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Original Article

ASSESSMENT OF THE EFFECT OF TREATMENT WITH NEOADJUVANT CHEMOTHERAPY ON BREAST CANCER AXILLARY LYMPH NODE

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Summary

Neoadjuvant chemotherapy is the standard of care for patients with locally advanced breast cancer (LABS). The aim of this study was to determine the effects of neoadjuvant chemotherapy on metastatic and nonmetastatic breast cancer axillary lymph nodes (ALNs). Seventy-seven patients with LABS and cytologically documented ALN metastases were treated in two prospective trials. Patients had breast surgery with level I and level II axillary dissection followed by additional chemotherapy or radiation treatment. Clinical nodal status was determined by physical examination and compared with histological and immunohistochemical findings. The regressive changes in primary tumor and ALNs were achieved using scoring systems. Objective clinical response in primary tumor was seen in 48.05% of patients. Histologic evidence of regression was noted in 46 cases (59.75%), with complete histologic regression achieved in 5 cases (6.49%). The number of cases according to the ALNs regression scoring system was as follows: 0 grade – viable metastases without effect – 21 patients (30%); 1 grade – cytotoxic effect in metastases – 20 patients (28.58%), 2 grade – micrometastasis – only3 patients (4.28%); 3 grade – no residual metastatic disease – 1 patient (1.43%); 4 grade – negative ALNs before and after treatment – 25 patients (35.71%). Nodular fibrotic area and iron-loaded macrophages suggested previous presence of tumor metastasis. There was significant relationship between histologic regression in the primary tumor and the presence of effect of cytotoxic agents on ALNs metastases ($r=0.9123$; $p<0.00001$). As chemotherapy is widely used in the treatment of breast carcinoma, pathologists should be aware of chemotherapy-induced changes in metastatic and in non-metastatic ALNs.

Key words: neoadjuvant chemotherapy, axillary lymph node, breast cancer

Introduction

Neoadjuvant or primary chemotherapy has assumed an increasing role in the management of patients with locally advanced breast cancer and is being prospectively evaluated in patients with earlier-stage disease [1]. Tumor down staging can allow breast-conserving surgery in patients for whom mastectomy is initially the only possibility for treatment.

Neoadjuvant chemotherapy allows for *in vivo* assessment of primary tumor response. On the other hand, the axillary nodal basin is the most common site of lymphatic metastases in breast cancer patients and suitable model for studying the effect of systemic cytotoxic therapy on lymphatic metastases and nonmetastatic ALNs.

The number of residual metastatic ALNs after primary chemotherapy has been established as an important prognostic factor for disease free survival [2-5].

Chemotherapy-induced changes in breast carcinoma have been evaluated in some reports.

Histomorphological changes in lymph nodes have not been evaluated in these studies [6-12].

In our study, morphological changes induced by chemotherapeutic agents in metastatic and nonmetastatic lymph nodes were evaluated.

Material and Methods

Between 1996 and 1999, seventy-seven consecutive patients with LABC were registered in two prospective trials of neoadjuvant chemotherapy utilizing 5-fluorouracil, epirubicin and cyclophosphamide (FEC) and cyclophosphamide, metotrexate and 5-fluorouracil (CMF) at the Oncology Center – Higher Medical Institute, Pleven, Bulgaria.

LABC was defined as histologically or cytologically documented stage IIA (T_2>4 cm), IIB, IIIA and IIIB breast cancer using the 1993 American Joint Committee on Cancer clasiffication system. Patients with primary inflammatory carcinomas were excluded.

The diagnosis was established by fine-needle aspiration, core-needle or incisional biopsy of the primary tumor. The node status of each patient was assessed using fine-needle aspiration biopsy.

A staging workup was performed at presentation and following three cycles of chemotherapy and including a complete history and physical examination, bilateral mammography, complete blood count, chest radiograph, ultrasonography of the abdomen, bone scan.

Each patient was entered prospectively into the protocol database and followed longitudinally.

The clinical response was assessed according to standard UICC criteria [13], and toxicity - according to WHO grading [14].

Morphologic evaluation

The pathologic response of primary tumor to neoadjuvant chemotherapy was classified using a semisquantative scoring system from 0 to 4: 0 – no effect; 1 – resorption and tumor sclerosis; 2 – minimal residual tumor only (<5 mm); 3 – residual in situ tumor only; 4 – no evidence of residual tumor [15].

The effect of treatment on ALNs was also graded from 0 to 4: 0 – viable metastases without therapeutic effect; 1 – evidence of metastases, but cytotoxic effect in tumor cells present, 2 – evidence of therapeutic effect (e.g., areas of necrosis, nodular fibrosis), but nests of tumor cells still present; 3 – evidence of therapeutic effect, no residual metastatic disease; 4 – non-metastatic lymph nodes before and after treatment.

At 3 months, following clinical and investigative reassessment, the patients treated by neoadjuvant chemotherapy received appropriate surgery and/or radiotherapy depending on the post-treatment clinical TNM staging (ypTNM).

Complete medical records of all patients were available for review at the time of this analysis.

Fine-needle aspiration specimens were examined with hematoxylin-eosin stains. Biopsy and mastectomy specimens were processed in a standardized manner, and hematoxylin and eosin-stained slides were prepared from formalin-fixed, paraffin embedded tissue samples.

Representative H&E slides of all pre- and post-treatment breast tissue pairs were available to be compared. The control group included 70 stage II-III breast cancer women who had been surgically treated with ALNs dissection alone. The latter group served as controls for histological observation on the post-treatment changes in ALNs only.

Immunoperoxidase staining was performed on paraffin-embedded section of selected axillary lymph nodes by use of streptavidin horseradish peroxidase method using primary monoclonal anti-cytokeratin cocktail (AE_1/AE_3 BioGenex), primary staining by aminoethyl carbazole, and counterstaining by hematoxylin. An additional adjacent serial on each block was stained with hematoxylin and eosin.

Data were analyzed using Statistica software (EPIINFO 6.0). The statistical significance level (P) was taken as a measure of the strength of evidence against the null hypothesis, and the level of $p \leq 0.05$ was considered statistically significant.

Results

Most of the patients had some clinical and pathological response of primary tumor to neoadjuvant chemotherapy. Objective clinical response was seen in 37 of 77 patients (48.05%) undergoing neoadjuvant chemotherapy, with complete clinical regression in 4 (4.19 %).

Histologic evidence of regression was noted in 46 cases (59.75%) of 77, with complete histologic regression achieved in 5 cases (6.49%).

Clinical response correlated only poorly with histologic evidence of regression (χ^2=0.1684; p=0.98).

Seventy patients underwent modified radical mastectomy with ALN dissection after primary chemotherapy. The median number of lymph nodes removed and sectioned was 17 (range 8 to 31). The response in the metastatic and non-metastatic ALNs is demonstrated in Figure 1.

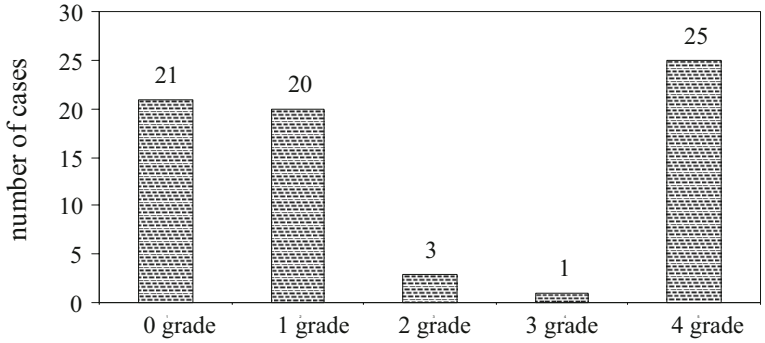

Figure1. Response of breast cancer ALNs to neoadjuvant chemotherapy

Only 1.43 percent of patients (n=1) had complete axillary conversion from cytologically documented axillary metastases to histologically negative ALNs status (3-rd grade) at lymph node dissection after chemotherapy treatment. These ALNs were serially sectioned and evaluated very cautiously on hematoxylin and eosin sections and by anticytokeratin staning. The primary tumor of this case was assessed as residual minimal ductal invasive cancer ($ypT_{1a}N_0$). In the site of the primary tumor a characteristic pattern of relatively cellular fibrous tissue with reactive lymphocytic infiltration, iron-loaded macrophages and scattered foci of tumor cells in between were observed.

In three cases (4.28%) 2-nd grade of histologic regression in ALNs was found. The characteristic pattern of changes in lymph nodes with metastases consisted of nodular hyaline fibrotic areas, and when present, scattered foci of tumor cells in between. Occasionally iron-loaded macrophages were found (Figure 2. A, B).

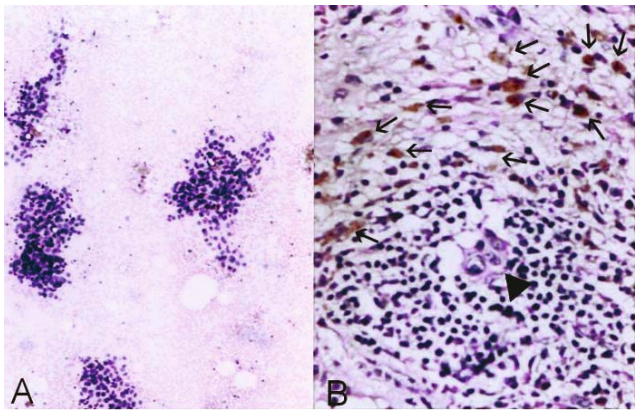

Figure 2. A – Fine needle aspiration-cytologic pattern of invasive carcinoma in ALN. H&E stain, 100x.
B – Histological slide after treatment – areas of fibrosis, iron-laden macrophages (arrows), nest of residual tumor cells in ALN (arrowhead) H&E stain, 400x

Immuhistochemical staining for cytokeratin in these cases confirmed the presence of tumor cells in association to nodal scarring.

In twenty cases (28.58%) therapeutic effect of cytostatic agents was established, but metastases were present (1-st regressive grade).

Cytologic changes in residual metastatic tumor cells were prominent and consisted of enlarged bizarre cells with both nuclear and cytoplasmic changes.

Nuclear changes consisted of enlargement of nucleolar surface area, marked polymorphism, a dust like chromatin pattern or hyperchromic nuclei, prominent nucleoli.

Cytoplasmic changes consisted of variable increased amounts of cytoplasm with bubbly vacuolization. In two cases prominent cytoplasmic vacuolization in metastatic tumor cells similar to these in primary tumor was found (Figure 3. A, B).

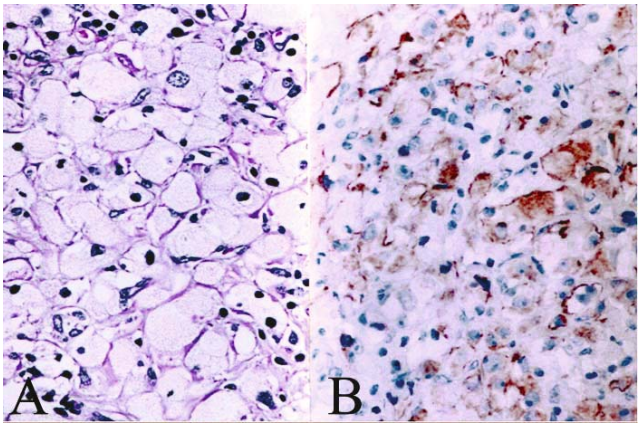

Figure 3. A – Prominent cytoplasmic vacuolization in metastatic ALN after treatment, nuclear pleomorphism H&E stain, 400x; **B** – Expression of epithelial markers by the tumor cells with prominent vacuolization Cytokeratin AE1/AE3 stain, 400x

These drug-induced changes in metastatic lymph nodes are similar to the changes observed in primary tumor. This type of changes was not observed in the control group ($p<0.001$) (Figure 4. A, B). There was a significant relationship between histologic regression in the primary tumor and the presence of effect of chemotherapeutic agents on lymph node metastases ($r=0.9123$; $p<0.00001$).

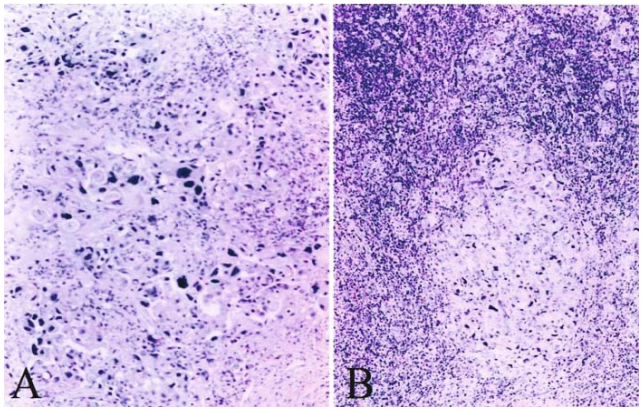

Figure 4. A – Morphologic changes-increased nuclear atypia in primary tumor H&E stain, 200x; B –Morphologic changes-increased nuclear atypia in metastatic ALN after treatment H&E stain,100x

Extended area of necrosis and cholesterol cleft deposits in primary tumor and in lymph node metastasis in two cases were found (Figure 5). In nineteen cases, focal fibrosis of the lymph nodes was also present that was similar to the desmoplasia observed in regressed primary tumor.

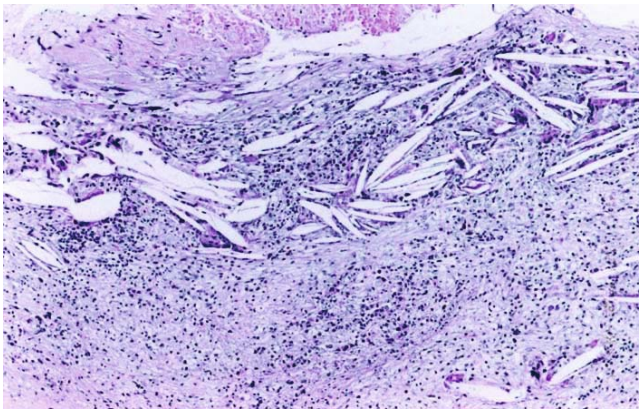

Figure 5. Extended area of necrosis and cholesterol crystals deposition in metastatic lymph node after chemotherapy, H&E stain, 100x

In 21 patients (30%) viable metastases without therapeutic effect were found.

In the node negative patients treated with cytotoxic therapy, the lymph nodes usually displayed considerable lymphoid depletion and relative expansion of intermediate sinusoids (Figure 6). In the twelve cases, focal fibrosis in otherwise completely negative lymph nodes was also present. Diffuse fibrosis was found in nine nodes negative before and after treatment cases. In the control group only six lymph nodes were hyalinized and fibrotic out of 320 metastatic lymph nodes ($p<0.001$).

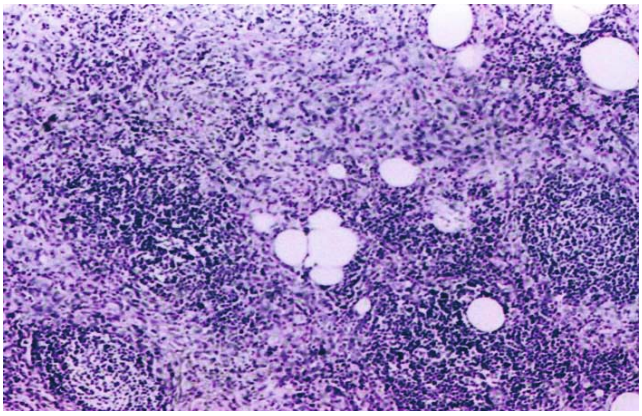

Figure 6. Non-metastatic lymph node after chemotherapy treatment showing lymphoid depletion and fibrosis H&E stain, 100x

Nodal sections from 20 patients who were originally identified as having negative ALNs at surgery (No) were reevaluated and histologically confirmed to be without metastases.

In 52.17% of ALNs negative control cases reactive hyperplastic changes were found. Breast cancer ALNs, treated with cytotoxic agents, lacked the characteristic reactive changes.

Discussion

Response to treatment is one of the most important indicators of therapeutic efficacy. Thus, the possibility of assessing this endpoint on a previously undisturbed primary tumor and ALNs is one of the main advantages of neoadjuvant chemotherapy.

A potential benefit of this assessment would be better monitoring of the response to the treatment and a more accurate definition of the patient population, which is likely to benefit from the treatment. Neoadjuvant chemotherapy is as effective as adjuvant chemotherapy. Additionally, the rate of breast conservation in operable disease can be increased [16].

There is a scarcity of literature describing the

histological changes following chemotherapy in breast cancer ALNs.

Most studies focus on the morphological effect on the primary tumor but the changes that may occur in the regional lymph nodes following therapy have not been described in detail. Our study identified several pathological findings in metastatic and in non-metastatic lymph nodes that are in agreement with previously described changes [17, 18].

We studied these changes in a group of seventy-seven patients who underwent incisional core- needle or fine needle biopsies of primary tumor and fine-needle aspiration of clinically positive ALNs for diagnosis and were then treated with three cycles of chemotherapy. We identified a subgroup of patients with apparently chemosensitive disease. The ratio of these patients with no metastatic disease or only micrometastasis ($\leq$ 2mm) left after chemotherapy in ALNs is very low, as compared to other authors [19, 20].

In conclusion, in these patients the typical morphological pattern consisted of nodular hyaline fibrotic area, iron-loaded macrophages, with an area of necrosis and tumor cells, when present, scattered in between. In these cells, cytoplasmic prominence and vacuolization was seen. As metastatic deposit might be found in hyalinized and fibrotic lymph nodes, post-chemotherapy ALNs dissection of breast carcinoma patients should be evaluated very cautiously. In many cases step sections and application of a sophisticated method as an immunohistochemical technique are required not to miss metastasis.

The main difficulty in assessing response to treatment is related to the fact that drug-induced morphological alterations were identified.

It is know that in lymphoid malignancies hyalinization and debris replace the neoplastic cells after chemotherapy [21-23]. Stromal fibrosis in the metastasis lymph nodes of breast, gastrointestinal tract and pancreatic carcinoma had been described. This may be the result of new collagen formation [24].

Necrosis in primary and in metastatic lymph nodes is accepted as the sign of tumor destruction. Some reports noted the importance of tumor necrosis in determining the effects of chemotherapy [25, 26]. Expanded areas of necrosis were found in two cases, similar to coagulative necrosis in primary tumor.

However, necrosis and fibrosis should be considered when evaluating the effects of chemotherapy in breast tissue and ALNs. Fibrosis and hyalinization were more prominent in the lymph nodes of the chemotherapy group than in the control group. These observations suggest that those types of changes are chemotherapy-induced.

Atrophic changes related to chemotherapy were recognizable in the non-metastatic lymph nodes. In addition, such cases usually displayed considerable lymphoid depletion and relative expansion of sinusoids.

Largely driven by the newer concept that breast cancer is a systemic disease from the onset, the management of invasive disease has undergone major changes.

Conclusion

Lately, a strong trend is seen towards neoadjuvant chemotherapy as the first line of systemic treatment in locally advanced breast cancer. Familiarity with chemotherapy-induced changes in the breast tissue and lymph nodes have considerable importance in the accurate interpretation of these specimens after treatment.

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