Prolonged Neoadjuvant Chemotherapy with GM-CSF in Locally Advanced Breast Cancer

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Abstract

Background. **Neoadjuvant chemotherapy improves survival in patients with locally advanced breast cancer (LABC). Usually three to four cycles of conventional-dose neoadjuvant chemotherapy are administered prior to local therapy, and another three cycles thereafter. In an attempt to improve results, we increased the dosages and applied GM-CSF, which, besides being a hematopoietic growth factor, has become increasingly known for its immunostimulatory effects, which might enhance the antitumor effect.**

Methods. **Forty-two patients with stage IIIA or IIIB breast cancer were treated with doxorubicin (A) (90 mg/m^2) and cyclophosphamide (C) (1,000 mg/m^2) at three-weekly intervals. In the second and fourth cycle a 10% dose reduction of both agents was applied. On the second day GM-CSF 250 µg/m^2/day was started and given for 10 days. Initially, some patients were treated with ≤ four cycles, but as the study progressed and toxicity appeared tolerable, six cycles were given whenever possible. After the chemotherapy, patients underwent surgery and postoperative radiotherapy.**

Results. **The response rate for the whole group to AC was 98% (95% confidence interval 94%-100%), with a clinical complete response rate of 50% (95% confidence interval 35%-65%). Six patients had a pathological complete response. Median follow-up from the start of chemotherapy is 49 months (range 10-100). The disease-free survival (DFS) at three years is 57% and the overall survival (OS) at three years is 79%. There is a significant trend for improved DFS ($p = 0.0000$) and OS ($p = 0.0002$) with increasing number of cycles.**

Conclusion. **The results of the present study with neoadjuvant dose-intensive AC chemotherapy and GM-CSF compare favorably with previous studies in patients with LABC. This is most apparent in patients who received six cycles of neoadjuvant chemotherapy. We hypothesize that these encouraging results are probably related to the prolonged presence of the primary tumor, and to the long-term administration of GM-CSF with the primary tumor and axillary lymph nodes in situ. Therefore, a randomized study is warranted. We already initiated an international randomized trial in patients with LABC in order to answer two questions. First, does prolonged neoadjuvant chemotherapy result in an improved DFS and OS in comparison with the conventional approach, and secondly, what is the effect of GM-CSF in this approach in comparison with G-CSF?**
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Introduction

Patients with locally advanced breast cancer (LABC) have a poor prognosis when treated with surgery and/or radiotherapy [1-3]. These tumors include stage IIIA and IIIB breast cancer according to the American Joint Committee on Cancer (AJCC) [4].

As a consequence of these poor results, neoadjuvant chemotherapy has been introduced. The aim of this approach is to render patients operable and to eradicate micrometastases at an early stage, when they are still sensitive to chemotherapy. Several studies have been performed; generally only three to four cycles of conventional-dose neoadjuvant chemotherapy are given prior to surgery and/or radiotherapy, often followed by adjuvant chemotherapy. The reported clinical response rate to neoadjuvant chemotherapy varies between 30% and 90%, with 10%-35% clinical

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complete remissions. The five-year overall survival (OS) rate is reported as being between 40% and 60% [5-8], while one study showed a five-year survival of 80% in patients with stage IIIA disease [9].

Increasing evidence for a steep dose-response effect in breast cancer of a number of chemotherapeutic agents [10], including doxorubicin (A) [11, 12] and cyclophosphamide (C) [13], two of the most effective agents in the treatment of breast cancer, has emerged over the last years. We have developed a dose-intensive regimen for AC in conjunction with GM-CSF as a hematopoietic growth factor. The latter was chosen because it also has immunostimulatory effects [14] which might enhance the antitumor effect of AC. The treatment schedule of AC with GM-CSF was first established in a dose-finding study [15]. Thereafter, in a phase II study in metastatic breast cancer patients we observed a high response rate [16]. In the present study, the treatment regimen was applied as neoadjuvant chemotherapy given prior to surgery and postoperative radiotherapy in patients with LABC. During the course of the study we extended the neoadjuvant chemotherapy from four to six cycles.

Patients and Methods

Patient Selection

Between 1990 and 1995, 42 patients with LABC were entered in this study. Patients with histologically or cytologically confirmed stage IIIA or IIIB breast cancer according to AJCC criteria [4] were eligible. Age below 65 years and a World Health Organization (WHO) performance status of ≤2 were additional entry criteria. Pretreatment assessment included a medical history, physical examination, electrocardiogram, chest x-ray, bone scan, ultrasound scan of the liver, left ventricular ejection fraction (LVEF) and baseline laboratory investigations (full blood count, serum creatinine and electrolytes, liver function tests, and urine analysis). Adequate bone marrow function (white blood cell count $\geq 4.0 \times 10^9$/l and platelet count $\geq 100 \times 10^9$/l), renal function (creatinine level ≤150 µmol/l) and hepatic function (bilirubin level ≤25 µmol/l) were required. Patients with a history of cardiovascular disease and/or a LVEF less than 50% were excluded. Written informed consent was required and the ethical and scientific review committees of the hospital approved the protocol.

Treatment

Treatment consisted of A 90 mg/m^2 and C 1,000 mg/m^2 by i.v. bolus injection. Cycles were repeated every 21 days. As established in a previous dose-finding study, a dose reduction of 10% relative to the previous dose level was applied in cycles 2 and 4 [15]. GM-CSF 250 µg/m^2/day was administered from days 2-11, in the first four patients as a continuous i.v. infusion and in the following 38 patients s.c. [17]. Initially, some patients received fewer than six cycles. However, when the study progressed and toxicity appeared tolerable, six cycles were administered whenever possible. While on treatment, all patients had a medical history, a physical examination, baseline laboratory tests, chest x-ray, and ECG before each cycle. Full blood count was performed three times a week and biochemical analysis weekly. Every second cycle and before the last cycle, a LVEF was performed. The next cycle of chemotherapy was delayed for one week if the white blood cell count was $\leq 3 \times 10^9$/l, the platelet count was $\leq 100 \times 10^9$/l, or in the presence of active infection, mucositis or a deterioration of the performance status to WHO grade 3 or 4. In case of a delay of two weeks and in case of a decline in LVEF below 50%, chemotherapy was discontinued. Four weeks after the last cycle of chemotherapy a mastectomy with axillary lymph node dissection was performed. Radiotherapy followed four to six weeks after mastectomy to a total dosage of 4005 cGy in 15 fractions given in 19-21 days to the thoracic wall, the internal mammary nodes, the axilla and the supraclavicular fossa. No additional chemotherapy or hormones were given.

Definition of Pathological Response

Methods of pathological examination have been described in detail elsewhere [18]. No residual tumor in the mastectomy specimen and axillary lymph nodes was defined as a pathological complete response. When only tumor was present at microscopic examination the response was graded as minimal residual disease. And when tumor was visible at macroscopic evaluation of the mastectomy specimen and axillary lymph nodes, the pathological response was graded as macroscopic disease.

Statistics

For survival analysis, disease-free survival (DFS), defined as time between end of radiotherapy and recurrent disease, and OS, defined as the time between start of chemotherapy and death from any cause, were used as follow-up parameters. The time to relapse was defined as the time between the end of radiotherapy and relapse. Kaplan-Meier [19] curves were plotted and were compared using the Mantel-Cox test [20-21].

Results

The pretreatment characteristics of the 42 patients entered are depicted in Table 1. All patients were assessable for response, toxicity and survival. A total of 227 cycles were given with a median of 6 (range 2-6) and a mean of 5.3 cycles.

Table 1. Patient characteristics

Total number of patients	42
Age (year) median (range)	47 (26-63)
Clinical stage	
IIIA	21
IIIB	21
Tumor diameter (cm) median (range)	9 (5-15)
Primary tumor	
T3	21
T4a	0
T4b	9
T4c	1
T4d	11
Nodal status	
N0	7
N1	15
N2	20
N3	0

Of the first 18 patients, treated when our experience with the chemotherapy schedule was limited, treatment was discontinued in 11 patients (61%) before six cycles were given. Reasons for early discontinuation were: a sepsis after the second cycle in one patient, general weakness in combination with a good clinical response (>50% reduction in tumor size) to the chemotherapy after four cycles in three patients and after five cycles in five patients, and a decline in LVEF below 50% after five cycles in two patients. When the study progressed, we administered six cycles whenever possible. Indeed, only seven of the next 24 patients (29%) received fewer than six cycles; six patients (25%) received five cycles, and one patient (4%) received four cycles. Reasons for discontinuation were persistent thrombocytopenia after five cycles in two patients, mucositis grade 4 after five cycles in one patient, and decline in LVEF after five cycles in three patients and after four cycles in one patient. Overall, 17 of 227 cycles were delayed for the following reasons: neutropenia (four cycles), thrombocytopenia (two cycles), mucositis (two cycles), general weakness (six cycles) and infection (three cycles).

Response and Survival

Forty-one out of 42 patients showed a clinical response (98%; 95% confidence interval 94%-100%). Twenty-one patients (50%; 95% confidence interval 35%-65%) achieved a clinical complete response, and 20 patients had a clinical partial response (48%; 95% confidence interval 33%-63%). One patient had stable disease. Usually, best response was observed after three cycles. At pathologic examination of the mastectomy specimen, six patients had a pathologic complete remission (14%), and 23 patients (55%) had only minimal residual disease, including three patients with only a few tumor cells in one lymph node. In 13 patients macroscopic tumor was still present. Details on pathologic examination are described elsewhere [18]. Clinical response in relation to pathological response is shown in Table 2. At a median follow-up of 49 months from the start of chemotherapy (range 10-100), 19 patients have relapsed, with a medium time to relapse of 15 months (range 2-47). Three patients, all with inflammatory breast cancer, had a local relapse. Not a single patient progressed during chemotherapy. Table 3 shows relapse according to number of chemotherapy cycles and stage of disease. DFS and OS at three years for the whole group were 60% and 79%, respectively (Fig. 1). DFS and OS according to number of chemotherapy cycles are shown in

Table 2. Clinical and pathologic response

Pathologic response	No. of patients	CCR	CPR	CSD
No residual carcinoma	6	5	1	
Minimal residual disease	23	12	11	
Macroscopic carcinoma	13	4	8	1
Axillary lymph nodes				
Negative	18	14	4	
1-3 positive	8	4	3	1
4-10 positive	15	3	12	
>10 positive	1	0	1	

CCR = Clinical complete response; CPR = clinical partial response, CSD = clinical stable disease.

Table 3. Relapse according to stage of disease and number of cycles

No. of cycles	Stage IIIA	Stage IIIB	IBC
≤4	2/2	2/2	1/1
5	3/6	0/3	3/4
6	4/13	1/5	3/6

IBC = Inflammatory breast cancer.

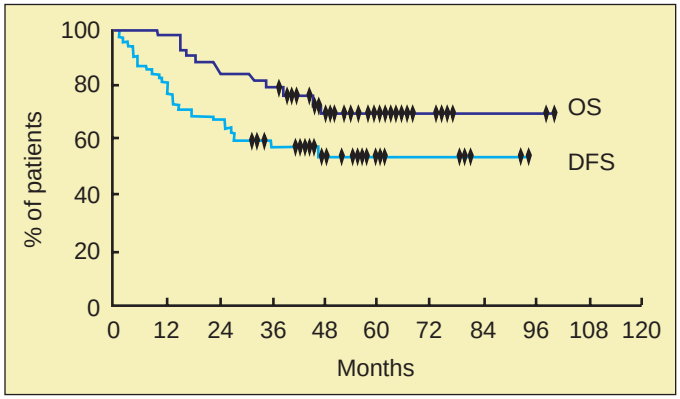

Figure 1. Overall survival (OS) and disease-free survival (DFS) of all patients.
♦ = *patients at risk.*

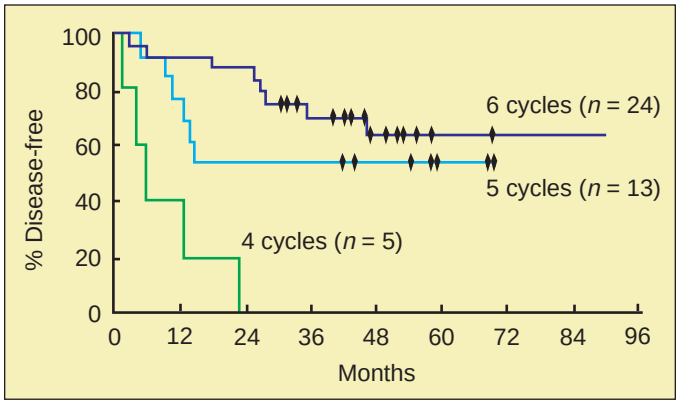

Figure 2. Disease-free survival according to number of cycles. ♦ = *patients at risk.*

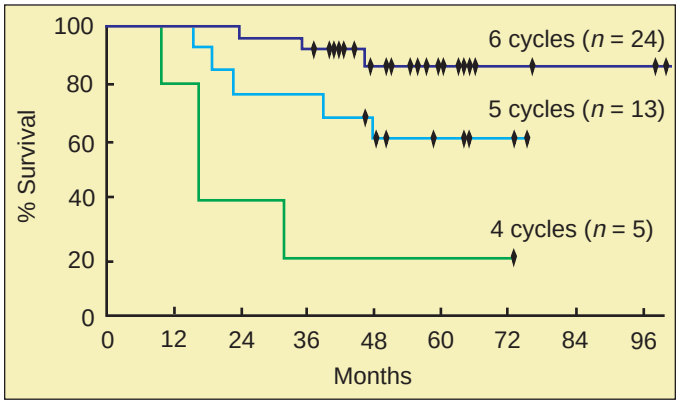

Figure 3. Overall survival according to number of cycles. ♦ = *patients at risk.*

Figures 2 and 3. Comparison of DFS and OS of patients who received four, five, or six cycles showed a significant trend for improved DFS ($p = 0.0000$) and OS ($p = 0.0002$) in patients who received more chemotherapy cycles.

Table 4. Toxicity of neoadjuvant doxorubicin and cyclophosphamide

Type of toxicity	Number of cycles	%
Neutrophil count <0.5 × 10^9/l	220	97
Thrombocytopenia ≥ grade 3	104	46
Red blood cell transfusions	61	26
Platelet transfusions	27	12
Neutropenic fever	18	8
Positive blood cultures	3	1
Mucositis ≥ grade 3	12	5
General weakness ≥ grade 3	20	9

Toxicity

Toxicity of this regimen is described in Table 4. Briefly, grade 3-4 neutropenia accompanied 97% of cycles. The nadir was between days 8 and 12 after the start of chemotherapy. Grade 3 or 4 thrombocytopenia occurred in 46% of cycles, mainly in the sixth cycle and only once in the first cycle. The majority of transfusions occurred in cycles four to six. Neutropenic fever requiring hospital admission and the administration of broad-spectrum antibiotics occurred in 18 cycles, but in only three cycles infection was confirmed by positive blood cultures. The organisms isolated were *Pseudomonas aeroginosa* in one cycle and a *Staphylococcus aureus* in two cycles. With the antiemetic regimen used, nausea and/or vomiting were usually mild, and it was never required to admit patients for alimentation. A remarkably low incidence of ≥grade 3 mucositis was seen (only in 5% of all cycles). The LVEF showed a decline below 50% in six patients. In these patients chemotherapy was discontinued—in one patient after four cycles and in five patients after five cycles. None of them developed clinical congestive heart failure during the follow-up. The LVEF was repeated after one year in all patients and no deterioration was observed.

Discussion

This regimen with neoadjuvant dose-intensive AC chemotherapy and GM-CSF showed a high response rate of 98%, with a clinical complete response of 50%. Initially, some patients received four cycles, but after 18 patients had been entered and toxicity appeared acceptable, the number of cycles was extended to six cycles whenever possible. The most frequent reason for discontinuation was a decline in LVEF to levels below 50%. During follow-up there was no deterioration of the LVEF and no patient developed cardiac failure, indicating that this scheme can be combined with chest wall radiation even in patients with left-sided breast cancer.

We observed a high response rate to neoadjuvant chemotherapy in comparison with previous studies on neoadjuvant chemotherapy in LABC, where response rates of 60%-80% have been reported, with 10%-30% clinical complete remissions [5-9]. Usually, conventional dosages of only three or four cycles of neoadjuvant chemotherapy are being applied followed by local treatment and sometimes post-operative chemotherapy. Pathologic complete response varied from 7% to 20% with the use of conventional neoadjuvant chemotherapy [22-24]. For dose-intensive induction regimens the pathological response varied from 17%-36% [25-30]. In our study six patients (14%) had a pathologically complete response, and 17 patients (40%) had only minimal residual microscopic disease, which makes a total of 54% of patients with major tumor reduction.

The five-year OS with conventional neoadjuvant chemotherapy has been reported to be in the range of 40%-60% [5-8], with one study reporting 80% OS after five

years in patients with stage IIIA disease only [9]. For the studies with dose-intensive neoadjuvant chemotherapy, five-year DFS and OS data are only available from the study of *Armstrong et al*. [27], who reported a five-year actuarial DFS of 58%, and a five-year actuarial OS of 75%. Until now, high-dose chemotherapy with peripheral stem cell support does not result in an enhanced DFS and OS in LABC patients [29-30]. In our study the median follow-up is relatively short, but it appears that actuarial five-year DFS and OS compare favorably to results reported thus far, particularly for those patients who received six cycles of neoadjuvant chemotherapy. These results suggest that prolonged treatment prior to local therapy improves DFS and OS. This has recently also been suggested by the results of *Merajver et al*. [31], who showed a high response rate with nine cycles of neoadjuvant chemotherapy in LABC patients. The survival data were, however, apparently lower in this study, where lower dosages of the chemotherapy were applied and no GM-CSF was used.

We hypothesize that our encouraging results are probably not only related to the 50% increase in the dose of chemotherapy, but also to the prolonged presence of the primary tumor, and to the long-term administration of GM-CSF with the primary tumor and axillary lymph nodes in situ. The administration of GM-CSF might have resulted in an immune response through stimulation of antigen presenting cells and cytotoxic T-cells in the draining lymph nodes of the primary tumor [32-33]. At present we are investigating local immunostimulatory effects of GM-CSF in axillary lymph nodes. Preliminary results show an increase of dendritic cells in lymph nodes of patients treated with neoadjuvant chemotherapy and GM-CSF compared with lymph nodes of patients who did not receive preoperative treatment or neoadjuvant chemotherapy without GM-CSF (manuscript in preparation). These findings can support this hypothesis.

Conclusion

Dose-intensive neoadjuvant chemotherapy with GM-CSF shows favorable results in patients with LABC. This is most apparent in patients who received six chemotherapy cycles. Based on our findings and those from recent preclinical studies, we hypothesize that it is more effective to administer chemotherapy in an extended neoadjuvant regimen taking advantage of concurrent biological and immunological processes in the primary tumor and its draining lymph nodes. We have initiated a randomized international study in LABC in Mexico, France, Spain and The Netherlands. The bifactorial design of this study allows testing of the effect of prolonged neoadjuvant treatment described in the present study in comparison with the conventional approach of three pre- and three postoperative chemotherapy cycles, as well as testing of the effect of GM-CSF versus G-CSF on the DFS and OS.

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