Human Pathology (2020) **104**, 54–65

ELSEVIER

Human PATHOLOGY

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Original contribution

Concordance of breast cancer biomarker status between routine immunohistochemistry/in situ hybridization and Oncotype DX qRT-PCR with investigation of discordance, a study of 591 cases☆,☆☆

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Received 13 May 2020; revised 20 July 2020; accepted 23 July 2020

Available online 3 August 2020

Keywords:
Breast cancer;
Estrogen receptor;
Progesterone receptor;
HER2;
Immunohistochemistry;
Oncotype DX;
Concordance

Summary Patients with estrogen receptor (ER)+/human epidermal growth factor receptor (HER)2−, lymph node− breast cancer with high recurrence risk benefit from adjuvant chemotherapy in addition to hormonal therapy. This study compares ER, progesterone receptor (PR), and HER2 status between routine immunohistochemistry (IHC)/in situ hybridization (ISH) and Oncotype DX (ODX) in 591 cases. ODX recurrence score (RS) and clinicopathologic features were compared between ER/PR-concordant and discordant cases. Hematoxylin and eosin (H&E) slides from ER discordant cases were reexamined. Concordance was high between ODX and IHC for ER status (580/591, 98.1%) and moderate for PR status (512/591, 86.6%). All 11 ER discordant cases were ER+ by IHC but ER− by ODX and high risk by ODX. Histologically, all of these cases were grade III invasive ductal carcinoma (IDC), except one case diagnosed as IDC with apocrine features. Although this case was grade I and ER/PR+ by IHC, this patient received chemotherapy because of high RS. Of 79 PR discordant cases, 60 were PR+ by IHC but PR− by ODX. Five hundred eighty-four cases had available HER2 data, with high negative agreement (580/582, 99.7%). However, both HER2+ cases by ISH were HER2− by ODX. Mean RS was higher for ER discordant than concordant cases (48.0 versus 17.1, $P < 0.0001$) and for PR discordant (IHC+/ODX−) than concordant cases (27.2 versus 16.7, $P < 0.0001$) with no significant differences in recurrence or metastasis. Overall, detection was more

☆ This work was presented at the 2020 USCAP conference.
☆☆ Disclosures: None.

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https://doi.org/10.1016/j.humpath.2020.07.022
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sensitive by IHC, and high RS of discordant cases suggests possible risk overestimation. Therapeutic decisions for discordant cases should continue to be based on clinicopathologic correlation and not oncotype alone.
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1. Introduction

Breast cancer treatment plans can now be refined for individuals based on biomarker status and recurrence risk. Approximately 70% of breast cancers are estrogen receptor positive (ER+), expressing ER alpha, which is involved in cancer proliferation and is the target of ERα antagonists in early stage ER+ breast cancer [1]. ER expression measured by immunohistochemistry (IHC) is known to be a useful predictor of prognosis and response to hormonal therapy [2,28], with ER+ status typically conferring a better prognosis. Other key biomarkers which characterize prognosis and influence treatment of early stage breast cancer include progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) [3,4]. PR expression is associated with enhanced response to hormonal therapy [5,6], whereas HER2 amplification is associated with worse prognosis but offers the possibility of treatment with HER2-targeted therapy [4,7].

In high-risk ER+ breast cancer, adjuvant chemotherapy may increase survival and decrease cancer recurrence [8]. Oncotype DX (ODX) is a 21-gene assay which is used to help place ER+/HER2−/lymph node− patients into recurrence risk categories and to predict benefit from chemotherapy [9,10]. In addition to generating a recurrence score (RS), ODX reports gene expression scores individually for ER, PR, and HER2 using quantitative reverse transcription polymerase chain reaction [11]. Further, expression of these biomarkers influences the overall ODX RS. Of note, PR-negative status has been shown to be closely associated with a higher ODX RS [12,13]. Thus, discrepancies in the reporting of the status of these biomarkers could result in faulty determination of prognosis and lead to harmful overtreatment or inappropriate withholding of hormonal therapy or chemotherapy [7,14,28]. This study examines the discordance of reported biomarker status between ODX and routine IHC/in situ hybridization (ISH). Discordant cases are further examined to evaluate ODX RS, patient clinicopathologic features, and histopathologic features.

2. Materials and methods

2.1. Case selection and biomarker status

The use of clinicopathological and histological data in this study was approved by the Institutional Review Board at University Hospitals Cleveland Medical Center. Early stage lymph node− breast cancer cases were reviewed from University Hospitals from the years 2008−2018, including 591 cases with available ER and PR status and ODX results. Of these cases, 584 had available HER2 status by both IHC/ISH and ODX. ER, PR, and HER2 status were compared between routine IHC/ISH and ODX.

ER and PR status were determined by IHC on biopsy specimens following the 2018 American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines. The antibodies used for IHC are listed in Table 1. All IHC markers were assessed by light microscopy. Scoring of immunostained slides was done according to the percentage of tumor cells exhibiting nuclear (ER and PR) and membrane (HER2) staining. Tumors were considered positive for ER or PR if there was at least 1% or more staining in tumor nuclei. HER2 status by IHC/ISH was evaluated using contemporaneous ASCO/CAP guidelines. Quantitative single-gene scores for ER, PR, and HER2 were reported by ODX as positive if the ER score was ≥6.5, PR score was ≥5.5, and HER2 score was ≥11.5. HER2 status was reported as negative if the HER2 score was <10.7, and equivocal if the HER2 score was between 10.7 and 11.4.

2.2. Clinicopathologic and histologic features

Clinicopathologic features were compared between ER and PR discordant and concordant cases including age, ODX RS, chemotherapy, hormonal therapy, recurrence, and metastasis. ODX RS ranges from 0 to 100 and is defined as low risk if 0−17, intermediate risk if 18−30, and high risk if 31−100 [9,11]. Tumor type and grade were evaluated for ER and PR discordant cases. Tumor grade was determined using the Nottingham modification of Bloom-Richardson system [15]. H&E slides from excision specimens of ER discordant cases were reexamined to assess amount of tumor, stroma, and other potentially discriminating

Table 1 Antibodies used for immunohistochemistry.

Antibody	Clone	Dilution	Source
Estrogen receptor	SP1	Predilute	Ventana
Progesterone receptor	IE2	Predilute	Ventana
HER2	4B5	Predilute	Ventana

Abbreviation: HER2, human epidermal growth factor receptor 2.

features, including tumor infiltrating lymphocytes, necrosis, and tumor heterogeneity.

2.3. Statistical analysis

Statistical analysis of ER/PR concordant and discordant clinicopathologic features was done using the IBM Statistical Package for Social Sciences (SPSS version 22 for Windows, IBM SPSS Inc, Chicago, IL). T-test or one-way ANOVA was performed for numerical data (ODX RS and age) and the χ^2 analysis for categorical data (chemotherapy, recurrence, and metastasis). $P \leq 0.05$ was considered statistically significant.

3. Results

3.1. ER concordance

Overall concordance of ER status between ODX and IHC was high (Table 2). Of the 591 cases, 580 cases, or 98.1%, were concordant for ER status. A total of 11 cases were discordant for ER status, and all of these cases were ER+ by IHC but ER− by ODX.

3.2. PR concordance

Concordance was moderately high for PR status with 512 of 591 cases, or 86.6% of cases, concordant between IHC and ODX (Table 3). Of the 79 PR discordant cases, 60 cases were PR+ by IHC but PR− by ODX, whereas 19 were PR− by IHC and PR+ by ODX.

3.3. ER and PR clinicopathologic features

Outcomes and clinicopathologic features were compared between ER/PR concordant and discordant cases including age, ODX RS, local recurrence and metastasis, and treatment with chemotherapy. Mean ODX RS was significantly higher for ER discordant cases than for ER concordant cases (48.0 ± 11.8 versus 17.1 ± 9.1, $P < 0.0001$) with 90.0% of patients with ER discordant tumors receiving chemotherapy versus 20.7% of those with ER concordant tumors (Table 4). Similarly, mean ODX RS was higher in IHC+/ODX− PR discordant cases than in PR concordant cases (Table 4; 27.2 ± 10.7 versus 16.7 ± 9.5, $P < 0.0001$). However, mean ODX RS of IHC−/ODX+ PR discordant cases (16.1 ± 4.2) was not significantly different from mean ODX RS of PR concordant cases (Table 4). Despite significantly higher RS, recurrence and metastasis were not significantly different between ER/PR discordant and concordant cases (Table 4).

Clinicopathologic features of hormone receptor discordant cases were further examined individually (Table 5, Table 6). For ER discordant cases (Table 5), patients were treated with chemotherapy in all but one case and hormonal therapy in all but two cases with available data. All ER discordant cases were grade III invasive ductal carcinoma (IDC), except case 446 which was diagnosed as grade I IDC with apocrine features. This case was discordant for both ER and PR status, testing positive by IHC but negative by ODX for both. Despite low tumor grade and positive hormone receptor status by IHC, this patient received chemotherapy because of a high ODX RS of 36. Further, all ER discordant cases had ODX RS designating high recurrence risk, ranging from 36 to 72. ER signal by IHC was generally low in ER discordant cases, with no ER staining intensities reported as strong, although percentage of tumor cells with ER staining was as high as 75%. Low ER positivity (≤10%) was seen in 3/11 ER discordant cases (Table 5). Similarly, PR discordant cases generally had low PR signal, but percentage of tumor cells stained was as high as 95% in 3 cases (Table 6). Interestingly, all 3 of these cases were lobular (2 cases) or had a component of lobular (1 case). Additionally, recurrence occurred in 5 PR discordant cases. Of these, 4 did not receive chemotherapy, although nearly half of the IHC+/ODX− PR discordant cases received chemotherapy. A total of 8 patients with PR discordant tumors did not receive hormonal therapy.

Table 2 ER status by immunohistochemistry and Oncotype DX.

	Oncotype ER+	Oncotype ER−
IHC ER+	579	11
IHC ER−	0	1

Abbreviations: IHC, immunohistochemistry; ER, estrogen receptor.

Table 3 PR status by immunohistochemistry and Oncotype DX.

	Oncotype PR+	Oncotype PR−
IHC PR+	471	60
IHC PR−	19	41

Abbreviations: IHC, immunohistochemistry; PR, progesterone receptor.

3.4. Histopathologic features

On examination of H&E slides from ER discordant case excision specimens sent for ODX testing, several histological features potentially contributing to discordant ER results were identified (Fig. 1A–E), whereas 2 cases (case 241, not shown, and case 373, Fig. 1 F) showed mainly highly extensive invasive tumor. Case 446 (Fig. 1 A) showed predominantly stroma and few tumor cells with apocrine features. Case 271 (not shown) similarly showed predominantly stroma. Case 581 (Fig. 1 B) was characterized by significantly heterogeneous tumor with areas of clear cytoplasm, eosinophilic cytoplasm, and chondromyxoid material. Three cases (250, 228, and 221;

Table 4 Clinicopathologic comparison summary of ER and PR concordant and discordant cases.

	Age (year, mean ± SD)	Oncotype score (mean ± SD)	Recurrence	Metastasis	Chemotherapy
ER Concordant N = 580	60.4 ± 10.2	17.1 ± 9.1	21 (3.7%)	15 (2.7%)	118 (20.7%)
ER Discordant N = 11	56.4 ± 7.5	48.0 ± 11.8	1 (10.0%)	0 (0.0%)	9 (90.0%)
ER Comparison *P*	0.194	<0.0001	0.321	1.0	<0.0001
PR Concordant N = 512	59.9 ± 10.5	16.7 ± 9.5	17 (3.4%)	15 (3.0%)	98 (19.5%)
PR Discordant (IHC + /ODX−) N = 60	62.8 ± 6.2	27.2 ± 10.7	5 (8.6%)	0 (0.0%)	26 (44.1%)
PR Discordant (IHC−/ODX +) N = 19	62.8 ± 10.7	16.1 ± 4.2	0 (0.0%)	0 (0.0%)	3 (15.8%)
PR Comparison *P*	0.075	<0.0001 (<0.0001[a])	0.094	0.305	<0.0001

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; IHC+/ODX−, discordant cases that are PR+ by immunohistochemistry and PR− by Oncotype DX; IHC−/ODX+, discordant cases that are PR− by immunohistochemistry and PR+ by Oncotype DX; SD, standard deviation.
[a] Statistically significant difference in mean oncotype score between PR concordant and IHC+/ODX− PR discordant cases by Bonferroni post hoc analysis.

Table 5 ER discordant case clinicopathologic features.

Case index	Age	Breast cancer type	Breast cancer grade	Hormonal therapy	Chemo	Recurrence	Mets	ODX score	ER (IHC)	ER intensity	PR (IHC)	PR intensity	ER (ODX)	PR (ODX)
195	48	IDC	III	yes	yes	no	no	38	5	w-m	0	NA	5.8	4.6
241	60	IDC	III	yes	yes	no	no	72	5	w-m	30	w	2.9	2.6
250	54	IDC	III	yes	yes	no	no	46	35	w-m	45	w	5.1	3.3
221	52	IDC	III	yes	yes	no	no	51	20	m	0	NA	5.4	3.2
228	46	IDC	III	yes	no (refused)	yes	no	40	75	m	70	s	5.8	6.5
271	60	IDC	III	–	–	–	–	40	40	m	25	m	6.0	5.4
418	66	IDC	III	no	yes	no	no	49	60	m	0	NA	5.6	3.2
373	59	IDC	III	no	yes	no	no	67	10	w	0	NA	4.2	3.2
446	70	IDC with apocrine features	I	yes	yes	no	no	36	30	w-m	5	w	6.0	3.8
581	50	IDC	III	yes	yes	no	no	40	55	w-m	<1	w	5.8	3.2
646	55	IDC	III	yes	yes	no	no	49	30	w	2	w	5.3	4.3

Abbreviations: IDC, invasive ductal carcinoma; chemo, chemotherapy treatment; -, data unavailable; mets, metastasis; ODX, Oncotype DX; ER, estrogen receptor; PR, progesterone receptor; IHC, percentage of stained cells by immunohistochemistry; w/m/s, weak/moderate/strong staining intensity; NA, not applicable; ER/PR (ODX), estrogen receptor and progesterone receptor score by Oncotype DX.

Fig. 1C–E) showed numerous tumor infiltrating lymphocytes. One of these excision specimens (case 228) had widespread background necrosis, and the excision of case 221 had areas of focal necrosis. Three of the excision specimens (cases 195, 418, and 646) were unavailable to be viewed as they were cases from outside hospitals.

3.5. HER2 concordance and clinicopathologic features

A total of 584 cases had available HER2 results for both ODX and IHC/ISH with high concordance for HER2− cases (580/582, 99.7% concordant). Of the 582 HER2− cases by IHC/ISH, the 2 discordant cases were HER2 equivocal by ODX. One of these cases, case 258 (Table 7), had an ODX HER2 score of 10.8 which is near the threshold for being called HER2 negative. Additionally, 2 cases were HER2+ by ISH, and both of these cases were HER2− by ODX (Table 7). One of these cases, case 669 had insufficient tumor to perform IHC and ISH on biopsy, but on excision, this case was group 3 HER2+ by 2018 ASCO/CAP guidelines as the HER2:CEP17 ratio was <2, and the HER2 copy number was ≥6 by 2 observers with IHC 2+. The other case, case 240, was group 1 HER2+ by

Table 6 PR discordant case clinicopathologic features.

Case index	Discordance	Age	Breast cancer type	Breast cancer grade	Hormonal therapy	Chemo	Recurrence	Mets	ODX score	ER (IHC)	ER intensity	PR (IHC)	PR intensity	ER (ODX)	PR (ODX)
241	IHC+/ODX−	60	IDC	III	yes	yes	no	no	72	5	w-m	30	w	2.9	2.6
250	IHC+/ODX−	54	IDC	III	yes	yes	no	no	46	35	w-m	45	w	5.1	3.3
271	IHC+/ODX−	60	IDC	I	−	−	−	−	40	40	m	25	m	6	5.4
446	IHC+/ODX−	70	IDC	I	yes	yes	no	no	36	30	w-m	5	w	6	3.8
646	IHC+/ODX−	55	IDC	III	yes	yes	no	no	49	30	w	2	w	5.3	4.3
190	IHC+/ODX−	61	ILC	II	yes	no	no	no	21	95	s	20	w	11.9	3.2
63	IHC+/ODX−	55	IDC	II	yes	yes	no	no	25	95	−	10	−	10.3	5.4
29	IHC+/ODX−	58	IDC	III	yes	yes	no	no	30	90	−	1	−	10.5	3.6
142	IHC+/ODX−	67	ILC	II	yes	yes	no	no	22	99	−	8	−	9.8	5.2
43	IHC+/ODX−	53	IDC	III	yes	yes	no	no	30	95	−	40	−	10.2	3.2
30	IHC+/ODX−	63	IDC-L	II	yes	yes	no	no	20	100	−	1	−	10.7	5.1
33	IHC+/ODX−	71	IDC	II	yes	no	yes	no	23	90	s	1	−	9.5	5
124	IHC+/ODX−	62	IDC	III	yes	yes	no	no	37	90	−	3	−	10.4	3.2
6	IHC+/ODX−	68	IDC	I	yes	no	no	no	18	95	s	2	m	11.3	5.2
165	IHC+/ODX−	64	IDC	III	yes	yes	no	−	26	95	−	5	−	10.9	3.2
239	IHC+/ODX−	58	IDC	II	yes	yes	no	no	33	95	s	5	m	12	3.8
243	IHC+/ODX−	65	ILC	II	yes	no	no	no	16	90	s	10	w-m	10.7	5.4
222	IHC+/ODX−	61	IDC	III	yes	yes	no	no	38	85	m	10	m	7.6	5.3
274	IHC+/ODX−	66	IDC-L	II	yes	no (refused)	yes	no	22	90	s	5	w	8.8	4.9
254	IHC+/ODX−	74	ILC	II	yes	no	yes	no	31	95	s	50	m	10	3.2
291	IHC+/ODX−	63	IDC	I	yes	no	no	no	24	99	s	70	m	10.1	3.2
322	IHC+/ODX−	64	ILC	II	yes	no	no	no	23	95	s	20	m	8.3	5.2
323	IHC+/ODX−	67	IDC	II	yes	no	no	no	12	95	s	10	s	12.5	4.3
301	IHC+/ODX−	66	IDC	I	yes	no	no	no	21	95	s	5	m	8.7	5.3
339	IHC+/ODX−	56	IDC	II	yes	yes	no	no	22	95	s	50	m	11.4	4.1
341	IHC+/ODX−	67	IDC	II	yes	yes	no	no	20	95	s	40	m	10.9	4.7
351	IHC+/ODX−	63	ILC	I	yes	no	no	no	22	95	s	5	m	9.3	5.4
398	IHC+/ODX−	55	IDC	II	no	no (refused)	no	no	29	95	s	20	w-m	10.2	4.8
430	IHC+/ODX−	69	ILC	II	yes	no	no	no	24	80	s	10	s	10.2	5.2
440	IHC+/ODX−	60	IDC	II	yes	no	no	no	34	90	s	10	s	6.8	3.6
492	IHC+/ODX−	61	IDC	III	yes	yes	no	no	34	95	s	10	m	9	5.1
497	IHC+/ODX−	70	IDC	III	yes	yes	yes	no	47	95	s	60	w-m	10.2	4.9
499	IHC+/ODX−	74	ILC	II	yes	no	no	no	22	95	s	95	s	7.9	5.1
523	IHC+/ODX−	76	IDC	II	yes	no	no	no	43	90	s	5	m-s	9.7	3.2
452	IHC+/ODX−	60	IDC	II	yes	yes	no	no	26	90	s	15	w-m	9.3	4.3
531	IHC+/ODX−	66	IMC	II	yes	no	no	no	19	95	−	2	−	11.3	3.3
545	IHC+/ODX−	78	ILC	II	yes	no	no	no	15	95	s	30	s	10.6	5.3
563	IHC+/ODX−	62	IDC	II	yes	no	no	no	17	95	s	25	m-s	11.3	5.4

573	IHC+/ODX−	61	IDC	II	yes	yes	no	no	25	95	s	10	s	9.1	5.1
443	IHC+/ODX−	50	IDC-L	II	yes	no	no	no	16	95	s	95	s	8.3	5.4
480	IHC+/ODX−	68	IDC	III	yes	yes	no	no	32	95	s	2	m	11.3	3.3
482	IHC+/ODX−	65	IDC	I	yes	no	no	no	20	95	s	2	w	10.8	3.7
484	IHC+/ODX−	60	IDC-L	II	yes	no (refused)	no	no	28	95	s	10	m	9.3	4.3
619	IHC+/ODX−	57	IDC-L	II	yes	no	no	no	27	95	s	5	m	8.9	5.3
626	IHC+/ODX−	63	IDC	II	no	yes	no	no	35	95	s	10	s	8.6	3.2
630	IHC+/ODX−	60	ILC	II	yes	no	no	no	22	95	s	95	s	11.1	4.4
633	IHC+/ODX−	58	IDC-L	II	no	no	no	no	23	95	s	50	m	10.8	5.1
589	IHC+/ODX−	63	IDC	II	yes	no	no	no	21	95	s	10	s	9.5	5
590	IHC+/ODX−	59	IDC	II	yes	no	no	no	23	95	s	10	s	12.4	4.2
597	IHC+/ODX−	63	IDC	III	no	yes	no	no	43	95	s	5	w	9.4	4.1
582	IHC+/ODX−	64	ILC	II	yes	no	no	no	16	98	s	10	w	10.8	5.2
609	IHC+/ODX−	56	IDC	III	yes	no	no	no	13	95	s	20	m-s	11.6	4.4
615	IHC+/ODX−	64	IDC	II	no	yes	no	no	25	95	s	3	w-m	11	3.2
641	IHC+/ODX−	66	IDC	II	no	no	no	no	21	90	s	2	w	10.5	5.4
664	IHC+/ODX−	52	IDC	II	no	no	yes	no	24	90	s	5	m	9.4	4.5
666	IHC+/ODX−	69	IDC	III	yes	yes	no	no	33	90	s	10	m	12.1	3.2
677	IHC+/ODX−	56	IDC	II	yes	yes	no	no	26	95	−	50	−	9.3	5
668	IHC+/ODX−	69	IDC	II	yes	no	no	no	9	95	−	5	−	11.5	4.5
669	IHC+/ODX−	55	IDC	III	yes	yes	no	no	35	100	s	5	m	12.5	5.4
682	IHC+/ODX−	77	IMC	II	no	no	no	no	21	95	s	10	s	11	4.9
199	IHC−/ODX+	63	IDC	I	yes	no	no	no	14	95	s	<1	w	9.3	5.7
94	IHC−/ODX+	68	IDC	II	yes	no	no	no	10	80	−	0	NA	10.9	8
21	IHC−/ODX+	41	IDC	II	yes	no	no	no	13	100	−	0	NA	10.1	6.2
143	IHC−/ODX+	80	ILC	II	yes	yes	no	no	22	99	−	0	NA	10	5.6
126	IHC−/ODX+	60	ILC	II	yes	yes	no	no	23	90	s	0	NA	9.6	6
38	IHC−/ODX+	65	IDC-L	II	yes	no	no	no	13	90	−	0	NA	10.6	7.9
91	IHC−/ODX+	58	IDC	I	yes	no	no	no	12	100	s	0	NA	10.7	5.5
312	IHC−/ODX+	61	ILC	II	yes	yes	no	no	14	100	s	0	NA	12.2	5.5
381	IHC−/ODX+	72	IDC-L	II	yes	no	no	no	18	95	s	0	NA	10.4	5.8
386	IHC−/ODX+	58	IDC	II	yes	no	no	no	10	95	s	0	NA	10.7	7.1
406	IHC−/ODX+	69	IDC-L	I	yes	no	no	no	15	99	s	<1	w	9.7	7.8
486	IHC−/ODX+	52	IDC	I	yes	no	no	no	19	95	s	0	NA	11.2	7.8
496	IHC−/ODX+	67	IDC-L	III	yes	no	no	no	22	95	s	0	NA	10.3	5.8
504	IHC−/ODX+	74	ILC	II	yes	no	no	no	20	95	s	<1	−	10.6	6.1

(*continued on next page*)

Table 6 (*continued*)

Case index	Discordance	Age	Breast cancer type	Breast cancer grade	Hormonal therapy	Chemo	Recurrence	Mets	ODX score	ER (IHC)	ER intensity	PR (IHC)	PR intensity	ER (ODX)	PR (ODX)
455	IHC−/ODX+	37	IDC	II	yes	no	no	no	19	95	s	0	NA	9.1	5.6
535	IHC−/ODX+	74	IDC	II	yes	no	no	no	18	95	m-s	<1	w	9.4	7.1
549	IHC−/ODX+	69	ILC	II	yes	no	no	no	10	95	s	0	NA	10	7.4
465	IHC−/ODX+	62	IDC-L	II	yes	no	no	no	18	80	m	0	NA	7.7	5.8
663	IHC−/ODX+	63	IDC-L	II	yes	no	no	no	16	70	w	0	NA	11.3	6.1

Abbreviations: IHC+/ODX−, discordant cases that are PR+ by immunohistochemistry and PR− by Oncotype DX; IHC−/ODX+, discordant cases that are PR− by immunohistochemistry and PR+ by Oncotype DX; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; IDC-L, mixed invasive ductal-lobular carcinoma; IMC, invasive mucinous carcinoma; chemo, chemotherapy treatment; -, data unavailable; mets, metastasis; ODX, Oncotype DX; ER, estrogen receptor; PR, progesterone receptor; IHC, percentage of stained cells by immunohistochemistry; w/m/s, weak/moderate/strong staining intensity; NA, not applicable; ER/PR (ODX), estrogen receptor and progesterone receptor score by Oncotype DX.

the 2018 guidelines as the HER2:CEP17 ratio was ≥2, and the HER2 copy number was ≥4. Thus, 2 cases in this study were positive for HER2 amplification by IHC/ISH, and both were reported as HER2− by ODX. Of the HER2 discordant cases in this study (Table 7), none had recurrence or metastasis to date, and all cases with available data received chemotherapy with oncotype scores ranging from 22 to 35.

4. Discussion

In this study of 591 cases, biomarker status concordance rates between routine IHC/ISH testing and ODX qRT-PCR were high for ER status and moderate for PR status. Negative agreement was high for HER2 status, but considering most HER2+ cancers are not sent for ODX testing, positive agreement was low as the two HER2+ cases by ISH were HER2− by ODX. Unique features of this study include detailed evaluation of clinicopathologic features and outcomes data in hormone receptor concordant and discordant cases. This analysis revealed that ODX RS and rates of treatment with chemotherapy were significantly higher in ER and PR discordant cases (positive by IHC and negative by ODX) than in concordant cases, yet no significant differences were observed in recurrence or metastasis rates between ER/PR concordant and discordant cases. All ER discordant cases were high risk by ODX, and all were diagnosed as grade III IDC, except one case notably diagnosed as grade I IDC with apocrine features which was discordant for both ER and PR status. Even though this tumor had a host of favorable prognostic features including low grade by the Nottingham grading system and positive status for both ER and PR by IHC, the high ODX RS of 36 led to chemotherapy treatment for this patient. In addition to being discordant for both ER and PR status between IHC and ODX, some possible causes of this grade I IDC case having a high ODX RS of 36 are low ER (30%) and PR (5%) staining by IHC or differences in morphology between the biopsy and excision specimen sent for ODX testing. This case was grade I on biopsy as it had predominant tubule formation, moderate nuclear pleomorphism, and <5 mitotic figures per 10 high power field. However, on excision, the tumor was grade II with moderate tubule formation and marked nuclear variation in size with prominent nucleoli and mitotic count <5 per 10 HPF. Therefore, the discrepancy in the grading between biopsy and excision suggests heterogeneity of the tumor based on morphology.

To investigate potential features contributing to discordance, this study examined H&E slides from ER discordant case excision specimens sent for ODX testing. Several of these excisions contained numerous tumor infiltrating lymphocytes with significant necrosis or stroma relative to tumor cells, and one slide showed distinct heterogeneity of tumor cells with chondromyxoid material. The presence of

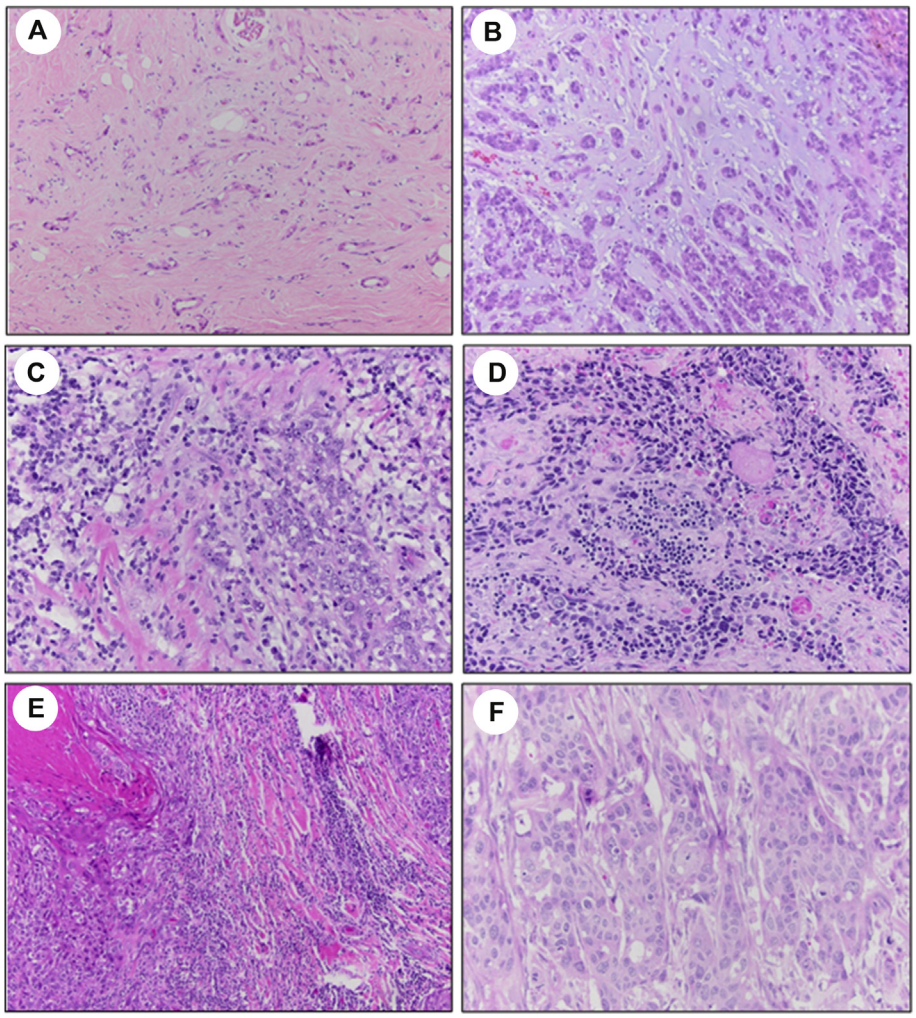

Fig. 1 H&E slide features of excision specimens from IHC/ODX ER discordant cases. A (original magnification × 100), case 446, shows prominent stroma, few tumor cells, and apocrine features. B (× 100), case 581, shows heterogeneous tumor with chondromyxoid material. C (× 400), case 250, shows numerous lymphocytes. D (× 400), case 228, shows numerous lymphocytes with background necrosis. E (× 100), case 221, shows lymphocytic infiltration with areas of focal necrosis. F (× 400), case 373, shows extensive invasive tumor. IHC, immunohistochemistry; ER, estrogen receptor.

many nontumor cells, such as stromal cells, cartilage, or inflammatory cells in the excision slide sent for ODX testing may introduce a large amount of nontumor RNA into the sample being tested and contribute to discordance of reported biomarker status between routine IHC and ODX. Previous work has shown that the presence of inflammatory cells and increased stromal cellularity in samples sent for oncotype testing may lead to artificially elevated ODX RS in low-grade breast cancer [16]. Additionally, consistent with our findings in ER discordant cases, a similar study of HER2 status concordance between fluorescence in situ hybridization (FISH) and ODX implicated heterogeneous HER2 amplification by FISH and small amounts of invasive tumor in the tissue sent for ODX testing as features related to discrepancy in HER2 status [17]. Further, as IHC is typically performed on biopsies, whereas ODX testing is typically performed on excision specimens, and tumor heterogeneity may further amplify discrepancies in results when testing tumor from different specimen types. Preanalytical issues may contribute to discordance of measured biomarker status such as differences in how well these different specimen types are fixed and biopsy site changes because of fibrosis and inflammation. Discordance may also be related to differences in the tests themselves as ODX uses qRT-PCR, whereas IHC works by detecting protein expression.

In this study, we identified higher detection rates of ER and PR expression by IHC compared with ODX as more biomarker-discordant cases were positive by IHC and negative by ODX rather than IHC-negative and ODX-positive. Similar findings implicating higher sensitivity of biomarker detection by IHC relative to ODX have been reported [2,18–20], although one study found that discordant cases were more commonly ER− by IHC and

Table 7 HER2 discordant case clinicopathologic features.

Case index	HER2 (IHC/ISH), Biopsy	HER2 (IHC/ISH), Excision	HER2 (ODX)	Chemo	Recurrence	Mets	ODX score	ER (IHC)	ER intensity	PR (IHC)	PR intensity	ER (ODX)	PR (ODX)
258	IHC: 1+ (−)	NA	10.8 (eq.)	yes	no	no	30	100	s	<1	w	12.5	3.4
339	IHC: 0 (−)	NA	11.4 (eq.)	yes	no	no	22	95	s	50	m	11.4	4.1
240	IHC: 2+ ISH: HER2:CEP17 ratio 3.8; HER2 copy number 5.5; CEP17 copy number 1.5 (+)[a]	ISH: HER2:CEP17 ratio 2.7; HER2 copy number 4.9; CEP17 copy number 1.8 (+)[a]	8.2 (−)	−	no	no	30	95	s	80	s	8.3	6.2
669	-[b]	IHC: 2+ ISH: HER2:CEP17 ratio 1.6; HER2 copy number 6.5; CEP17 copy number 4.1 (+)[a]	9.4 (−)	yes	no	no	35	100	s	5	m	12.5	5.4

Abbreviations: HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization; (−), HER2 negative; (+), HER2 positive; (eq.), HER2 equivocal; ER/PR/HER2 (ODX), estrogen receptor/progesterone receptor/HER2 score by Oncotype DX; chemo, chemotherapy treatment; -, data unavailable; mets, metastasis; w/m/s, weak/moderate/strong staining intensity by IHC.

[a] HER2 status is shown based on 2018 guidelines.

[b] Case 669 had insufficient tumor to perform IHC and ISH on biopsy.

ER+ by ODX [21]. These differences could be related to variations in IHC protocols as the previously mentioned study used less sensitive antibodies and tissue microarray analysis, which involves performing IHC on smaller samples. Further, concordance of reported biomarker status in this study between IHC and ODX was high for ER status (98.1%) and slightly lower for PR status (86.6%), a finding consistent with several other studies reporting ER concordance ranging from 91 to 100% and slightly lower PR concordance ranging from 88 to 94.2% [2,18−21] (Table 8).

Two cases in our study were HER2+ by ISH, and both of these cases were HER2− by ODX. HER2+ cases are not usually sent for ODX testing; however, these cases were because one of the cases (case 669) had insufficient tumor in the biopsy specimen for IHC and ISH testing, whereas the other case (case 240) was HER2 equivocal by IHC on the biopsy specimen and was sent for ODX testing before ISH results could be obtained. ISH results on the excision were HER2+ with a HER2:CEP17 ratio of 2.7 and HER2 copy number 4.9, but by ODX analysis of the same block, the tumor scored strongly negative for HER2. This discrepancy may be due, at least in part, to features observed on reexamination of the excision specimen including abundant fibrosis, tumor infiltrating lymphocytes, and heterogeneity.

Table 8 IHC/Oncotype DX hormone receptor status concordance in other studies.

Study	Number of cases	ER concordance	PR concordance
Neely et al.	610	98.9%	90%
Kraus et al.	464	98.9%	94.2%
Park et al.	265	98.9%	91.3%
O'Connor et al.	80	100%	94%
Badve et al.	776	91%	88%
Present study	591	98.1%	86.6%

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; IHC, immunohistochemistry.

Discrepancies in reported HER2 status as seen in these cases have been repeatedly demonstrated in other studies showing high false-negative rates of HER2 detection by ODX qRT-PCR [17,18,20,22]. In a quality assurance study of HER2 status concordance involving 843 cases, Dabbs et al. identified 23 HER2 equivocal cases by IHC/FISH of which all were reported as HER2− by ODX and 36 HER2+ cases by IHC/FISH of which only 10 were reported as HER2+ by ODX with 12 as HER2 equivocal and 14 as HER2− [22]. In a study of 610 cases by Neely et al. 5 cases were HER2+ by IHC/FISH, but only one of these was reported as HER2+ by ODX, whereas 2 were reported as HER2− and 2 as equivocal [18]. Further, 14 of their 15 HER2 equivocal cases by IHC/FISH were HER2− by ODX [18]. Similarly, Park et al. reported 0% positive agreement for HER2 status between routine testing and ODX [20], and Dvorak et al. reported 50% positive agreement [17]. Possible factors contributing to the large discrepancies in these studies are tumor features, including heterogeneity and low tumor cellularity with extensive nontumor tissue, which may contribute to variability in ODX results between samples as ODX uses PCR-based methodology which measures RNA irrespective of morphology. Thus, the presence of nearby nontumor cells and heterogeneity could affect the quantitative results of ODX, whereas IHC analysis is performed on morphologically intact tissue in which

positive results can be visually confirmed to be derived from the tumor cells. This is not the case for ODX testing as tissue processing for RT-PCR involves homogenizing the tissue for analysis, yielding hormone receptor location and heterogeneity of the tissue undetectable. Discordance of HER2 results may also be because of where cutoffs are applied for ISH versus ODX RT-PCR. Cutoffs are not expected to perfectly align between the 2 testing modalities, thus borderline ISH cases may be discrepant when compared with ODX. Variability in IHC, such as protocol differences for cases sent from outside institutions, as well as variability in ODX testing may also contribute to discrepancy [17,18,20,22].

Some sources of variability in IHC results include differences in fixation, staining interpretation, or antibody clones used. For example, previous studies show that the SP1 ER clone is more sensitive than the 1D5 and 6F11 clones [23,24]. At our institution, we use the SP1 ER antibody clone, and fixation time is standardized between 6 and 72 h, measures which likely minimize variability in IHC results and optimize IHC sensitivity. Similar to IHC, ODX RT-PCR testing also has limitations because of preanalytical factors including variation in probe and primer selection, reagents, RNA extraction, reverse transcription, and PCR protocols and machines [21]. These are all factors that could contribute to discordant results between ODX and IHC. Although each test has its limitations, there are several advantages to using IHC rather than ODX for ER, PR, and HER2 testing. IHC is less expensive with shorter turnaround time, it is easier to perform, it offers better evaluation of morphology, and the results of this study support that it measures ER, PR, and HER2 expression with higher sensitivity. Thus, ODX has high concordance with IHC for ER status and moderate concordance for PR status, but IHC has additional advantages and is clinically validated by prospective trials for routine use in measuring ER, PR, and HER2 status.

The discordance of biomarker status is important clinically as discrepancies in results reported by IHC/FISH and ODX impact individual patients and oncologists. Discrepancies create uncertainty among clinicians about which test results to use which could lead to suboptimal treatment. ER, PR, and HER2 status are strong predictors of treatment response in women with breast cancer. Semiquantitative IHC and FISH are the techniques validated by ASCO/CAP to measure expression of these biomarkers, and the routine utilization of IHC and FISH for measuring ER, PR, and HER2 status has undergone validation in clinical trials [25−28]. More recently, ODX RT-PCR molecular testing reports began to include measures of ER, PR, and HER2 expression, but biomarker expression measured by ODX RT-PCR has not been similarly validated in prospective trials. This is important clinically as there are reported cases of patients not receiving trastuzumab because of negative HER2 status by ODX and not receiving hormonal therapy because of negative ER status by ODX despite positive status by routine IHC/FISH testing [20]. This is additionally problematic given that the Oncotype RS was validated for use in ER+ breast cancer only [11]. Further, ASCO/CAP guidelines recommend a cutoff of $\geq$1% staining by IHC for reporting positive hormone receptor status as tumors with as few as 1% positive staining confer better prognosis with hormonal therapy than those with <1% ER/PR staining [25]. Thus, it is critical to detect hormone receptor status with high sensitivity for each individual patient not only to accurately predict RS and benefit from chemotherapy treatment but also to guide hormonal and targeted therapies. Similarly, as ER and PR expression levels have been shown to correlate with time to recurrence [29], an individual's particular levels of ER and PR expression determine their benefit from hormonal therapy, which must be weighed with an individual's risk factors for experiencing side-effects of hormonal therapy. In our study, ER expression varied from 5 to 75% in the ER discordant (IHC+/ODX-) group.

Additionally, as PR IHC provides prognostic information for ER+ breast cancer, and PR negativity is associated with higher ODX risk scores, our PR discordance results suggest that IHC for PR is helpful to capture a more complete picture of risk particularly in cases for which PR is discordant between IHC and ODX when ODX risk score may be falsely elevated. PR status by IHC also provides useful information for ER− cases to determine if these patients might still benefit from hormonal therapy. Although ER status is a well-known and important predictor of benefit from hormonal therapy, there is evidence that ER status alone does not adequately allow for selection of patients who could benefit from hormonal therapy. One study showed that PR positivity by IHC in ER− cases further increased benefit from tamoxifen with 54% risk reduction compared to 21% risk reduction for ER−/PR− cases [14]. Therefore, to better select which patients should receive hormonal therapy, both ER and PR expression measured by IHC should be determined.

The main shortcoming of this study is that it is retrospective and thus not experimentally controlled to fully elucidate the relationship between biomarker status discordance and outcomes. Specifically, many of the ER/PR discordant cases were high risk by ODX RS and thus received chemotherapy which may have impacted observed rates of recurrence and metastasis. Additionally, variability in ODX results in this study may be because of excision features such as those identified on ER discordant H&E slides including marked tumor heterogeneity, necrosis, limited tumor cellularity of the sample undergoing testing, or presence of numerous cells with non-tumor RNA.

This study determines ER, PR, and HER2 concordance between IHC and ODX for cases at University Hospitals Cleveland Medical Center, providing quality assurance for our institution as well as contributing 591 additional cases to the literature. In comparison to similar previous studies, our study is unique as it adds in depth analysis of clinicopathologic features, including age, Oncotype risk score, treatment, recurrence, and metastasis, comparing clinical

features and outcomes data between concordant and discordant cases to investigate the clinical impact of discordance. It also examines these features more closely for each individual discordant case alongside IHC and ODX expression values. This study also provides additional examination of histologic features present in ER discordant case excision specimens which may predict discordance.

In conclusion, at our institution, ODX and IHC displayed high concordance for ER status and moderate concordance for PR status, with more sensitive detection by IHC. HER2 concordance between ODX and IHC/ISH was high for HER2− cases, whereas 2 HER2+ cases by ISH were both reported as HER2− by ODX, consistent with more sensitive detection by ISH similarly reported in other studies. High ODX RS of discordant cases suggest possible risk overestimation without significant differences in rates of recurrence or metastasis between concordant and discordant cases. Our findings suggest that discordance between IHC/ISH for ER, PR, and HER2 and ODX parameters is rare, but does occur, and analyzing the reasons for differences may help to explain the discrepancy. Further study is needed to investigate the role of specimen type in discordance, and therapeutic decisions for discordant cases should continue to be based on clinicopathologic correlation and not oncotype alone.

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