

Primary systemic therapy does not eradicate disseminated tumor cells in breast cancer patients

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Abstract

Introduction The presence of disseminated tumor cells in the bone marrow of breast cancer patients has proven to be an independent prognostic factor. The aim of this study was to investigate the status of tumor cell dissemination after primary systemic therapy in relation to therapy response.

Methods Bone marrow aspirates were obtained from 120 patients after completion of primary systemic therapy. Disseminated tumor cells were detected by immunocytochemistry using the APAAP method. Bone marrow status was correlated with clinicopathological factors as well as tumor response to primary systemic therapy.

Results Sixty out of 120 patients had disseminated tumor cells in their bone marrow aspirates (50%). Response rates were 18% for pathologic complete remission, 52% for partial remission, 28% for no change and 3% for progression. Despite complete remission, 36% of these patients were bone marrow positive. In the partial remission group, the positivity rate was 48%. About 61% of patients with stable disease had disseminated tumor cells in their bone marrow. A trend to higher positivity rates was observed in the poor responder group compared to responders (61% vs. 38%, $P = 0.1$).

Conclusion Primary systemic therapy does not completely eradicate disseminated tumor cells in the bone marrow of breast cancer patients. The biological role of persistent disseminated tumor cells needs to be further investigated to optimize current and future therapeutic strategies.

Keywords Breast cancer · Primary systemic therapy · Disseminated tumor cells · Neoadjuvant therapy

Introduction

Neoadjuvant or primary systemic therapy (PST) has become an accepted alternative to traditional adjuvant therapy as studies have confirmed that its effect on long-term survival is similar to that of adjuvant chemotherapy [1–3]. Large clinical trials have reported complete clinical remission rates of 30–60% depending on the type of chemotherapy [4–7]. While initially the treatment of choice for locally advanced tumors (T3, T4), the use of PST in patients with smaller T1 and T2 tumors has been the focus of intense discussions (NIH and St.Gallen Consensus Panels). One major advantage of PST is that it offers the opportunity to assess first-hand the clinical chemosensitivity of the individual tumor, allowing for further risk-stratification and early identification of chemo-resistant cancers. The effect of both adjuvant and PST on long-term survival has been attributed to its ability to eradicate microdisseminated tumor cells that could potentially develop into distant metastases. Disseminated isolated tumor cells, that do not qualify as clinically apparent metastases have been detected in different tissues of patients with breast cancer and most other cancers [8–10]. About 35–40%

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of breast cancer patients without distant metastases have disseminated tumor cells (DTC) in their bone marrow (BM) at the time of primary therapy. Numerous studies show that patients with positive tumor cell detection in the BM have a worse prognosis when compared to patients with a negative BM-aspirate [11–13], findings that have recently been confirmed by metanalysis [14]. It remains unclear how PST influences tumor cell dissemination or metastatic potential of disseminated tumor cells. However, as the effect of PST on microdisseminated tumor cells is considered crucial for the long-term benefit of such therapies, we look at its effect on the incidence of cytokeratin positive cells in breast cancer patients.

Patients and methods

All patients receiving PST at our institution were eligible for this study. Patients with locoregional relapse or distant metastases were excluded. About 120 patients agreed to participate in the study and were included in the final analysis. All patients gave informed written consent. After completion of PST patients underwent BM aspiration under general anesthesia at the time of surgery. Histological diagnosis of breast carcinoma was established prior to chemotherapy by high speed microbiopsy. Clinical tumor size and nodal status were assessed by palpation, sonography and mammography before chemotherapy or hormone therapy. The absence of distant metastases was confirmed by sonographic and radiographic examinations. Both patients treated with endocrine therapy and chemotherapy were included in current trials and received standardized treatment. Patients in the chemotherapy group mostly received taxane containing chemotherapies, most commonly four cycles of epirubicine/cyclophosphamide followed by four cycles of a taxane over a total of 24 weeks. Before starting each cycle, clinical examination, breast palpation, breast-sonography and mammography was performed. Three to four weeks after completion of the final cycle of PST, surgery and BM aspiration was performed. About 15–20 ml of BM was aspirated from the anterior iliac crest ipsilateral to the afflicted breast under general anesthesia before start of the surgical procedure. Unilateral aspiration was chosen because during follow-up visits, aspirations are performed under local anesthesia. After preparation of cytopspins, immunocytochemical staining and automated detection of enriched disseminated tumor cells was performed. Detailed laboratory protocols controls are described elsewhere [15]. Leukocytes from healthy blood donors

were used as negative controls. The presence of one or more pancytokeratin positive cells per cytopspin, based on the criteria established by the International Society for Cellular Therapy was evaluated as positive [16]. All final pathology reports were reviewed. Pathologic assessment included the surgical breast specimen as well as removed axillary lymphnodes. Only patients with no remaining tumor were considered to have pathological complete response (pCR). For stratification and statistical evaluation, the patients were divided in four groups (PD: progressive disease, NC: no change, PR: partial remission and CR: complete remission) based on their response to PST. CR was defined as pathologic complete disappearance of any tumorous tissue and limited to this group. Patients with clinical complete remission who were found to have residual cancer on histology were included in the partial remission group. PR was defined as a decrease in tumor size of at least 50%, NC as stable tumor size to a reduction in tumor size of less than 50%. Progressive disease was defined as an increase in tumor sizes of more than 25%. The relationship between BM status and categorical or dichotomous clinicopathological factors in the four subgroups was analyzed by chi-squared test. Statistical analysis was performed by SPSS (Version 11.5). *P*-values less than 0.05 were considered statistically significant.

Results

Basic patient characteristics of the study population are outlined in Table 1. After completion of primary systemic therapy and at the time of surgery, 60 (50%) patients had a positive BM, i.e. detectable tumor cells by immunocytochemistry. The number of detected cells ranged from 1 to 100 cells per 2×10^6 mononuclear cells (mean: 2.4 cells per 2×10^6 mononuclear cells for all patients, 4.9 cells for DCT-positive patients.). Figure 1 shows a cytokeratin-positive disseminated tumor cell of a patient after PST. Presence of disseminated tumor cells was not correlated to any of the clinicopathological factors including tumor size, nodal status, grading, histology, hormone receptor or HER2 status (Table 1). No difference of positivity rates in BM was seen between different treatment groups (endocrine versus taxane based versus anthracycline based therapy) ($P = 0.553$). Table 2

The majority of patients ($n = 62$, 52%) showed a partial remission of the primary tumor. Complete remission, defined as the absence of detectable malignant tissue at the primary site was observed in 22 (18%) cases. In 33 (28%) cases the tumor size

Table 1 Basic characteristics

	Total	BM pos (%)	<i>P</i> -value
Total	120	60 (50%)	–
<i>Menopausal status</i>			
Pre	57	29 (51%)	0.855
Post	63	31 (49%)	
<i>Tumor size before therapy</i>			
T1	5	4 (80%)	0.488
T2	56	29 (51%)	
T3	25	11 (42%)	
T4	34	16 (50%)	
<i>Tumor size after therapy</i>			
ypTo	21	8 (38%)	0.489
ypT1	41	22 (54%)	
ypT2	41	20 (49%)	
ypT3-4	16	10 (63%)	
<i>Nodal status</i>			
yNo	67	33 (49%)	0.30
yN1	37	16 (43%)	
yN2-3	16	11 (69%)	
<i>ER status</i>			
Neg	34	16 (47%)	0.685
Pos	86	44 (51%)	
<i>PR status</i>			
Neg	55	24 (44%)	0.200
Pos	65	36 (55%)	
<i>HER2 status</i>			
Neg	85	45 (53%)	0.315
Pos	35	15 (43%)	
<i>Grading</i>			
I–II	82	44 (54%)	0.230
III	36	15 (39%)	
<i>Histology</i>			
ductal	78	36 (46%)	
lobular	26	13 (50%)	
others	16	9 (69%)	0.258
<i>Surgery</i>			
BCT	64	33 (52%)	0.714
Mastectomy	56	27 (48%)	
<i>Primary systemic therapy</i>			
Endocrine therapy	16	7 (44%)	0.553
Chemotherapy	104	53 (51%)	

Table 2 Correlation between bone marrow status and response to primary therapy

Therapy response	<i>N</i> = 120		BM positive <i>N</i> = 60
<i>Poor responder</i> ^b	36	30%	22 (61%)
Progressive disease ^a	3	3% (2,5)	2 (67%)
Stable disease ^a	33	28%	20 (61%)
<i>Responder</i> ^b	84	70%	38 (45%)
Partial remission ^{a,c}	62	52%	30 (48%)
Complete remission ^{a,c,d}	22	18%	8 (36%)

^a Comparison of positivity rates between SD, PR, PD und CR: *P* = 0.32

^b Comparison of positivity rates poor responder versus responder *P* = 0.11

^c Comparison of positivity rates between CR and poor responders *P* = 0.07

^d pathologic complete remission (pCR)

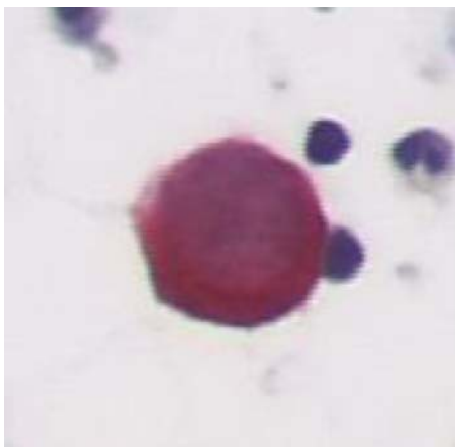
^e includes clinical complete remission with pathologic residual disease

remained unchanged and 3 (3%) patients showed progression during PST.

A trend towards a higher positivity rate in patients with a poor response to PST (progressive disease/stable disease) compared to those with partial and complete remission was seen but did not reach statistical significance (*P* = 0.11). The positivity rates in patients with NC or PD were 61% and 69%, respectively. In contrast, positive BM findings were seen in 48% and 36% of patients with PR and CR, respectively.

Discussion

PST is thought to become a more common form of treatment for primary breast cancer in the future. One key aspect of PST is the correlation between tumor response and prognosis [17, 18]. Patients who underwent PST and showed a complete pathologic remission generally have a better prognosis than those with incomplete or even no response [19]. Other prognostic factors for breast cancer patients who underwent PST are well known from the adjuvant setting and include grading, tumor size as well as nodal status before and after therapy. This study looks at another established prognostic factor in breast cancer: the presence of CK-positive cells thought to be disseminated tumor cells (DTC) in the BM of patients who underwent PST. Numerous studies have shown that patients with positive tumor cell detection in the BM have a worse prognosis when compared to patients with a negative aspirate. Numerous single center studies ranging from 350 to over 800 patients with a median follow-up of up to 12 years have shown the prognostic significance of

**Fig. 1** Cytokeratine-positive cell from bone marrow aspirate

DTCs [20–24]. A recent pooled analysis including more than 4,700 patients confirmed these findings [14]. Further studies have shown that while adjuvant systemic therapy reduces the number of CK-positive patients, neither adjuvant chemotherapy nor adjuvant endocrine therapy can completely eliminate the presence of CK positive cells from the BM [25–28]. In fact, patients with a persistence of CK positive cells after chemotherapy have a worse prognosis than those who were able to clear all CK-positive cells from their BM [29]. Because of these findings, identification and characterization of disseminated tumor cells have become important areas of research. Particularly characterization of disseminated cells after conventional adjuvant or primary systemic therapy could identify patients who are likely to benefit from novel therapeutic concepts such as specific antibody-based therapies against target cell-surface antigens. Examples for such treatment-modalities in different stages of development or clinical application are antibody-therapies against HER2, Ep-CAM (17-1A) and uPA-R surface antigens [30].

Our study shows that the rate of CK positive cells in patients after similar chemotherapy but without prior removal of the tumor is surprisingly high. In fact, patients included in our study showed a slightly higher rate of disseminated cells in the BM as would be expected in similar patients before initiation of either surgical and/or systemic treatment.

Different explanations are possible: First, contrary to the adjuvant situation it is not clear if disseminated tumor cells detected after treatment survived chemotherapy or if they are new cells. During PST, tumor cell shedding could conceivably continue until the final removal of the tumor, creating a constant source of passive shedding or active dissemination. It has been technically difficult to routinely describe the exact nature of individual cytokeratin positive cells in the BM of breast cancer patients. While some of them appear to be viable disseminated tumor cells, others could be necrotic or apoptotic cells. These could be the result of passive cell shedding by the primary tumor, rather than the result of an active pre-metastatic process. As such, we would indeed expect a higher rate of “shedded”, but clinically insignificant cells in the bone marrow of patients who underwent systemic chemotherapy prior to tumor removal. Another explanation is the concept of tumor cell dormancy. The success of chemotherapy in both primary or adjuvant settings aimed at proliferating cell populations, may be limited by the fact that many of the residual tumor cells present in bone marrow could be nonproliferative or dormant [31]. Finally, DTCs might differ from the local tumor population more than previously thought. Klein

et al. found that the genomic characteristics of disseminated tumor cells differ widely from primary tumor cells of the same patient. In fact, disseminated single tumor cells in bone marrow may evolve independently from the primary tumor altogether [32] and as such could respond differently to chemotherapy.

We find an interesting though not statistically significant correlation between tumor response and presence of DTC's. Such a correlation, if confirmed in a larger patient population, would point towards a negative prognostic impact of persistent DTC's after chemotherapy as already confirmed in the adjuvant setting and would further strengthen the concept of DTC-monitoring as a marker for systemic residual disease.

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