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# Clinico-pathologic predictors of patterns of residual disease following neoadjuvant chemotherapy for breast cancer

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## Abstract

Among breast cancer patients treated with neoadjuvant chemotherapy (NAC) who do not experience a pathologic complete response (pCR), the pattern of residual disease in the breast varies. Pre-treatment clinico-pathologic features that predict the pattern of residual tumor are not well established. To investigate this issue, we performed a detailed review of histologic sections of the post-treatment surgical specimens for 665 patients with stage I-III breast cancer treated with NAC followed by surgery from 2004 to 2014 and for whom slides of the post-NAC surgical specimen were available for review. This included 242 (36.4%) patients with hormone receptor (HR)+/HER2− cancers, 216 (32.5%) with HER2+ tumors, and 207 (31.1%) with triple negative breast cancer (TNBC). Slide review was blinded to pre-treatment clinico-pathologic features. pCR was achieved in 7.9%, 37.0%, and 37.7%, of HR+/HER2− cancers, HER2+ cancers, and TNBC respectively ($p < 0.001$). Among 389 patients with residual invasive cancer in whom the pattern of residual disease could be assessed, 287 (73.8%) had a scattered pattern and 102 (26.2%) had a circumscribed pattern. In both univariate and multivariate analyses, there was a significant association between tumor subtype and pattern of response. Among patients with HR+/HER2− tumors, 89.4% had a scattered pattern and only 10.6% had a circumscribed pattern. In contrast, among those with TNBC 52.8% had a circumscribed pattern and 47.2% had a scattered pattern ($p < 0.001$). In addition to subtype, histologic grade and tumor size at presentation were also significantly related to the pattern of residual disease in multivariate analysis, with lower grade and larger size each associated with a scattered response pattern ($p = 0.002$ and $p = 0.01$, respectively). A better understanding of the relationship between pre-treatment clinico-pathologic features of the tumor and pattern of residual disease may be of value for helping to guide post-chemotherapy surgical management.

In current clinical practice almost all patients with invasive breast cancer receive some form of systemic therapy (i.e., chemotherapy, HER2-targeted therapy, endocrine therapy), the details of which depend upon the tumor subtype [1]. While this is often given after surgical removal of the tumor (i.e., in the adjuvant setting), systemic therapy after a core needle biopsy diagnosis of carcinoma but prior to definitive surgery (i.e, in the neoadjuvant setting) is being used increasingly, particularly for patients with triple negative and HER2-positive breast cancers [2, 3].

A recent meta-analysis of 10 randomized clinical trials that included over 4700 patients showed no differences in distant recurrence or breast cancer mortality rates for patients treated with adjuvant versus neoadjuvant chemotherapy (NAC) [4]. Therefore, in clinical practice, the choice between adjuvant and neoadjuvant chemotherapy in any given patient is based on a variety of radiologic, clinical and pathologic factors. There are several benefits to the neoadjuvant approach [2, 3, 5–8]. First, NAC can result in reducing the local disease burden (downstaging) so that non-operable tumors become operable; patients initially requiring mastectomy can be treated with breast conserving surgery (BCS); and patients initially requiring an axillary

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dissection can be managed with sentinel lymph node biopsy. Second, NAC provides an opportunity for in vivo assessment of tumor response to treatment permitting tailoring of further systemic therapy based on response. Third, the extent of response to NAC is a prognostic factor for all breast cancer subtypes with the best outcomes seen among patients in whom a pathologic complete response (pCR) is achieved [8]. Finally, treatment response in clinical trials of NAC is used by the U.S. Food and Drug Administration as a criterion supporting approval of new drugs.

Among patients treated with NAC who do not achieve a pCR, an understanding of the pattern and distribution of residual disease in the breast is of importance since this may have an influence on the risk of both loco-regional and systemic recurrence. This information could, therefore, be of use in guiding further local and systemic therapy. In particular, knowledge of the patterns of residual disease may be of value in identifying which patients may be adequately managed by BCS with limited margin widths versus wider margins, and which patients would be best served by mastectomy after NAC.

In this study we evaluated the patterns of residual disease in the breast in 665 patients with breast cancer who underwent BCS or mastectomy following NAC and related these patterns to pre-treatment clinico-pathologic features. We also sought to determine if alterations in tumor cells and histologic features of the tumor bed after NAC varied with pre-treatment tumor subtype.

## Materials and methods

Patients with stage I-III breast cancer treated with NAC followed by BCS or mastectomy between 2004 and 2014 were retrospectively identified from institutional databases. Among 987 cases identified, histologic sections of the post-treatment surgical specimen were available for review for 665. Clinico-pathologic features abstracted from the databases included age, clinical tumor size and nodal status, clinical and/or radiographic multifocality or multicentricity at presentation, histologic type, histologic grade, estrogen receptor (ER), progesterone receptor (PR) and HER2 status of the tumor in the pre-treatment core needle biopsy, and type of NAC. ER/PR and HER2 assay results were scored and reported according to the relevant ASCO-CAP guidelines at the time of diagnosis. Of note, some of these patients were treated before publication of the initial ASCO-CAP ER/PR and/or HER2 guidelines [9, 10]. Nevertheless, during the time preceding publication of the ASCO-CAP ER/PR guideline [9] (i.e., prior to 2010), at our institution ER/PR results were categorized as positive when at least 1% of tumor cells showed nuclear staining for ER and PR (and further categorized as low positive when between 1% and 10% of cells showed nuclear staining). In addition, prior to publication of the initial HER2 testing guideline in 2007 [10], cases at our institution were considered HER2+ if they showed 3+ staining by immunohistochemistry (IHC) or 2+ staining by IHC and HER2 gene amplification by FISH.

Hematoxylin and eosin (H and E)-stained sections of the post-treatment surgical specimens were reviewed blinded to the pre-treatment clinical features and to the histologic features and receptor status of the tumor in the pre-treatment biopsy. Residual tumor in the post-treatment surgical specimen was categorized into one of four patterns based on review of all histologic sections, supplemented by the gross description in the pathology report and the results of imaging studies (Figs. 1 and 2). Pattern A was assigned to tumors in which there was a single, confined focus of residual invasive carcinoma present within the tumor bed, with little or no treatment-related fibrosis within the tumor nodule itself. The peripheral edges of the nodule could be smooth or irregular, but the nodule was discrete. Pattern B was characterized by residual tumor that was present in a confined, circumscribed area of the tumor bed, but in which the carcinoma was separated into smaller nests by varying amounts of treatment-related fibrosis. The presence of sections with only treatment-related fibrosis in between sections containing residual tumor precluded assignment to either of these two patterns. In pattern C, there were two or more distinct clusters of residual carcinoma present in one or more sections. Cases with tumor present on more than one section had to be separated by at least one intervening slide of only treatment-related fibrosis to be categorized as pattern C. Tumors assigned to pattern D were characterized by tumor cells singly and in small nests haphazardly distributed across a broad area of tumor-related fibrosis in multiple slides. For the purposes of analysis, patterns A and B were considered "circumscribed" and patterns C and D were considered "scattered". Cases that showed no or minimal response to therapy and cases with inadequate gross descriptions were not assigned a category and were excluded from analysis.

The histologic features evaluated for the residual invasive carcinoma in the surgical specimens included histologic type, histologic grade, lymphovascular invasion, retraction artifact around tumor cell nests, stromal tumor infiltrating lymphocytes (TILs) assessed using the published guidelines of the TILs Working Group [11, 12], and treatment effects on tumor cells. Treatment effects included moderate/marked nuclear atypia, cytoplasmic eosinophilia, cytoplasmic vacuolization/foaminess, and a histiocyte-like appearance. Residual in situ carcinoma was assessed for nuclear grade, architectural patterns, comedo necrosis, and treatment effects on tumor cells as for invasive tumor cells. In addition, luminal obliteration by fibrosis, prominent

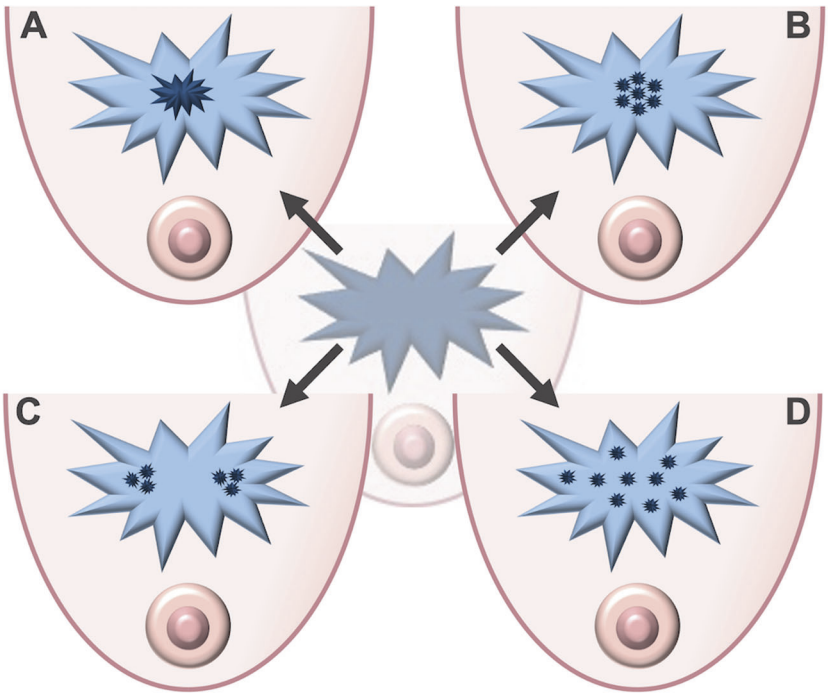


**Fig. 1 Schematic representation of patterns of residual disease following neoadjuvant chemotherapy.** **a** Solitary, confined focus of residual tumor with little or no intervening treatment-related fibrosis. **b** Tumor cell nests are separated by small areas of treatment-related fibrosis but are confined to a circumscribed area. **c** Scattered, clustered foci of residual tumor, with large intervening areas of treatment-related fibrosis. **d** Diffusely scattered tumor cells, singly and in small clusters, with prominent associated treatment-related fibrosis.

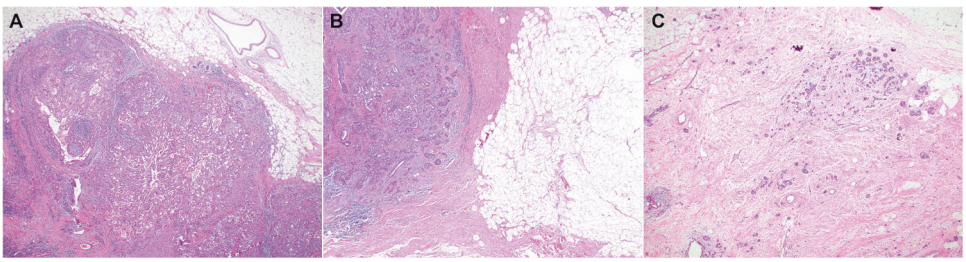


**Fig. 2 Histologic images of patterns of residual tumor. a** In this case there is a single, circumscribed focus of residual invasive carcinoma with a patchy associated lymphoid infiltrate. This pattern of residual tumor corresponds to that depicted in Fig. 1a. **b** In this case the residual tumor is composed of nests of tumor cells, separated by treatment-related fibrosis, but the tumor cell nests are present in a confined, circumscribed area. This pattern of residual tumor corresponds to that depicted in Fig. 1b. **c** In this case there are widely scattered residual tumor cells, in small clusters and as single cells, broadly distributed across a fibrotic tumor bed. This pattern corresponds to that depicted Fig. 1d.

calcifications and thickened basement membrane were evaluated in the residual in situ component. The tumor bed was evaluated for the presence of edema, fibrosis/scarring, stromal elastosis, stromal mucin, myxoid change, stromal lymphocytes [12], foamy macrophages, hemosiderin-laden macrophages, and stromal hemosiderin deposition. Non-neoplastic breast tissue was evaluated for the presence of features consistent with treatment effects (i.e., lobular atrophy and epithelial atypia).

Data were analyzed using SPSS statistical software (IBM Corporation, Armonk, New York). Ratios were compared using Pearson chi-square or Fisher exact tests, as appropriate. Means were compared using Student's $t$ test. Two-sided $p$-values of <0.05 were considered statistically significant. A binary logistic regression model was fitted to identify factors associated with a scattered pattern of response post-NAC. The goodness-of-fit logistic regression was performed using the Hosmer–Lemeshow test.

This study was approved by the Institutional Review Board of the Dana-Farber/Harvard Cancer Center.

## Results

Among the 665 cases with slides available for review, 278 were lumpectomy specimens and 387 were mastectomy specimens. The median and mean patient age was 49 years (range 22–86 years), median and mean tumor sizes were 3.0 cm and 3.5 cm, respectively (range 0.6–14.0 cm), 383 (57.6%) patients presented with clinically positive axillary

lymph nodes, and 216 (32.5%) had multifocal or multicentric disease. On the pre-treatment core needle biopsy 600 carcinomas (90.2%) were invasive ductal carcinoma, 18 (2.7%) were grade 1, 175 (26.3%) were grade 2, 469 (70.5%) were grade 3, and 375 (56.4%) were hormone receptor (HR) positive. Based on the combination of HR and HER2 status, 242 cases (36.4%) were HR+/HER2−, 216 (32.5%) were HER2+, and 207 (31.1%) were HR−/HER2− (triple negative, TNBC). Regarding NAC regimens, 93.4% of patients (621/665) received an anthracycline and/or taxane-based regimen and 94.4% (204/216) of patients with HER2+ tumors received HER2-targeted therapy.

A pCR (defined as no residual invasive carcinoma in the breast or lymph nodes; ypT0N0 or ypTisN0) was seen in 177 patients (26.6%). The rate of pCR was significantly related to tumor subtype and was seen in 19 of 242 HR+/HER2− tumors (7.9%), 80 of 216 HER2+ tumors (37%), and 78 of 207 TNBC (37.7%), ($p < 0.001$).

Among the remaining 488 cases, 57 showed no response or only minimal response to treatment, 12 had an insufficient gross description in the surgical pathology report to permit accurate assessment of residual disease pattern, 10 had residual carcinoma only in lymphovascular spaces, and 20 had residual disease in lymph nodes only with no residual invasive carcinoma in the breast. These 99 cases were omitted from further analysis leaving 389 cases (143 lumpectomies and 246 mastectomies) with residual invasive carcinoma in the breast in which the pattern of residual disease could be assessed. Of these 389 cases, 102 (26.2%) had a circumscribed pattern of residual disease (Fig. 1, patterns A or B) and 287 (73.8%) had a scattered pattern of residual disease (Fig. 1, patterns C or D).

The relationship between clinico-pathologic features at presentation and pattern of residual disease is shown in Table 1. Larger tumor size, clinically positive lymph nodes, and focality at presentation were all significantly associated with a scattered pattern of residual disease ($p = 0.001$, $p = 0.04$, and $p = 0.02$, respectively). The pattern of residual tumor was also significantly related to tumor grade and receptor profile. A scattered pattern was more frequent among grade 1 or 2 cancers than among grade 3 tumors (90.8% vs 62.6%, $p < 0.001$). Among patients with HR+ cancers, 86.1% had a scattered pattern of residual disease while only 13.9% had a circumscribed pattern. Conversely, among those with HR− tumors, 52.8% had a circumscribed pattern of residual tumor whereas 47.2% had a scattered pattern ($p < 0.001$). When stratified by both HR and HER2 status, a circumscribed pattern of residual disease was seen in 54.8% of TNBC, 29% of HER2+ tumors, and 10.6% of HR+/HER2− tumors. Conversely, a scattered pattern of residual disease was seen in 89.4% of HR+/HER2− tumors, 71% of HER2+ tumors and 45.2% of TNBC ($p < 0.001$). Of note, among patients with a scattered pattern of residual disease, a widely scattered pattern (Fig. 1d) was significantly more common in patients with HR+/HER2− tumors than in those with TNBC (86.4% vs 59.5%, $p = 0.001$). While the number of cases in each subgroup is relatively small, HER2+ tumors that were HR+ were more similar in their pattern of residual disease to HR+/HER2− tumors (scattered pattern in 60 of 77 cases, 77.9%), whereas HER2+ tumors that were HR− had a pattern of residual disease more similar to TNBC (circumscribed pattern in 14/30 cases, 46.7%), and this difference was statistically significant ($p = 0.02$). The pattern of residual disease was not significantly related to patient age, or chemotherapy regimen in either the population as a whole (Table 1) or within any of the breast cancer subtypes as defined by receptor profile (data not shown). In addition, there was no significant relationship between histologic tumor type and pattern of residual disease.

Given that the pattern of residual disease among patients with TNBC was almost equally divided between circumscribed and scattered patterns ($n = 51$ and $n = 42$, respectively) we evaluated the features associated with the pattern of residual tumor in the TNBC subgroup alone. The only feature significantly related to pattern of residual disease

**Table 1** Pattern of residual disease related to clinico-pathologic features at initial presentation among 389 patients with evaluable residual disease in the breast.

| | Circumscribed ($n = 102$) | Scattered ($n = 287$) | $p$ value |
|---|---|---|---|
| Age (mean, years) | 48 | 49 | 0.05 |
| Tumor size (mean, cm) | 31.3 | 37.1 | **0.001** |
| Clinical node status | | | **0.04** |
| Positive ($n = 236$) | 53 (22.5%) | 183 (77.5%) | |
| Negative ($n = 153$) | 49 (32.0%) | 104 (68.0%) | |
| Chemotherapy | | | 0.988 |
| Anthracycline and/or taxane-based ($n = 366$) | 96 (26.2%) | 270 (73.8%) | |
| Other ($n = 23$) | 6 (26.1%) | 17 (73.9%) | |
| Focality | | | **0.02** |
| Unifocal ($n = 246$) | 69 (28.0%) | 177 (72.0%) | |
| Multifocal ($n = 94$) | 28 (29.8%) | 66 (70.2%) | |
| Multicentric ($n = 49$) | 5 (10.2%) | 44 (89.8%) | |
| Histologic grade[a] | | | **<0.001** |
| 1 or 2 ($n = 152$) | 14 (9.2%) | 138 (90.8%) | |
| 3 ($n = 235$) | 88 (37.4%) | 147 (62.6%) | |
| Hormone receptor status | | | **<0.001** |
| Positive ($n = 266$) | 37 (13.9%) | 229 (86.1%) | |
| Negative ($n = 123$) | 65 (52.8%) | 58 (47.2%) | |
| Tumor subtype | | | **<0.001** |
| HR+/HER2− ($n = 189$) | 20 (10.6%) | 169 (89.4%) | |
| HER2+ ($n = 107$) | 31 (29.0%) | 76 (71.0%) | |
| Triple-negative ($n = 93$) | 51 (54.8%) | 42 (45.2%) | |

*HR* hormone receptor.

[a] Two patients did not have grade information for the pre-treatment biopsy.

among patients with TNBC was multicentricity at initial presentation. All seven patients with multicentric disease at presentation who had residual tumor had a scattered pattern of residual disease; in contrast, a scattered pattern was seen in 26 of 62 (41.9%) patients with unifocal disease and in 9 of 24 patients (37.5%) with multifocal disease at presentation $p = 0.009$). The associations between larger tumor size and node positive status at presentation with a scattered pattern of residual tumor in patients with TNBC were not significant ($p = 0.07$ and $p = 0.09$, respectively). There was no significant relationship between patient age, chemotherapy regimen, or histologic grade and pattern of residual disease among TNBC patients.

On multivariate analysis, histologic grade 1 or 2 and HR+ status were independently associated with a scattered pattern of residual tumor, with the greatest effect among patients with HR+/HER2− tumors. Multifocality at presentation was associated with a borderline significant increase in the odds of a scattered pattern of residual tumor, but the 95% confidence intervals are wide, and multicentricity showed no significant association. In addition, larger tumor size was associated with a higher likelihood of a scattered pattern of residual disease. In particular, for every 1 mm increase in tumor size, there was a 2% increase in the odds of a scattered pattern of residual tumor (Table 2).

Among cases with residual invasive carcinoma in the breast, several treatment-related changes in the invasive carcinoma cells were significantly related to tumor subtype (Table 3). In particular, moderate/marked nuclear atypia, cytoplasmic vacuolization/foaminess, and resemblance of tumor cells to histiocytes were all most common in TNBC (Fig. 3). In contrast, among 390 cases with residual ductal carcinoma in situ (DCIS) in the post-treatment surgical specimen, none of the treatment-related changes in the DCIS differed significantly by subtype. Overall, the mean percent stromal TILs among cases with residual invasive carcinoma was 16.8% (range 0–100%). High stromal TILs, defined as a percentage of stromal TILs greater than the mean, was more common in residual TNBC than in the other breast cancer subtypes (Table 3).

Several histologic features of the tumor bed varied significantly by tumor subtype (Table 4). In particular, foamy macrophages, hemosiderin-laden macrophages, and stromal hemosiderin deposition were each more common in the tumor bed of TNBC than in the tumor bed of other subtypes. Conversely, stromal elastosis, stromal myxoid change and stromal mucin were more frequent in the tumor bed of HR+/HER2− tumors than in other subtypes (Fig. 4).

The frequency of treatment-related changes in non-neoplastic breast tissue (i.e., lobular atrophy and epithelial atypia) did not differ significantly among subtypes and was seen in 42 TNBC cases (20.3%), 65 HER2+ cases (30.1%), and 60 HR+/HER2− cases (24.8%) ($p = 0.07$).

**Table 2** Multivariate analysis relating clinico-pathologic features to likelihood of scattered pattern of residual tumor.

| | OR | 95% CI | *p* value |
|---|---|---|---|
| Tumor size (per mm) | 1.022 | 1.005–1.040 | **0.013** |
| Nodal status | | | |
| Negative | 1 (ref) | | |
| Positive | 1.135 | 0.655–1.967 | 0.651 |
| Focality | | | |
| Unifocal | 1 (ref) | | |
| Multifocal | 2.742 | 0.984–7.644 | 0.05 |
| Multicentric | 0.975 | 0.527–1.804 | 0.94 |
| Histologic grade | | | |
| 3 | 1 (ref) | | |
| 1 or 2 | 2.894 | 1.468–5.704 | **0.002** |
| Tumor subtype | | | |
| Triple-negative | 1 (ref) | | |
| HR−/HER2+ | 1.344 | 0.563–3.211 | 0.505 |
| HR+/HER2+ | 3.275 | 1.589–6.750 | **0.001** |
| HR+/HER2− | 6.673 | 3.374–13.197 | **<0.001** |

*OR* odds ratio, *CI* confidence interval, *HR* hormone receptor.

## Discussion

In this study, we evaluated the patterns of residual invasive carcinoma among 389 patients with stage I-III breast cancer treated with NAC who had evaluable residual disease in the post-treatment surgical specimen. This study is the largest and most detailed to date relating clinico-pathologic features of breast cancers at presentation to pattern of residual tumor, alterations in tumor cells, and histologic features of the tumor bed after NAC. Our results indicate that several clinico-pathologic features at presentation including tumor size, histologic grade, and particularly tumor subtype as defined by hormone receptor and HER2 status are significantly associated with the pattern of residual carcinoma in the breast among patients who do not experience a pCR.

It has long been recognized that among patients with breast cancer treated with NAC who do not achieve a pCR, the histologic pattern of residual carcinoma in the breast varies [13–15]. In many cases, the residual tumor is present as scattered tumor cells within the tumor bed, singly and in small nests. In other cases, a more confined, circumscribed area of residual tumor is present with or without associated treatment-related fibrosis [13–15]. However, the clinico-pathologic features at presentation that predict the pattern of residual disease after NAC have not been well-defined. A better understanding of the relationship between pre-treatment clinical, pathologic and biologic features of the tumor and pattern of residual disease may be of particular value for helping to guide post-chemotherapy surgical management.

**Table 3** Treatment effects in invasive tumor cells and stromal TILs among 468 cases with residual invasive disease in the breast related to tumor subtypes.

| | HR+/HER2− ($n = 216$) | HER2+ ($n = 127$) | TNBC ($n = 125$) | $p$ value |
|---|---|---|---|---|
| Moderate/marked nuclear atypia | 97 (44.9%) | 55 (43.3%) | 92 (73.6%) | **<0.001** |
| Cytoplasmic vacuolization/foaminess | 110 (50.9%) | 72 (56.7%) | 100 (80.0%) | **<0.001** |
| Tumor cells resembling histocytes | 33 (15.3%) | 21 (16.5%) | 35 (28.0%) | **0.01** |
| High stromal TILs[ab] | 34 (15.9%) | 33 (27.3%) | 67 (54.5%) | **<0.001** |

*HR* hormone receptor, *TNBC* triple negative breast cancer, *TILs* tumor infiltrating lymphocytes.

[a]High TILs defined as greater than average percentage of TILs in cases with residual invasive disease in the breast (cutoff 16.8%).

[b]Ten patients with residual carcinoma only in lymphovascular spaces did not have stromal TILs assessed and were excluded.

**Table 4** Tumor bed changes related to breast tumor subtype.

| | HR+/HER2− ($n = 242$) | HER2+ ($n = 216$) | TNBC ($n = 207$) | $p$ value |
|---|---|---|---|---|
| Fibrosis/scarring | 241 (99.6%) | 209 (96.8%) | 206 (99.5%) | **0.014** |
| Foamy macrophages | 39 (16.1%) | 60 (27.8%) | 67 (32.4%) | **<0.001** |
| Hemosiderin laden-macrophages | 110 (45.5%) | 110 (50.9%) | 136 (65.7%) | **<0.001** |
| Hemosiderin deposition | 132 (54.5%) | 106 (49.1%) | 146 (70.5%) | **<0.001** |
| Stromal elastosis | 94 (38.8%) | 67 (31.0%) | 51 (24.6%) | **0.005** |
| Myxoid change | 46 (19.0%) | 29 (13.4%) | 17 (8.2%) | **0.004** |
| Stromal mucin | 20 (8.3%) | 11 (5.1%) | 1 (0.5%) | **0.001** |

*HR* hormone receptor, *TNBC* triple negative breast cancer.

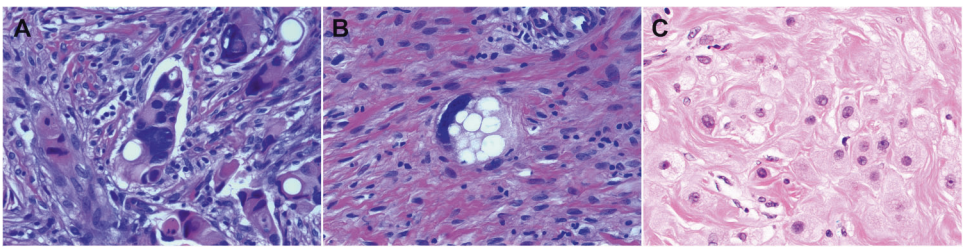


**Fig. 3 Treatment effects in invasive carcinoma cells.** (**a**) Marked cytologic atypia, (**b**) Prominent cytoplasmic vacuolization (**b**), (**c**) Tumor cells with an appearance similar to foamy histiocytes.

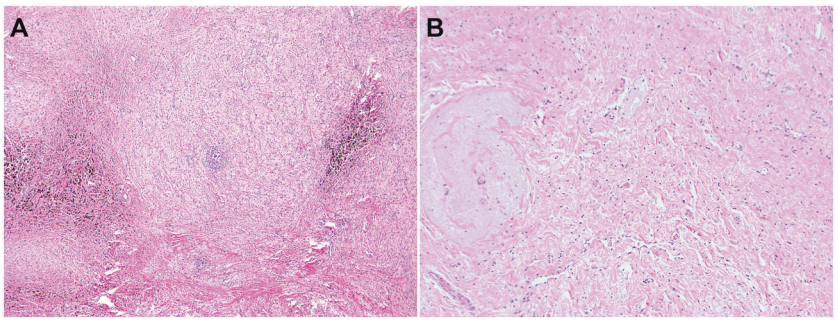


**Fig. 4 Tumor bed features related to tumor subtype.** (**a**) Foamy histiocytes and stromal hemosiderin deposition were more common in the tumor bed of triple negative breast cancers, (**b**) Stromal elastosis was more commonly seen in the tumor bed of HR+/HER2− cancers.

Given that there is no standardized or universally accepted classification system to characterize the histologic patterns of residual tumor following NAC, we developed a schema for categorizing the pattern of residual carcinoma that was based upon histologic examination of all available H and E-stained sections, supplemented by the gross description in the pathology report and the results of imaging studies (Fig. 1). Using our schema, 74% of patients had a scattered pattern of residual disease and 26% had residual tumor confined to a circumscribed area. Of note, we found that in univariate analysis features at presentation that significantly correlated with a scattered pattern of residual disease were larger tumor size, positive lymph nodes, lower histologic grade and HR+ status. In multivariate analysis, lower histologic grade and HR+ status were independently associated with a scattered pattern of residual tumor.

Among patients with HR+ disease at diagnosis, regardless of HER2 status, a scattered pattern of residual disease was seen in 86% of cases, whereas only 14% had a circumscribed pattern of residual tumor. In contrast, among patients with HR− tumors, 53% had a circumscribed pattern of residual cancer and 47% demonstrated a scattered pattern of residual disease. These differences were even more striking when comparing HR+/HER2− tumors to TNBC.

It is difficult to compare the results of our study to those of prior studies that have reported on patterns of residual tumor in the breast after NAC due to differences in patient selection, NAC treatment protocols, and proportion of patients treated with BCS versus mastectomy, as well as lack of central pathology review in some prior studies, and, as noted above, the lack of a standardized system to classify patterns of residual disease. Even when the same terms are used by different authors to categorize residual tumor patterns, comparisons between studies are difficult due to a lack of detailed definitions. For example, while terms such as "unifocal" and "multifocal" have been used by several authors to characterize patterns of residual tumor or tumor regression after NAC, in some studies these terms are not defined sufficiently to determine if they are being used to describe radiologic, macroscopic (gross pathologic), or histologic patterns of residual carcinoma.

Two prior studies have found a significant relationship between tumor subtype as defined by receptor profile and pattern of residual disease among patients who did not experience a pCR after NAC. In one study of 351 patients, 180 of 192 HR+ cases had a "non-circumscribed" pattern of residual disease, compared with 59 of 107 HR− cases (92% vs 55%, $p < 0.001$) [16]. While these results are based on evaluation of histologic sections, there are no histologic descriptions of what constituted a "circumscribed" or "non-circumscribed" pattern of residual tumor. In addition, this study focused only on the relationship between initial receptor status and patterns of residual tumor; other features at presentation were not evaluated to assess their relationship to the pattern of residual disease. In another study of 346 patients treated with BCS following NAC the authors reported that "multifocal regression" (defined as "invasive tumor present as single cells or clusters of cells spread over a fibrotic area" and analogous to our "scattered" pattern of residual tumor) was significantly more frequent in HR+ tumors than in HR− tumors. In particular, among patients with residual disease after NAC, "multifocal regression" was seen in 60 of 115 of patients with HR+ tumors compared with 30 of 87 with HR− tumors (52.2% vs 34.5%, $p = 0.01$) [17]. However, the goal of that study was to relate pattern of residual tumor to the risk of ipsilateral breast tumor recurrence (IBTR) after breast conservation therapy rather than to identify features at presentation predictive of pattern of residual tumor. In contrast to the two studies cited above [16, 17], a study of 90 mastectomy specimens from patients who had been treated with NAC did not find a relationship between initial receptor status and pattern of residual tumor. In that study [18], Wang et al. described three patterns of residual disease using sub-serial whole breast sectioning: solitary (type I), multifocal/patch-like (type II), and main residual tumor with satellite lesions at least 1.0 cm away (type III). These patterns were found in 61%, 33% and 6% of cases, respectively. While the type II and III patterns were associated with larger primary tumor size at presentation, there was no significant association between HR or HER2 status and residual tumor pattern. Similarly, in another study of 106 post-NAC specimens, Zombori and Cserni found no significant relationship between what the authors referred to as "regression inhomogeneity" and tumor subtype [17, 19–21].

We also found significant differences in treatment-related changes in tumor cells and alterations in the tumor bed according to pre-treatment tumor subtype. Moderate/marked nuclear atypia, cytoplasmic vacuolization/foaminess, resemblance of tumor cells to histiocytes, and a higher number of stromal TILs were all most common in TNBC. With regard to changes in the tumor bed, we found that both foamy and hemosiderin-laden macrophages and stromal hemosiderin deposition were all more common in the tumor bed of TNBC than in the tumor bed of other subtypes. Conversely, stromal elastosis, myxoid change, and mucin were more frequent in the tumor bed of HR+/HER2− tumors than in other subtypes. Only one prior study has documented differences in histologic features of the tumor bed in relationship to the pre-treatment receptor status. Lee et al found that while fibrosis in tumor bed was more often seen in HR+ tumors, necrosis was more frequently observed in the tumor bed of HR− tumors. In addition, these authors reported that foamy and hemosiderin-laden macrophages in the tumor bed were more commonly seen in TNBC than in other subtypes [16], results similar to ours.

In summary, our results indicate that tumor size, histologic grade, and particularly tumor subtype are significantly and independently associated with the pattern of residual carcinoma among breast cancer patients treated with NAC who do not achieve a pCR. Whether the patterns of residual disease and their predictors are of clinical importance in guiding further local therapy and/or predicting the risk of subsequent distant recurrence, loco-regional recurrence or ipsilateral breast tumor recurrence requires evaluation in clinical outcome studies.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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