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Acute and Late Toxicity Following Adjuvant High-Dose Chemotherapy for High-Risk Primary Operable Breast Cancer

A Quality Assessment Study

Inge M. Svane, Keld M. Homburg, Claus Kamby, Dorte L. Nielsen, Ole Roer, Dorte Sliffsgaard, Hans E. Johnsen and Steen W. Hansen

From the Departments of Oncology (I.M. Svane, C. Kamby, D.L. Nielsen, O. Roer, D. Sliffsgaard, S.W. Hansen) and Haematology (H.E. Johnsen), Herlev Hospital, University of Copenhagen, Denmark and the Department of Clinical Immunology, Odense University Hospital, Denmark (K.M. Homburg)

Correspondence to: Inge Marie Svane, Department of Oncology, Herlev Hospital, University of Copenhagen, DK-2730 Herlev, Denmark. Fax: + 45 44 530 176. E-mail: inge.m.svane@dadlnet.dk

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From 1996 to 2000, high-dose chemotherapy with haematopoietic stem-cell support was used as an adjuvant treatment strategy for management of primary high-risk breast cancer patients with more than five positive nodes. This single institution study included 52 women aged ≤ 56 years with primary operable breast cancer and ≥ 6 tumour-positive axillary lymph nodes. The treatment regimen consisted of at least three initial courses of FEC (5-fluorouracil, epirubicin, cyclophosphamide) followed by high-dose chemotherapy (cyclophosphamide, thiotepa, carboplatin) supported by autologous peripheral blood stem-cell reinfusion. This study focuses on quality control including evaluation of toxicity, supportive therapy and assessment of the stem-cell products. Cytokeratin 19 positive cells were found in the stem-cell product from 3/37 patients. Data regarding organ toxicity were used for evaluation of short- and long-term side effects. Substantial acute toxicity and frequent catheter-related infections were found. Long-term toxicities included reduced lung diffusion capacity (n = 36), fatigue (n = 14), arthralgia/myalgia (n = 10), neurotoxicity (n = 9) and memory loss (n = 4). However, most toxicities were grade 1–2 and reversible within two years. No treatment-related death occurred. Within a median follow-up of 30 months (range, 11–57), 25% of the patients had relapsed. Recurrence-free survival was 75% and overall survival was 88% three years after the start of treatment. Overall, high-dose chemotherapy was relatively well tolerated, with manageable toxicity and an acceptable requirement of supportive therapy. Until now, high-dose chemotherapy has not proven superior to conventional-dose adjuvant chemotherapy, therefore it is necessary in the future to focus on well-designed randomized studies.

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High-dose chemotherapy (HDC) with bone marrow or peripheral stem-cell transplant for treatment of breast cancer became the focus of high hopes when phase II trials indicated a significant improvement in survival among high-risk, node-positive as well as metastatic patients (1, 2). However, there is still a lack of substantial data from randomized phase III trials documenting that this treatment regimen is superior to conventional treatment (3–8). Apparently, a major obstacle has been the reluctance of oncologists to conduct the necessary randomized trials and the reluctance of patients to participate, possibly due to conviction of the benefits of high-dose therapy. Thus, although HDC has been used extensively as an adjuvant treatment strategy, it should still be regarded as an experimental treatment (9, 10).

Recently, the available data from randomized studies on high-dose chemotherapy for breast cancer have been reviewed (11–13). The most optimistic results were found in a Dutch study (5) indicating a reduction of relapse and an almost 10% survival advantage after 3 years. A final analysis of an earlier, smaller, randomized phase II study has recently been published indicating a reduction of relapse but showing no significant difference in overall survival or disease-free survival rates after 7 years (14). Comparable data from the CALGB-Intergroup study were presented by Peters et al. at ASCO in 2001 (7). Although the overall benefit from HDC appears to be marginal, it cannot be ruled out that there might be larger benefit in a small subgroup (13).

A significant objection to the use of high-dose chemotherapy and stem-cell support has been the adverse

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effects of this treatment on both short- and long-term survival (6). Therefore, we find it relevant more thoroughly to document the morbidity, as well as the requirement of supportive therapy associated with high-dose treatment. In this study we focus on feasibility, tolerability and use of supportive therapy in relation to HDC with peripheral stem-cell transplant.

Supportive reinfusion of autologous peripheral blood stem cells is integrated in the HDC regimen, initially aiming to increase the level of end-stage blood circulating cells, thereby reducing the risk of acute side effects, such as infections, bleeding or anaemia and ultimately aiming to re-establish the haematopoiesis. Stem-cell and tumour-cell quantification in the graft may be important for adequate evaluation of the treatment (15–18). Thus, quality assessment of the stem-cell graft was included in the study.

MATERIAL AND METHODS

Study design

This is a report from a prospective single institution treatment of unselected patients with primary operable breast cancer who entered an adjuvant high-dose chemotherapy programme at Herlev University Hospital, Denmark, from January 1996 to April 2000.

Data regarding tolerability and toxicity were collected prospectively and the CTC toxicity criteria were used for evaluation. Data related to the primary diagnostic outcome were obtained from the individual case records.

Patients treated

The selection criteria on entry were: patients with histologically proven primary breast cancer who had undergone primary tumour excision (mastectomy or lumpectomy) and axillary lymph node dissection (levels I and II) showing ≥ 6 tumour-positive lymph nodes; age ≤ 56 years.

Primary surgery and staging procedures were conducted according to the uniform guidelines (19, 20).

The performance status of all the patients was 0. Pretreatment evaluation included physical examination, chest x-ray, bone scan, unilateral bone marrow biopsy, ECG, estimation of cardiac ejection fraction (MUGA) scan, determination of lung diffusion and ^{51}Cr-EDTA-clearance, and blood samples for evaluation of liver, renal function as well as haematologic parameters. Patients with distant metastases were excluded.

A total of 58 evaluable patients were treated. Six patients were excluded from the analysis. In two patients, involvement of the bone marrow was demonstrated before initiation of high-dose chemotherapy; two patients were excluded from high-dose chemotherapy after three cycles of FEC (cyclophosphamide, 5-fluorouracil, epirubicin) because of renal impairment as determined by reduced Cr-EDTA-clearance; one patient suffered from life-threatening catheter-related infection during FEC, and one patient abandoned high-dose chemotherapy because of toxicity caused by FEC. Thus, a total of 52 patients were evaluable for the present analysis. The pre-transplant characteristics of the included patients are presented in Table 1.

Standard-dose induction chemotherapy

All patients received at least 3 cycles of FEC I.V. on day 1 at 3-week intervals: cyclophosphamide (500 mg/m^2), 5-fluorouracil (500 mg/m^2), epirubicin (90 mg/m^2). Ten of the patients received a 4th cycle of FEC prior to stem-cell mobilization and HDC, because, owing to logistic planning, the time period from the last FEC to initiation of high-dose chemotherapy was getting to be too long.

High-dose chemotherapy

After peripheral blood stem cell (PBSC) harvest, patients received HDC starting after a median of 26 days (range, 19–54) after the last course of FEC. The regimen consisted of cyclophosphamide (1.5 g/m^2), thiotepa (125 mg/m^2), carboplatin (200 mg/m^2) given daily on four consecutive days and supplemented with Mesnum (1.5 g/m^2) for five days (21).

PBSC was reinfused 48 h after completion of chemotherapy. Starting the following day, G-CSF 5 g/kg/day was administered until the ANC (neutrophil count) was $> 1 \times 10^9$ /L for three consecutive days.

Table 1

Clinical and pathological characteristics of patients at primary diagnosis

Patient characteristics	n	%
No. of evaluable patients	52	100
Age in years, median	48 (30–56)	
Menopausal status		
Pre-/Post-/Unknown	37/14/1	71/27/2
Primary tumour location		
Left/Right/Bilateral	21/29/2	40/56/4
Tumour size, median (mm)	37.5 (9–130)	
Histology		
Ductal/Lobular/Other	46/2/4	88/4/8
Histologic grade[1]		
I/II/III/ungradable	4/28/16/4	8/54/30/8
Hormone receptor status		
ER+/PgR+	14	27
ER+/PgR−	14	27
ER−/PgR+	5	10
ER−/PgR−	18	34
Unknown	1	2
Axillary lymph nodes		
No. of removed nodes, median	16 (7–37)	
No. of involved nodes, median	10 (6–36)	

[1] According to Bloom & Richardson (20).

Supportive therapy

A prophylactic antiemetic regimen was used during high-dose chemotherapy; ondansetron 16 mg I.V. given twice daily plus metoclopramide 200 mg or metopimazine 35 mg/m^2 given as a 24-h continuous I.V. infusion. Metoclopramide and metoprimazine were given alternately in order to minimize adverse events. All patients received prophylactic antibiotic (ceftazidime) as long as the neutrophil cell count was below 0.5×10^9/L. Patients seropositive for herpes simplex virus were prophylactically treated with acyclovir until the neutrophil count was $> 0.5 \times 10^9$/L for more than two days.

For maintenance of a platelet count of at least 10×10^9/L, irradiated platelet transfusions were administered and irradiated red blood cells were given to maintain haemaglobin concentrations above 5.5 mmol/L. In periods with absolute neutropenia (ANC $< 0.5 \times 10^9$/L), patients were accommodated in private hospital rooms.

Supplementary therapy

Between 4 and 12 weeks after high-dose therapy the patients received radiation therapy (2 Gy $\times$ 24) to the chest wall and regional lymph nodes using standard radiation techniques (22). An axillary shield was used in 45 patients with extensive lymph node dissection (> 9 nodes removed). One patient refused radiotherapy for psychological reasons. Furthermore, 12 out of 28 hormone receptor-positive patients were treated with tamoxifen.

Blood samples

Patients were followed, with weekly blood cell counts taken during FEC treatment and daily blood cell counts during hospitalization for high-dose chemotherapy. Data from these blood samples were used to describe the haematologic toxicities.

Quality assessment of the stem-cell graft

Peripheral blood stem cell harvest. PBSCs were mobilized using recombinant human granulocyte colony-stimulating factor (G-CSF; Filigrastim) 10 g/kg/day, subcutaneously, for 10 days starting on the day following the 3rd cycle (4th cycle) of FEC (23). Leukapheresis was normally performed 10–13 days after the last course of FEC, triggered by a CD34+ number ≥ 20/L blood. In 40 patients, only one leukapheresis was performed, whereas in the remaining patients two or three leukaphereses were performed on consecutive days to attain a sufficient autograft with $\geq 2 \times 10^6$ CD34+ cells/kg (24). One patient underwent leukapheresis seven times. In 11 of the patients the autograft consisted of purified CD34+ stem cells isolated from the leukapheresis product using magnetically activated cell selection (25).

Detection of contaminating tumour cells. Samples of 1×10^7 mononuclear cells (MNC) harvested from the leukapheresis products were assessed for contaminating tumour cells. In order to concentrate the carcinoma cells, a negative selection for CD45+ cells and a positive selection for epithelial cells were performed using immunomagnetic beads (26, 27). Cultured tumour cells served as positive controls and MNC from healthy volunteer blood donors served as negative controls for the analyses.

The samples were split into two for confirmatory testing by immunocytochemistry (ICC) (28, 29) and reverse transcriptase-polymerase chain reaction (RT-PCR) (30). The targets were the epithelial cytokeratin proteins CK8, −18 and −19 and CK19 mRNA, respectively (31).

Statistics

Descriptive statistics are reported as frequencies, medians and ranges. Association between CD34+ count and primary and secondary clinical endpoints was analysed by calculating the correlation coefficients according to Pearson's method. A paired t-test was used for analysis of treatment-induced changes in organ functions. Resulting p-values < 0.05 were regarded as statistically significant.

Relapse-free and overall survival was calculated according to the Kaplan–Meier method (32). Relapse-free survival was defined as time from date of surgery to first evidence of progressive disease. Overall survival was defined as time from surgery to death.

Data were analysed as of April 1 2001. All statistical analyses were performed using SPSS software packages (Chicago, IL).

RESULTS

Data were obtained on 52 breast cancer patients regarding toxicity (during induction chemotherapy, high-dose chemotherapy and at two years of follow-up), supportive treatment, recovery and relapse/survival.

Induction chemotherapy

Non-haematologic toxicity. Patients were treated with FEC as an induction therapy prior to high-dose chemotherapy, and the toxicities were all well known. Thus, the majority of patients experienced grade 0–2 toxicity, with the exception of alopecia; only one patient had grade 3 nausea and three patients had grade 3 vomiting.

Patients were provided with a central double lumen Hickmann leukapheresis catheter through which the chemotherapy was administered. Eighteen patients had catheter-related toxicity during induction chemotherapy (Table 2). Fourteen cases of catheter-related infections were observed; localized infection was observed in 9 patients, bacteraemia in 5 patients, an in 9 patients removal of the catheter was necessary in order to control the infection. Thrombosis was seen in 4 patients and resulted in removal of the catheter in 2 cases. Furthermore, 7

Table 2

Catheter-related toxicity

	No. of patients		
	Induction chemotherapy	High-dose chemotherapy	Total
Catheter-related infections	14	5	19
Local infection	9	1	10
Bacteraemia	5	4	9
Removal of catheter	9	5	14
Thrombosis	4	0	4
Removal of catheter	2	0	2

patients suffered grade 1–2 pain associated with the catheter.

Haematologic toxicity. Leukocyte nadir with counts lower than 1×10^9/L were found in 6 out of 52 patients during the courses of FEC; 27 patients (52%) had a granulocyte nadir with counts lower than 0.5×10^9/L, while 4 patients (8%) had a platelet nadir with counts below 50×10^9/L. Twenty patients had anaemia with haemoglobin values of less than 6.0 mmol/L after the third course of FEC.

High-dose chemotherapy

Non-hematologic toxicity. The significant non-haematologic toxicities according to the CTC criteria during high-dose chemotherapy are summarized in Table 3. No toxic deaths were observed. Infections were observed in 12 of the patients (23%), 5 of them catheter-related (Table 2); localized infection in 1 patient and bacteraemia in 4 patients. Infection was caused by *Staphylococcus albus* in all 5 patients and the catheter had to be removed in order to control the infection. In total, 23 catheter-related events were reported in the 52 patients during treatment, leading to removal of the catheter in 16 cases. Other local infections consisted of cystitis, pneumonia, enteritis and eruptions of herpes zoster (n = 1) and herpes simplex (n = 1). Verified bacteria included *Klebsiella pneumoniae* (n = 1) and Clostridium (n = 1).

Grade 3 nausea was described in 25 patients, 15 patients had grade 3 vomiting and 16 patients had grade 3 diarrhoea; no grade 4 vomiting or diarrhoea was observed. In 14 of the patients (27%) the HDC was associated with pain, including abdominal pain (n = 10), myalgia/arthralgia (n = 5) and headache (n = 3). Renal function was affected in three patients; one patient had transient oliguria due to hypotension (grade 3), one had an increase in se-creatinine and one patient had grade 3/4 renal failure, requiring transient haemodialysis. One patient developed a pulmonary embolism and dyspnoea (grade 4) during high-dose chemotherapy.

Haematologic toxicity. As expected, the high-dose regimen induced pronounced bone marrow suppression. Haemopoietic recovery was generally rapid; ANC recovered to $> 0.5 \times 10^9$/l after a median of 9 days (range, 7–28), while platelet recovered to a count $> 50 \times 10^9$/L after a median of 13.5 days (range, 9–82).

Post-transplant, the patients were supported with transfusions of concentrated red blood cells and platelets. Patients received a median 6 units of red blood cells (range, 2–58). Five patients were transfused with more than 10 units of red blood cells. Furthermore, the patients received a median 3.5 units of platelets (range, 0–61). Thirteen patients did not require platelet transfusion, while four patients were transfused with more than 10 units of

Table 3

Non-haematologic toxicity during high-dose chemotherapy

	No. of patients						
Toxicity grade	0	I	II	III	IV	Present not graded	Not described
Nausea	2	9	10	25	0	5	1
Vomiting	5	10	12	15	0	6	4
Diarrhoea	7	8	12	16	0	7	2
Stomatitis	13	11	13	9	0	5	1
Infections	40	8	2	2	0	0	0
Dermatitis	14	10	14	8	0	4	2
Neuropathy	51	0	0	0	0	0	1
Dizziness	48	3	1	0	0	0	0
Bleeding	38	2	1	1	0	0	0
Pain	36	6	7	1	0	2	0

platelets. The requirement of multiple platelet and red blood cell transfusions was coincident.

In 34 patients (65%), leukopenia was connected with fever $>38°C$ lasting for a median of 3.5 days (range, 0–30) and only 3 patients had fever for more than 10 days. Prophylactic or therapeutic antibiotic treatment was applied in the majority of patients for a median of 8.5 days (range, 0–41), however, 15 patients needed antibiotic treatment for more than 10 days.

The majority of patients (47/52) were discharged from hospital before day 28 after initiation of high-dose chemotherapy (median = 20, range, 16–106). Three patients were discharged more than 40 days after initiation of high-dose chemotherapy.

Quality assessment of the stem-cell graft

For evaluation of efficacy of the stem-cell reinfusion, the number of CD34+ cells in the stem-cell product was analysed in all patients. The patients received a median of 8.0×10^8 CD34+ stem cells (range, $1.5–17.2 \times 10^8$) corresponding to a median of 10.0×10^6 CD34+ stem cells/kg (range, $2.2–31.0 \times 10^6$). Assessment of coherence between CD34+ cell content in the graft and primary clinical endpoints such as transfusion of blood components and interventional antibiotic treatment as well as recovery parameters (secondary endpoints) was carried out. No statistically significant correlation was found between reinfused CD34+ cell count/kg and requirement of transfusion or antibiotics, duration of fever or recovery of ANC. However, when the total number of reinfused CD34+ stem cells was used, a significant ($p = 0.02$) correlation with platelet recovery was seen.

For safety evaluation, the content of carcinoma cells in the stem-cell graft was analysed in 37 consecutive patients. Two different assays, RT-PCR and immunocytochemistry (ICC), were used to demonstrate contaminating tumour cells.

Cells positive for the tumour marker cytokeratin 19 were found in the stem-cell product from 3 of the 37 patients (8%); one of the patients had a positive marker only in RT-PCR. Patients positive in the ICC, had tumour cell counts close to the detection level of 10^{-7} to 10^{-6}, that is between 1 and 3 tumour cells in the sample. One of the three patients had an early relapse. However, owing to the few positive events observed, evaluation of the influence on clinical outcome was not possible.

Toxicity in the 2-year follow-up period after high-dose chemotherapy

Temporary and permanent toxicity according to the CTC criteria was recorded during follow-up. Thirty-eight patients were evaluable, while 14 patients who died before two years or were not followed for two years were still defined as non-evaluable. Temporary adverse effects were observed in 33 of the patients, including grade 1–2 fatigue (n = 12), grade 1–2 arthralgia/myalgia (n = 8) and transient neurotoxicity consisting of vertigo (n = 1) and grade 1–2 paraesthesia (n = 6). Pulmonary symptoms including recurrent infections and mild dyspnoea were reported in 3 patients as well as transient, reduced memory in 2 patients. Localized herpes zoster eruptions were observed in 9 patients. Finally, 10 patients reported adverse effects related to surgery and radiotherapy.

Irreversible side effects of the treatment were observed in 12 of the patients, including neurotoxicity (2 patients with grade 1 paresthesia), fatigue (2 patients), reduced memory (2 patients) and mild to moderate muscle and joint pain (2 patients). The four remaining patients had individual problems including symptomatic (grade 2) reduction in pulmonary function, aggravation of existing hearing impairment, dental problems and weakness of the arm/shoulder, probably caused by surgery.

As a measure of recovery, working ability was evaluated. Patients followed for a shorter time than the evaluation period and patients who had relapsed or had died before the time of evaluation were defined as non-evaluable. Six months after completion of treatment, 12 out of 37 evaluable patients (32%) regained normal working ability and 14 (38%) regained partial working ability, while 11 patients were unable to work. After 12 months, 20 out of 32 patients (63%) had normal and 9 (28%) partial working ability, whereas 3 patients were still unable to work. After 24 months, all evaluable patients were able to work. Thus, 21/24 (88%) achieved normal working ability and 3 patients (12%) had partial working ability.

Treatment-induced changes of pulmonary, renal and cardiac function

The patients were submitted to evaluation of pulmonary, renal and cardiac function before treatment with FEC, before HDC and during follow-up. The follow-up evaluation was done after a median of 34 and 110 days from the time of initiation of HDC.

Pulmonary evaluation included assessment of lung diffusion capacity (DL). Prior to treatment 11 patients had a DL < 80% of the normal value; these patients had continuous abnormal values during treatment and follow-up. The remaining patients all had normal diffusion capacity prior to treatment. After FEC the number of patients with DL < 80% was increased to 24 and a significant ($p < 0.001$) reduction of the mean DL was seen (Fig. 1a). HDC induced a further decrease in DL ($p < 0.001$) and at the time of the first follow-up 36 patients had a DL < 80%. Twelve of the 36 affected patients had a grade 1 reduction and 22 patients had a grade 2 reduction. Two patients had grade 3 reduction of DL, but both patients had subnormal (grade 2/3) DL prior to treatment. At the time of the second follow-up, 28 of the patients continued to have subnormal DL. The regaining of normal or close to normal DL within two years was registered in 17 of the

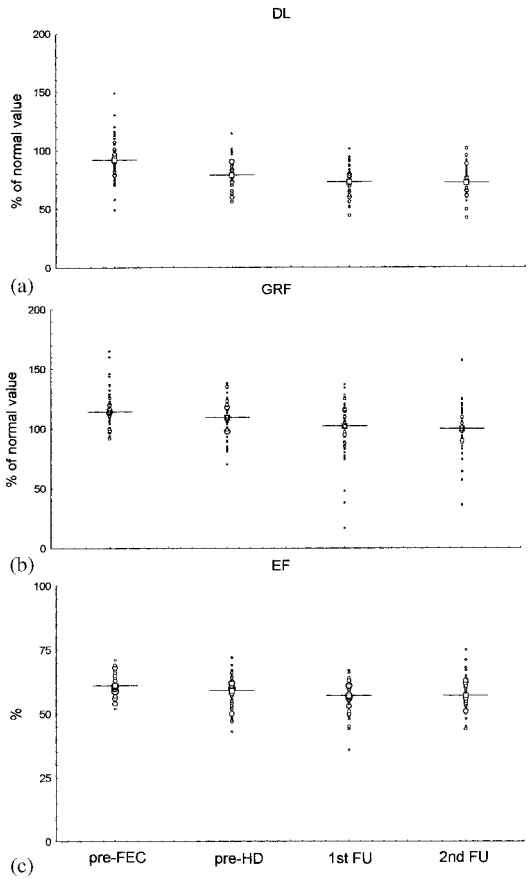

Fig. 1. Treatment-induced changes in pulmonary, renal and cardiac functions. The size of the dots indicates the number of patients with the same value. — Indicates the median values. (a) Lung diffusion capacity (DL) evaluated by DL_{CO}. (b) Glomerular filtration rate (GFR) evaluated by Cr-EDTA-clearance. (c) Ejection fraction (EF) evaluated by MUGA-scan.

patients, 9 continued to have subnormal DL and 10 patients were non-evaluable.

Cr-EDTA-clearance was used for evaluation of the renal function (GFR). Prior to treatment with FEC all patients had normal values. During FEC a significant ($p = 0.002$) reduction of the mean GFR was seen (Fig. 1b), followed by a further decrease during HDC ($p = 0.001$). The majority of patients retained GFR values within the normal reference interval, but three patients developed moderately reduced GFRs ($< 75\%$ of the normal value). In one patient the renal function was moderately affected after FEC, and this patient developed temporary renal failure during high-dose treatment. She regained 33% of normal renal function and had normal se-creatinine during follow-up.

A MUGA-scan was used for evaluation of cardiac ejection fraction (EF). All patients had a normal MUGA-scan before FEC treatment. During FEC a significant ($p = 0.001$) reduction of the mean EF was seen (Fig. 1c), whereas the reduction in EF caused by HDC was minor and non-significant. For the majority of patients, the observed decrease in EF was within the reference interval and a clinically relevant reduction in EF ($< 46\%$) was observed in only four patients during follow-up. In one of these patients the reduction had already been observed prior to HDC.

Relapse and survival

At the time of the analysis, the median follow-up (from day of surgery) of the patients for survival was 35 months (range, 13–65) and median follow-up of patients for progression was 30 months (range, 11–57). A total of 13 patients (25%) relapsed, 8 cases occurring within two years of primary diagnosis (Fig. 2). Eleven (21%) of the patients died of breast cancer during follow-up, 6 of them within two years of surgery. Relapse-free survival (RFS) and overall survival (OS) were calculated according to the Kaplan–Meier method with the actuarial RFS of 75%, and OS of 88% three years after the start of treatment.

The anatomical pattern of relapse is presented in Fig. 3. Seven patients with early relapse, defined as < 1 year after end of treatment, all had distant metastases (including contralateral lymph nodes), while 3 out of 6 patients with relapse > 1 year after end of treatment had local recurrence.

No association between the parameters for treatment intensity (haematologic and non-haematologic toxicity,

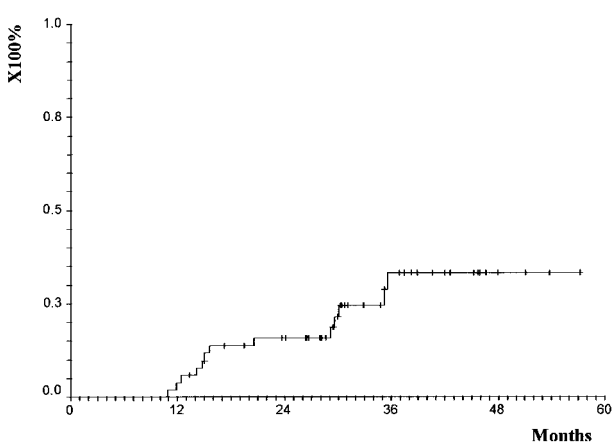

Fig. 2. Kaplan–Meier plot of recurrence after primary diagnosis (surgery) (n = 52).

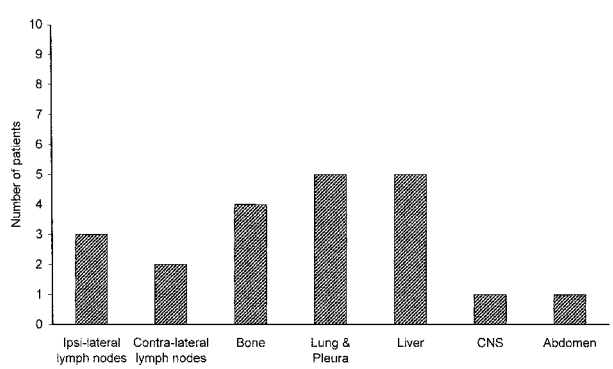

Fig. 3. Pattern of relapse of disease during follow-up (n = 13).

recovery data) and recurrence of disease was found (data not shown).

DISCUSSION

During the period 1996 to 2000 a total of 52 women with primary, high-risk breast cancer were treated with adjuvant high-dose therapy at our institution. The patients constitute a homogeneous population as staging, surgery, selection and treatment were performed according to uniform guidelines (19, 20, 33). Detailed information regarding treatment toxicity and supportive therapy has been recorded on this group during treatment and two years of follow-up.

From these data we evaluated the haematologic and non-haematologic toxicity associated with therapy. We found that FEC treatment was associated with minor, predictable toxicity (34, 35). Catheter-related complications were reported as a prevailing clinical problem involving systemic infections and venous thrombosis, often requiring removal of the catheter. The frequency of catheter-related complications reported in our patients (23 events in 52 patients) is not unusually high, as others have reported frequencies of more than 60% (23). However, even though catheter complications were not directly associated with the HDC, as they often emerged during FEC, they contributed significantly to the iatrogenic morbidity connected with the programme.

The high-dose regimen was not associated with toxic deaths but two patients suffered from life-threatening, acute organ toxicity comprising severe renal failure and pulmonal embolism. Non-life-threatening acute toxicities including nausea, vomiting and diarrhoea were common, as reported elsewhere (23, 33). However, although morbidity was pronounced during a relatively short period of high-dose therapy, the intensive use of newer antiemetics and anti-diarrhoeal agents made the regimen tolerable for most patients.

Long-term toxicity included fatigue, arthralgia/myalgia and both cerebral and peripheral neurotoxicity; most toxicities were grade 1–2 and were reversible within two years.

Even though cognitive deficits have not been systematically assessed in this study, the registered loss of memory could be an indication of cognitive impairment, as HDC has been found to increase this risk (36). Knowledge concerning the fundamental mechanisms of chemotherapy-induced cognitive dysfunction is still insufficient. Recently, a study of late effects of HDC on cognitive function was carried out (37) for comparison of neuropsychological and neurophysiological parameters. Neurophysiological disturbances were found more frequently after HDC, but no correlation with neuropsychological performance was found in that cohort of patients.

Working ability is another measure of recovery from treatment as well as an indicator of the severity of cognitive impairment. One year after HDC, only three of the evaluable patients were still unable to work. The recovery-time for working ability seems to be acceptable as the patients also received radiotherapy during this period. Furthermore, the regained working ability indicates a less pronounced cognitive impairment. On the other hand, if HDC is to be implemented as an adjuvant therapy for younger breast cancer patients, the central neurotoxicity associated with the specific regimens should be thoroughly investigated, as long-term cognitive impairment may have a major influence on the quality of life of patients.

One of the most prevalent side effects of HDC and radiotherapy was pulmonary toxicity, as a reduction in lung diffusion capacity was measured in nearly 75% of the patients (38). However, the clinical relevance was marginal, as only three patients presented with pulmonary symptoms during follow-up and only one patient had persistent clinical affection of the pulmonary function.

The main toxicity of HDC is myelo-suppression and, as anticipated, all patients had periods of absolute neutropenia and thrombocytopenia. On the other hand, rapid haematopoietic recovery after reinfusion of autologous blood stem cells was seen in the vast majority of patients, and only four patients had prolonged periods of neutro- and thrombocytopenia. Concordant with earlier findings (33), neutrophils recovered prior to platelets sustained by the administration of G-CSF, and the need for transfusions was acceptable. Thus, we find that autologous stem-cell support and the use of G-CSF have reduced the problems of cytopenia to an acceptable level.

Quality assessment of the stem cell graft is important for an adequate evaluation of the HDC regimen (15). When evaluating the efficacy of the stem-cell reinfusion, we found no correlation between the CD34+ cell count/kg and requirement of transfusion or duration of recovery. Only when using the total CD34+ cell number a significant correlation with platelet recovery was observed. The minimal number of CD34+ cells necessary for clinical engraftment is not known and may vary depending on the composition of stem-cell subsets in the graft. However, it has been shown that a graft content of more than $5–10 \times 10^6$ CD34+ cells/kg body weight ensures a rapid recovery (39). Consequently, as the vast majority of patients in this study had CD34+ count/kg above this limit, it could explain the lack of significant influence on recovery time.

Identification of contaminating carcinoma cells in the stem-cell graft was also used as a clinically relevant parameter for evaluation of the HDC regimen. Although such contamination may not be directly responsible for disease recurrence, indirectly it may be a marker for an ineffective induction therapy (40–42).

Surprisingly, only three patients had CK-positive tumour cells identified in the graft, indicating that contamination of stem-cell products with carcinoma cells above the detection limit of one out of 10^7 to 10^6 is an infrequent

phenomenon in this category of breast cancer patients. Owing to the low number of CK-positive patients in the study, we were unable to evaluate whether carcinoma cells in the stem-cell product predict clinical outcome.

We found a recurrence-free survival of 75% and an overall survival of 88% three years after start of treatment. These data are in line with those from a comparable retrospective study presented at a meeting of the American Society of Clinical Oncology (ASCO) in 2002, which showed a survival advantage compared to conventional therapy (43). However, as patients were not randomized and the median follow-up is less than three years, no conclusions should be drawn regarding the efficacy of adjuvant high-dose chemotherapy.

Recently, data available from small randomized studies on high-dose chemotherapy for breast cancer showed no significant difference in overall survival or disease-free survival rates (4, 14), but indications of a reduction of relapse have been found in several studies (5, 7, 14). Therefore, at the moment, the superiority of HDC as opposed to conventional chemotherapy appears, at best, marginal. Follow-up data from large randomized studies (5) must be awaited before reliable conclusions can be drawn on whether subgroups of patients might derive some benefit from HDC (13).

Our main objectives were to assess both the clinical and technical quality of the HDC strategy. Apart from two isolated cases of severe renal and pulmonary toxicity, the high-dose treatment was relatively well tolerated, without serious organ toxicity and without toxic deaths. The present chemotherapy regimen was found to be feasible with manageable toxicity and an acceptable requirement of supportive therapy in patients with localized breast cancer. Thus, with respect to toxicity, this regimen may also be applicable to other malignant diseases.

Despite the unfulfilled expectations, there is little doubt that the future will bring new dose-intensive chemotherapy strategies in the attempt to control cancer progression and dissemination. Patients treated with dose-intensive chemotherapy are assumed to have maximal reduced disease and a complementary effect of further chemotherapy is not likely. Therefore, new anticancer treatment strategies such as cancer vaccination therapy and monoclonal antibody therapy are potential candidates in the treatment of minimal residual disease.

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