Myelotoxicity as a Prognostic Factor in Patients with Advanced Breast Cancer Treated with Chemotherapy: A Pooled Analysis of Two Randomised Trials Conducted by The Hellenic Cooperative Oncology Group

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Abstract. *Background: A number of studies have shown that absence of myelotoxicity during chemotherapy is associated with worse outcome for various types of cancer, including carcinoma of the breast. The aim of this study was to determine whether myelosuppression in patients being treated with chemotherapy for advanced breast cancer has an impact on their prognosis. Patients and Methods: A retrospective review was conducted of a series of 475 patients with advanced breast cancer enrolled in two randomised trials, who received first-line chemotherapy. The impact of severe (grade 3 or 4) hematological toxicity on survival and time to disease progression was assessed. Results: When severe myelotoxicity was evaluated as a whole, a significant negative association for time to disease progression and a trend for a worse survival were demonstrated. In multivariate analysis, hematological toxicity retained its significance as an independent negative prognostic factor for time to disease progression. Conclusion: Our findings do not confirm the results of previous studies which have demonstrated a better outcome for patients experiencing hematological toxicity during treatment.*

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Key Words: Myelotoxicity, chemotherapy, prognosis, advanced, breast cancer.

The impact of chemotherapy dose and dose intensity on the outcome of patients with breast cancer remains a matter of debate. A number of retrospective clinical trials have shown a positive correlation between the dose or dose intensity of chemotherapy and the outcome of patients with early breast cancer (1, 2), whereas others have failed to show a similar association (3). Likewise, the results of the studies

prospectively addressing the role of dose and dose intensity in the adjuvant setting have not been consistent (4-7). Although a few trials have demonstrated a dose-response effect in advanced disease (8), other controlled studies have not shown a benefit in outcome when patients received a higher dose of chemotherapy (9).

Moreover, body surface area-based (BSA) dosing of chemotherapy does not account for the complex processes of cytotoxic drug elimination and this could lead to an unpredictable variation in efficacy (10, 11). Myelotoxicity during treatment might indicate both the level of exposure and the susceptibility of tissues to chemotherapy. The potential importance of tailoring the dose of chemotherapy based on hematological toxicity in breast cancer patients receiving adjuvant treatment has been suggested by Bergh *et. al.* (12, 13).

With the assumption that myelotoxicity might represent an important biological marker of the effect of chemotherapy, a number of trials have investigated the possible association between hematological toxicity and treatment outcome. Saarto *et al.* (14) reported that the leukocyte nadir during adjuvant chemotherapy with cyclophosphamide, doxorubicin and oral ftorafur (CAFt) in patients with breast cancer was significantly correlated with improved distant disease-free (DDFS) and overall survival (OS). Furthermore, a similar report from the same group demonstrated that a low leukocyte nadir during the adjuvant chemotherapy with cyclophosphamide, methotrexate and fluorouracil (CMF) was associated with a long DDFS in univariate, but not in multivariate analysis (15). In another trial investigating the prognostic effect of myelosuppression on survival after adjuvant chemotherapy (CMF) for breast carcinoma, patients experiencing grade 3 and 4 myelotoxicity had a significantly better outcome in univariate analysis. This observation was found to be of borderline significance in multivariate analysis (16). Cameron *et al.* (17) reviewed the case notes of 750 women treated with the CMF regimen and they found that 45% of patients who had grade 2 and 3 neutropenia had a 10% absolute survival advantage over those with no neutropenia. Similar findings suggesting a correlation between hematological toxicity and favorable outcome in patients with early breast cancer have also been reported in other studies (18). Data regarding the predictive or prognostic role of toxicity in metastatic breast cancer are limited. In a randomized clinical trial in patients with advanced breast cancer (ABC), a relationship between hematological and gastrointestinal toxicity and therapeutic efficacy was demonstrated with a superior survival and response rate recorded for patients with such toxicity (19). Concerning other types of cancer, a number of studies have also shown that absence of myelotoxicity is associated with worse outcome (20-24).

The purpose of this study was to determine whether myelosuppression in patients receiving chemotherapy for metastatic breast carcinoma affects the prognosis of the disease. To address the question, we conducted a retrospective review of a series of patients with ABC participating in two randomised trials, who received first-line chemotherapy.

Patients and Methods

Five hundred and ten patients with ABC who participated in two randomized clinical trials (11B/97 and 11B/99) conducted by the Hellenic Cooperative Oncology Group (HeCOG) (25, 26) were candidates to participate in this retrospective study. Seven patients who never started chemotherapy and 3 patients for whom no data on toxicity were available were excluded. The aim of this study was to explore the effect of severe myelotoxicity on patient outcome. Therefore, it was decided to include only patients who survived long enough to experience severe myelotoxicity in the analysis. Since the maximum treatment duration (first infusion to last infusion) was 4 months, day 121 was set as the landmark day. Patients who died within 4 months from the day of randomization were excluded from the survival analysis, while those experiencing a disease progression earlier than 4 months after their randomization were not included in the time to disease progression (TTP) analysis. Consequently, the study population comprised 475 patients with metastatic breast cancer enrolled in these two studies. Both trials were approved by the HeCOG Protocol Review Committee and Institutional Review Boards in participating institutions. Written informed consent was obtained from all patients. Eligibility criteria and dose modifications were the same across the studies.

The first study (11B/97) compared the efficacy of two different schedules of epirubicin and paclitaxel, as first-line chemotherapy, in patients with ABC (25). From October 1997 until May 1999, 183 eligible patients entered the study. Chemotherapy in group A (93 patients) consisted of four cycles of epirubicin at a dose of 110 mg/m^2 followed by four cycles of paclitaxel at a dose of 225 mg/m^2 in a 3-hour infusion. All cycles were repeated every 2 weeks with granulocyte colony-stimulating factor (G-CSF) support. The therapeutic regimen in group B (90 patients) consisted of epirubicin (80 mg/m^2) immediately followed by paclitaxel (175 mg/m^2 in a 3-hour infusion) every 3 weeks for six cycles.

The second study (11B/99) compared the survival between patients with ABC being treated with epirubicin/paclitaxel or paclitaxel/carboplatin chemotherapy (26). From January 1999 to April 2002, 327 eligible patients were randomized to receive either paclitaxel 175 mg/m^2 in a 3-hour infusion followed by epirubicin 80 mg/m^2 (group A: 163 patients) or paclitaxel, as in group A, followed by carboplatin at an area under the curve (AUC) of 6 mg x min/ml (group B: 164 patients) every 3 weeks for six cycles.

Assessment of hematological toxicity. In both studies, complete biochemistry and full blood count (FBC) were measured on the day of treatment. FBC was repeated between cycles in the case of fever, hemorrhagic manifestations, or severe mucositis. The frequency of observation for the blood count parameters was the same across studies and study arms. In cases of granulocytopenia or thrombocytopenia on the first day of the cycle, treatment was delayed until the absolute neutrophil count was 1,500/μl or higher and the platelet count was 100,000/μl or higher, respectively. Toxicity criteria were those adopted by the World Health

Organization (WHO). Patients randomized to group A in the first study (11B/97) were given G-CSF (filgrastim, 5 μg/kg daily) prophylactically on days 2 to 10 of each cycle.

Statistical analysis. Survival was defined as the time from day 121 to each patient's death or last contact. TTP was defined as the time from day 121 to documented disease progression, death, or last contact. Death from the disease without documentation of disease progression was considered as an event in the TTP analysis.

Anemia, thrombocytopenia and neutropenia were recorded according to WHO criteria and severe myelotoxicity was defined as the presence of severe (grade 3 or 4) anemia, thrombocytopenia or neutropenia at least once during chemotherapy. Patient and treatment characteristics in different myelotoxicity categories were compared by means of chi-square test for categorical variables and the non-parametric Mann-Whitney test for continuous variables. Survival and TTP were estimated using the Kaplan-Meier method, and survival curves for patients with severe myelotoxicity *versus* all other patients were compared using the log-rank test stratified by treatment group. For both survival time and TTP, multivariate Cox regression analysis was performed. The following variables were included in the models: treatment status (completion *vs*. discontinuation), estrogen receptor (ER)-progesterone receptor (PR) status (positive *vs*. negative), menopausal status (post- *vs*. premenopausal), maintenance hormonal treatment (HT) (yes *vs*. no), performance status (PS) on the Eastern Cooperative Oncology Group scale (1 or 2 *vs*. 0), presence of visceral metastases (yes *vs*. no), presence of bone metastases (yes *vs*. no), number of metastatic sites (2 or ≥3 *vs*. 1), myelotoxicity (yes *vs*. no), adjuvant chemotherapy, adjuvant hormonal therapy and group of first-line treatment. In order to identify the subclass of significant variables in the presence of treatment group, a backward selection procedure with exclusion criterion p=0.10 was used. In cases of significant myelotoxicity effect, interaction terms for treatment groups with myelotoxicity were added in the final model if found significant (p<0.05). In order to explore the effect of each toxicity separately, Kaplan-Meier estimates were calculated for anemia, thrombocytopenia and neutropenia categories (severe *vs*. non-severe). Cox regression models for TTP and survival were re-fitted including anemia, thrombocytopenia and neutropenia instead of myelotoxicity. Additional Cox regression analysis was performed considering three categories of myelotoxicity (absent: grade 0, mild: grade 1 or 2, and severe: grade 3 or 4). Statistical analysis was performed using SPSS 11.01 (SPSS, Chicago, IL, USA). All statistical tests were two-sided at the 0.05 level of significance.

Results

For the 475 patients included in the analysis, patient and tumor characteristics are listed in Table I. Between patients with severe myelotoxicity and all other patients, there were no significant differences in terms of those characteristics. The median duration of follow-up was 44.4 months (range, 0.1-75.2 months). Four hundred and thirty-six patients had not relapsed before the landmark day of 4 months and were thus included in the TTP analysis. Among these 436 patients, 367 either relapsed or died from the disease without prior documented progression and the median TTP was 8 months (range, 0.1-75.2 months; 95% CI 6.2-7.9 months). Moreover,

Table I. *Selective patient and tumor characteristics*.

	Myelotoxicity			
	Absent or mild		Severe (Grade 3 or 4)	
N	391		84	
Age (years)				
Median	58		55	
Range	26-78		33-77	
	N	%	N	%
Performance status				
0	269	69	50	60
1	102	26	27	32
2	20	5	7	8
Menopausal status				
Premenopausal	119	30	28	33
Postmenopausal	272	70	56	67
ER status				
Negative	121	31	21	25
Positive	215	55	52	62
Unknown	55	14	11	13
PR status				
Negative	144	37	35	42
Positive	187	48	37	44
Unknown	60	15	12	14
Adjuvant CT				
No	210	54	43	51
Yes	181	46	41	49
Adjuvant HT				
No	204	52	46	55
Yes	187	48	38	45
Adjuvant RT				
No	275	70	55	65
Yes	116	30	29	35
Sites of metastases				
Locoregional				
Axillary nodes	108	28	22	26
Skin	68	17	14	17
Breast	60	15	14	17
Supraclavicular nodes	69	18	15	18
Distant				
Bones	172	44	49	58
Visceral	250	64	63	75
Soft tissue/nodes	88	23	14	17
Abdomen/ascites	7	2	2	2
Other breast	19	5	2	2
Pleural effusion	29	7	6	7
Locoregional only	47	12	7	8
Distant only	209	53	45	54
Locoregional and distant	135	35	32	38
Metastatic sites				
1 metastatic site	113	29	15	18
2 metastatic sites	117	30	26	31
≥ 3 metastatic sites	161	41	43	51

ER, estrogen receptor; PR, progesterone receptor; CT, chemotherapy; RT, radiotherapy; HT, hormonal therapy.

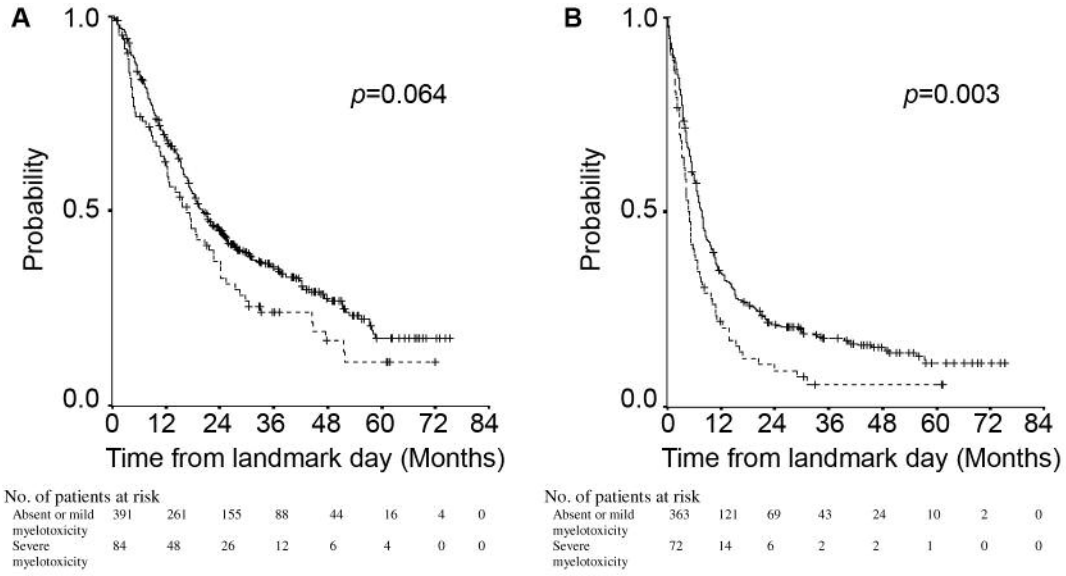

Figure 1. *Kaplan-Meier curves for the survival (A) and time to disease progression (TTP) (B) of patients with grade 3 or 4 myelotoxicity (dashed line) and all other patients.*

328 patients had died (325 from the disease; 3 from unrelated causes: 2 from stroke and 1 from cardiac arrest) and the median survival was 19.4 months (range, 0.1-75.2 months; 95% CI 16.9-21.8 months).

In terms of toxicity, both regimens in the above studies were generally well tolerated and serious (grade 3 or 4) adverse events were infrequent. Overall, 84 patients (18%) experienced at least one severe myelotoxicity event during their treatment. More specifically, 20 patients (4%) developed severe anemia, 10 patients (2%) presented severe thrombocytopenia, while 69 patients (15%) experienced severe neutropenia.

In study 11B/97, G-CSF administration was required in all group A patients according to the protocol. In group B, 61% of patients received G-CSF, mainly for maintaining dose intensity, *i.e.* to administer subsequent cycles on time, and an additional 21% of patients in group B for severe toxicity. In study 11B/99, G-CSF administration was required in 73 patients (45%) in group A and 79 patients (48%) in group B, mainly for maintaining dose intensity. Dose intensity of epirubicin and paclitaxel, but not carboplatin, was significantly lower in patients with severe myelotoxicity. Median relative dose intensity (RDI) of epirubicin was 0.87 (range, 0.17-1.1) in patients with grade 3 or 4 myelotoxicity and 0.97 (range, 0.24-1.4) in those without such a toxicity ($p<0.001$). Likewise, RDI of paclitaxel was 0.87 (range, 0.20-1.15) and 0.97 (range, 0.1-1.49), respectively ($p<0.001$).

When any severe myelotoxicity was investigated as a whole, a significant negative association with TTP and a trend for negative association with survival were demonstrated. The median TTP was 5 months (range, 0.3-61.4 months; 95% CI 4.0-6.1 months) in patients with grade 3 or 4 myelotoxicity and 7.7 months in patients without severe toxicity (range, 0.1-75.2 months; 95% CI 6.8-8.6 months) ($p=0.003$). Furthermore, median survival was 16.6 months in patients who developed grade 3 or 4 myelotoxicity (range, 0.4-71.9 months; 95% CI 11.2-22 months) *versus* 20 months (range, 0.1-75.2 months; 95% CI 16.9-23.2 months) for those without toxicity ($p=0.064$). Kaplan-Meier curves for survival and TTP of patients with severe myelotoxicity and all other patients are shown in Figure 1.

According to the results of the multivariate analysis, myelotoxicity was not proven to be an independent prognostic factor for survival. Including treatment group in the model, positive ER-PR status [hazard ratio (HR) 0.75, 95% CI 0.57-0.98, $p=0.032$] and maintenance hormonal therapy (HR 0.65, 95% CI 0.51-0.84, $p=0.001$) implied a lower hazard for death, while impaired PS (HR 1.39, 95% CI 1.08-1.79, $p=0.010$), presence of visceral metastases (HR 1.40, 95% CI 1.07-1.82, $p=0.014$) and presence of more than 3 metastatic sites (HR 1.53, 95% CI 1.13-2.06, $p=0.005$) were identified as predictors of worse survival. In the presence of the above prognostic factors, group A of the 11B/99 study had a significantly worse survival compared to group B of the same study (HR 1.37, 95% CI 1.08-1.79, $p=0.038$) (Table II).

For the TTP, presence of visceral metastases (HR 1.50, 95% CI 1.17-1.91, $p=0.001$), presence of more than 3 metastatic sites (HR 1.66, 95% CI 1.25-2.21, $p<0.001$) and maintenance hormonal therapy (HR 0.65, 95% CI 0.49-0.78,

Table II. *Multivariate Cox regression model for survival.*

	Hazard ratio	95% CI	*p*-Value
ER-PR			
Negative	1	-	-
Positive	0.75	0.57-0.98	**0.032**
Performance status			
0	1	-	-
1 or 2	1.39	1.08-1.79	**0.010**
Visceral metastases			
No	1	-	-
Yes	1.40	1.07-1.82	**0.014**
Number of metastatic sites			
1	1	-	-
2	1.11	0.80-1.53	0.541
≥3	1.53	1.13-2.06	**0.005**
Maintenance HT			
No	1	-	-
Yes	0.65	0.51-0.84	**0.001**
Treatment group			
11B/99 group B	1	-	-
11B/99 group A	1.37	1.08-1.79	**0.038**
11B/97 group A	1.23	0.87-1.73	0.236
11B/97 group B	1.25	0.87-1.79	0.225

CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor; HT, hormonal therapy.

Table III. *Multivariate Cox regression model for the time to progression.*

	Hazard ratio	95% CI	*p*-Value
ER-PR			
Negative	1	-	-
Positive	0.78	0.60-1.00	0.053
Myelotoxicity			
No	1	-	-
Yes	3.07	1.92-4.89	**<0.001**
Visceral metastases			
No	1	-	-
Yes	1.50	1.17-1.91	**0.001**
Number of metastatic sites			
1	1	-	-
2	1.18	0.86-1.60	0.303
≥3	1.66	1.25-2.21	**<0.001**
Maintenance HT			
No	1	-	-
Yes	0.65	0.49-0.78	**<0.001**
Treatment group			
11B/99 group B	1	-	-
11B/99 group A	1.76	1.31-2.38	**<0.001**
11B/97 group A	1.27	0.89-1.82	0.193
11B/97 group B	1.40	0.93-2.11	0.103
Treatment status			
Discontinuation	1	-	-
Completion	0.64	0.40-1.04	0.074
Myelotoxicity by			
11B/99 group B	1	-	-
11B/99 group A	0.31	0.15-0.65	**0.002**
11B/97 group A	0.46	0.19-1.12	0.087
11B/97 group B	0.47	0.22-1.00	0.050

CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor; HT, hormonal therapy.

$p<0.001$) were identified as independent prognostic factors for patients' survival (Table III). Within group B of study 11B/99, the presence of severe myelotoxicity was significantly associated with a higher risk of disease progression (HR 3.07, 95% CI 1.92-4.89, $p<0.001$). Effect of severe myelotoxicity was not significant in group A or B of the 11B/97 study nor in group A of the 11B/99 study. This result is illustrated in Figure 2.

Using three categories of myelotoxicity: absent (grade 0), mild (grade 1 or 2) and severe (grade 3 or 4) in a Cox regression analysis, results were similar. The effect of myelotoxicity on survival was still insignificant, while severe myelotoxicity was negatively associated with TTP in group B of the 11B/99 study (HR 3.17, 95% CI 1.87-5.38, $p<0.001$). All other prognostic factors in the final models for both survival and TTP remained unchanged.

Furthermore, the effect of severe anemia and severe thrombocytopenia on both survival and TTP was significant ($p=0.033$, $p<0.001$, $p<0.001$ and $p=0.008$, respectively), whereas the effect of severe neutropenia did not play a role in patients' outcome (data not shown).

Discussion

Despite the increasing use of novel targeted agents, cytotoxic chemotherapy remains the mainstay of anticancer treatment and it is likely that it will continue to be used in the years to come. There is concern regarding the impact of hematological toxicity after treatment on the outcome of cancer patients receiving chemotherapy (27, 28). The aim of the current study was to determine whether myelosuppression in patients being treated with chemotherapy for ABC has an impact on the prognosis of the disease. In the present study, we found a negative association between severe myelotoxicity and TTP and a trend for a worse survival. Hematological toxicity retained its significance as a negative prognostic factor in the multivariate Cox regression model for TTP. For patients who experienced severe anemia or thrombocytopenia during treatment, a negative association with TTP and survival was also established, but the number of such patients was relatively small (4% and 2% , respectively).

Regarding the current results, it could be argued that patients who developed grade 3 or 4 toxicity might represent a more "ill" population consisting of women with less favorable characteristics such as worse PS and thus a worse outcome for those patients would be reasonable. Such a hypothesis could not explain our findings, since in the multivariate analysis, myelotoxicity was found to be significant, taking into account the PS. Furthermore, there were no significant differences between the two groups in terms of PS and other patient or tumor characteristics. Moreover, the lower RDI of epirubicin and paclitaxel in the

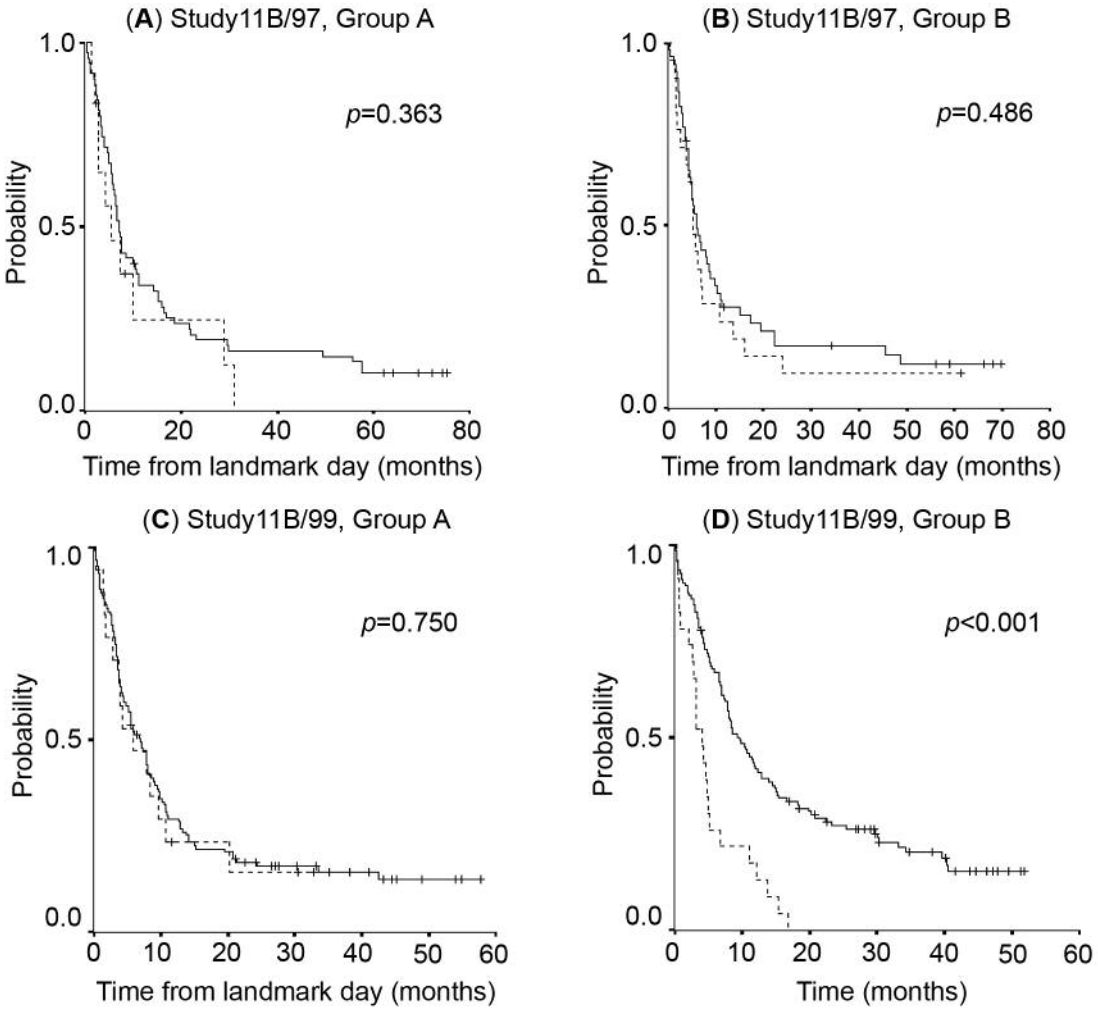

Figure 2. *Kaplan-Meier curves for time to disease progression (TTP) of patients with grade 3 or 4 myelotoxicity (dashed line) and all other patients, within each treatment group.*

group of patients with severe toxicity could only partially explain the worse outcome, since the observed difference in RDI seems quite small to be clinically meaningful in terms of efficacy. Furthermore, the possibility of metastatic disease to the bone marrow, which is usually associated with poor prognosis, could not be excluded in those patients experiencing severe hematological toxicity, especially thrombocytopenia.

Our findings do not confirm the results of previous studies concerning mainly patients with early (14-18) but also those with metastatic disease (19). Moreover, our results contradict those reported in a recently published analysis of three randomised trials in patients with advanced non-small cell lung cancer (24). In this study, neutropenia during chemotherapy was associated with increased survival of patients. Furthermore, the presence in general (even grade 1 or 2) and not the severity of neutropenia was prognostic for better outcome. In our study, a similar association was not confirmed. In contrast, a trend for shorter TTP was shown in patients experiencing severe neutropenia (data not shown). The use of G-CSF in a large proportion of patients in our study could potentially affect the results, but when analysis was repeated after excluding the patients who received G-CSF prophylactically (group A in the first study), the results did not change (data not shown). Furthermore, when Cox regression analysis was performed considering three categories of myelotoxicity: absent (grade 0), mild (grade 1 or 2) and severe (grade 3 or 4), the results did not change, suggesting that only severe and not mild hematological toxicity was related to prognosis.

Currently, we are not able to provide a definitive explanation for the association demonstrated in our study, but our findings support the negative prognostic role of severe myelotoxicity in patients with advanced carcinoma of the

breast. The hypothesis of escalating the dose of chemotherapy in order to tailor treatment based on myelotoxicity could be a conclusion of the reports in patients with early disease, assuming that hematological toxicity constitutes a biological indicator of dose intensity. However, according to our findings, expanding the individual planning of chemotherapy doses to patients with metastatic breast carcinoma is not justified. Moreover, it is well known that the development of myelosuppression is influenced both by chemotherapy and patient characteristics, including age, general condition, comorbidities, previous chemotherapy or radiotherapy, and tumor involvement of the bone marrow and, hence, the association of myelotoxicity with the activity of the treatment is not always direct.

In summary, the findings of the current study do not confirm those of previous studies which have shown a better outcome for patients developing hematological toxicity during chemotherapy. In addition, our results provide some evidence that severe myelotoxicity during treatment might be associated with worse prognosis in patients with metastatic breast cancer. Given the absence of prospective data, further studies designed to address this issue are warranted.

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Received March 12, 2008
Revised June 20, 2008
Accepted July 3, 2008