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INTENSIVE CYCLIC CHEMOTHERAPY AND TRANSPLANTATION OF AUTOLOGOUS PERIPHERAL BLOOD PROGENITOR CELLS (PBPC) OR WHOLE BLOOD IN HIGH-RISK BREAST CANCER — FOLLOW UP AT 10 YEARS

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Aim: **The main aim of our paper is to contribute to objectification of currently widely discussed results of overall survival (OS), disease free survival (DFS) and time from relapse to tumor progression (TTP) in women with breast cancer.** ***Methods:*** **Forty consecutive patients fulfilling the eligibility criteria were admitted to the study. Fifty-six women were included in the control group. All patients received 6 cycles of adjuvant intensive cyclic combined chemotherapy with epirubicin 150 mg/m^2 and cyclophosphamide 1250 mg/m^2 (EC) applied each 14 days. To overcome haematological toxicity transplantations of autologous peripheral blood progenitor cells (PBPCs) or whole blood enriched of PBPC were used.** ***Results:*** **We found statistically significant difference in OS regardless of the stage of the disease to the benefit of women treated by intensive cyclic EC chemotherapy when compared with the control group. In evaluation of DFS no statistically significant difference was found in survival between the control group and the group with all stages of the disease. TTP in women without relation to the stage was statistically significantly longer than in the control group.** ***Conclusion:*** **In our study intensive cyclic EC chemotherapy did not show better curative effect when compared with conventional dosage chemotherapy.**

Key Words: **intensive cyclic chemotherapy, overall survival, disease free survival, time to tumor progression, PBPC, whole blood, breast cancer.**

The idea of the benefit of increasing dose intensity was based on two known hypotheses. The first one, Goldie-Coldman's hypothesis, postulates that spontaneous development of drug resistance following exposition to cytotoxic agents has a measurable velocity. The bigger the tumor size, the greater is the number of resistant clones. The result is that early and frequent intensive treatment may prevent the development of resistance and ineffective of oncologic therapy.

The second is Norton-Simon hypothesis on Gomperzian's tumour growth kinetics , presumes that a small tumor grows more quickly than a large one. The kinetics of tumor growth is non-exponential. The more the cancer cells are destroyed by chemotherapy, the more quickly they are renewed [1–4].

In 1994, with regard to the state of knowledge at that time, we started our program of breast cancer treatment in women at our department. First by high-dose chemotherapy and later by intensive cyclic chemotherapy with support of autologous peripheral haemopoietic stem cell transplantation (PBPC). Intensive cyclic chemotherapy and its conception was based on the presumption that only one even highly effective chemotherapy course (and dose) is mostly not effective enough for eradication of very sensitive tumors [5–6]. Therefore, it was rightly supposed that administration of more courses of intensive cyclic chemotherapy could be more effective than the conventional dosage [3].

The strategy of intensive sequential chemotherapy was designed on the presumption that chemotherapy with two consecutive regimens and with non-crossed resistance would bring much better effect. Bonadonna et al. [7] documented the benefit of sequential administration in the study with doxorubicin and CMF applied sequentially and alternatively to 405 women with breast cancer and more than 4 positive lymph nodes. The cumulative dosage of cytostatic agents was the same in both arms, but better result was ascribed to higher dose-intensity in the arm with sequential treatment [7].

The original motive of our study was based on the results published by Bastholt et al. [8]. They investigated the efficacy and toxicity of epirubicin given to female patients with metastatic breast cancer. The presented outcomes suggested a linear "dose-response" relation for antracyclin-based regimens, though a threshold effect could not be eliminated [8].

Here we present the results of treatment in a group of 40 women observed for 10 years. All patients received 6 cycles of adjuvant intensive cyclic combined chemotherapy with epirubicin 150 mg/m^2 and cyclophosphamide 1250 mg/m^2 (EC) applied every 14 days. At the beginning of the treatment phase there was a great problem with haematological toxicity. To overcome this serious complication autologous bone marrow transplantation and, later on, autologous peripheral

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Abbreviations used: EC – epirubicin and cyclophosphamide; OS – overall survival; DFS – disease free survival; PBPCs – peripheral blood progenitor cells; TTP – time from relapse to tumor progression.

blood progenitor cell transplantation (PBPCs) or administration of whole blood enriched with PBPC were used. Haemopoiesis reparation after EC regimen with PBPC or whole blood transfusion was sufficient, with no necessity to reduce chemotherapy dosage [9].

The main aim of our paper is to contribute to objectification of currently widely discussed results of overall survival (OS), time from complete remission to the first signs of relapse — disease free survival (DFS) and time from relapse to tumor progression (TTP) in women with breast cancer. According to our results, we think that we cannot clearly demonstrate better curative effect with intensive cyclic chemotherapy when compared with the control group treated by conventional chemotherapy. Our study, however, repeatedly shows that using both high-dose or cyclic intensive chemotherapy — we must be very careful in interpretation outcomes, and partial success does not entitle us to make premature conclusions.

PATIENTS AND METHODS

Forty consecutive patients fulfilling the eligibility criteria were admitted to the study. The mean age was 44.8 years (range 31–56 years). Fifty–six women were included in the control group, their average age was 55.4 years (range 31–77 years) (Table). Informed consent was obtained from each of the patients prior to treatment. Follow up was 10 years (range 108–125 months).

Eligibility criteria for admission to the protocol. Patients with stage II breast cancer with more than 10 positive axillary lymph nodes and conservative surgery with axillary lymph node dissection were eligible for admission. These patients had biopsy-proven locally advanced or inflammatory breast carcinoma, stages III and IV but no mastectomy or metastatic disease without CNS and BM involvement. The performance status was 0 or 1 (WHO scale). The patients had no previous or concomitant malignancy, no significant cardiac disease, normal blood count (pretreatment neutrophils $> 2 \times 10^9$/l, platelets $> 100 \times 10^9$/l), and normal basic biochemical parameters. They had not received previous chemotherapy.

Mobilization procedures and chemotherapeutic regimen. All patients received six cycles of chemotherapy at 14-day intervals consisting of epirubicin at the dose of 150 mg/m^2 and cyclophosphamide at the dose of 1250 mg/m^2 (EC) on day 1. In the first cycle, 24 h after chemotherapy, mobilization was started with a s. c. application of G-CSF (Roche, Basel Switzerland) at the dose of 5 μg/kg/day for the next 13 days. In all remaining cycles, G-CSF was given from day 7 after chemotherapy. In the first cycle of intensive chemotherapy, leukaphereses were performed on days 11, 12 and 13, and whole blood collection was performed on day 14. On day 15 the second cycle of chemotherapy was applied and whole blood, taken the day before, was reinfused (day 2 of the second cycle). In the third cycle whole blood was collected on day 13, chemotherapy was applied on day 14, and whole blood was reinfused on day 15. In the fourth to sixth cycles, no whole blood was collected, and cryopreserved leukapheresed PBPC for haematopoietic rescue were used instead.

PBPC harvest and application. PBPC were collected by leukaphereses during G-CSF administration in the first chemotherapy cycle. The leukaphereses were performed using an automated COBE Spectra continuous blood flow cell separator (COBE BCT, Lakewood, CO) with the following parameters: blood volume processed 10,000–12,000 ml (median 11,000 ml) per procedure and inlet flow rate 60–70 ml/min. The original COBE MNC program was used, software version 5.1 with quick start. The inlet/AC ratio was 1.12. PBPC were cryopreserved with 10% DMSO in a stabilization solution containing dextran, sucrose, dextrose, and anticoagulants. HSA was added to a final concentration of 1%. The product was frozen in a Kryo-10 programmable freezer (Planner, Biomed, UK) with a controlled rate of freezing (–1 K/min to –90 ˚C, –5 K/min to –150 ˚C). After freezing, PBPC were stored in liquid nitrogen at –196 ˚C. Thawing was performed in a waterbath at 37 ˚C, and the suspension was infused into a central venous catheter directly from the freezing bags (Baxter Fenwal, Deerfield, IL) without removal of the cryoprotectant.

Whole blood collection. In the first and second cycles, whole blood was collected on the last day of each cycle into standard blood collection bags (Japan Medical Supply [JMS] *(S)* PTE, Ltd., Singapore) containing 50 ml of CPDA solution as an anticoagulant. The total volume of whole blood was 450 ml. The bags were stored in a refrigerator at the tissue bank at 4 ˚C for 2 days before being reinfused after chemotherapy in the next cycle.

Flow cytometry and colony assays. Mononuclear cells (MNC) expressing the CD34 surface membrane antigen were quantified by flow cytometry using the Con IgG I-FITC antibody and the CD34 FITC-conjugated 8G12 antibody (HPCA-2 FITC) (Becton Dickinson, Mountain View, CA). Cytofluorometric analysis was performed using a FACScan (Becton Dickinson) after lysis of the erythrocytes with ammonium chloride-EDTA. The minimum number for each test was 10,000, and the count of CD34+ cells was performed in a wide gate. However, it was possible to exclude the debris and the erythrocytes by using the forward scatter characteristics CD45 fluorescence dot plot. Haematopoietic progenitor cells (HPCs) were determined by CFU-GEMM, BFU-E, and CFU-GM. Isolated purified MNC were plated at a concentration of 2×10^5/ml. All semisolid cultures were performed in duplicate and stimulated with 50 ng/ml GM-CSF, IL-3 and 3 IU/ml EPO. CFU, GM, CFU-GEMM, and BFU-E colonies were counted after 14 days of incubation in 5% CO_2, and 5% O_2, at 37 ˚C. The medium consisted of IMDM supplemented with 10% BSA, 20% FBS, 100 μg 3% glutamine, 100 μg penicillin + streptomycin, 135 μg 7.5% $NaHCO_3$, and 0.9% methylcellulose. Aliquot volumes of 1 ml were plated in duplicate in 35 mm Petri dishes. Only aggregates with more than 40 cells were considered colonies and were scored using an inverted

Olympus OSP-2 microscope after 14 days of CFU-GM or after 18 days of BFU-E and CFU-GEMM incubation at 37 °C in a 5% CO_2, fully humidified atmosphere.

Statistical analysis. The main end points for the comparison of the three treatments were overall survival, disease-free survival and time to tumor progression. Overall survival (OS) was calculated from diagnosis determination to death from any cause. Data on patients known to be alive at the time of the analysis were censored at the time of their last follow-up visit. Disease-free survival (DFS) was calculated from diagnosis determination to relapse from any cause. Data on patients known to be alive and without a relapse at the time of analysis were censored at the time of their last follow-up visit. Time to tumor progression (TTP) was calculated from relapse determination to time of tumor progression from any cause. Data on patients known to be alive at the time of an analysis were censored at the time of their last follow-up visit. All treatment comparisons are based on the intention-to-treat principle. The Kaplan-Meier method was used to estimate curves for OS, DFS and TTP, and comparisons were made with the use of the log-rank test. Cox proportional-hazards models were fitted in order to estimate hazard ratios and confidence intervals. Differences in the overall treatment comparison and the treatment comparison of the control group were with 95% confidence intervals. All p values are based on two-side tests.

RESULTS

Mobilization procedure — mean yields of collected PBPC and whole blood. In all 40 treated patients, the mean WBC count before EC and G-CSF administration was 6.40 x 10^9/l (range 2.30–12.10 x 10^9/l). They decreased by day 8 to 1.20 x 10^9/l (range 0.60–2.70 x 10^9/l) and increased again to 12.60 x 10^9/l (range 1.10–42.60 x 10^9/l) by day 10. The peak value of WBC 50.10 x 10^9/l (range 21.10–88.00 x 10^9/l) appeared by day 14. The mean number of CD34+ cells in peripheral blood before chemotherapy was 3.70 x 10^7/l (range1.30–9.20 x 10^7/l). The first peak was observed on day 4 (mean 9.10 x 10^7/l, range 0.20–17.40 x 10^7/l), a decrease with minimal values was seen on day 8 (mean 1.39 x 10^7/l, range 0.20–2.90 x 10^7/l), and the second peak appeared on day 14 (mean 64.40 x 10^7/l, range 31.20–134.40 x 10^7/l). The mean number of circulating progenitors (CFU-GM) in peripheral blood before chemotherapy was 2.10 x 10^5/l (range 0.90–5.30 x 10^5/l). After cytostatics and G-CSF, the numbers increased with the first peak by day 4 (mean 15.7 x 10^5/l, range 0.7–70.0 x 10^5/l) and decreased to minimal values by day 8 (mean 1.82 x 10^5/l, range 0–4.30 x 10^5/l), and the second peak was observed by day 14 (mean 179.10 x 10^5/l, range 19.10–383.30 x 10^5/l). The mean number of circulating progenitors (BFU-E) in peripheral blood before chemotherapy was 4.20 x 10^5/l, range 2.20–9.80 x 10^5/l). After cytostatics and G-CSF, their numbers increased with the first peak by day 4 (mean 29.7 x 10^5/l, range 3.70–139.0 x 10^5/l) and decreased to minimal values by day 8 (mean 2.32 x 10^5/l, range 1.12–8.20x10^5/l), and the second peak was observed by day 14 (mean 402.7 x 10^5/l, range 59.60–813.40 x 10^5/l). The mean number of circulating progenitors (CFU-GEMM) in peripheral blood before chemotherapy was 0.50 x 10^5/l (range 0–1.40 x 10^5/l). After cytostatics and G-CSF, their numbers increased with the first peak by day 4 (mean 2.7 x 10^5/l, range 0.7–9.0 x 10^5/l) and decreased to minimal values by day 8 (mean 0.22 x 10^5/l, range 0–1.20 x 10^5/l), and the second peak was observed by day 14 (mean 22.10 x 10^5/l, range 1.60–51.30 x 10^5/l). Absolute peak levels of circulating CFU-GM, BFU-E, CFU-GEMM, CD34+ cells and leukocytes in peripheral blood were seen approximately on day 14. PBPC harvesting was started as soon as there was evidence of a considerable increase in WBC count after the chemotherapy-induced nadir. This usually occurred 10 days after application of cytostatics. Altogether three leukaphereses were performed, and the maximum harvest was achieved on day 13. On day 14 (peak incidence of CFU-GM, BFU-E, and CFU-GEMM), the median increment of absolute values of CFU-GM over the baseline was approximately 853-fold, BFU-E was 959-fold, CFU-GEMM was 44.2-fold, and CD34+ cells was 17.4-fold.

Application of progenitors and haematopoietic recovery. The mean values of applied progenitors per cycle were CD34+ cells 1.52 x 10^6/kg, CFU-GM 1.18 x 10^5/kg, BFU-E 2.54 x 10^5/kg, and CFU-GEMM 0.31 x 10^5/kg in the courses supported by whole blood and CD34+ 2.04 x 10^6/kg, CFU-GM 1.59 x 10^5/kg, BFU-E 2.87 x 10^5/kg, and CFU-GEMM 0.34 x 10^5/kg in the leukapheresis-supported courses. The applied dose of cytostatics and intervals were identical in all patients. Leukopenia in patients supported with whole blood (second and third cycles) vs leukapheresed PBPC (fourth through to sixth cycles) was as follows: grade 4, 13/6 (38.2%/17.6%), grade 3, 19/23 (55.9%/70.6%), and grade 2, 1/4 (2.9%/11.8%), respectively. Thrombocytopenia was grade 4, 11/6 (32.4%/17.6%), grade 3, 10/7 (29.4%/20.6%), grade 2, 7/13 (20.6%/38.2%), and grade 1, 6/6 (17.6%/17.6%), respectively. In all patients, the mean number of platelet transfusions applied either as whole blood or leukapheresed PBPC was 3 vs 2 (range 2–6 vs 0–4) per cycle. The mean number of erythrocyte transfusions was 7 vs 2 (range 4–8 vs 1–4) per cycle. The difference in the number was not significant at $p < 0.001$, whereas in erythrocyte transfusions, the difference at $p < 0.001$ was statistically significant because Hb levels did not exceed 105 g/l, which was why the number of erythrocyte transfusions increased.

Evaluation of clinical results. We performed a follow-up analysis of all patients. The follow-up period was 10 years (range 108–126 months). In the group with intensive cyclic chemotherapy (EC) responses in high-risk patients previously treated with surgery and then with adjuvant chemotherapy (n = 5) were not evaluated. The response rate (RR) in patients with locally advanced or inflammatory breast carcinoma (n = 10) was 80%, CR was 80%, and PR was 10%. No response to therapy

was observed in 10%. In women with metastatic disease (n = 17), RR was 76.4%, CR was not achieved, and PR was 58.8%. Two patients died during the therapy. The control group consisted of 56 women with breast cancer and adjuvant FAC chemotherapy — fluorouracil, doxorubicin, cyclophosphamide, in 6 cycles (85,7%) . CMF — cyclophosphamide, methotrexate, fluorouracil (10.7%) — 6 cycles in 66,7% and weekly doxorubicin (3.6%) — 10 cycles given to all patients. The control group was made up of 56 women with breast cancer, 85.7% of whom received 6 cycles of FAC chemotherapy (fluorouracil, doxorubicin, cyclophosphamide), 10.7% received CMF (cyclophosphamide, methotrexate, fluorouracil; 6 cycles in 66.7% of this group), and 3.6% received 10 cycles of weekly doxorubicin in adjuvant setting. The response rate (RR) in patients with locally advanced or inflammatory breast carcinoma (n = 26) was 88.5%, CR was 80.7%, and PR was 19.3%. No response to therapy was observed in 3 patients. In women with metastatic disease (n = 19), RR was 52.6%, CR was not achieved, and PR was 47.3%. Evaluation of RR results in the group with intensive cyclic EC chemotherapy and in the control group showed that the difference of 80% vs 88.5% (p = 0.870) in the group with locally advanced or inflammatory breast carcinoma was not statistically significant. CR of 80% vs 80.7% was not statistically significant. PR of 10% vs 19.3% (p = 0.514) was on the border of significance for the control group. The difference in RR in women with metastatic disease was 77.4% vs 52.6% (p = 0.326). This difference was statistically significant. CR was not achieved in any group with metastatic carcinoma. PR was 58.8% vs 47.3% (p = 0.427). This difference was on the border of statistical significance. Furthermore, we found that OS, regardless of the stage of the disease, was significantly different (p = 0.0186) for the benefit of women treated by intensive cyclic EC chemotherapy with haemopoietic support (PBPC or whole blood) in comparison with the control group. Evaluation of survival shows that the maximum difference in OS is in the period of 5–7 years (range 2.8–8.6 years) and after that time the differences are not so significant (Fig. 1, *a*). However, when we divided the patients into groups according to the stage of the disease and compared them with the identical control groups, we found that there was no statistical difference (p = 0.996) in women with stage II, the difference was very small (p = 0.035) in the group with stage III and similar it was in the group with stage IV (p = 0.0352 (Fig. 1, *b*, *c*, *d*). Here, it is not clear which group more or less contributed to this seemingly good result of OS (Fig. 1, *b*, *c*, *d*). Probably, an important role played the fact that maximum good results in OS were achieved between the years 5 and 7 of follow-up. (Fig. 2, *a*). Evaluation of DFS showed no statistically significant difference in survival (p = 0.6703) between the controls and the patients regardless of the stage of the disease (Fig. 2, *a*) When DFS was compared in patients with stage II, this difference was also not significant (p = 0.7909) and similar result was found in stage III (p = 0.8579) and stage IV (p = 0.6581) (Fig. 2, *b*, *c*, *d*). Yet, a very interesting outcome was noted when we compared TTP. When TTP was compared in all treated patients without relation to the stage of the disease, it was statistically significantly longer ($p < 0.001$) than in the control group (Fig. 3, *a*). Accordingly it was seen at TTP evaluation in patients with stage II and III ($p < 0.001$) (Fig. 3, *b*, *c*). This statistical difference was somewhat smaller in stage IV (p = 0.0265) (Fig. 3, *d*). In the relapse

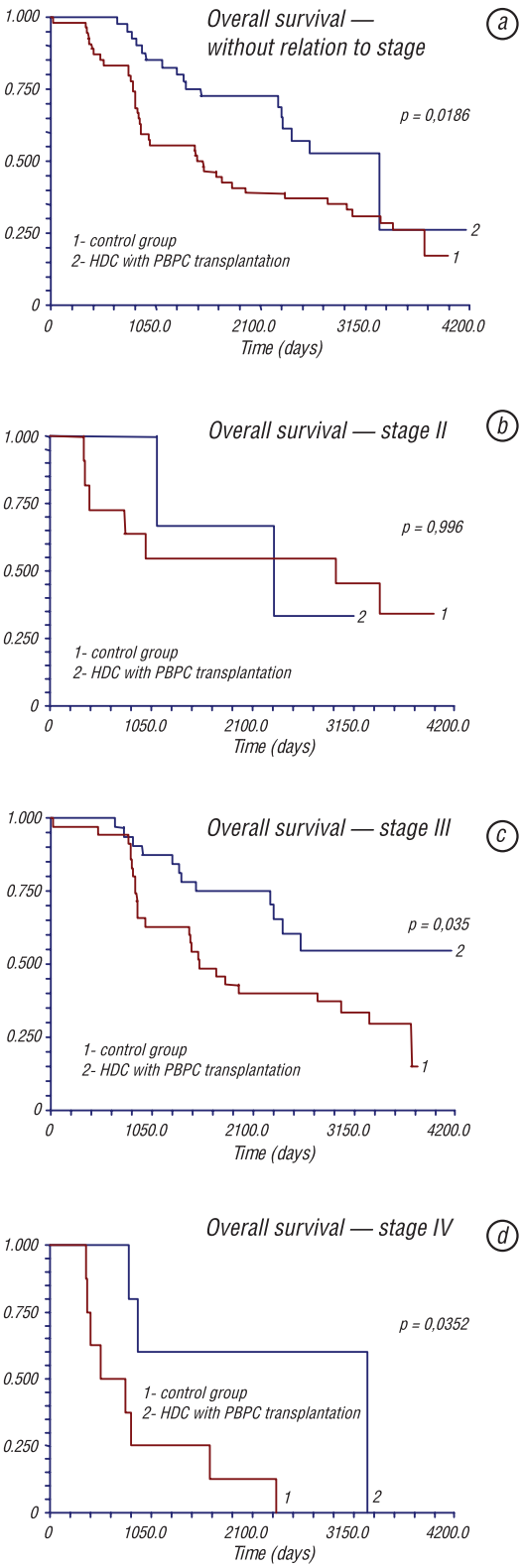

Fig. 1. Kaplan — Meier survival curves

period in the 2nd and 3rd line 47.5% of women received combined chemotherapy with paclitaxel/docetaxel at the beginning of therapy and combination of chemotherapy and immunotherapy (paclitaxel/docetaxel and transtuzumab) was applied to 10% of women that were first treated with intensive EC chemotherapy. This was in contrast with the control group where only 5.4% of patients were treated by a combination of chemotherapy and immunotherapy. In addition, hormonal treatment was changed in all patients with hormone-dependent carcinomas after recurrence of the disease. The changes were as follows: EC group — anastrozol 42.5%, letrozol 15%, control group — anastrozol 12.5%, letrozol 5.4%. 95% of patients from the group with prior intensive chemotherapy and only 19.6% of patients from the control group were given tamoxifen.

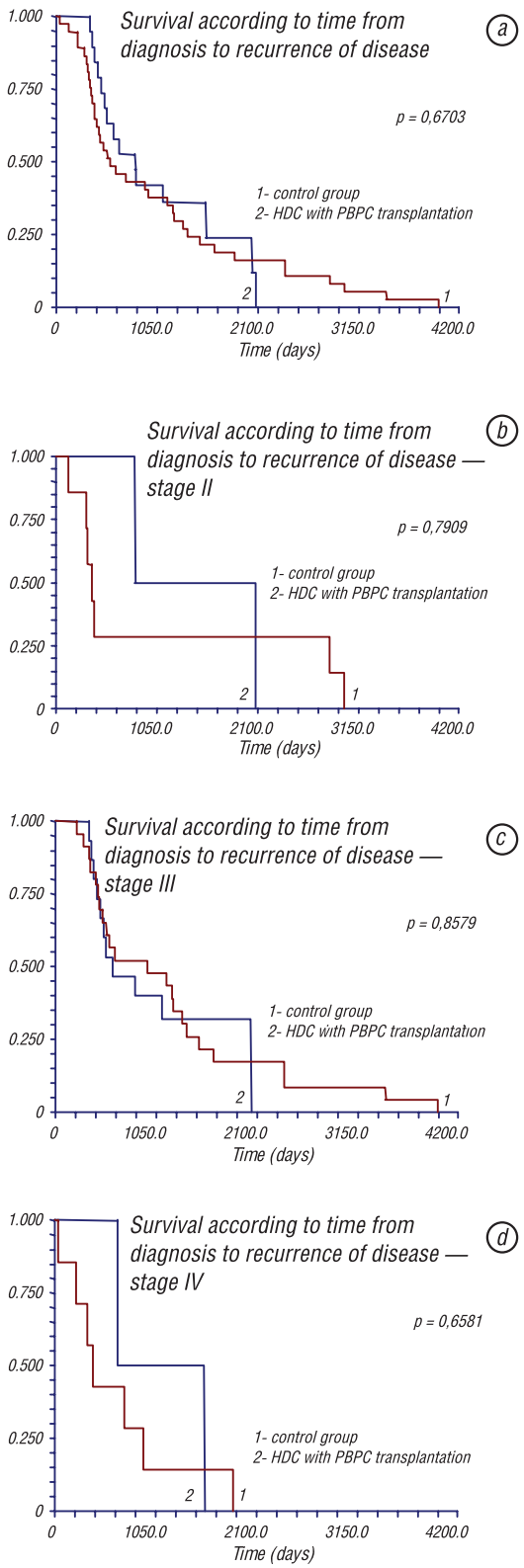

Fig. 2. Kaplan — Meier survival curves

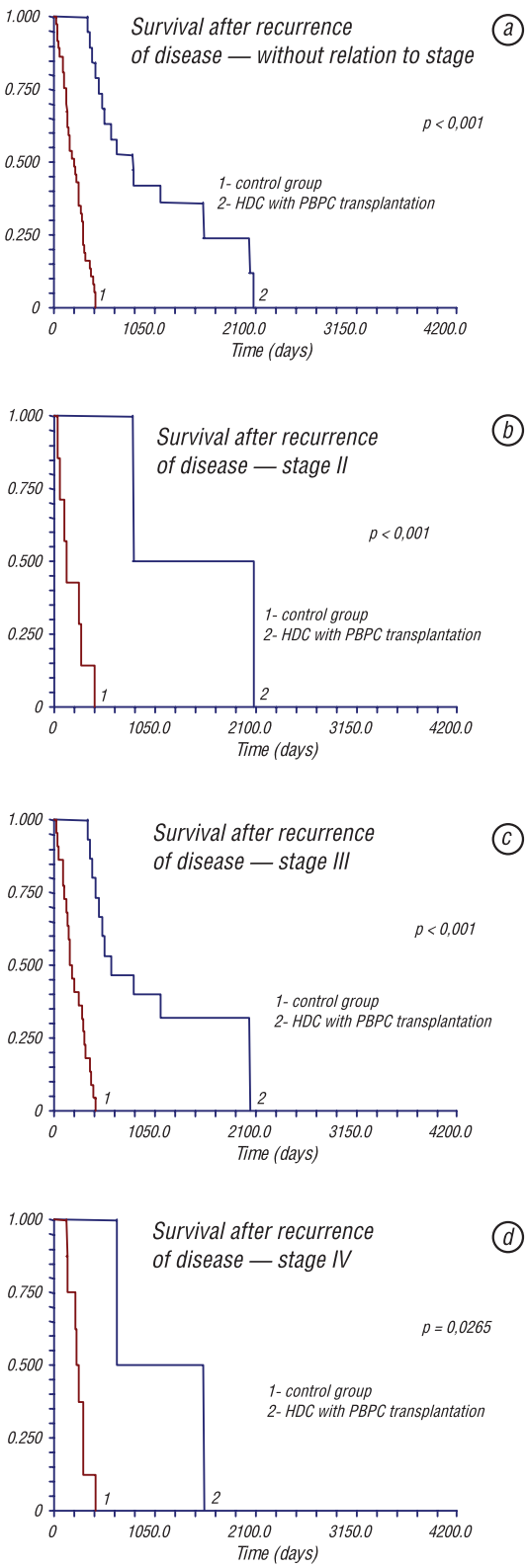

Fig. 3. Kaplan — Meier survival curves

Table. Patients treated with intensive cyclic chemotherapy and control group

	Control	ICCH
Number of patients	56	40
Mean age (years)	55.4 (range 31–77)	44.8 (range 31–56)
ER+/PR+	11	9
Stage II breast cancer (⩾ 10 positive axillary lymph nodes)	11	13
Stage III and IV breast cancer (locally advanced breast carcinoma)	26	10
Metastatic breast carcinoma	19	17

Notes: ICCH – intensive cyclic chemotherapy – epirubicin plus cyclophosphamide; ER+/PR+ – patients with estrogen-positive receptor and progesterone-positive receptor; Stage II, III and IV – American Joint Committee on Cancer Staging.

DISCUSSION

The idea about benefit from increasing dose intensity is based on two known hypotheses, namely, Goldie-Coldman's hypothesis and Norton-Simon's hypothesis [1–2]. A number of studies were then conducted to verify these presumptions, the aim being to demonstrate the influence of high-dose chemotherapy on tumour treatment. Here we wish to remind some important studies which in different ways also influenced our approach and further decision-making just before starting the treatment of women with breast cancer [6].

One hundred and two women with primary breast cancer and more than 10 positive axillary lymph nodes were admitted to the pilot study of Peters and his group. All patients were treated with high doses of cyclophosphamide, cis-platin and carmustin with autologous bone marrow support [10]. In this study, DFS after 2.5 years was 72%, which shows a survival benefit in comparison with patients having conventional chemotherapy [10]. This study represented a certain stimulus because, at that time, the number of patients with solid tumours treated with high-dose chemotherapy notably increased . The primary idea of our study was based on this paper, but also on the results published by Bastholt and colleagues as they investigated the efficacy and toxicity of epirubicin at the doses of 40, 60, 90 and 135 mg/m^2 applied to women with metastatic breast cancer every 3 weeks. They observed an increase in the number of responses and prolongation of TTP with raising dosage of epirubicin from 40 mg/m^2 to 90 mg/m^2, but not at higher doses. This study concluded that the dosage of 90 mg/m^2 is the optimal tri-weekly dose because it was not confirmed that doses higher than 90 mg/m^2 improve outcomes in any way [8]. This was also one of the reasons why we chose intensive cyclic combined EC chemotherapy with haemopoietic support [11]. Over the next few years many other studies were performed which verified the relation between dose and dose intensity.

For example, here we can mention the Cancer and Leukemia Group B (CALGB) study in which 1572 patients were analysed. The authors conclude that the effect may depend on the dose, or that there may be a threshold dose below which the clinical effect is significantly worse. Both DFS and OS showed an upward trend to improve with higher dose intensity, but the difference between groups was not statistically significant [12]. In 1997 Bearman et al. published their experience with high-dose chemotherapy and stem cell support as an adjuvant therapy for 54 women with 4–9 positive axillary nodes. Even though the result of DFS evaluation was promising, the authors themselves admit that a longer follow-up is necessary to compare survival time in these patients with historical controls treated by standard-dose chemotherapy [13]. Gianni et al. published 5-year follow-up results of their sequential high-dose chemotherapy programme with stem cell support applied as adjuvant therapy in women having breast cancer stage II or III with ⩾10 positive lymph nodes. DFS and OS were analysed on an intent-to-treat basis. The difference in DFS was statistically significant between groups (p = 0.04) but not in OS. The difference was on the border of statistical significance (p = 0.05). The main difference in treatment was probably radiotherapy of the breast or chest wall in patients with high-dose chemotherapy, which may be, at least in part, the cause of some differences found between the treated groups [14].

After a period of time when some studies showed both — good and worse therapeutic results, it was necessary to analyse the achieved results responsibly. Generally, it was accepted that more accurate results can be achieved only in large randomized studies [15]. In 1998 the NCI (National Cancer Institute) gave priority to four randomized trials with HDC and stem cell support [15].

We found out that in evaluation of our results we had to include other factors which could influence the gathered data. There were several such factors in our group of 40 women treated by intensive cyclic EC chemotherapy. It was the fact that the majority of patients received hormonal therapy with tamoxifen — 95% vs 19.6% in the control group. In addition, hormonal treatment was changed in all patients with hormone-dependent tumours after recurrence: EC group — anastrozol 42.5%, letrozol 15%, control group — anastrozol 12.5%, letrozol 5.4% of patients. Furthermore, all women that received intensive cyclic chemotherapy underwent breast radiotherapy as opposed to 85.7% of women in the control group. But what we really think is that our results significantly influenced administration of paclitaxel/docetaxel chemotherapy in 47.5% of patients in 2nd and 3rd line following cancer relapse as well as the results with combined chemotherapy and immunotherapy (paclitaxel/docetaxel and transtuzumab) given to 10% of the patients after breast cancer recurrence and who were first treated by intensive cyclic EC chemotherapy. In contrast to this, only 5.4% of the patients in the control group were given combined chemotherapy and immunotherapy.

In 1995 Bonadonna et al. published the results of their study, which showed good therapeutic results of high-dose chemotherapy in women with advanced breast cancer. This treatment was associated with better survival in DFS and OS evaluation in comparison with standard chemotherapy and shortened intervals between cycles. Patients with high-dose chemotherapy had better a 4-year survival rate according to evaluation of DFS than those who received conventional chemotherapy (60% vs 44%). Moreover, the group with high-dose chemotherapy also showed better OS (75% vs 70%) [7]. However, here we also have to remind that

these and other results need to be assessed with caution because no group received taxans. We think that conclusions in this study might have been influenced, just as it was in our study. This was confirmed by our TTP result showing a clinically significant effect with application of taxans alone or taxans in combination with immunotherapy. Today we know that increasing the dose with prophylaxis of growth factors has no benefit in adjuvant treatment of breast cancer. It is clear now, that prospective clinical trials with high-risk patients and high-dose chemotherapy with bone marrow or peripheral stem cell transplantations did not demonstrate statistically significant benefit. In the clinical trial CALGB 9082 (Cancer and Leukemia Group B) 785 women with more than 10 positive lymph nodes were evaluated and neither OS nor DFS were better in the transplanted group [16]. A Rodenhuis´ study included 855 patients with more than 3 lymph positive nodes who received 5 cycles of FEC chemotherapy or 4 standard cycles of FEC chemotherapy plus one dose-density regimen with transplantation. Though a short improvement in 5-year DFS (59% vs 65%) was noted, it was without statistical significance. Only subanalyses demonstrated a slight benefit in younger patients with more than 10 positive nodes, HER-2 negative and lower grade of the disease [17]. Even more precise analysis did not indicate negative factors such as hormonal independence, HER-2 negativity, lower grade etc., influencing the benefit of intensive cyclic EC chemotherapy.

Presently, results of some current trials show that administration of high-dose chemotherapy may have a specific importance in treatment of primary advanced — inflammatory [18, 19] and metastatic breast cancer. For example, in 2006 Vredenburgh et al. published a study in which they assessed OS in 425 women with metastatic breast cancer after high-dose chemotherapy with haemopoietic support. The patients received 2–4 cycles of inductive combined chemotherapy with doxorubicin, 5-fluorouracil and methotrexat (AFM). Women in complete remission were randomized into the group with high-dose combined chemotherapy (cyclophosphamide, cisplatin and carmustine) plus haemopoietic support. The follow-up period was 5 years. Comparison of OS women after inductive chemotherapy and after HDC was 3.8 vs 9.7 months with benefit for HDC ($p < 0.006$). The authors here prove that salvage HDC may prolong survival in cases of metastatic breast cancer [20].

This and other results could be a good reason to conduct another clinical trials. However, the present results do not justify the use of high-dose chemotherapy within the framework of standard adjuvant therapy as it is still a curative regimen for clinical trials [21–23].

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REFERENCES

1. **Norton L, Simon R.** Tumor size, sensitivity to therapy and the design of treatment schedules. Cancer Treat Rep 1977; **61**: 1307–17.

2. **Goldie JH, Coldman AJ.** A mathematic model for relating the drug sensitivity of tumors to their spontaneous mutation rate. Cancer Treat Rep **1979; 63**: 1723–33.

3. **Weiss RB.** Introduction: Dose-intensive therapy for adult malignancies. Semin Oncol 1999; **26**: 1–5.

4. **Simon R, Norton L.** The Norton-Simon hypothesis: designing more effective and less toxic chemotherapeutic regimens. Nat Clin Pract Oncol 2006; **3**: 406–7.

5. **Crown J.** High-dose chemotherapy of adult solid tumors. 23rd ESMO congress, 1988, J, Norton L, Simon R. The Norton-Simon hypothesis revisited. Cancer Treat Rep 1986; **70**: 163–9.

6. **Filip S, Petera J, Odrážka K, Bláha M.** A current look at high-dose chemotherapy in breast cancer. Neoplasma 2000; 47: 261–8.

7. **Bonadona G, Zambetti M, Valagussa P.** Sequential or alternating doxorubicin and CMF regimens in breast cancer with more than three positive nodes. Ten-year results. JAMA 1995; **273**: 542–7.

8. **Bastholt L, Dalmark M, Gjedde SB, Pfeiffer P, Pedersen D, Sanbberg E, Kjaer M, Mouridsen HT, Rose C, Nielsen OS, Jakobsen P, Bentzen SM.** Dose-response relationship of epirubicin in the treatment of postmenopausal patients with metastatic breast cancer: a randomised study of epirubicin at four different dose levels performed by the Danish Breast Cancer Cooperative Group. J Clin Oncol 1996; **14**: 1146–55.

9. **Filip S, Bláha M, Odrážka K, Měříčka P, Vávrová J.** Application of whole blood and peripheral blood progenitor cells (PBPC) and new strategies for rescue after intensive cyclic chemotherapy in high-risk breast cancer. J Hematotherapy Stem Cell Research 2000; **9**: 31–8.

10. **Peters WP, Ross M, Vredenburgh JJ, Meisenberg B, Marks LB, Winer E, Kurtzberg J, Bast RC Jr, Jones R, Shpall E.** High-dose chemotherapy and autologous bone marrow support as consolidation after standard-dose adjuvant therapy for high-risk primary breast cancer. J Clin Oncol 1993; **11**: 1132–43.

11. **Filip S, Bláha M, Petera J, Vávrová J, Knížek J.** Peripheral progenitor cells (PBPC) in supportive care after high-dose chemotherapy in breast cancer. Neoplasma 2001; **48**: 39–47.

12. **Wood WC, Budman DR, Korzun AH, Cooper MR, Younger J, Hart RD, Moore A, Ellerton JA, Norton L, Ferree CR.** Dose and dose intensity of adjuvant chemotherapy for stage II, node-positive breast carcinoma. N Engl J Med 1994; **30**: 1253–9.

13. **Bearman SI, Overmoyer BA, Bolwell BJ, Taylor CW, Shpall EJ, Cagnoni PJ, Mechling BE, Ronk B, Baron AE, Prudy MH, Roos M, Jones RB.** High-dose chemotherapy with autologous peripheral blood progenitor cell support for primary breast cancer in patients with 4–9 involved axillary lymph nodes. Bone Marrow Transplant 1997; **20**: 931–7.

14. **Gianni AM, Siena M, Bregini M, Di Nicola M, Orefice S, Cusumano F, Salvadori B, Luini A, Greco M, Zucali R, Rilke F, Zambetti M, Valagussa P, Bomadona G.** Efficacy, toxicity, and applicability of high-dose sequential chemotherapy as adjuvant treatment in operable breast cancer with 10 or more involved axillary nodes: five-year results. J Clin Oncol 1997; **15**: 2312–21.

15. **Subramanyan S, Kennedy MJ.** Breast cancer: High-dose therapy. Oncologist 1998; **3**: 289–99.

16. **Hurd DD, Peters WP.** Randomized, comparative study of high-dose (with autologous bone marrow support) versus

low-dose cyclophosphamide, cisplatin, and carmustine as consolidation to adjuvant cyclophosphamide, doxorubicin, and fluorouracil for patients with operable stage II or III breast cancer involving 10 or more axillary lymph nodes (CALGB Protocol 9082). Cancer and Leukemia Group B. J Natl Cancer Inst Monogr 1995; **19**: 41–4.

17. **Rodenhuis S, Bontenbal M, Beex LV, Wagstaff J, Richel DJ, Nooij MA, Vest EE, Hupperets P, Van Tinteren H, Peterse HL, Tenvergert EM, De Vries EG; Netherlands Working Party on Autologous Trnasplantation in Solid Tumors.** High-dose chemotherapy with hematopoietic stem-cell rescue for high-risk breast cancer. N Engl J Med 2003; **349**: 7–16.

18. **Veyert C, Levy C, Chollet P, Merrouche Y, Roche H, Kerbart P, Fumoleau P, Fargeot P, Clavere P, Chevallier B.** Inflammatory breast cancer outcome with epirubicin-based induction and maintenance chemotherapy: ten-year results from the French Adjuvant Study Group GETIS 02. Cancer 2006; **107**: 2535–44.

19. **Kroger N, Frick M, Gluz O, Mohramann S, Metzner B, Jackisch C, Ko Y, LindemannI HW, Meier CR, Lohrann HP, Ruffert U, Hanel M, Bodenstein H, Neubauer A, Ehninger G, Wolf HH, Kolbe K, Burock K, Zander AR, Nitz U.** Randomized trial of single compared with tandem high-dose chemotherapy followed by autologous stem-cell transplantation in patients with chemotherapy-sensitive metastatic breast cancer. J Clin Oncol 2006; **24**: 3919–26.

20. **Vredenburgh JJ, Coniglio D, Broawater G, Jones RJ, Roos M, Shpall JE, Hussein A, Rizzieri D, Marks BL, Gilbert C, Affronti ML, Moore S, Mc Donald C, Petros WP, Peters WP.** Consolidation with high-dose combination alkylating agents with bone marrow transplantation significantly improves disease-free survival in hormone-insensitive metastatic breast cancer in complete remission compared with intensive standard-dose chemotherapy alone. Biol Blood Marrow Transpl 2006; **12**: 195–203.

21. **Hanrahan EO, Broglio K, Frye D, Buzdar AU, Theriault RL, Valero V, Bosser DJ, Singletary SE, Strom EA, Gajewski JL, Champlin RE, Hortobagyi GN.** Randomized trial of high-dose chemotherapy and autologous hematopoietic stem cell support for high-risk primary breast cancer: follow-up at 12 years. Cancer; **106**: 2327–36.

22. **Vogl DT, Stadtmauer EA.** High-dose chemotherapy and autologous hematopoietic stem cell transplantation for metastatic breast cancer: a therapy whose time has passed. Bone Marrow Transpl 2006; **37**: 985–7.

23. **Ahmad K.** High-dose chemotherapy for advanced breast cancer. Lancet Oncol 2006; **7**: 19.

ИНТЕНСИВНАЯ ЦИКЛИЧЕСКАЯ ХИМИОТЕРАПИЯ И ТРАНСПЛАНТАЦИЯ АУТОЛОГИЧНЫХ КЛЕТОК-ПРЕДШЕСТВЕННИКОВ ПЕРИФЕРИЧЕСКОЙ КРОВИ ИЛИ ЦЕЛЬНОЙ КРОВИ БОЛЬНЫМ РАКОМ МОЛОЧНОЙ ЖЕЛЕЗЫ — ДЕСЯТИЛЕТНИЙ МОНИТОРИНГ

Цель: **объективизация широко обсуждаемых результатов по общей выживаемости (ОВ), периода ремиссии (ПР) и продолжительности периода от рецидива к опухолевой прогрессии (ПРОП) у больных раком молочной железы.** ***Методы:*** **обследовано 40 больных, отвечающих установленным критериям отбора, и 56 здоровых женщин, составивших контрольную группу. У всех больных проведено 6 циклов адъювантной интенсивной циклической комбинированной химиотерапии (эпирубицин — 150 мг/м^2 и циклофосфамид — 1250 мг/м^2 (ЭЦ)), проводимой каждые 14 дней. Для преодоления гематологической токсичности применяли трансплантацию клеток-предшественников периферической крови (КППК) или цельной крови, обогащенной КППК.** ***Результаты:*** **выявлены статистически значимые различия в ОВ, не зависящие от стадии заболевания, у пациенток, получавших интенсивную циклическую химиотерапию ЭЦ, по сравнению с контрольной группой. При оценке ПР не выявлено статистически значимых различий выживаемости между контрольной группой и больными во всех стадиях заболевания. ПРОП у пациенток независимо от стадии заболевания статистически значимо выше, чем таковой контрольной группы.** ***Выводы:*** **по результатам исследования, интенсивная циклическая химиотерапия ЭЦ не имеет лечебных преимуществ по сравнению со стандартной дозовой химиотерапией.**

Ключевые слова: **интенсивная циклическая химиотерапия, общая выживаемость, период ремиссии, период до опухолевой прогрессии, клетки-предшественники периферической крови, цельная кровь, рак молочной железы.**