Caught in the Middle: Discrepancies Between Immunohistochemistry and Oncotype DX in Hormone Receptor Assessment Leading to Triple-Negative Reclassification -Tumour Heterogeneity or Just Low ER Expression?

V. Angelis, A. Zamani, A Grobbelaar, P. Hall, M. Phillips, P. Schmid, K. Hawkesford, F. Irakleidis, M. Thorat, S. Ledwidge, L. Begolli, Z .Ullah, D. Ryan, P Makhija, K. Silauskas and M. Warren .

Background

Discrepancies in the assessment of ER, PR, and HER2 status by immunohistochemistry (IHC) and Oncotype DX (ODX) can substantially impact treatment decisions. Variations in assay results that alter tumour molecular subtype may lead to changes in recommended adjuvant therapy. Here, we present a series of cases exploring whether discrepancies in ER expression reflect tumour heterogeneity or misclassification. We demonstrate how reclassification impacted systemic treatment decisions and genetic testing eligibility, highlighting the importance of these discrepancies in personalising breast cancer care.

Methods

- Retrospective analysis
- Single institution – St Bartholomew's Hospital
- Included all 686 patients who underwent ODX testing at our centre between 2020 and 2025
- Data extracted from ODX reports (recurrence score, qualitative and quantitative assessments of *ESR1*, *PGR*, and *ERBB2* receptor genes mRNA levels) and histopathology reports (receptor protein status determined by standard IHC staining on diagnostic biopsy specimens [1])
- Each patient's diagnostic biopsy slides reviewed blind by a breast pathologist and ER status by IHC re-scored
- ER receptor protein expression quantified using QuPath digital automated image analysis
- ER status by IHC conducted on the resection specimen tumour block which was used for ODX testing
- Results of ER IHC on diagnostic biopsy blind review and resection tissue scoring compared

Results

- 12 patients (1.7%; 12/686) demonstrated discrepancy, with a negative ESR1 gene expression score on ODX and ER IHC positive score (4-8) (Table 1)
- All 12 cases (100%) confirmed to be either ER low positive (ER 3, 4) or ER negative (ER 0, 2) by IHC on the resected tissue (Table 1).
- All discordant tumours were grade 3 invasive ductal carcinomas presenting in women aged 30–77 years.
- 3/12 patients (25%) had a germline BRCA1 mutation.

Table 1. Comparison of original ER score, reviewer ER score and ODX results for 12 discrepant breast cancers

	On resection			IHC - original	Oncotype DX			IHC - blind review	
Patient	Histological type	Grade	TNM stage	ER score on diagnostic biopsy*	Recurrence score	ER gene score†	ER status	ER score on diagnostic biopsy*	ER score on resection specimen*
1	IDC, medullary like	3	pT1b pN0	8	49	3.7	Negative	4	0
2	IDC, NST	3	pT1c pN0	5	44	4.6	Negative	3	4 (3 on review)
3	2 tumours: IDC, NST & IDC, medullary like	3	pT1c pN0	8	41	5.1	Negative	8, 3	4 (3 on review)
4	IDC with bizarre pleomorphic cells	3	pT3 pN0	6	65	5.3	Negative	5	3
5	IDC, NST	3	pT2 pN0	4	56	5.3	Negative	3	4
6	IDC, NST	3	pT1c pN0	6 (referral case)	49	5.5	Negative	ND	0
7	IDC with spindle cell metaplastic component	3	pT2 pN0	5	68	5.8	Negative	4	0
8	IDC, NST	3	pT2 pN0	5	43	5.9	Negative	4	4
9	IDC, medullary like	3	pT1c pN0	5	52	6.3	Negative	5	3
10	IDC, NST	3	pT2 pNX	4	68	6.4	Negative	3	N/a
11	IDC, medullary like	3	pT0 pN1a	4	58	6.4	Negative	4	N/a
12	IDC, medullary like	3	pT2 pN0	6	57	6.5	Negative	4	N/a

Table 1. Key: IDC – invasive ductal carcinoma; NST – no special type; ND – not done; n/a not available (Oncotype test performed on biopsy not resection). *ER status by Allred score: Allred score 5-8 = ER positive; Allred score 3, 4 = ER low positive; Allred score 0-2 = ER negative [1]. †An Oncotype ER score of 6.5 and below is ER negative.

Figure 1. Heterogeneity and multifocality – reasons for reclassification

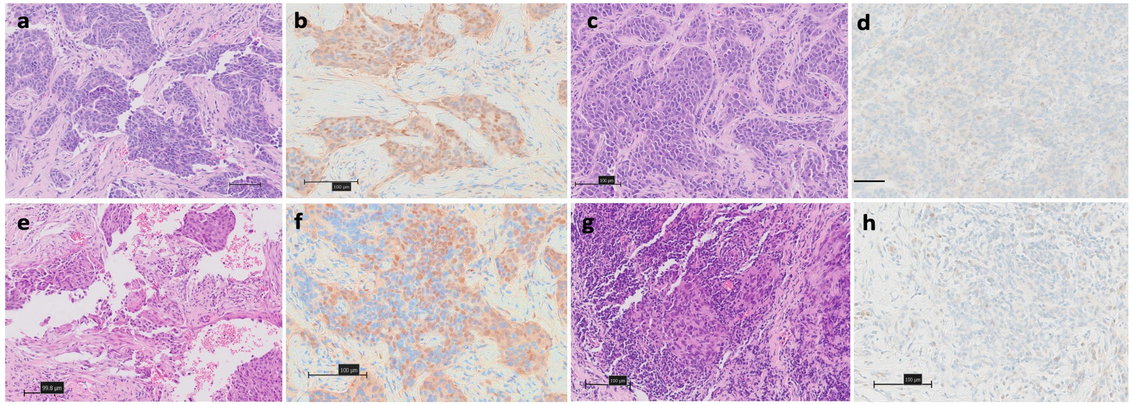
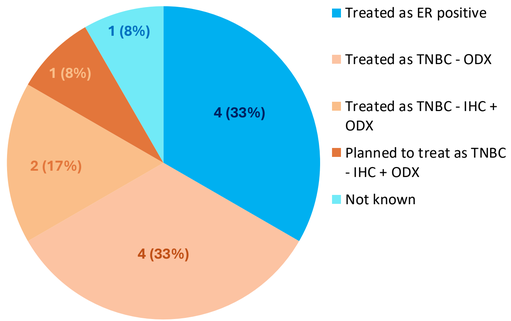


Figure 1. Photomicrographs to show the heterogeneous morphology and ER immunohistochemical staining of discrepant breast cancer cases (1) Invasive ductal carcinoma with intratumoural morphological heterogeneity on H&E (a,b) and corresponding areas on ER immunohistochemistry (c,d) and (2) Multifocal invasive ductal carcinoma with one ER8 tumour focus shown on H&E (e) and ER IHC (f) and second ER0 tumour focus shown on H&E (g) and IHC (h). In both cases the resection block on which ODX testing performed corresponded to the ER0 area on the biopsy. All images x 200 magnification.

Figure 2. Impact of reclassification on adjuvant systemic therapy treatment decisions



Results continued

Reclassification as ER low positive or triple negative breast cancer (TNBC)

- In 7/12 cases (58.3%), tumours displayed heterogeneous ER IHC staining on blind review of the ER IHC diagnostic biopsy slides, with predominantly ER negative tumour present on the resection block (Figure 1)
- In 3/12 cases (25%), diagnostic biopsies originally reported as ER positive on the basis of highest-staining hotspots were reclassified as overall ER low-positive on review (Table 1)
  - 2/3 (66.7%) also exhibited heterogeneity of ER IHC staining
- 1/12 cases had multifocal breast cancer with one ER positive (score 8) and one ER low positive tumour (score 3) with the Oncotype test performed on the ER low positive tumour (Figure 1).

Impact on systemic treatment decisions

- 4/12 patients (33.3%) treated with ER-positive adjuvant chemotherapy regimens (Figure 2)
- 7/12 patients (58.3%) planned for treatment with TNBC regimens, with 6/12 (50%) proceeding to treatment (Figure 2; one patient was not fit for chemotherapy and died following metastatic recurrence)
- 10/12 patients (83.3%) continue follow-up at our centre, with no cases of recurrence or death

Conclusions

Discordance between IHC and ODX ER status is rare (1.7%). Most discrepancies were attributable to intratumoural heterogeneity of ER protein expression in biopsies with predominantly ER negative sub-clones in resection samples. Repeat IHC confirmed these resected cases as ER low-positive or negative in concordance with ODX, confirming a higher-risk subgroup.

Our findings as well as data from several breast receptor RT-PCR studies [2,3,4] also support the potential for subtype reclassification of breast cancers initially classified as ER low positive (score 3,4), with low or negative ODX *ESR1* gene scores as similar in biological behaviour and chemotherapy response to ER negative tumours. This could help guide personalisation of adjuvant treatment strategies and prompt germline mutation testing for BRCA following reclassification of tumours as TNBC.

Figure 2. Out of the 12 patients with IHC/ODX discordance, 6 patients were treated with adjuvant TNBC chemotherapy regimens. In 2 cases, this was on the basis of both an ER low positive score on original diagnostic biopsy IHC and a negative ER gene score on ODX. In the remaining 4 cases, this decision was on the basis of the ODX alone (all 4 cases were reported ER positive on original diagnostic IHC). One patient was planned for treatment as TNBC on the basis of both ER low positive original IHC and ODX; however, she was subsequently not fit for chemotherapy. 4 patients were treated with ER positive regimens. One patient transferred care to their local centre and their adjuvant chemotherapy treatment decision is not known.

References [1] Deb R et al. Dataset for histopathological reporting of breast disease in surgical excision specimens of breast cancer (2024) G148-Dataset-for-histopathological-reportingof-breast-disease-in-surgical-excision-specimens-of-breast-cancer.pdf [2] Badr NM, Zaakouk M, Zhang Q, Kearns D, Kong A, Shaaban AM. Concordance between ER, PR, Ki67, and HER2-low expression in breast cancer by MammaTyper RT-qPCR and immunohistochemistry: implications for the practising pathologist. Histopathology. 2024 Sep;85(3):437-450 [3] Wegscheider AS et al. Comprehensive and Accurate Molecular Profiling of Breast Cancer through mRNA Expression of ESR1, PGR, ERBB2, MKI67, and a Novel Proliferation Signature. Diagnostics (Basel). 2024 Jan 23;14(3):241 [4] Chen HL et al. Impact of estrogen receptor expression level on response to neoadjuvant chemotherapy and prognosis in HER2-negative breast cancers. BMC Cancer. 2023 Sep 8;23(1):841.