Fisher's exact test). No such differences in dose intensity or toxicity were seen in the PC arm.

4. Discussion

In multivariate analysis after adjustment for known prognostic factors, we found linear associations between pretreatment elevated neutrophil count and short overall and progression-free survival. As there was no such association for the lymphocyte count, the relationship between neutrophil-lymphocyte ratio and overall survival was also found, however it was to some degree weak and non-linear. As a consequence, we consider that absolute neutrophil count may better serve as a prognostic factor. An optimal cut-off value for the relationship between neutrophil count and overall survival was identified as 4500/mm^3 (corrected hazard ratio, 1.67; 95%CI, 1.09-2.54). In the VGD arm, the low-neutrophil group (<4500/mm^3) tended to have a lower incidence of severe non-hematological toxicity and tolerated longer administration of the chemotherapeutic agents compared with the high-neutrophil group. However, no such association was found in the PC arm, and pretreatment neutrophil

count was equally predictive of prognosis in both treatment arms when analysed separately. We therefore do not consider it likely that the pretreatment neutrophil count serves as an indicator of intolerance to chemotherapy, rather than as an indicator of poor prognosis.

A number of studies in the last two decades have suggested an association between the neutrophil count or neutrophil-lymphocyte ratio and the prognosis of cancer patients,[7-16] although no acceptable explanations for the mechanisms underlying these observed associations have been proposed. Moreover, although neutrophilia often accompanies the diagnosis of cancer, the causes of neutrophilia in cancer patients are not fully understood, and are likely to be the result of a combination of factors. One obvious cause of neutrophilia is paraneoplastic production of myeloid growth factors by cancer cells themselves. Granulocyte-colony stimulating factor (G-CSF) is a growth factor that acts selectively on bone marrow granulocytic lineage cells, and is considered to play a central role in granulopoiesis. Administration of G-CSF was reported to increase bone marrow neutrophil precursors and shorten bone marrow transit time in mice and humans,[20-22] resulting in marked increases in the production of neutrophils. Granulocyte-macrophage colony stimulating factor (GM-CSF) and macrophage-colony stimulating factor are other examples of hematopoietic growth factors that cause neutrophilia by *in vivo* administration.[23,24] A variety of

non-hematopoietic malignant tumours including mesothelioma,[25] squamous cell carcinoma of the oropharynx,[26] melanoma,[27] glioblastoma,[28] and carcinoma of the lung[29] have been reported to secrete G-CSF or GM-CSF and cause significant leucocytosis. Although there have been several reports of the existence of autocrine growth loops for G-CSF and GM-CSF in non-hematopoietic tumour cells, implying G-CSF- and GM-CSF-producing tumours are more aggressive,[30,31] the relationship between paraneoplastic production of myeloid growth factors and prognosis remains unclear. Furthermore, considering the linear relationship we observed between pretreatment neutrophil count and survival in this study, ectopic production of myeloid growth factors, which often causes marked neutrophilia, does not seem to be the sole reason for the observed association between neutrophil count and prognosis.

Other possible factors that cause neutrophilia are coexistent infection and cancer-related inflammation. In this study, patients with active infection were excluded based on the eligibility criteria of the trial, and there is no clear reason to assume the existence of latent infection as the cause of neutrophilia and poor prognosis.

The association between cancer and inflammation was initially pointed out during the 19th century. However, recent advances in understanding of tumour

biology have stimulated renewed interests in searching for links between cancer and inflammation.[3-6] Today, it is widely accepted that chronic inflammation contributes to the initiation and progression of cancer. Furthermore, it is now known that inflammatory processes almost always accompany cancer, and persistence of chronic inflammation-like processes within cancer tissue causes suppression of anti-tumour immunity by several mechanisms such as activation of type 2 T-helper responses, recruitment of regulatory T cells and activation of the chemokine system, and result in promotion of cancer growth and metastasis. Thus, inflammation may result in the aggressive growth of a tumour. The cytokines interleukin (IL)-6 and tumour necrosis factor-alpha (TNFα), which are implicated in the pathogenesis of cancer-related inflammation as well as of acute inflammatory processes, are also known to induce neutrophilia.[32-34] It is possible that the neutrophil count at diagnosis indicates the severity or nature of inflammation occurring within the tumour, and thus reflects prognosis. In a recent report, a proportion of patients with metastatic cancer were shown to have IL-6-mediated elevation in serum cortisol levels. This may partly explain the neutrophilia of cancer patients, although its contribution to outcome is not yet known.[35]

We did not measure inflammatory markers such as C-reactive protein or hemogram of total white cell count in this study. However, we are investigating

correlations between several cytokines and prognosis in a correlative study of another clinical trial (ClinicalTrials.gov identifier NCT00616031).

Besides inflammation in cancer tissue, host factors may influence the prognosis of cancer patients. It is now known that lifetime exposure to infectious diseases and other sources of inflammation is not only related to the pathogenesis of cancer, but also plays an important role in aging and influences longevity.[36,37] Aging is a complex process, and numerous genes are known to have associations with longevity.[38] Polymorphisms of the genes that encode proteins involved in inflammatory processes (e.g., IL-1, IL-6, IL-10 and TNFα) are suspected to affect aging and longevity. Given the close relationship between cancer and inflammation, it is natural to speculate that genetic polymorphisms in inflammation-related genes may also influence host responses to cancer and prognosis; peripheral neutrophil count may be an indicator of this association.

Another possibility is that neutrophil directly down-regulates host cellular immunity against cancer, thereby affecting the prognosis. *In vitro* studies showed that neutrophils suppress the cytolytic activity of lymphocytes and natural killer cells when co-cultured with neutrophils and lymphocytes from normal healthy donors; the degree of suppression was proportional to the number of neutrophils added.[39-41] The clinical relevance of these effects seen in

in vitro studies is currently unknown. The biological basis for the multi-factorial and complex association is also unknown, and merits further research.

5. Conclusion

Using the dataset from a randomised controlled trial, we have confirmed that pretreatment peripheral blood neutrophil count is an independent prognostic factor in patients with advanced NSCLC receiving modern chemotherapy. The results need to be investigated for generalisability in other populations. Since neutrophil count is easily measured at low cost, it may be a useful predictor of prognosis in clinical practice. Considering the strength of the association reported here, neutrophil count should be taken into account as a stratification factor in future randomised clinical trials of patients with advanced NSCLC.

Conflict of interest

Kaoru Kubota has received honoraria from Eli Lilly, Sanofi-Aventis, and Chugai. All other authors declared no conflicts of interest.

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Table 1. Baseline patient characteristics (n=388)

Characteristics	No.	%
Age, years, Median (Range)	65 (33-81)	
Sex		
Male	276	71
Female	112	29
Smoking history		
Nonsmokers	96	25
Former smokers	107	28
Current smokers	168	43
Unknown	17	4
Stage		
IIIB	68	18
IV	320	82
Histologic type		
Squamous cell	76	20
Adenocarcinoma	274	70
Others	38	10
ECOG Performance status		
0	154	40
1	234	60
Weight loss (from 6months before enrolment)		
< 5%	317	82
≥ 5%	71	18
LDH		
Normal (< ULN)	279	72
High (≥ ULN)	109	28
Bone metastases		
No	280	72
Yes	108	28
Liver metastases		
No	357	92
Yes	31	8
Skin metastases		
No	379	98
Yes	9	2
Neutrophils, /mm^3, Median (range)	4304 (205-17100)	
Lymphocytes, /mm^3, Median (range)	1386 (243-4200)	
Monocytes, /mm^3, Median (range)[a]	404.2 (0-1620)	
Red blood cells, x10^4/mm^3, Median (range)	420 (286-579)	
Platelets, x10^4/mm^3, Median (range)[b]	26 (11-380)	

ULN: upper limit of normal

a: One missing value

b: Two missing values

Table 2. Multivariate Cox regression analysis for neutrophil, lymphocyte and monocyte counts

Factors	Overall survival				Progression-free survival			
	Hazard Ratio[a]	95%CI	p	p[b]	Hazard Ratio[a]	95%CI	p	p[b]
Neutrophil count (/mm^3)								
Quartile 1 (< 3278)	1.00	–	–	.0008	1.00	–	–	.024
Quartile 2 (< 4304)	1.25	0.86–1.82	.251		1.19	0.88–1.61	.258	
Quartile 3 (< 5873)	1.76	1.22–2.53	.002		1.32	0.97–1.78	.076	
Quartile 4 (≥ 5873)	1.94	1.35–2.79	.0003		1.61	1.18–2.19	.003	
Lymphocyte count (/mm^3)								
Quartile 1 (< 1082.3)	1.00	–	–	.251	1.00	–	–	.545
Quartile 2 (< 1386.1)	1.14	0.81–1.61	.438		1.10	0.82–1.47	.535	
Quartile 3 (< 1821.8)	0.83	0.58–1.19	.303		0.88	0.65–1.20	.424	
Quartile 4 (≥ 1821.8)	1.13	0.80–1.59	.495		0.95	0.70–1.28	.732	
Neutrophil-lymphocyte ratio								
Quartile 1 (< 2.093)	1.00	–	–	.011	1.00	–	–	.040
Quartile 2 (< 2.914)	1.42	0.98–2.05	.065		1.39	1.02–1.88	.035	
Quartile 3 (< 4.744)	1.83	1.27–2.62	.001		1.50	1.09–2.06	.012	
Quartile 4 (≥ 4.744)	1.56	1.09–2.24	.015		1.48	1.09–2.02	.013	
Monocyte count (/mm^3)								
Quartile 1 (< 289.9)	1.00	–	–	.381	1.00	–	–	.969
Quartile 2 (< 402.3)	0.93	0.65–1.32	.674		1.05	0.78–1.41	.755	
Quartile 3 (< 550.4)	1.07	0.75–1.52	.712		0.99	0.72–1.35	.924	
Quartile 4 (≥ 550.4)	1.26	0.89–1.78	.203		1.04	0.76–1.42	.792	

CI: confidence interval

a: Adjustment for sex, smoking, stage, ECOG PS, weight loss, LDH, bone metastases, liver metastases and skin metastases

b: P values for global association

Table 3. Cutpoint analysis for neutrophil count and overall survival

Neutrophil count (Cut-off points, /mm^3)	Uncorrected Hazard Ratio[a]	Uncorrected p-value
2500	1.95	.016
3000	1.78	.001
3500	1.40	.021
4000	1.57	.0007
4500	**1.72[b]**	**<.0001[c]**
5000	1.49	.002
5500	1.51	.002
6000	1.46	.008
6500	1.75	.0004
7000	1.62	.005
7500	1.59	.015
8000	1.88	.004
8500	1.86	.007
9000	1.78	.017
9500	1.89	.009

a: (hazard of death in patients on or above the cut-off point) divided by (hazard of death in patients below the cut-off point), after adjustment for sex, smoking, stage, ECOG PS, weight loss, LDH, bone metastases, liver metastases and skin metastases

b: Corrected hazard ratio: 1.67 (95%CI, 1.09-2.54)

c: Corrected P=0.0009

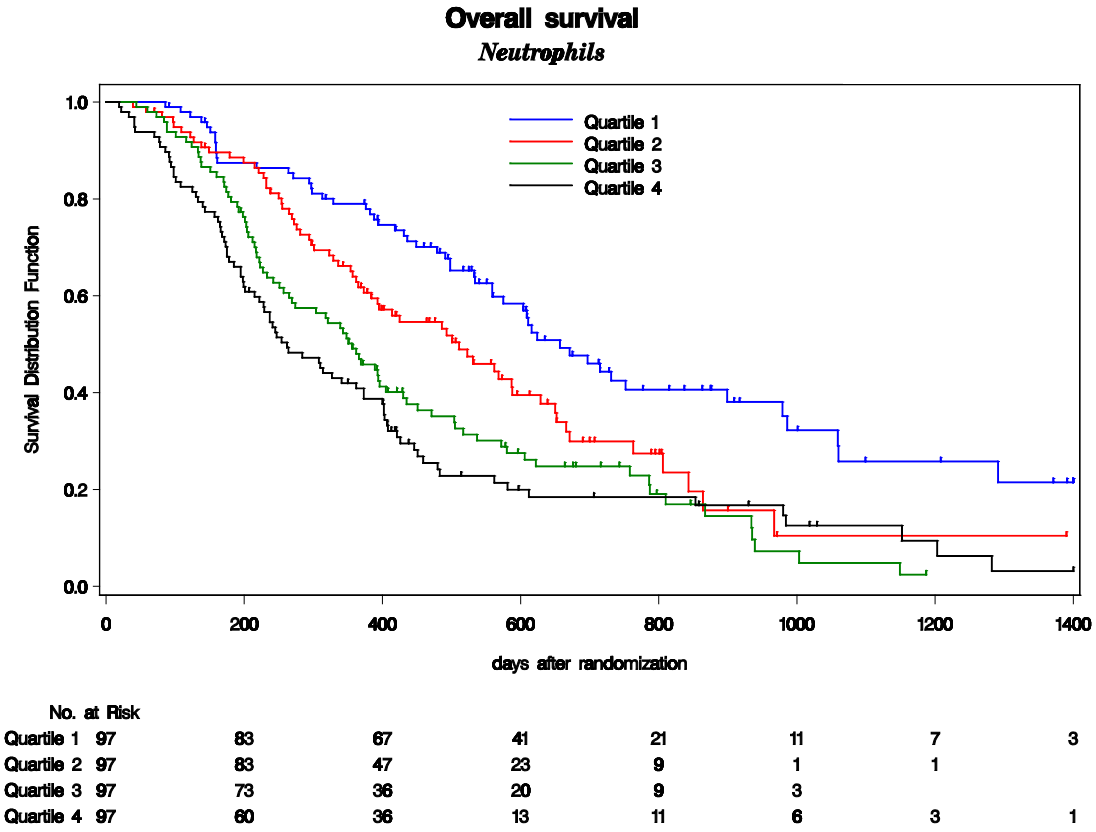
Table 4. Prognostic factor analysis for overall survival using proportional hazards regression model without variable selection

Factors	Hazard Ratio	95%CI	P-value
Performance status			
0	1.00	-	-
1	2.03	1.54-2.67	<0.0001
Neutrophil count			
$< 4500/mm^3$	1.00	-	-
$\geq 4500/mm^3$	1.72	1.34-2.19	<0.0001
LDH			
Normal	1.00	-	-
High	1.57	1.20-2.05	0.001
Smoking history			
Non/Former smokers	1.00	-	-
Current smokers	1.56	1.18-2.06	0.002
Liver metastases			
No	1.00	-	-
Yes	1.62	1.08-2.43	0.020
Sex			
Male	1.00	-	-
Female	0.74	0.54-1.02	0.064
Weight loss			
< 5%	1.00	-	-
≥ 5%	1.30	0.96-1.76	0.092
Skin metastases			
No	1.00	-	-
Yes	1.78	0.85-3.72	0.124
Bone metastases			
No	1.00	-	-
Yes	1.21	0.90-1.63	0.204
Stage			
IIIB	1.00	-	-
IV	1.24	0.88-1.75	0.222

Figure legends

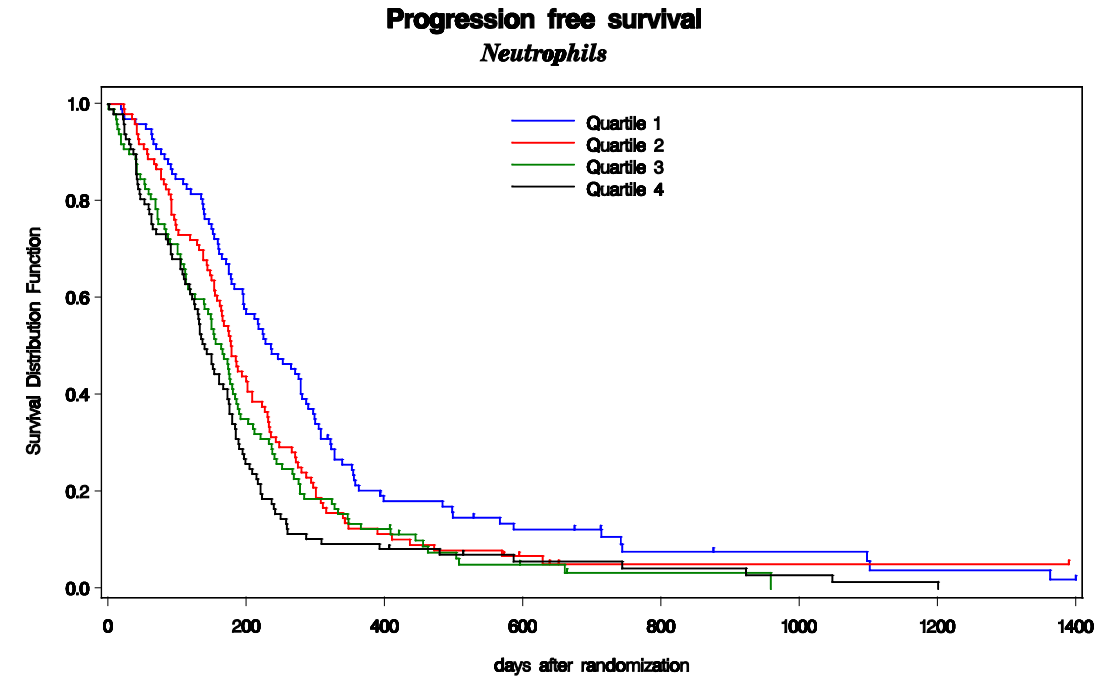
Figure 1. Kaplan-Meier estimates according to quartiles for the effect of pretreatment neutrophil count on (A) overall survival (B) progression-free survival.

Figure 2. Kaplan-Meier estimates according to optimal cut-off point (4500/mm^3) for the effect of pretreatment neutrophil count on overall survival.



No. at Risk								
Quartile 1	97	83	67	41	21	11	7	3
Quartile 2	97	83	47	23	9	1	1	
Quartile 3	97	73	36	20	9	3		
Quartile 4	97	60	36	13	11	6	3	1

Figure 1A



No. at Risk								
Quartile 1	97	56	16	10	5	4	2	1
Quartile 2	97	42	10	4	1	1	1	
Quartile 3	97	34	12	3	1			
Quartile 4	97	25	8	4	3	2	1	

Figure 1B

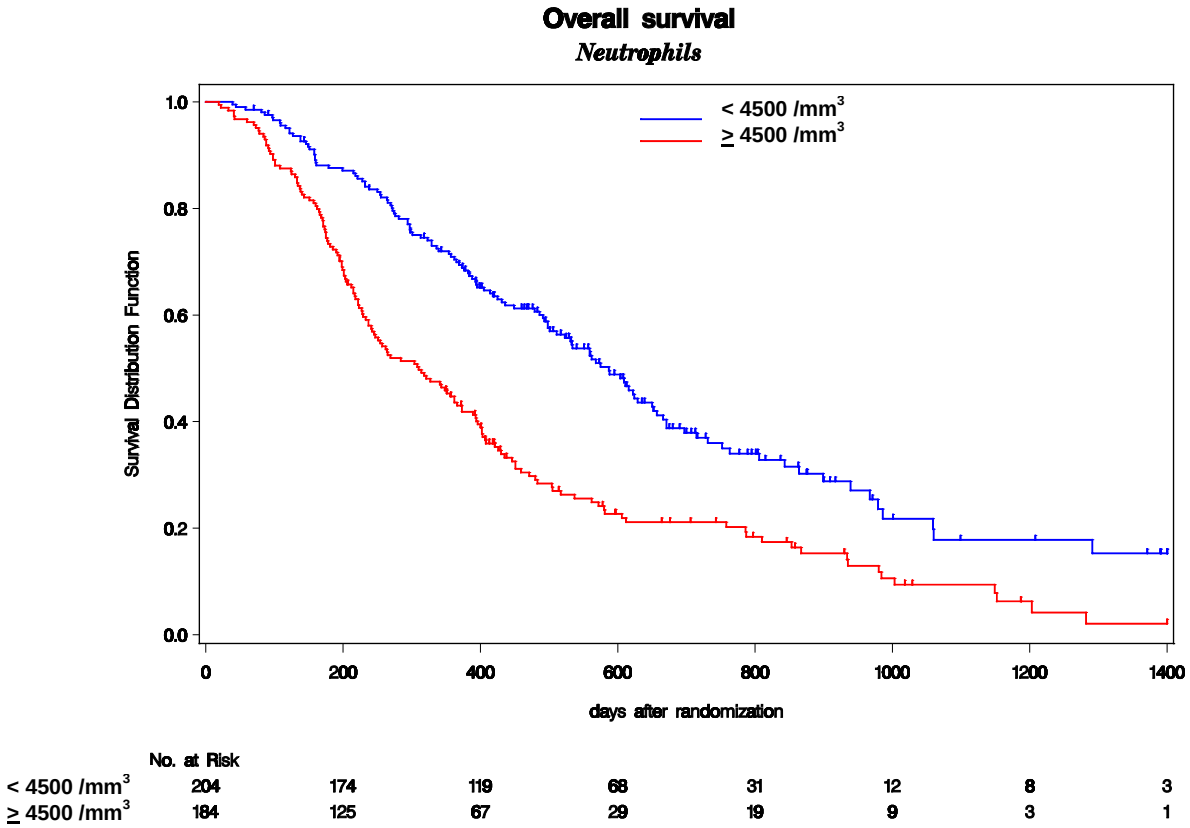

Overall survival
Neutrophils
< 4500 /mm3
≥ 4500 /mm3
Survival Distribution Function
1.0
0.8
0.6
0.4
0.2
0.0
0
200
400
600
800
1000
1200
1400
days after randomization
No. at Risk
< 4500 /mm3 204 174 119 68 31 12 8 3
≥ 4500 /mm3 184 125 67 29 19 9 3 1

Figure 2