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Morphological and molecular changes following neoadjuvant endocrine therapy of oestrogen receptor-positive breast cancer: implications for clinical practice

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Morphological and molecular changes following neoadjuvant endocrine therapy of oestrogen receptor-positive breast cancer: implications for clinical practice

Aims: Neoadjuvant endocrine therapy (NAET) is used in the management of oestrogen receptor (ER)-positive breast cancer. The optimal method for histological assessment of response and the effect of NAET on the tumour morphology, grade and molecular profile remain unclear. The aim of this study is to investigate the NAET effect on tumour type, grade and molecular profile by analysing a well-characterised cohort of breast cancer samples in a single large UK tertiary referral centre, and to provide guidance on the pathological assessment of those lesions to inform adjuvant management and prognosis.

Methods and results: A single large-institution cohort of 132 patients who received NAET over a 13-year period was identified. Comprehensive clinical, histopathological and follow-up data were collected. A detailed histological review of a subset with residual post-

treatment carcinoma was undertaken. Two carcinomas (both of the lobular type) achieved complete pathological response. Central scarring was seen in 49.3% of tumours post-treatment. Significant changes in tumour type (41.6%), tumour grade (downgrading in one-third of tumours), and progesterone receptor (PR) expression (22.3%), with a switch to PR-negative status in 17.6% of cases, were observed. The last of these was associated with an absence of tumour-infiltrating lymphocytes ($P = 0.005$). Ten per cent of cases showed a change in HER2 expression ($P = 0.002$). The median patient survival was 60 months, and downgrading of tumours was associated with better overall survival ($P = 0.05$).

Conclusions: We propose a histological method for assessment of residual carcinoma following NAET, and recommend repeat ER/PR/HER2 testing to inform management and prognosis.

Keywords: aromatase inhibitors, breast carcinoma, neoadjuvant endocrine therapy (NAET), pathological response

Introduction

Endocrine therapy is increasingly being used upfront for the treatment of breast cancer, either as primary

therapy for patients unsuitable for chemotherapy/surgery,^{1,2} or as a neoadjuvant treatment option for hormone receptor-positive breast cancer. Neoadjuvant endocrine therapy (NAET) can reduce tumour size to allow breast conservation, and also influence the management decision on chemotherapy.³ Patients with hormone receptor-positive breast cancer have shown overall clinical response rates ranging from 36% to 51% for tamoxifen, and from 38% to 70% for

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aromatase inhibitors (AIs).^{4–11} A UK multicentre audit of neoadjuvant therapies showed considerable variation in the uptake rate and practice of NAET, with patchy information on the pathological response rate.¹² During the coronavirus disease 2019 pandemic, patients with oestrogen receptor (ER)-positive HER2-negative early breast cancer received NAET in order to postpone surgical treatment, which was prioritised for the more urgent and aggressive tumours such as triple-negative and HER2-positive cancers.^{13,14} Therefore, it has become essential for pathologists and the multidisciplinary teams to be familiar with the handling, reporting and management of patients receiving NAET.

Currently, the preoperative endocrine prognostic index (PEPI) is the only available index that relates the response to therapy to risk of relapse, but it is not currently in use in routine practice. It is based on the assessment of tumour size, nodal status, Ki67 level, and ER Allred score. Patients with a low pathological stage and a favourable biomarker profile (PEPI score of 0) showed a lower rate of relapse, indicating that adjuvant chemotherapy can be omitted, unlike in those with a high pathological stage disease at surgery and a poor biomarker profile.³ There is sparsity of data on the histological changes that follow NAET regarding tumour profile and hormone receptor expression. Unlike for neoadjuvant chemotherapy (NACT), there are no guidelines to assess the pathological response after NAET, and NACT reporting systems are not validated for use in the endocrine setting. Therefore, there is inconsistency of assessment and histological reporting of post-NAET tumours.¹⁵ In this study, we aimed to investigate the NAET effect on tumour type, grade and molecular profile by analysing a well-characterised cohort of tumour samples in a single large UK tertiary referral centre, and to provide guidance on the pathological assessment of those lesions to inform adjuvant management and prognosis.

Patients and methods

Female patients who underwent NAET for invasive breast carcinoma at Queen Elizabeth Hospital Birmingham, UK were identified from the clinical databases.

STUDY GROUP

Included in this study were patients with primary ER-positive operable breast carcinoma who received

NAET followed by breast surgery in the period between November 2007 and December 2019. The treatment was standardised according to the national and local protocols, and the average duration of treatment was 6 months. Patients who did not undergo surgery (primary endocrine therapy) were excluded. Surgical specimens were sampled thoroughly, similarly to the NACT specimen sampling. Where possible, a marker clip was inserted before therapy to indicate the site of the tumour. When no tumour could be identified macroscopically, specimen X-ray scanning was performed to identify a marker clip, radiological calcification, and/or residual tumour.

DATA COLLECTION

Comprehensive clinical data, including patient age, ethnicity, type of NAET treatment, type of surgery, and overall survival (OS), were collected. The following pathological data, when available, were identified from the pathology reports on both pretreatment core biopsy samples and residual tumours: tumour type, tumour grade, including individual scores for tubule formation, nuclear pleomorphism, and mitoses, ER and progesterone receptor (PR) status, HER2 status, and pathological response. An immunohistochemical score of 3+ or a 2+ fluorescence *in-situ* hybridisation (FISH)-positive score were considered to indicate HER2 positivity, according to the UK guidelines.¹⁶ The response was classified into pathological complete response (pCR) (no residual invasive carcinoma in the breast and axillary nodes), minimal residual disease ($\leq 10\%$ residual invasive carcinoma), pathological partial response ($>10\%$ residual invasive carcinoma with histological evidence of tumour response), and no response (no evidence of tumour response). A detailed histological review of tumour morphology, grade, tumour cellularity (assessment of the percentage of average cancer cellularity across the largest cross-section of the residual tumour bed), scarring pattern (central versus diffuse fibrosis), with central scarring defined as central acellular fibrous area surrounded by residual tumour cells,¹⁵ margin status (pushing versus infiltrating) and architecture of the tumour (nodular versus diffuse) was undertaken on 75 postoperative tumour sections by two pathologists (N.M.B. and A.M.S.), including a specialist breast pathologist. Tumour-infiltrating lymphocytes (TILs) were also assessed in the residual post-treatment carcinoma according to the International Immuno-Oncology Biomarker Working Group on Breast Cancer guidelines. Stromal TILs (TILs that

were not in direct contact with the tumour nests or cells) were evaluated. Immune infiltrates outside of the tumour borders, e.g. in adjacent normal tissue or ductal carcinoma *in situ*, were not included in the assessment.¹⁷

IMMUNOHISTOCHEMICAL STAINING

Immunohistochemical staining was performed for the characteristic areas of tumours from microscopically selected samples (regions), based on examining the standard (haematoxylin and eosin) staining. Dewaxing of the slides was performed in the PT link Dako (Glostrup, Denmark) automated immunohistochemistry system for hormonal receptors while Roche Ventana Ultra (Basel, Switzerland) machine was used for HER2 staining. The slides were processed in target retrieval solution for 70 min at 97°C [Cell Conditioning Solution 1 (CC1) for 64 min at 95°C for HER2 staining]. The next CC1 blocking step was performed with peroxidase inhibitor, and was preceded and followed by washing. The slides were incubated with the primary antibody [ER, clone EP1 (Dako), RTU; PR, clone PgR 1294 (Dako), RTU; and HER2, clone 4D5]. After washing, slides were incubated in FLEX/horseradish peroxidase (HRP) for a 30-min blocking step and in FLEX 3,3'-diaminobenzidine (DAB) for 10 min, each with washing before and after [for HER2 staining, an HRP multi-timer was used instead for an 8-min blocking step (Roche; antibody diluent with casein), and DAB was added for 8 min with washing before and after, followed by copper sulphate solution for 4 min]. Finally, slides were embedded in haematoxylin for 15 min.

STATISTICAL ANALYSIS

Statistical analysis was performed with the IBM SPSS package V.24. Analysis for pretreatment and post-treatment categorical variables, including tumour type, grade, ER/PR Allred score, and HER2 expression, was performed with the chi-square test. Receptor status was also dichotomised into negative and positive by use of a cut-off value of an Allred score for >2 for ER/PR and an HER2 immunohistochemical score of 3+ (or 2+ FISH-positive) to define positivity. The non-parametric Kruskal–Wallis test was used to assess the relationship between cellularity as a continuous variable and response to therapy. The Kaplan–Meier method was used for survival analysis. OS was defined as the duration in months between the date of diagnosis and the date of last follow-up or

death. A *P*-value of ≤ 0.05 was considered to be significant.

Results

A total of 132 patients fulfilled the inclusion criteria. The neoadjuvant regimen predominantly comprised AIs (96.2%), with the rest of the patients receiving tamoxifen. The pretreatment clinicopathological characteristics of all patients are summarised in Table 1. The majority (96.2%) of the patients were aged >50 years (median, 73 years; range, 40–93 years), and they were mostly Caucasian (84.1%). On pretreatment biopsy, the tumours were predominantly no special type (NST) carcinoma (63.6%) and showed grade 2 differentiation (74.1%). All tumours were ER-positive; 88.6% were PR-positive, and 6.8% were HER2-positive. The few HER2-positive patients were given NAET, and not chemotherapy or anti-HER2 therapy, because of either age/comorbidities or patient choice. pCR was achieved in two cases (2.6%), and 18.2%, 75.3% and 3.9% of patients showed minimal residual disease, pathological partial response and no pathological response, respectively. The two cases in which pCR was achieved were patients of Caucasian ethnicity, aged 74 years and 58 years. Both were diagnosed with invasive classic lobular carcinoma of grade 2, were strongly positive for hormone receptors (Allred scores for ER were 8/8 for both; PR scores were 6/8 and 7/8, respectively), and had negative HER2 status.

It is of note that patients who received AIs were statistically significantly more likely to show histological evidence of tumour response than those who received tamoxifen (97.2% versus 75%, *P* = 0.03). Tumour stage 2 (ypT2) was seen in 64.8% of the patients. Breast-conserving surgery was achieved in 67.4% of the patients. Sentinel lymph node biopsy was performed in 68.8% of the patients, with nodal metastasis being seen in 49.6%.

HISTOLOGICAL TUMOUR TYPE

The two invasive carcinomas that showed pCR were of the lobular type. There was a change in histological type following NAET in 55 of 132 cases (41.6%), and this was statistically highly significant (*P* = 0.001), with an increase in tubular carcinoma of 3% and a decrease in the mixed subtypes of 1.5%. Details of the histological types before and after NAET are shown in Table 2.

Table 1. Clinicopathological features of the study cohort (pretreatment)

Criterion	No. (%)
Age (years)	
>50	127 (96.2)
≤50	5 (3.8)
Ethnicity	
Caucasian	111 (84.1)
Asian	11 (8.3)
Black	3 (2.3)
Other	7 (5.3)
Invasive tumour type	
No special type	84 (63.6)
Lobular carcinoma	22 (16.7)
Other (mucinous, tubular) carcinoma	9 (6.8)
Mixed ductal and lobular carcinoma	17 (12.9)
Tumour grade*	
I	18 (13.7)
II	97 (74.1)
III	16 (12.2)
Progesterone receptor	
Positive	117 (88.6)
Negative	15 (11.4)
HER2	
Positive	9 (6.8)
Negative	123 (93.2)
Tumour stage[†]	
T1	30 (24.5)
T2	79 (64.8)
T3	13 (10.7)
Lymph node stage[‡]	
N0	63 (50.4)
N1	39 (31.2)
N2	12 (9.6)
N3	11 (8.8)

Table 1. (Continued)

Criterion	No. (%)
Type of treatment	
Aromatase inhibitors	127 (96.2)
Tamoxifen	5 (3.8)
Type of surgery	
Breast-conserving surgery	89 (67.4)
Mastectomy	43 (32.6)

*Grading was not applicable in one core, owing to a very small amount of tumour.

[†]Tumour size was assessed radiologically before treatment (data for 10 cases were not available).

[‡]Data of seven cases were not available.

TUMOUR GRADE

Table 3 summarises tumour grades before NAET and after NAET. Downgrading (a decrease in the overall grade by at least one grade) was seen in 30.4% of cases, and upgrading (increase in overall grade by at least one grade) in 15.6% ($P = 0.01$). The downgrading was predominantly due to a decrease in the mitotic count ($P < 0.001$) and an increase in tubule formation ($P = 0.05$), and was significantly associated with better survival. Grade 2 and 3 tumours constituted 85.9% of cases in pretreatment core biopsies. This proportion decreased to 69.5% following treatment. The proportion of grade 1 carcinoma increased from 14% to 30.4% after NAET.

HISTOLOGICAL FEATURES OF RESPONSE TO NAET

Of the 75 available post-treatment surgical excisions, 77.3% showed infiltrative tumour margins. Central scarring was seen in 49.3% of cases (Figure 1A). Tumour cellularity ranged from 1% to 90%, with an average of 40.6%. Lymphocytic infiltration was seen in 25.3% of cases. A nodular architecture of the tumour comprising multiple adjacent foci was seen in 32% of cases (Table 4). Reduced tumour cellularity was significantly related to achieving response to therapy ($P < 0.001$) (Figure 1B,C).

ER EXPRESSION

Following treatment, one of 130 available tumour pairs (0.7%) changed profile from ER-positive to ER-negative. Five cases (Allred scores of 6 and 7) showed

Table 2. Details of tumour type before and after neoadjuvant endocrine therapy (NAET)

Pre-NAET tumour type	Post-NAET tumour type						Total
	NST carcinoma	Lobular carcinoma	Mucinous carcinoma	Tubular carcinoma	Mixed carcinoma	No residual	
NST carcinoma	63	4	3	6	8	0	84
Lobular carcinoma	8	10	0	0	2	2	22
Mucinous carcinoma	3	1	1	0	1	0	6
Tubular carcinoma	1	1	0	0	1	0	3
Mixed carcinoma	11	2	0	1	3	0	17
Total	86	18	4	7	15	2	132

NST, no special type.

Table 3. The distribution of tumour grade in pretreatment core biopsies and post-treatment residual invasive carcinoma

Pre-NAET grade	Post-NAET grade			Total
	I	II	III	
I	11	6	1	18
II	27	54	13	94
III	1	11	4	16
Total	39	71	18	128*

NAET, neoadjuvant endocrine therapy.

*Two specimens had no residual disease, and grading was not applicable in another two, owing to the very small amount of tumour in one core and residual disease in the other.

an increase in ER expression by at least one score. Ten cases (Allred score of 8) showed reduced ER expression by at least one score.

PR EXPRESSION

PR status showed a highly significant change between pretreatment and post-treatment tumour samples in 22.3% of cases ($P < 0.001$). Twenty-three cases (17.6%) changed profile from PR-positive to PR-negative (Figure 1D,E), and five changed profile from PR-negative to PR-positive (3.8%). Further variation in the Allred score without affecting the final PR status was seen in 69 cases (52.2%) (Table 5). Retention of the same PR status following therapy correlated with better response to therapy ($P = 0.018$). A decrease in the PR Allred score was significantly associated with an absence of lymphocytic infiltration ($P = 0.005$).

HER2 EXPRESSION

One hundred and fourteen HER2-negative tumours (87.7%) and another three HER2-positive (2.3%) tumours retained the same profile following NAET treatment. A discordant HER2 status following treatment was seen in 13 of 130 cases (10%). Five cases changed profile from HER2 overexpression to HER2-negative (3.8%). Eight cases changed profile from HER2-negative to HER2-positive following treatment (6.1%). This change was statistically highly significant ($P = 0.002$).

LYMPH NODE RESPONSE

Pretreatment lymph nodes were assessed with imaging and cytology/biopsy sampling. Following NAET, 49.6% of tumours showed histologically confirmed nodal metastasis. The nodal metastasis showed similar histological features of regression to the breast carcinoma, particularly the associated fibrosis. No significant association between nodal status and patient outcome was found in this cohort.

PATIENT SURVIVAL

OS ranged from 8 months to 137 months, with a median of 60 months (interquartile range 36–84, 95% confidence interval 57–67). Downgrading of tumours following NAET was associated with better OS ($P = 0.05$). Patients with no change in their PR status following NAET had longer mean survival than those whose tumours had lost PR expression following treatment (107.3 months and 91.7 months, respectively). However, this difference did not reach

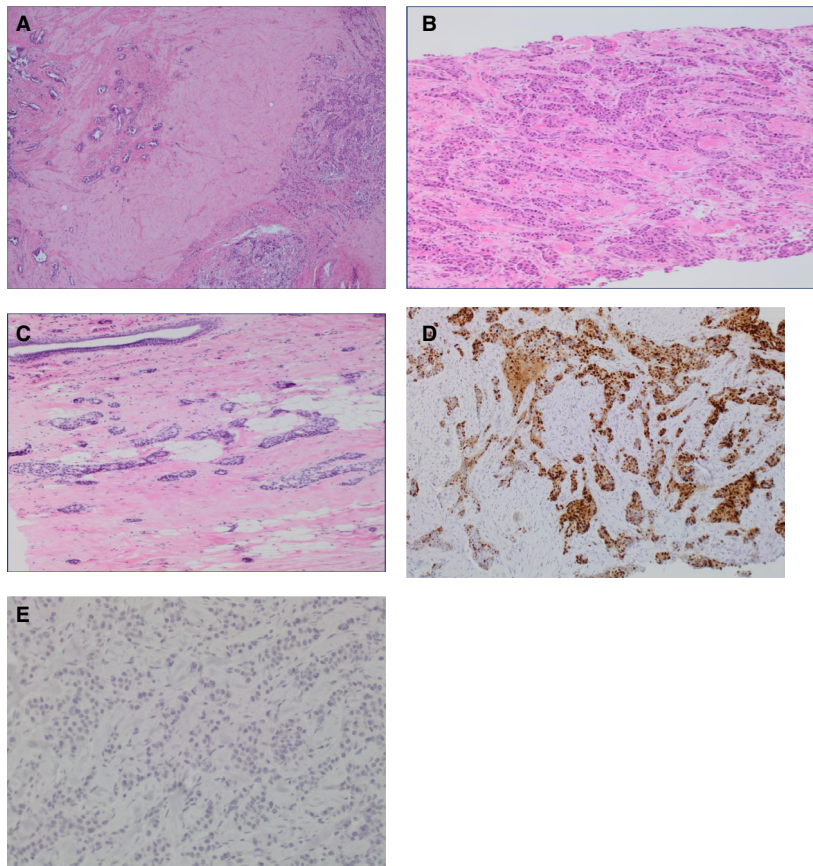


Figure 1. Histological features of response to neoadjuvant endocrine therapy. A, Post-treatment surgical excision showing central fibrous scarring with peripheral viable tumour cells. B, High tumour cellularity pretreatment. C, Low tumour cellularity post-treatment, same tumour. D, Progesterone receptor (PR)-positive invasive carcinoma of no special type (pretreatment). E, The tumour switched to a PR-negative status following neoadjuvant endocrine therapy.

statistical significance. No significant association was found between other histological parameters and OS.

Discussion

Little is known about the effect of NAET on breast tumour characteristics. In this study, we report significant changes in histological tumour type, grade, cellularity and receptor status following NAET. We show that tumours tended to acquire more specialised phenotypes, with a change of 21 of 84 of NST carcinomas (25%) to other special tumour types, including tubular, lobular, mucinous and/or mixed types. Downgrading of invasive carcinoma was associated with better OS.

The variation in histological type, grade, hormone receptor and HER2 status is intriguing. Although this may reflect a genuine change in the tumour phenotype as a result of NAET, the effects of sampling and/or tumour heterogeneity should also be considered.

For example, the change of eight lobular carcinomas and three mucinous carcinomas to NST carcinoma is likely to reflect a pretreatment mixed phenotype and/or tumour heterogeneity. It is plausible that the limited core biopsy sampled a different histological type/profile to the full excision. It is also possible that various tumour profiles responded differently to NAET, with the least responsible/resistant phenotype remaining as residual carcinoma. We therefore recommend repeat testing of the residual carcinoma for ER/PR/HER2, as a switch from a negative to a positive result would provide treatment options for patients. The final molecular profile may be a better indicator of prognosis than the pretreatment sample. In the current study, tumours that retained their PR profile were associated with better survival. A change in PR status from positive to negative may be an early indication of endocrine resistance.

NAET has an antiproliferative effect on breast tumour cells, which is associated with reduced

Table 4. Details of the histological findings of 75 residual carcinoma following neoadjuvant endocrine treatment

Parameter	No. (%)
Scarring pattern	
Central	37 (49.3)
Diffuse	38 (50.7)
Tumour margin	
Infiltrative	58 (77.3)
Well circumscribed	17 (22.7)
Architecture of the tumour	
Nodular	24 (32)
Diffuse	51 (68)
Lymphocytic infiltration	
Positive	19 (25.3)
Negative	56 (74.7)
Pathological response*	
Pathological complete response	2 (2.6)
Minimal residual disease	14 (18.2)
Pathological partial response	58 (75.3)
No response	3 (3.9)

*Data about pathological response was available for 77 cases.

Table 5. Progesterone receptor (PR) Allred score before and after neoadjuvant endocrine treatment (NAET)

PR pre-NAET	PR post-NAET								NA	Total
	0	1	3	4	5	6	7	8		
0	10	0	0	1	1	1	0	1	0	14
2	0	0	1	0	0	0	0	0	0	1
3	0	0	2	0	0	0	0	1	0	3
4	1	0	0	2	0	2	0	0	0	5
5	3	0	0	1	6	1	0	1	0	12
6	1	0	1	2	1	1	1	1	1	9
7	5	0	1	2	1	1	1	0	1	12
8	13	1	7	5	11	8	4	27	0	76
Total	33	1	12	13	20	14	6	31	2	132

NA, not applicable, two cases achieved pathological complete response.

expression of the proliferation marker Ki67 in patients showing response to therapy irrespective of NAET regimen.^{18–20} A previous study reported downgrading of breast carcinomas following either AI or tamoxifen treatment. This was associated with fewer mitoses with AI therapy, whereas tamoxifen induced more tubule formation.¹⁸ Here, we report a significant reduction in mitotic activity and more prominent tubule formation resulting in downgrading of one-third of the tumours following AI treatment. In addition, pCR of two invasive lobular carcinomas was achieved. In the UK, Ki67 is not investigated routinely in breast carcinomas with and without prior therapy, according to the NHS Breast Screening Programme guidelines.²¹

In the current study, a minor effect of NAET on ER expression was seen, with only one case changing to ER-negative status. The effect of NAET on ER expression has not been well documented in the literature, and the data so far have been conflicting, with reports suggesting a reduction^{19,20} or no significant effect following neoadjuvant AI treatment.²²

Data from the IMPACT trial showed reductions in PR levels by 41% and 82% after 2 weeks and 12 weeks of anastrozole therapy, respectively, whereas tamoxifen resulted in increased expression after 2 weeks, followed by a return to pretreatment levels at 12 weeks.²² A reduction in PR expression following NAET has also been documented in previous studies.^{18,23} It has long been thought that PR positivity is a prerequisite for a favourable response to endocrine therapy.²⁴ PR status significantly improved outcome prediction over ER status alone for adjuvant endocrine therapy in two large breast cancer databases,²⁴ and is considered to be an indication of an intact ER signalling pathway.^{25,26} Subsequently, PR was shown to be down-regulated by growth factors, indicating that it is a potential surrogate for breast cancer tumour activity.²⁵ The IMPACT trial showed more marked reductions in Ki67 levels in PR-positive tumours.²² Our reported decrease in PR levels in 69.8% of cases following treatment may be an indication of suppression of the ER signalling pathway and a lower likelihood of further tumour response to endocrine therapy than for tumours that retain PR expression. Although there was no correlation between this change and OS, we observed that patients with no change in their tumour PR status had longer mean survival than those who lost PR expression following NAET. This might be a reflection of the poor prognostic effect of loss of PR expression following NAET.

The change in HER2 expression following NAET is a novel finding. Crosstalk between the ER and HER2

pathways has been described,²⁷ and a change in ER/PR/HER2 expression following NACT has been well documented.^{28,29} The precise mechanisms by which residual tumours change profile remain unknown. A recent concept of transcriptional plasticity/adaptation to allow tumour cells to escape the treatment effects has been proposed.³⁰ HER2 signalling members were shown to reduce ER expression at both the mRNA and protein levels.³¹ ER was also shown to stimulate down-regulation of HER1 and HER2 expression.^{32,33} Thus, a combination of AIs and trastuzumab correlated with longer progression-free survival than the use of AIs alone in HER2-positive tumours.³⁴ In the neoadjuvant setting, potent anti-HER2 treatment with trastuzumab and lapatinib combined with letrozole in patients with locally advanced HER2-positive/ER-positive breast carcinoma resulted in a pCR rate of 21%.³⁵ Long follow-up for those patients who showed a change in tumour profile is warranted to confirm the clinical significance of this finding.

The pCR rate reported in this study is remarkably low. pCR in the NAET setting has uniformly been reported to be <10%, and this was confirmed in a recent meta-analysis.³⁶ Morphological changes of tumour regression were evident following NAET, and included decreased tumour cellularity and increased fibrosis.^{15,18} In the current study, central fibrous scarring was a frequent finding, being identified in almost half of the cases. Thomas *et al.* were the first to document the appearance of central scarring; this occurred in 58.5% of their cohort treated with hormone therapy as compared with systemic chemotherapy (4%). In their study, central scarring was associated with a clinical reduction in tumour volume.¹⁵ We did not find a correlation between central scarring and the pathological response to therapy or patient survival.

Although lymphocytic infiltration was not a common feature following NAET, we observed that the absence of lymphocytic infiltration was significantly related to reduced PR expression in post-treatment samples. This highlights a potential link between the tumour immune response and PR, suggesting a prognostic role for TILs in the NAET setting. Infiltrating immune cells in pretreatment cores were shown to be associated with poor response to neoadjuvant AIs.³⁷

Downgrading of tumours correlated with longer OS. However, no significant correlation was found between other studied clinical or histological parameters and survival in our cohort. As indicated by a comprehensive meta-analysis of neoadjuvant studies, correlation with survival as an endpoint for NAET studies may be challenging. Possible reasons include

the recommended use of adjuvant endocrine therapy, with variable adherence, the potential use of adjuvant chemotherapy, and the long, indolent course of the ER-positive tumours and their low early recurrence rate.³⁶

There are some similarities in and differences between the histological response following NACT and that following NAET. Whereas some histological features, such as fibrosis, decreased cellularity, and lymphocytic infiltration, are common to both NACT and NAET responses, we confirmed that central scarring was characteristic of the NAET response and is seen in approximately half of the cases, whereas this feature is uncommon following NACT. This observation was first reported by Thomas *et al.*,¹⁵ and has been confirmed in this study. The changes in tumour morphology, grade and ER/PR/HER2 status have previously been reported following NACT in several studies. However, the proportion of carcinomas showing ER conversion (from positive to negative) following NACT (5.7%,³⁸ 12.4%,³⁹ and 5.2%²⁸) is much higher than that seen after NAET in the current study (one case, 0.77%).

Table 6. Recommended microscopic features to include in the histological reporting of post-neoadjuvant endocrine therapy tumours

Histological features
Tumour type
Tumour grade
Tumour margin: pushing/infiltrative
Central scarring: present/absent
Tumour cellularity (%)
Tumour-infiltrating lymphocytes ¹⁷
Repeat receptor testing
Estrogen receptor
Progesterone receptor
HER2
Pathological response
Pathological complete response
Minimal residual disease (≤10% residual invasive carcinoma with evidence of tumour response)
Partial response (>10% residual invasive carcinoma with evidence of tumour response)
No histological evidence of response

In conclusion, we report significant changes in tumour morphology, PR expression and HER2 expression following NAET. Downgrading of invasive carcinomas post-treatment is associated with better survival. Central fibrous scarring is a common finding, and lymphocytic infiltration seems to play a role in association with the reduced PR expression following therapy. The data highlight the importance of thorough histological assessment of the post-treatment surgical specimens. We recommend detailed pathological examination of the residual post-NAET tumours, including tumour grade, the presence/absence of central scarring, and lymphocytic infiltration, and repeat hormone receptor and HER2 testing (Table 6). The changes in hormone receptor and HER2 status may be prognostic and predictive of response to therapies that otherwise would not be offered to patients on the basis of the pretreatment tumour profile. Standardised reporting of these increasingly encountered complex specimens is required to allow comparison of results among institutions globally and to help in the collection of high-quality histological and outcome data to inform future patient management.

Conflicts of interest

The authors state that they have no conflicts of interest.

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Author contributions

N.M. Badr collected the clinicopathological data, performed the statistical analysis, and wrote the manuscript. A.M. Shaaban conceived the original idea, supervised the findings, and reviewed the manuscript. All authors discussed the results and contributed to the final version of manuscript.

References

- Mathew J, Asgeirsson KS, Jackson LR, Cheung KL, Robertson JFR. Neoadjuvant endocrine treatment in primary breast cancer—review of literature. *Breast* 2009; **18**: 339–344.
- Barroso-Sousa R, Silva DDAFR, Alessi JVM, Mano MS. Neoadjuvant endocrine therapy in breast cancer: current role and future perspectives. *Ecancermedicalscience* 2016; **10**: 609.
- Ellis MJ, Tao Y, Luo J *et al.* Outcome prediction for estrogen receptor-positive breast cancer based on postneoadjuvant endocrine therapy tumor characteristics. *J. Natl. Cancer Inst.* 2008; **100**: 1380–1388.
- Eiermann W, Paepke S, Appfelstaedt J *et al.* Preoperative treatment of postmenopausal breast cancer patients with letrozole: a randomized double-blind multicenter study. *Ann. Oncol.* 2001; **12**: 1527–1532.
- Smith IE, Dowsett M, Ebbs SR *et al.* Neoadjuvant treatment of postmenopausal breast cancer with anastrozole, tamoxifen, or both in combination: the Immediate Preoperative Anastrozole, Tamoxifen, or Combined with Tamoxifen (IMPACT) multicenter double-blind randomized trial. *J. Clin. Oncol.* 2005; **23**: 5108–5116.
- Fontein DBY, Charehbili A, Nortier JWR *et al.* Efficacy of six month neoadjuvant endocrine therapy in postmenopausal, hormone receptor-positive breast cancer patients—a phase II trial. *Eur. J. Cancer* 2014; **50**: 2190–2200.
- Kaufmann M, von Minckwitz G, Mamounas EP *et al.* Recommendations from an international consensus conference on the current status and future of neoadjuvant systemic therapy in primary breast cancer. *Ann. Surg. Oncol.* 2012; **19**: 1508–1516.
- Semiglazov VF, Semiglazov VV, Dashyan GA *et al.* Phase 2 randomized trial of primary endocrine therapy versus chemotherapy in postmenopausal patients with estrogen receptor-positive breast cancer. *Cancer* 2007; **110**: 244–254.
- Cataliotti L, Buzdar AU, Noguchi S *et al.* Comparison of anastrozole versus tamoxifen as preoperative therapy in postmenopausal women with hormone receptor-positive breast cancer: the Pre-Operative 'Arimidex' Compared to Tamoxifen (PROACT) trial. *Cancer* 2006; **106**: 2095–2103.
- Masuda N, Sagara Y, Kinoshita T *et al.* Neoadjuvant anastrozole versus tamoxifen in patients receiving goserelin for premenopausal breast cancer (STAGE): a double-blind, randomised phase 3 trial. *Lancet Oncol.* 2012; **13**: 345–352.
- Santa-Maria CA, Camp M, Cimino-Mathews A, Harvey S, Wright J, Stearns V. Neoadjuvant therapy for early-stage breast cancer: current practice, controversies, and future directions. *Oncology* 2015; **29**: 828–838.
- Whitehead I, Irwin G, Bannon F *et al.* The NeST (Neoadjuvant systemic therapy in breast cancer) study: National Practice Questionnaire of United Kingdom multi-disciplinary decision making. *BMC Cancer* 2021; **21**: 90.
- <https://associationofbreastsurgery.org.uk/media/296462/abs-ukbcg-statement-may-20-final.pdf> (accessed September 2020).
- <https://www.breastsurgeons.org/docs/statements/ASBrS-Resource-Guide-on-Endocrine-Therapy-in-the-COVID-19-Pandemic.pdf> (accessed September 2020).
- Thomas JS, Julian HS, Green RV, Cameron DA, Dixon MJ. Histopathology of breast carcinoma following neoadjuvant systemic therapy: a common association between letrozole therapy and central scarring. *Histopathology* 2007; **51**: 219–226.
- Rakha EA, Pinder SE, Bartlett JMS *et al.* Updated UK Recommendations for HER2 assessment in breast cancer. *J. Clin. Pathol.* 2015; **68**: 93–99.
- Salgado R, Denkert C, Demaria S *et al.* The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. *Ann. Oncol.* 2015; **26**: 259–271.
- Miller WR, Dixon JM, Macfarlane L, Cameron D, Anderson TJ. Pathological features of breast cancer response following neoadjuvant treatment with either letrozole or tamoxifen. *Eur. J. Cancer* 2003; **39**: 462–468.

19. Harper-Wynne CL, Sacks NP, Shenton K *et al.* Comparison of the systemic and intratumoral effects of tamoxifen and the aromatase inhibitor vorozole in postmenopausal patients with primary breast cancer. *J. Clin. Oncol.* 2002; **20**: 1026–1035.
20. Makris A, Powles TJ, Allred DC *et al.* Changes in hormone receptors and proliferation markers in tamoxifen treated breast cancer patients and the relationship with response. *Breast Cancer Res. Treat.* 1998; **48**: 11–20.
21. Ellis I, Al-Sam S, Anderson N *et al.* Pathology reporting of breast disease in surgical excision specimens incorporating the dataset for histological reporting of breast cancer. Royal College of Pathologists, June 2016.
22. Dowsett M, Ebbs SR, Dixon JM *et al.* Biomarker changes during neoadjuvant anastrozole, tamoxifen, or the combination: influence of hormonal status and HER-2 in breast cancer—a study from the IMPACT trialists. *J. Clin. Oncol.* 2005; **23**: 2477–2492.
23. Maeda S. Changes of estrogen receptor, progesterone receptor and HER2 expression in breast cancer with/without neoadjuvant chemotherapy or endocrine therapy. *J. Clin. Oncol.* 2019; **37**(15 Suppl.); e14699.
24. Bardou VJ, Arpino G, Elledge RM, Osborne CK, Clark GM. Progesterone receptor status significantly improves outcome prediction over estrogen receptor status alone for adjuvant endocrine therapy in two large breast cancer databases. *J. Clin. Oncol.* 2003; **21**: 1973–1979.
25. Cui X, Zhang P, Deng W *et al.* Insulin-like growth factor-I inhibits progesterone receptor expression in breast cancer cells via the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin pathway: progesterone receptor as a potential indicator of growth factor activity in breast cancer. *Mol. Endocrinol.* 2003; **17**: 575–588.
26. Jensen EV, DesSombre ER. Steroid hormone binding and hormone receptors. In Holland JF, Frei Spaceiiiqq E, Bast RC Jr *et al.* eds. *Cancer medicine*, 4th edn. Baltimore, MD: Williams and Wilkins, 1996; 1049–1060.
27. Giuliano M, Trivedi MV, Schiff R. Bidirectional crosstalk between the estrogen receptor and human epidermal growth factor receptor 2 signaling pathways in breast cancer: molecular basis and clinical implications. *Breast Care* 2013; **8**: 256–262.
28. Gahlaut R, Bennett A, Fatayer H *et al.* Effect of neoadjuvant chemotherapy on breast cancer phenotype, ER/PR and HER2 expression—implications for the practising oncologist. *Eur. J. Cancer* 2016; **60**: 40–48.
29. Rossi L, Verrico M, Tomao S *et al.* Expression of ER, PgR, HER-2, and Ki-67 in core biopsies and in definitive histological specimens in patients with locally advanced breast cancer treated with neoadjuvant chemotherapy. *Cancer Chemother. Pharmacol.* 2020; **85**: 105–111.
30. da Silva-Diz V, Lorenzo-Sanz L, Bernat-Peguera A, Lopez-Cerda M, Munoz P. Cancer cell plasticity: impact on tumor progression and therapy response. *Semin. Cancer Biol.* 2018; **53**: 48–58.
31. Guo S, Sonenshein GE. Forkhead box transcription factor FOXO3a regulates estrogen receptor alpha expression and is repressed by the Her-2/neu/phosphatidylinositol 3-kinase/Akt signaling pathway. *Mol. Cell. Biol.* 2004; **24**: 8681–8690.
32. Lee AV, Cui X, Oesterreich S. Cross-talk among estrogen receptor, epidermal growth factor, and insulin-like growth factor signaling in breast cancer. *Clin. Cancer Res.* 2001; **7**(12 Suppl.); 4429s–4435s.discussion 4411s–4412s.
33. Newman SP, Bates NP, Vernimmen D, Parker MG, Hurst HC. Cofactor competition between the ligand-bound oestrogen receptor and an intron 1 enhancer leads to oestrogen repression of *ERBB2* expression in breast cancer. *Oncogene* 2000; **19**: 490–497.
34. Kaufman B, Mackey JR, Clemens MR *et al.* Trastuzumab plus anastrozole versus anastrozole alone for the treatment of postmenopausal women with human epidermal growth factor receptor 2-positive, hormone receptor-positive metastatic breast cancer: results from the randomized phase III TAnDEM study. *J. Clin. Oncol.* 2009; **27**: 5529–5537.
35. Rimawi MF, Mayer IA, Forero A *et al.* Multicenter phase II study of neoadjuvant lapatinib and trastuzumab with hormonal therapy and without chemotherapy in patients with human epidermal growth factor receptor 2-overexpressing breast cancer: TBCRC 006. *J. Clin. Oncol.* 2013; **31**: 1726–1731.
36. Spring LM, Gupta A, Reynolds KL *et al.* Neoadjuvant endocrine therapy for estrogen receptor-positive breast cancer: a systematic review and meta-analysis. *JAMA Oncol.* 2016; **2**: 1477–1486.
37. Dunbier AK, Ghazoui Z, Anderson H *et al.* Molecular profiling of aromatase inhibitor-treated postmenopausal breast tumors identifies immune-related correlates of resistance. *Clin. Cancer Res.* 2013; **19**: 2775–2786.
38. Dede DS, Gumuskaya B, Guler G, Onat D, Altundag K, Ozisik Y. Evaluation of changes in biologic markers ER, PR, HER 2 and Ki-67 index in breast cancer with administration of neoadjuvant dose dense doxorubicin, cyclophosphamide followed by paclitaxel chemotherapy. *J. BUON* 2013; **18**: 366–371.
39. Yang YF, Liao YY, Li LQ, Xie SR, Xie YF, Peng NF. Changes in ER, PR and HER2 receptors status after neoadjuvant chemotherapy in breast cancer. *Pathol. Res. Pract.* 2013; **209**: 797–802.