Evaluation and Reporting of Breast Cancer after Neoadjuvant Chemotherapy

Fang Fan*

Department of Pathology and Laboratory Medicine, University of Kansas School of Medicine, Kansas City, KS, USA

Abstract: Neoadjuvant chemotherapy (NAC) applied prior to surgical excision of the tumor has become a frequently used therapeutic approach in patients with operable and inoperable breast cancer. Such chemotherapy alters the morphology of breast cancers and normal breast tissue. Thus it is important for pathologists to become familiar with these changes and to know how to handle and assess breast specimens after neoadjuvant chemotherapy. Evaluation of the therapeutic response and measurement of residual disease is important in the pathologic assessment of the final surgical resection specimens because these data help in predicting patient's chances for cure and survival, and also provide guidance for further therapy. This review will discuss treatment effects and demonstrate how to evaluate, sample and measure residual breast cancer in excision specimens including axillary lymph nodes.

Keywords: Breast cancer, chemotherapy, histopathology.

INTRODUCTION

Neoadjuvant chemotherapy, implying that the patient will receive chemotherapy before the complete surgical removal of the carcinoma, has been of recent the standard of care for the treatment of locally advanced breast cancer [1]. One of the major benefits of neoadjuvant chemotherapy is that it can diminish large cancers enabling the surgeon to remove the residual tumor by a more limited operation, such as lumpectomy, instead of mastectomy. The response to neoadjuvant chemotherapy may provide some indication about the potential response of the tumor to further treatment and in general may be informative about the biology of the carcinoma under treatment. Since the recent data and a meta-analysis of the published results showed no difference between neoadjuvant therapy and adjuvant therapy in terms of survival and overall disease progression, neoadjuvant chemotherapy can be offered as a standard treatment or as an alternative to adjuvant treatment to all patients who are expected to receive chemotherapy for their cancers [2].

Assessment of the therapeutic response and measurement of residual disease in the breast and/or axillary lymph node is important because it may predict survival and provide guidelines for further therapy [3, 4]. Complete clinical regression does not imply complete pathologic response (cPR). Between 60-80% of patients considered to have a clinical complete response have residual tumor detected by pathologists in the surgical specimens. On the other hand, about 20% of patients with clinically suspected residual disease have complete pathologic response after microscopic examination [5]. Therefore, pathologic assessment of the final surgical resection specimen is still the gold standard for determining a complete response.

*Address correspondence to this author at the Department of Pathology, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66160, USA; Tel: 913 588 1184; Fax: 913 588-8780;
E-mail: FFan@kumc.edu

HANDLING OF SURGICAL RESECTION SPECIMENS AFTER NEOADJUVANT CHEMOTHERAPY

Before the examination and sampling of the surgical resection specimen it is absolutely essential to obtain as many clinical data as possible, including the radiologic report. Under ideal conditions mammography X-Rays should be sent to the pathologist together with the surgical specimen, or the pathologist should have access to the mammograms through the hospital interdepartmental information technology system. The essential data include:

- The histologic diagnosis on the pre-treatment core biopsy.
- Axillary lymph node status.
- The length of chemotherapy and the drugs that were used.
- The size and location of the tumor prior and after chemotherapy.
- Clinical and radiologic impression of the treatment response.

Mastectomy Specimen

The mastectomy specimen should be received fresh with a mark indicating the axillary tail. Detailed clinical information including the pretreatment tumor size and location and post-treatment radiologic imaging finding are absolutely essential before examining the specimen. If lesions are thought to be multiple it is imperative to have the mammogram or at least a detailed radiology report describing the mammography data.

The posterior surface (deep margin) of the mastectomy specimen is inked. The specimen is serially sectioned at a 5 mm interval from the posterior surface leaving the skin intact. The cut surface is examined for evidence of tumor (tumor bed), residual tumor and previous biopsy site, especially at the locations corresponding to the radiology report. Grossly, the tumor bed appears as a poorly defined

1874-3757/09

fibrotic area or simply fibrotic streaks; the residual tumor appears as fleshy nodules or areas. The tumor bed size and distance to margins should be measured. If a patient has had an excellent response to neoadjuvant chemotherapy, a gross lesion may not be detected, and the specimen may be sent to radiology for X-Ray to identify the previous biopsy clip (if was placed previously).

The sampling method and number of blocks taken vary among institutions and are dependent on the size of the specimen and the size of the lesion. In general, the previous tumor bed should be sampled extensively; additional or entire sampling may be necessary if the initial sections don't show microscopic residual tumor.

Lumpectomy Specimen (Wide Local Excision)

The lumpectomy specimen should be received fresh and oriented. The specimen should be measured in three dimensions and inked in six colors marking superior/inferior, medial/lateral and anterior/posterior margins. Then the specimen is serially sectioned perpendicular to the long axis of the specimen at a 3 mm interval. The slices are laid out and carefully examined. If a gross lesion or tumor bed is seen, the lesion size and distance to all six margins are recorded. Any close margins (less than 2 mm) should be reported to the surgeon right away for possible immediate re-excisions. The sampling method and the number of blocks taken vary among institutions and are dependent on the size of the specimen and the size of the lesion. In general (at our institution), if no gross lesion/tumor bed is seen, the entire specimen is submitted to look for microscopic residual tumor. If there is a gross lesion/tumor bed, then the entire lesion is submitted and its distance to margins documented. Only representative sections of the remaining grossly unremarkable specimen are submitted.

Axillary Lymph Nodes

The axillary lymph node sample may include sentinel lymph node or axillary lymph node dissection after neoadjuvant chemotherapy. The specimen is handled the same way as for lymph node in the non-neoadjuvant setting. Axillary lymph nodes are usually smaller and atrophic therefore more difficult to identify after neoadjuvant chemotherapy.

CHEMOTHERAPY-INDUCED MORPHOLOGIC CHANGES

Changes in Benign Breast Tissue

Benign breast tissue show significant atrophy of the terminal ductal lobular units (TDLU) after neoadjuvant chemotherapy [6]. This includes reduction of the lobular acini (Fig. **1**), lobular sclerosis and the attenuation of the lobular/ductal epithelium (Fig. **2**). The attenuation of the epithelial lining may make the myoepithelial cells appear prominent (Fig. **3**). Sometimes, there are scattered atypical epithelial cells in the TDLU containing variably enlarged hyperchromatic nuclei and vacuolated cytoplasm (Fig. **4**). Signs of cell proliferation are absent and there is no mitotic activity.

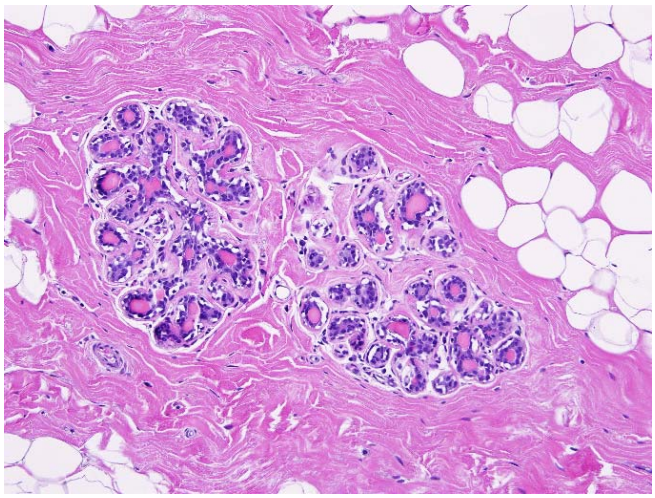


Fig. (1). The normal breast terminal ductal lobular unit (TDLU) shows atrophy and diminished acini unit after neoadjuvant chemotherapy (H&E, x100).

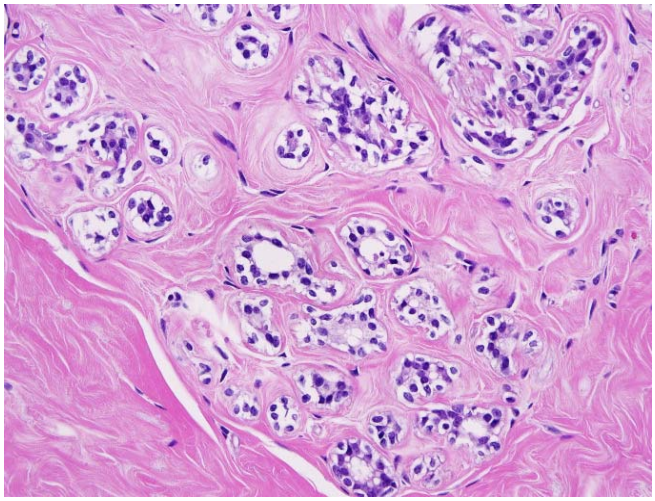


Fig. (2). There is intralobular sclerosis and attenuation of the ductal/lobular epithelium (H&E, x400).

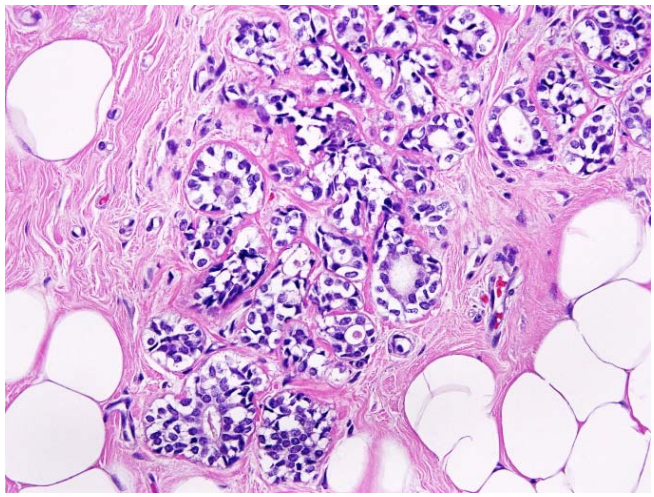


Fig. (3). The attenuation of the epithelium makes the myoepithelial cells appear prominent; not to be mistaken for lobular neoplasia. (H&E, x400).

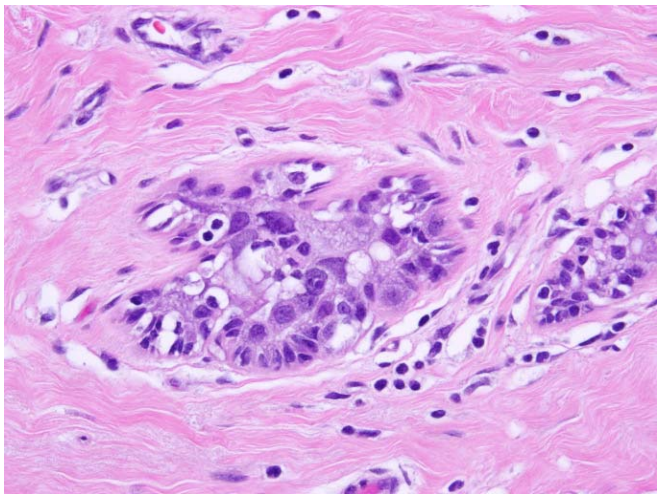


Fig. (4). Neoadjuvant chemotherapy may make the epithelial cells of normal duct appear atypical containing enlarged nuclei, prominent nucleoli and vacuolated cytoplasm (H&E, x600). However, cell proliferation and mitotic activity are absent.

Changes in Breast Cancer

The neoadjuvant chemotherapy effect is recognized as a fibrous or fibromyxoid area containing patchy lymphocytes, histiocytes, and absence of normal breast ducts and TDLUs (Fig. **5**). Hemosiderin laden macrophages and foreign body giant cells are also present in the tumor bed representing previous biopsy site. When the tumor bed is extensively sampled and no tumor cells are identified, this is termed complete pathologic response.

If residual cancer cells are present, they may be seen as infiltrating cords and nests (Fig. **6**), or sparse and singly dispersed cells mimicking histiocytes. On the other hand, collections of histiocytes may resemble residual tumor cells. Immunohistochemical stains with cytokeratin and CD68 will help in differentiating tumor cells from histiocytes (Fig. **7**). Residual tumor nests may show marked retraction artifact in the fibrous stroma mimicking lymphovascular invasion; immunohistochemical stain for lymphatic channel marker D2-40 may be useful to distinguish tissue retraction from lymphatic invasion (Fig. **8**). Specimens containing residual tumor cells are labeled as showing signs of a *partial pathologic response.*

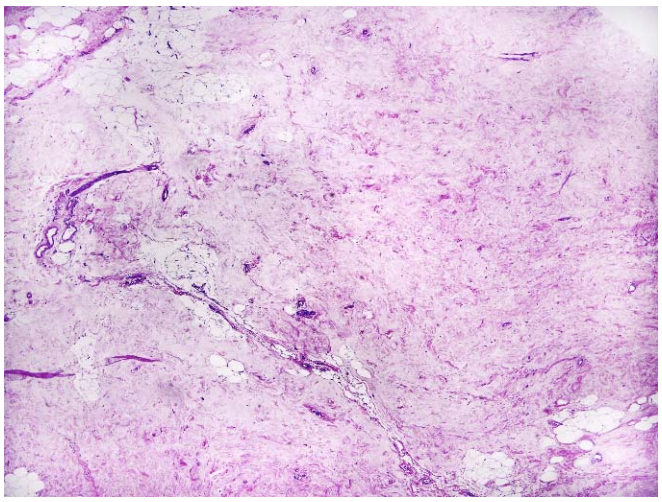
Residual cancer cells surviving chemotherapy may show a spectrum of changes, which are evident in the invasive as well as the in-situ component of the tumor. Most common chemotherapy effects include nuclear hyperchromasia, nuclear pleomorphism and cytoplasmic changes such as hypereosinophilic cytoplasm and vacuolization (Fig. **9**).

The size or extent of the residual breast cancer is measured as the largest contiguous focus of residual carcinoma or the number of tumor foci encompassing the area of tumor bed. The margins of residual tumor (DCIS and invasive carcinoma) should be evaluated and distance reported.

Changes in Lymph Nodes

Axillary lymph nodes may become small and atrophic after neoadjuvant chemotherapy. Microscopically, lymph

(**A**)



(**B**)

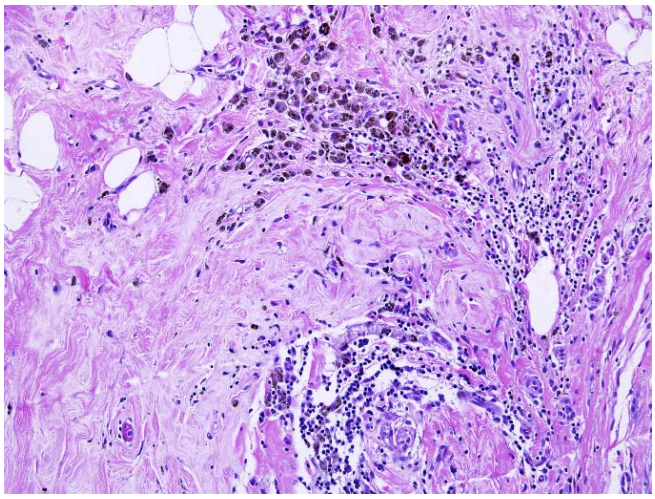


Fig. (5). (**A**) The neoadjuvant chemotherapy effect is characterized by a fibromyxoid area devoid of normal breast ducts and terminal ductal lobular unit (H&E, x200). (**B**) Patchy lymphocytes and hemosiderin-laden macrophages are often seen in the tumor bed (H&E, x400).

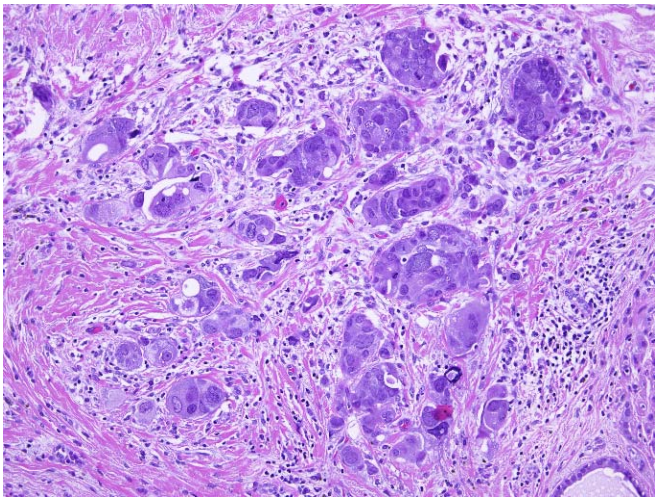
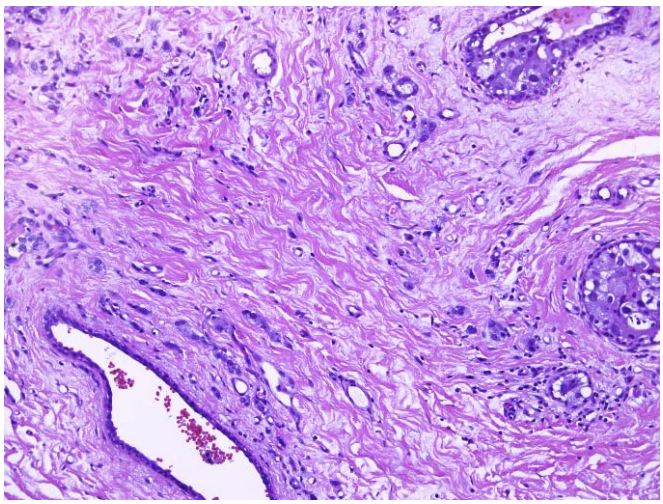


Fig. (6). Residual invasive ductal carcinoma is present, associated with a background treatment effects (patchy lymphocytes and fibrosis) (H&E, X200).

(**A**)



(**B**)

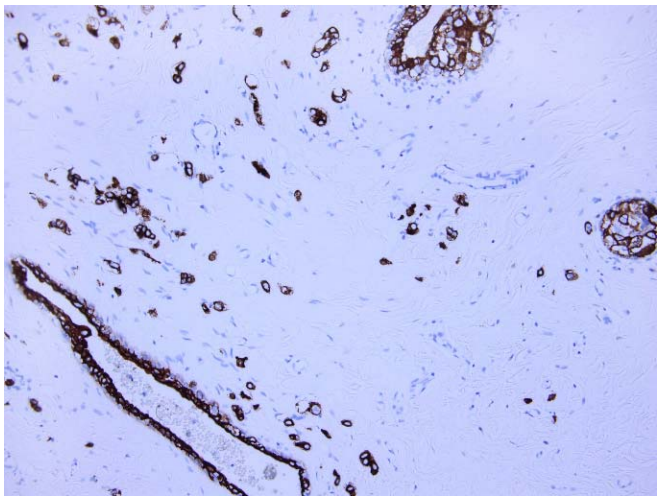


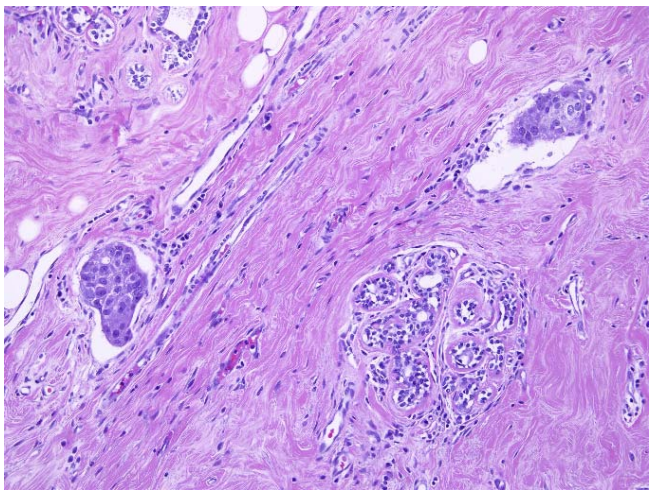
Fig. (7). (**A**) Residual tumor cells are sparse and singly dispersed mimicking histiocytes (H&E, X400). (**B**) The diagnosis is confirmed by positive immunohistochemical stain for Pan-cytokeratin (Immunohistochemical stain, X400).

nodes may show depletion of lymphocytes, fibrosis and collections of histiocytes (Figs. **10**, **11**). The latter two features are indications of prior metastases that have responded completely to chemotherapy. Efforts should be made to identify residual tumor cells in these lymph nodes and report the presence or absence of treatment effects (Fig. **12**).

PATHOLOGY REPORT

The pathology report should be as detailed as possible. Many institutions are using a synoptic reporting format recommended by the Association of Directors of Anatomic and Surgical Pathology (ADASP) [7]. The American College of Surgeons- Commission on Cancer (ACS-CoC) requires that the pathology reports of institutions approved by them include scientifically validated data presented in a checklists, which have been approved and distributed by the College of American Pathologist on their website. Dynamic templates of such checklists have been developed to reduce reporting errors, improve work efficiency and increase compliance [8].

(**A**)



(**B**)

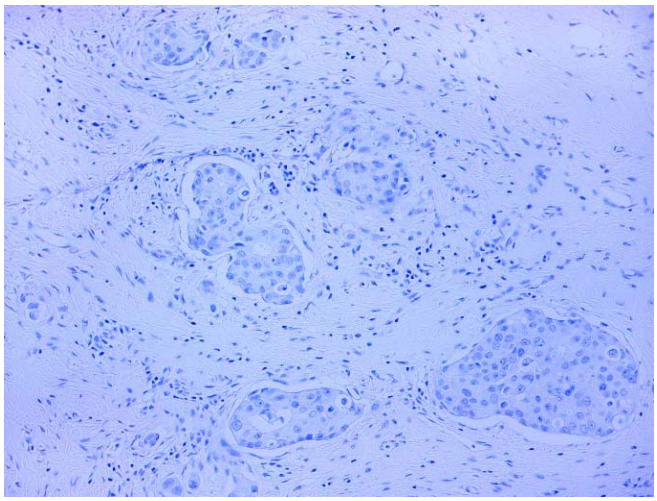


Fig. (8). (**A**) Residual invasive carcinoma may have marked retraction artifact in the fibrous stroma concerning for lymphatic channel invasion (H&E, X 200). (**B**) Immunohistochemical stain for D2-40 is useful in the interpretation (Immunohistochemical stain, X200).

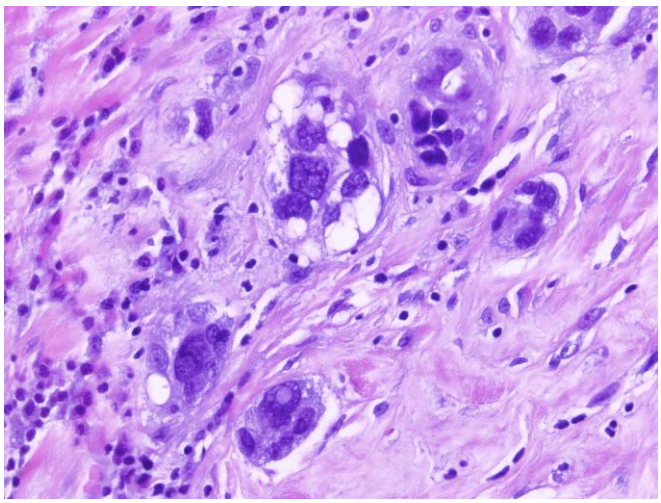


Fig. (9). Residual tumor cells may show treatment effects including hyperchromatic and pleomorphic nuclei and vacuolated nuclei and cytoplasm (H&E, X400).

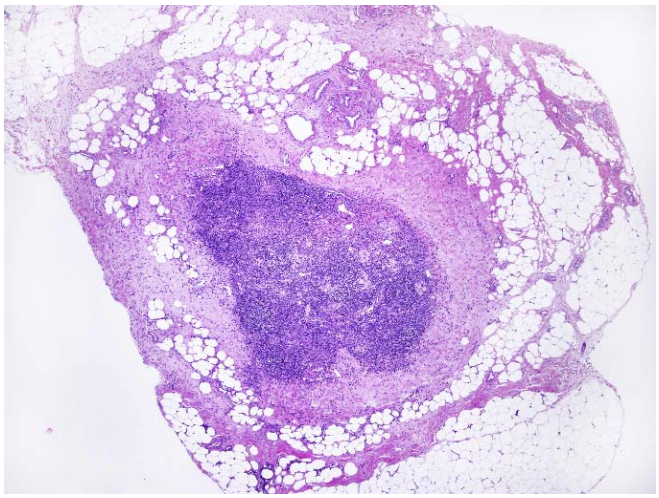
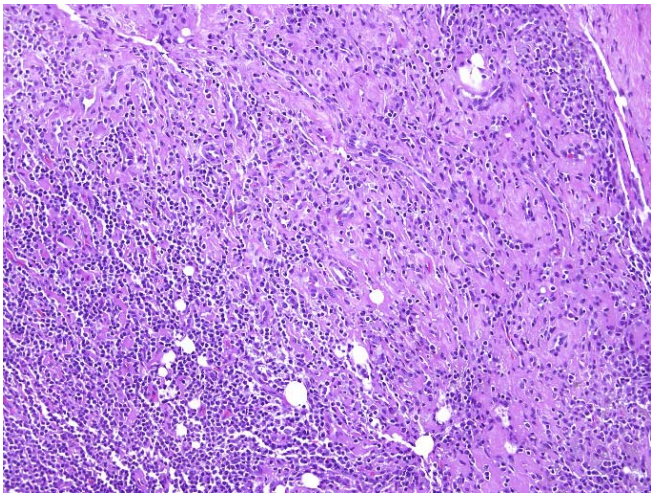


Fig. (10). After neoadjuvant chemotherapy, a benign lymph node becomes atrophic showing depletion of lymphocytes and fibrosis (H&E, X 200).

(**A**)



(**B**)

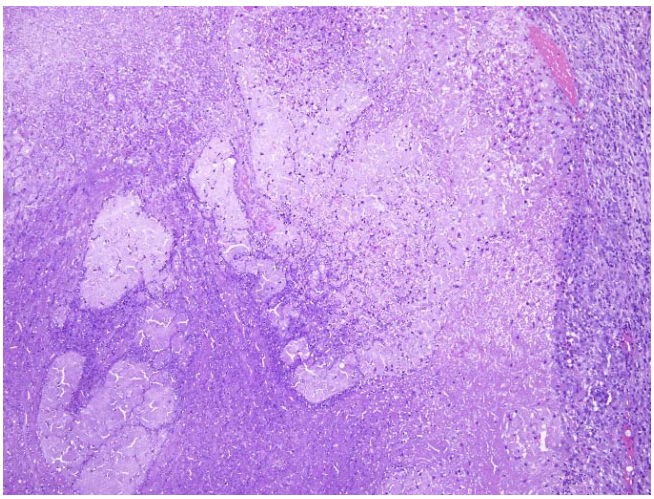
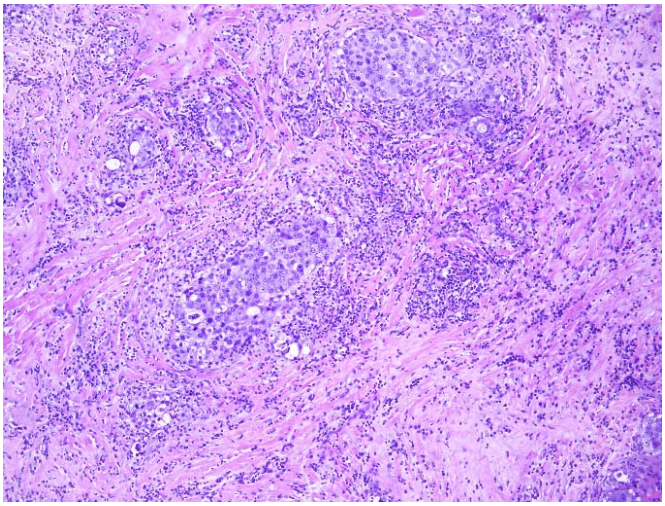


Fig. (11). (**A**) The presence of fibrosis and collections of histiocytes in the lymph node are features indicating of prior metastases (H&E, x400). (**B**) Sometimes, the lymph node shows extensive necrosis with no viable residual tumor cells identified (H&E, X 400).

(**A**)



(**B**)

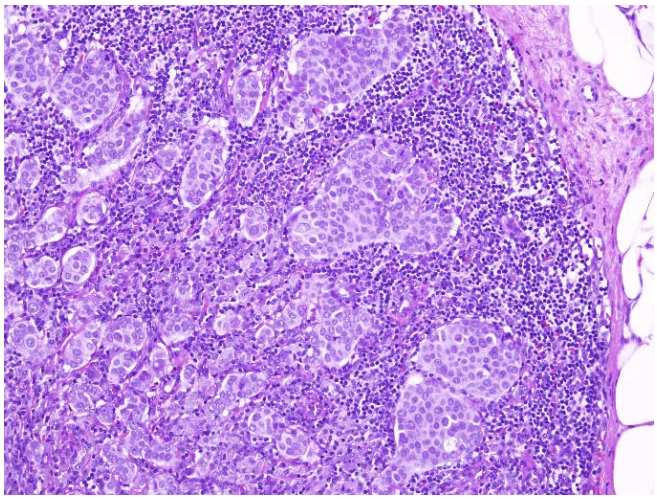


Fig. (12). (**A**) Residual tumor nests are identified in the lymph node associated with background treatment effects (H&E, x 600). It should be reported as such in the pathology report. (**B**) Lymph node metastasis showing no treatment effect (H&E, X 200).

Grading of Pathologic Response

The degree of neoadjuvant chemotherapy effect should be assessed and reported because it has significant prognostic value. Several systems have been proposed to record pathologic response of breast cancer to treatment. We will briefly mention two systems here: the Miller-Payne grading system [9] and the one proposed by Pinder *et al*. [10].

The Miller-Payne system for classification of neoadjuvant chemotherapy treated breast cancers is as following:

Grade 1: No change or some alteration to individual malignant cells, but no reduction in overall cellularity

Grade 2: A minor loss of tumor cells, but overall cellularity still high; up to 30% loss

Grade 3: Between an estimated 30% and 90% reduction in tumor cells

Grade 4: A marked disappearance of tumor cells such that only small clusters of widely dispersed individual cells remain; more than 90% loss of tumor cells

Grade 5: No malignant cells identifiable in sections from the site of the tumor; only vascular fibroelastotic stroma remains often containing macrophages; however, DCIS may be present

The Miller-Payne [9] system does not include the assessment of axillary lymph nodes. In the system proposed by Pinder *et al*. [10], they recommend recording the pathologic response as following:

1. Complete pathologic response, either no residual carcinoma or no residual invasive tumor but DCIS present.
2. Partial response to therapy, either (i) minimal residual disease/near total effect (e.g. < 10% of tumor remaining) or (ii) evidence of response to therapy but with 10-50% of tumor remaining or (iii) > 50% of tumor cellularity remains evident, when compared with the previous core biopsy sample, although some features of response to therapy present.
3. No evidence of response to therapy.

They also recommend a similar reporting system in post-neoadjuvant lymph node samples:

1. No evidence of metastatic disease and no evidence of changes in the lymph nodes.
2. Metastatic tumor not detected but evidence of response/down-staging, e.g. fibrosis.
3. Metastatic disease present but also evidence of response, such as nodal fibrosis.
4. Metastatic disease present with no evidence of response to therapy.

It should be mentioned that all these systems need further approval for validity and reproducibility; they rely on extensive sampling of the tumor bed. Each institution should adopt a system to report the degree of treatment response of breast cancer to neoadjuvant chemotherapy in the pathology report.

Other Pathological Parameters

Other pathologic parameters that should be included in a pathology report after breast cancer neoadjuvant chemotherapy are the same as that for pretreatment breast cancer and include tumor type, size, grade, margins, and biomarkers (ER/PR/Her-2).

Staging

The current recommendation of the American Joint Committee on Cancer (AJCC) for staging of breast cancers is based on pretreatment examination and radiologic findings, and the postoperative pathologic assessment of the resected tumor and regional lymph nodes. Proposals have been made to modify this approach and use other systems for staging of tumors in patients who have received neoadjuvant chemotherapy [11]. However, as of today there is no consensus for a need to change the existing staging system or to develop a new staging system for tumors treated by neoadjuvant chemotherapy.

REFERENCES

[1] Schwartz GF, Hortobagyi GN, Masood S, *et al*. Proceedings of the consensus conference on neoadjuvant chemotherapy in carcinoma of the breast, April 26-28, 2003, Philadelphia, PA. Hum Pathol 2004; 35: 781-4.

[2] Kaufmann M, von Minckwitz G, Bear HD, *et al*. Recommendations from an international expert panel on the use of neoadjuvant (primary) systemic treatment of operable breast cancer: new perspectives 2006. Ann Oncol 2007; 18: 1927-34.

[3] Symmans WF, Peintinger F, Hatzis C, *et al*. Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy. J Clin Oncol 2007; 25: 4414-22.

[4] Penault-Llorca F, Abrial C, Raoelfils I, *et al*. Comparison of the prognostic significance of Chevallier and Sataloff's pathologic classifications after neoadjuvant chemotherapy of operable breast cancer. Hum Pathol 2008; 39: 1221-28.

[5] Fisher ER, Wang J, Bryant J, *et al*. Pathobiology of preoperative chemotherapy: findings from the National Surgical Adjuvant Breast and Bowel (NSABP) protocol B-18. Cancer 2002; 95: 681-95.

[6] Schnitt SJ, Collins LC. Biopsy Interpretation of the Breast Treatment Effects. Philadelphia, PA: Lippincott Williams and Wilkins 2009; pp. 435-39.

[7] Association of Directors of Anatomic and Surgical Pathology (ADASP). Recommendations for the reporting of breast carcinoma. Am J Clin Pathol 1995; 104: 614-9.

[8] Qu Z, Ninan S, Almosa A, *et al*. Synoptic reporting in tumor pathology: Advantages of a web-based system. Am J Clin Pathol 2007; 127: 898-903.

[9] Ogston KN, Miller ID, Payne S, *et al*. A new histological grading system to assess response of breast cancers to primary chemotherapy: Prognostic significance and survival. Breast 2003; 12: 320-27.

[10] Pinder SE, Provenzano E, Earl H, Ellis IO. Laboratory handing and histology reporting of breast specimens from patients who have received neoadjuvant chemotherapy. Histopathology 2007; 50: 409-17.

[11] Jeruss JS, Mittendorf EA, Tucker SL, *et al*. Staging of breast cancer in the neoadjuvant setting. Cancer Res 2008; 68: 6477-81.

Received: May 15, 2009 Revised: June 15, 2009 Accepted: July 5, 2009