Pathologist's Practice Patterns in Breast Cancer Biomarker Testing After Neoadjuvant Chemotherapy

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Background:

• Neoadjuvant chemotherapy (NAC) prior to surgical excision is associated with decreased tumor size and improved surgical outcomes in the treatment of breast cancer in a subset of patients.

• Previous studies report changes in biomarker status following NAC; however, there are currently no guidelines regarding repeat testing, and it is performed at the discretion of the attending pathologist.

• Our aim was to evaluate the impact of tumor characteristics on post-NAC repeat testing and repeat testing practice patterns among pathologists.

Design:

• A retrospective search identified cases from 2012-2015 with invasive breast carcinoma diagnosed via biopsy and subsequently treated with NAC and surgical resection.

• Biomarker studies for each needle core biopsy included estrogen receptor (ER) immunohistochemistry (IHC), progesterone receptor (PR) IHC, and both HER2 IHC and HER2 fluorescence in situ hybridization (FISH).

• Repeat testing of at least 1 biomarker was performed on a subset of excisions per pathologist's preference.

• Tumor characteristics of cases with repeat testing were compared to those without repeat for the cohort and per pathologist.

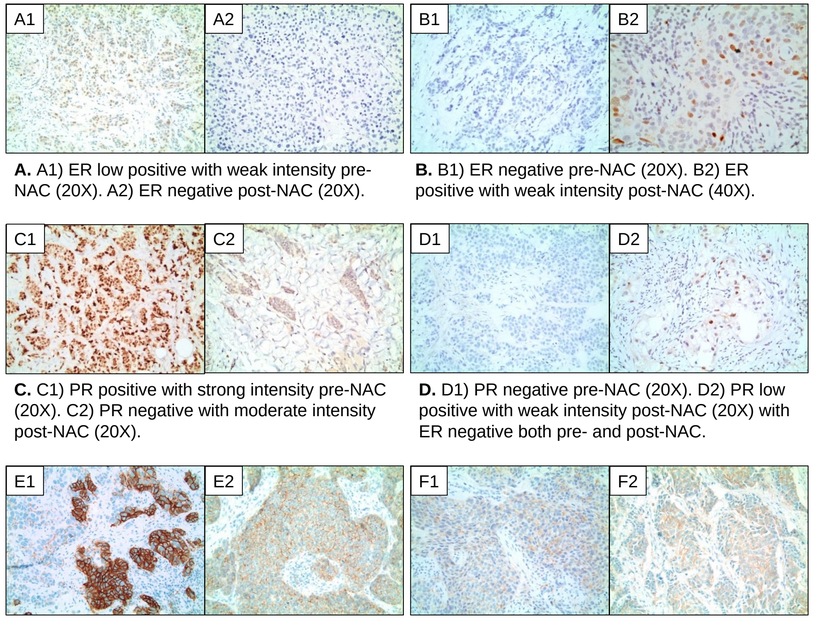
• Statistical analysis was performed via unequal variances 2 tailed 2 sample T testing and $\chi 2$ testing.

Table 1. Tumor characteristics of cases without and with repeat biomarker testing.

		Biopsy				Resection	
	Age (y)	Median Grade	ER Negative	PR Negative	HER2 Negative	≥ypT1a	≥ypN1mi
No Repeated Biomarkers (n = 69)	51.5	3	45%	54%	68%	75%*	54%
Biomarkers Repeated							
ER (n = 75)	51.8	3	47%	60%	77%	92%*	55%
PR (n = 73)	52.0	3	48%	60%	79%	93%*	53%
HER2 (n = 77)	52.2	3	51%	60%	84%	94%*	56%

* Statistically significant ($p \leq 0.01$)

Figure 1. Photomicrographs of biomarkers pre- and post-NAC.



A. A1) ER low positive with weak intensity pre-NAC (20X). A2) ER negative post-NAC (20X).

B. B1) ER negative pre-NAC (20X). B2) ER positive with weak intensity post-NAC (40X).

C. C1) PR positive with strong intensity pre-NAC (20X). C2) PR negative with moderate intensity post-NAC (20X).

D. D1) PR negative pre-NAC (20X). D2) PR low positive with weak intensity post-NAC (20X) with ER negative both pre- and post-NAC.

E. E1) HER2 positive (IHC 3+; FISH ratio 3.9, copy 12.4) with heterogeneity (weak staining on left, strong staining on right) pre-NAC (20X). E2) HER2 equivocal (IHC 2+; FISH ratio 1.43, copy 4.6) post-NAC (20X).

E. F1) HER2 negative (IHC 1+; FISH ratio 1.98, copy 3.2) pre-NAC (20X). F2) HER2 equivocal by IHC but positive by FISH (IHC 2+; FISH ratio 2.4, copy 3.6) post-NAC (20X).

Table 2. Biomarker status of pre-NAC biopsy cases.

Pre-NAC Biomarker Status	% (n) without repeat	% (n) with repeat
ER+/PR+/HER2-	36% (25)	27% (22)
ER+/PR-/HER2-	1% (1)	10% (8)
ER-/PR-/HER2+	12% (8)	5% (4)
ER+/HER2+	17% (12)	13% (11)
ER-/PR-/HER2-	32% (22)	42% (35)
Other	1% (1)	4% (3)

Table 3. Clinically significant biomarker status changes.

Patient	Change	Pre-NAC Biopsy	Post-NAC Resection	Therapy Change	Adjuvant Addition
1	ER- to ER+	ER- (0%)	ER+ (15%, weak)	Y	Anastrazole
2	ER- to ER+	ER- (0%)	ER+ (2%, weak)	Y	Tamoxifen
3	PR- to PR+ (with ER- in Bx)	PR- (0%)	PR+ (1%, weak)	Y	Tamoxifen
4	PR- to PR+ (with ER- in Bx)	PR- (0%)	PR+ (30%, weak)	D	Endocrine therapy delayed due to pregnancy
5	HER2- to HER2+	HER2- (IHC 1+; FISH ratio 1.98, copy 3.2)	HER2+ (IHC 2+; FISH ratio 2.4, copy 3.6)	Y	Trastuzumab

Table 4. Comparison of pathologist practice patterns regarding tumor characteristics in cases without repeat biomarker testing vs with repeat.

		Biopsy		Resection	
Pathologist	Age (y)	Tumor Grade	Triple Positive	≥ypT1a	≥ypN1mi
1 & 2					
Biomarkers Not Repeated (n = 7)	50.0	3	57%*	57%*	43%
Biomarkers Repeated (n = 38)	55.1	3	13%*	92%*	61%
3 & 4					
Biomarkers Not Repeated (n = 23)	52.1	2	9%	83%	70%
Biomarkers Repeated (n = 9)	49.4	3	0%	78%	44%
5 & 6					
Biomarkers Not Repeated (n = 39)	51.3	3	15%	74%*	41%
Biomarkers Repeated (n = 36)	50.2	3	6%	97%*	50%

* Statistically significant ($p \leq 0.01$)

Results:

• 152 NAC treated breast resections were diagnosed by 6 pathologists (Pathologists 1-6), of which 55% (n = 83) had repeat testing of at least 1 biomarker (**Table 1, Table 2, Figure 1**).

• Clinically significant biomarker status changes included ER- to ER+, PR- to PR+ (with ER- on biopsy), and HER2- to HER2+ (**Table 3**).

• There was no impact of age, grade, triple positivity on biopsy, or size or node status at resection on biomarker stability post-NAC.

• For the cohort, repeat testing was more common in tumors ≥ypT1a (for ER, PR, and HER2, $p \leq 0.01$). There was no impact of patient age, tumor grade, or node status.

• 3 practice patterns of repeat testing were identified ($p \leq 0.01$) (**Table 4**):

1. Repeat testing in the majority of cases (pathologists 1 and 2, 84% repeated 38/45)
2. Repeat testing in the minority of cases (pathologists 3 and 4, 28% repeated, 9/32)
3. Repeat testing in approximately half of cases (pathologists 5 and 6, 48% repeated, 36/75)

• In practice pattern 1 (frequent retesting), tumors without repeat testing were more likely to be triple positive ($p < 0.01$).

• In practice pattern 2 (infrequent retesting), post-NAC tumor size (≥ypT1a) did not impact frequency of repeat testing ($p = 0.76$).

Conclusion:

• Repeat testing was more common in larger tumors (≥ypT1a), but there was significant variability in pathologist practice patterns.

• Further study of factors that predict biomarker stability post-NAC is needed to create guidelines for repeat testing and allow for more uniform testing practices.