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Quantitative measurement of HER2 expression in breast cancers: comparison with "real world" HER2 testing in a multi-center Collaborative Biomarker Study (CBS) and correlation with clinicopathological features

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Abstract

Background: Accurate determination of HER2 status is critical in determining appropriate therapy for breast cancer patients. The HERmark® assay is a novel method to quantitatively measure HER2 total protein expression (H2T) in breast cancer. In this study, we compared HERmark H2T with central laboratory HER2 retesting and local (site reported) HER2 testing of formalin-fixed, paraffin-embedded (FFPE) breast cancer tissues. The quantitative total HER2 measurements (H2T) by HERmark and results of local HER2 tests were correlated with tumor pathohistological characteristics and overall survival of breast cancer patients.

Methods: 232 FFPE breast cancer tissues were provided by 11 CBS study sites for HER2 testing by the HERmark assay and central laboratory IHC re-testing was performed in blinded fashion. Local HER2 immunohistochemistry and/or fluorescence *in situ hybridization* (FISH) results and valid HERmark H2T and central HER2 IHC results were obtained in 192 cases for analysis.

Results: H2T showed a significant correlation with central HER2 IHC staining intensity (P<0.0001). The concordance rates of positive and negative HERmark status (excluding equivocal) with those of local HER2 status determined by the CBS sites, and with those of central HER2 IHC status were 84% (Kappa=0.68) and 96% (Kappa=0.91), respectively. Higher H2T levels significantly correlated with higher tumor grade (P=0.007) and negative ER/PR status (P=0.002). Twenty-six (14%) cases showed discordant (conversion of negative and positive) results between local HER2 status and HERmark status. Of the discordant cases, HERmark significantly agreed with H-score of central HER2 IHC retesting (P=0.014), as compared with local HER2 status. The concordant negative group (*local HER2 negative/H2T low*) demonstrated better overall survival (OS) (HR=0.198, P=0.0001), compared to that of concordant positive group (*local HER2 positive/H2T high*). The concordant negative group also showed better OS than that of discordant *local HER2 negative/H2T high* group (HR=0.065, P=0.0003), but showed no significant difference in OS as compared to that of discordant *local HER2 positive/H2T low* group (HR=1.774, P=0.499). In 24 cases (13%) considered to be "triple negative" by local HER2, ER and PR testing, HERmark re-classified 4 cases (17%) as HER2 positive.

Conclusions: H2T by HERmark yields a continuum of quantitative HER2 protein measurements that shows an excellent correlation with central HER2 IHC retesting and confirms the known correlations between HER2 expression with tumor grade and ER/PR status. OS results of concordant HER2 positive or negative groups (between local HER2 testing and HERmark H2T) confirmed that HER2 positive patients (excluding adjuvant trastuzumab therapy) have worse OS than patients with HER2 negative disease. However, in the HERmark and local HER2 discordant groups, OS appeared to track better with H2T by HERmark and not with the local HER2 status. Novel quantitative HER2 measurements may identify patients with false (+) and (-) HER2 status by local HER2 testing and may provide added clinical value to routine "real world" HER2 testing.

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Quantitative measurement of HER2 expression in breast cancers: comparison with "real world" HER2 testing in a multi-center Collaborative Biomarker Study (CBS) and correlation with clinicopathological features

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Background

Accurate assessment of tumor HER2 status is critical in determining appropriate therapy for breast cancer patients. The HERmark® Breast Cancer Assay is a novel method to quantitatively measure HER2 total protein expression (H2T) in breast cancer. In this multi-center Collaborative Biomarker Study (CBS), we compared HERmark H2T with local (site-reported) HER2 testing and central laboratory HER2 retesting of formalin-fixed, paraffin-embedded (FFPE) breast cancer tissues. The quantitative total HER2 measurements by HERmark and results of local ("real world") HER2 testing were correlated with tumor histopathological characteristics and overall survival of breast cancer patients.

Methods

Collaborative Biomarker Study (CBS)

The HERmark CBS was a retrospective biomarker study with the primary objective of comparing quantitative H2T by HERmark with conventional HER2 testing methods, such as immunohistochemistry (IHC) and/or fluorescence *in situ* hybridization (FISH), and describing concordance and discordance between HER2 testing methods. Secondary objectives of the study included correlating HER2 results by HERmark versus local HER2 testing with clinical outcomes, including outcomes in patients with discordant HER2 results by HERmark versus locally determined HER2 status.

Tissue Samples

Each site was instructed to identify approximately 50% HER2 positive and 50% HER2 negative breast cancer cases for the study. 232 FFPE breast cancer samples, originally collected between January 2000 and May 2005, were freshly cut and prepared as slides by 11 CBS study sites. HER2 testing by the HERmark assay and central laboratory IHC re-testing was performed in blinded fashion. Local HER2 IHC and/or FISH results, site-reported clinical HER2 status (based on IHC or combination of IHC and FISH results), and HERmark H2T and central HER2 IHC results were obtained in 192 cases for analysis. Patient demographic and tumor characteristic data were provided by CBS study sites after completion of the HERmark assay. Subsequent central HER2 IHC re-testing was also performed.

The HERmark® Breast Cancer Assay

H2T was quantified using the HERmark assay as previously described (*Huang et al. Am J Clin Pathol 134:303, 2010*). H2T was quantified through the release of a fluorescent tag ("V" for "VeraTag® reporter," Figure 1) conjugated to a HER2 monoclonal antibody ("Ab8"). The antibody is paired with a biotinylated second HER2 mAb ("Ab15"). Upon illumination with red light, an avidin-linked photosensitizer molecule (PM) produces singlet oxygen ($^1O_2$) which cleaves tags (V) in close proximity. Signals (V) are quantified by capillary electrophoresis and normalized to invasive tumor area on the FFPE tissue section. The continuous H2T results are categorized as HERmark negative, HERmark equivocal, and HERmark positive with pre-defined H2T analytical cutoff values (*Huang et al. Am J Clin Pathol 2010;134:303*) for the determination of HERmark HER2 status. A pre-defined HERmark clinical cutoff (*Lipton et al. Cancer 2010;116:5168*) was used to determine tumor H2T low and H2T high patient groups in overall survival analysis.

Central laboratory HER2 IHC retesting

Central laboratory HER2 IHC retesting was performed by the Center for Molecular Biology and Pathology (CMBP, Laboratory Corporation of America, Inc., Research Triangle Park, NC), using the HercepTest™ (Dako, Glostrup, Denmark).

The HERmark® Assay

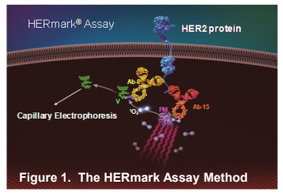


Figure 1. The HERmark Assay Method

Patient / Tumor Characteristics

Parameter	No.	% (range)
Sample Size	**194**	
Median Length of Follow-up (months)	193	67.1 (14.8 - 302.8)
Median Age (yrs)		51 (27 - 84)
< 40	21	11%
40-49	66	34%
50-59	50	26%
≥ 60	57	29%
Menopausal Status		
Premenopausal	75	39%
Perimenopausal	8	4%
Postmenopausal	96	49%
Not reported	15	8%
Tissue Source		
Primary breast	187	96%
Other *	7	4%
Median Tumor Size (cm)	185	2.1 (0.4 - 14)
Not reported	9	
Tumor Grade		
Grade 1 (well)	17	9%
Grade 2 (moderate)	49	25%
Grade 3 (poor)	93	48%
Not reported	35	18%
Stage at Diagnosis		
I	46	24%
II	91	47%
III	40	21%
IV	13	7%
Not reported	4	2%
Nodal Status at Diagnosis		
Node positive	89	46%
Node negative	66	34%
Not reported	39	20%
HER2 Status (reported)		
Positive	83	43%
Negative	110	57%
Equivocal	1	1%
HER2 IHC (reported)		
3+	73	38%
2+	29	15%
IHC 2+ / FISH positive	*2*	*7%*
IHC 2+ / FISH negative	*17*	*59%*
IHC 2+ / FISH N/R	*10*	*34%*
1+	32	16%
0	60	31%
HER2 FISH (reported)		
Positive	24	35%
Negative	44	65%
Hormone Receptor (ER/PR) Status		
Positive	141	73%
Negative	53	27%
ER and PR Status (reported)		
ER (+), PR (+)	110	57%
ER (+), PR (-)	30	15%
ER (-), PR (+)	1	1%
ER (-), PR (-)	53	27%
Adjuvant HER2-targeted Therapy		
No	174	90%
Yes†	20	10%
Metastatic HER2-targeted Therapy		
No	174	90%
Yes‡	20	10%

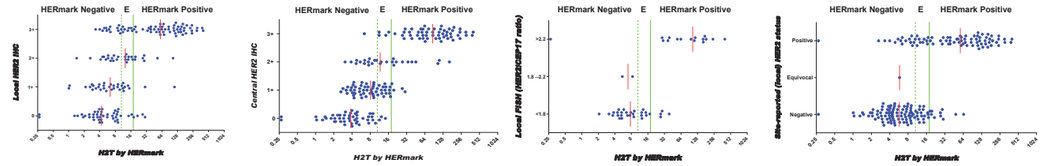
* Other tissue source: skin, supraclavicular, sentinental lymph node, axillary lymph node, ovary, lung, chest wall

† One patient rec'd lapatinib; 19 rec'd trastuzumab

‡ All patients rec'd trastuzumab

Results

Figure 2. Comparison of HERmark with local HER2 IHC, central HER2 IHC, local HER2 FISH, and site-reported HER2 status



* HERmark equivocal (E) zone is defined within the two green vertical lines. Short vertical red line indicates median of a distribution.

Table 1. Concordance of HERmark with local HER2 IHC, central HER2 IHC, local HER2 FISH, and site-reported (local) HER2 status

	Local HER2 IHC								Central HER2 IHC							
	Negative		Equivocal		Positive		Total IHC		Negative		Equivocal		Positive		Total IHC	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
HERmark Negative	63	69%	14	15%	14	15%	91	51%	86	86%	13	13%	1	1%	100	52%
HERmark Equivocal	9	38%	8	33%	7	29%	24	13%	18	75%	4	17%	2	8%	24	13%
HERmark Positive	6	9%	7	11%	52	80%	65	36%	4	6%	23	34%	41	60%	68	35%
Total	*78*	*43%*	*29*	*16%*	*73*	*41%*	*180*	*100%*	*108*	*56%*	*40*	*21%*	*44*	*23%*	*192*	*100%*
Overall concordance	*68% , (63+43+2)/180*								*68% , (86+41)/192*							
Kappa (CI 95%), overall	*0.483 (0.380 to 0.587); Weighted Kappa= 0.561*								*0.470 (0.375 to 0.566); Weighted Kappa= 0.626*							
*Concordance, excluding Eqv.**	*85% , (63+52)/(63+6+14+52)*								*96% , (86+41)/(86+4+1+41)*							
Kappa (CI 95%), excluding Eqv.	*0.703 (0.583 to 0.822)*								*0.914 (0.841 to 0.988)*							

	Local HER2 FISH (HER2/CEP17 ratio)								Site-reported clinical HER2 status							
	<1.8		1.8-2.2		>2.2		Total FISH		Negative		Equivocal		Positive		Total HER2	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
HERmark Negative	19	86%	2	9%	1	5%	22	46%	83	83%	1	1%	16	16%	100	52%
HERmark Equivocal	7	100%	0	0%	0	0%	7	15%	16	67%	0	0%	8	33%	24	13%
HERmark Positive	4	21%	0	0%	15	79%	19	40%	10	15%	0	0%	58	85%	68	35%
Total	*30*	*63%*	*2*	*4%*	*16*	*33%*	*48*	*100%*	*109*	*57%*	*1*	*1%*	*82*	*43%*	*192*	*100%*
Overall concordance	*77% , (19+0+15)/48*								*73% , (83+0+56)/192*							
Kappa (CI 95%), overall	*0.493 (0.295 to 0.691); Weighted Kappa= 0.594*								*0.519 (0.417 to 0.621); Weighted Kappa= 0.589*							
*Concordance, excluding Eqv.**	*87% , (19+15)/(19+4+1+15)*								*84% , (83+58)/(83+10+16+58)*							
Kappa (CI 95%), excluding Eqv.	*0.742 (0.534 to 0.951)*								*0.682 (0.570 to 0.794)*							

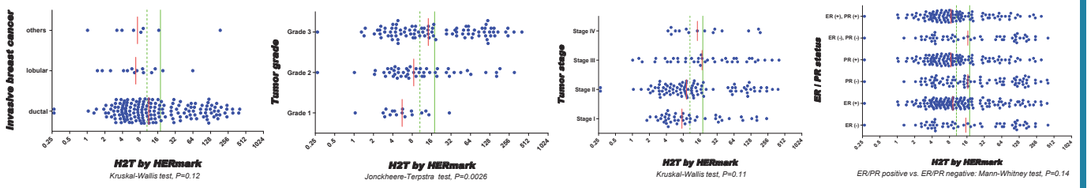
* Equivocal (Eqv.) cases from both tests were excluded. - Central IHC status is defined per ASCO/CAP guidelines for HER2 testing (Wolff et al. JCO 2007;25:118). - Site-reported clinical HER2 status was submitted by study site as patient's final HER2 status based on local IHC and/or local FISH.

Table 2. HER2 reclassification of site-reported triple negative* cases by HERmark

	N	%
HERmark negative	19	79%
HERmark equivocal	1	4%
HERmark positive	4	17%
Total	*24*	*100%*

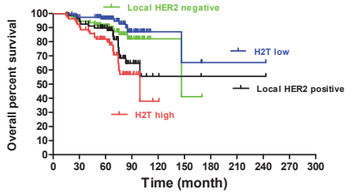
* 13% (24/192) cases of this cohort were determined as triple negative (HER2 -, ER -, PR-) by study sites.

Figure 3. Correlation of HERmark H2T with tumor histologic type, tumor grade, tumor stage, and ER/PR status.



Kruskal-Wallis test, P=0.12 | Jonckheere-Terpstra test, P=0.0026 | Kruskal-Wallis test, P=0.11 | ER/PR positive vs. ER/PR negative: Mann-Whitney test, P=0.14

Figure 4. Overall Survival (OS) by local HER2 status vs. HERmark H2T

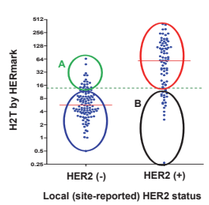


Survival Comparison	HR	P (log-rank test)
HER2 positive vs. HER2 negative	1.778	0.0984
H2T high vs. H2T low	5.614	<0.0001

Patients	N	Median Survival (month)
HER2 positive	82	Undefined
HER2 negative	108	146.7
H2T high	80	99.1
H2T low	110	Undefined

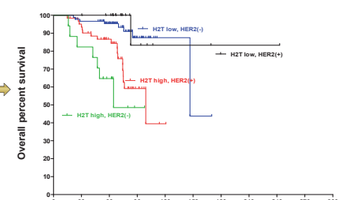
- Local ("real-world") HER2 status (HER2 negative or positive) was determined and reported by study site using IHC, FISH, or both assays, at physician's discretion.
- H2T low and H2T high were defined by a pre-determined H2T clinical cutoff of 13.8 (*Lipton et al. Cancer 2010;116:5168*).
- Undefined = median (50%) overall survival not yet reached.

Figure 5. H2T vs. local HER2 status



Patient groups	HER2 testing results	N	%
Concordant positive	H2T high, HER2 (+)	63	33%
Concordant negative	H2T low, HER2 (-)	91	48%
Discordant group A	H2T high, HER2 (-)	17	9%
Discordant group B	H2T low, HER2 (+)	19	10%
Total		190	100%

Figure 6. Overall Survival (OS) by HER2 groups



Survival Comparison	HER2 groups	HR	P (log-rank test)
H2T high, HER2 (+) vs. H2T low, HER2 (-)	Concordant positive vs. Concordant negative	4.733	0.0002
H2T high, HER2 (+) vs. H2T high, HER2 (-)	Concordant positive vs. Discordant group A	0.4112	0.1064
H2T high, HER2 (+) vs. H2T low, HER2 (+)	Concordant positive vs. Discordant group B	3.209	0.0237
H2T high, HER2 (-) vs. H2T low, HER2 (-)	Discordant group A vs. Concordant negative	41.54	<0.0001
H2T low, HER2 (+) vs. H2T low, HER2 (-)	Discordant group B vs. Concordant negative	0.8245	0.4435

Results

- The HERmark assay provides quantitative measurement of total HER2 expression (H2T) over a wide dynamic range (~ 3 logs).
- HERmark showed good general concordance with routine "real-world" HER2 testing (IHC and FISH) (Table 1).
- However, as expected, concordance (excluding equivocal) between HERmark and central lab IHC was higher compared to that between HERmark and local IHC (96% and 85% concordant, respectively) (Table 1).
- Of the 24 (13%) triple negative cases as reported at the local level (HER2 negative, ER negative, and PR negative), 4 (17%) were reclassified as HERmark HER2 positive (Table 2).
- Higher H2T levels correlated with higher tumor grade (JT test, P=0.0026) and trended with negative ER/PR status (Mann-Whitney P=0.14) (Figure 3).
- High H2T (>13.8) by HERmark significantly correlated (HR=5.6, P<0.001) with poor overall survival (OS) whereas HER2 positive status by routine (local testing) only trended with OS (HR 1.78, P=0.098) in this cohort of breast cancer patients, most of whom (90%) did not receive HER2 targeted therapy (Figure 4).
- The observed discrepancy in OS based on different HER2 classification methods (Figure 4) appears to be due to misclassification of HER2 status by routine (local) testing (Figure 5).
- In HER2 status discordant cases (Figure 6, green and black lines) between real-world (local) HER2 status and HERmark H2T, H2T appears to be more accurate as indicated by better correlation with OS (prognostic) between H2T and concordant HER2 status, compared with that between local HER2 status and concordant HER2 status.

Conclusions

- Our study confirms prior reports that HER2 status determined by central lab testing appears to be more reliable than local "real world" HER2 results.
- Quantitative HER2 total protein expression (H2T) by HERmark enriched the identification of both HER2 positive and negative breast cancers in this study and may provide added clinical value to "real world" HER2 testing.
- Poor overall survival noted in the high H2T discordant cases may identify a cohort of HER2 positive breast cancers (local HER2 negative) that could benefit from HER2-targeted therapies. Future trials to test this hypothesis are warranted.

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