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Toward standard HER2 testing of endometrial serous carcinoma: 4-year experience at a large academic center and recommendations for clinical practice

Natalia Buza[1], Diana P English[2], Alessandro D Santin[2] and Pei Hui[1]

[1]Department of Pathology, Yale University School of Medicine, New Haven, CT, USA and [2]Department of Obstetrics, Gynecology, and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA

HER2 overexpression and/or amplification have been reported in endometrial serous carcinoma, suggesting that HER2 may be a promising therapeutic target. However, there is considerable variation in the reported rates of HER2 overexpression and amplification, likely—at least in part—resulting from variability in the testing methods, interpretation, and scoring criteria used. Unlike in breast and gastric cancer, currently there are no established guidelines for HER2 testing in endometrial carcinoma. A total of 108 endometrial carcinoma cases—85 pure serous carcinomas and 23 mixed endometrial carcinomas with serous component—were identified over a 4-year period. All H&E and HER2 immunohistochemical slides were reviewed and HER2 FISH results (available on 52 cases) were retrieved from pathology reports. HER2 immunohistochemical scores were assigned according to the FDA criteria and the current breast ASCO/CAP scoring criteria. Clinical information was retrieved from the patients' medical records. Thirty-eight cases (35%) showed HER2 overexpression and/or gene amplification, 20 of which (53%) had significant heterogeneity of protein expression by immunohistochemistry. Lack of apical membrane staining resulting in a lateral/basolateral staining pattern was observed in the majority of HER2-positive tumors. Five of the HER2-positive cases (13%) demonstrated discrepant immunohistochemical scores when using the FDA *versus* ASCO/CAP scoring system. The overall concordance rate between HER2 immunohistochemistry and FISH was 75% (39/52) when using the FDA criteria, compared with 81% (42/52) by the ASCO/CAP scoring system. In conclusion, in this largest comprehensive study, 35% of endometrial serous carcinoma harbors HER2 protein overexpression and/or gene amplification, over half of which demonstrate significant heterogeneity of protein expression. The current breast ASCO/CAP scoring criteria provide the highest concordance between immunohistochemistry and FISH. Assessment of HER2 immunohistochemistry on multiple tumor sections or sections with large tumor areas is recommended, due to the significant heterogeneity of HER2 protein expression.
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The significance of human epidermal growth factor receptor 2 (HER2/*Neu*, ERBB2) amplification and HER2 protein overexpression has been well established in the pathogenesis and targeted therapy of breast cancer and more recently in gastric and gastroesophageal junction carcinomas.[1–3] Tumor-specific HER2 testing guidelines have been developed to reflect the unique biological features of each of these tumor types and to predict the clinical response to HER2-targeted therapy.[4–7] Although HER2/*neu* has been studied in endometrial cancer for over 15 years, standard testing methods or scoring guidelines are yet to be developed. The reported rates of HER2 overexpression in endometrial serous carcinoma range between 14 and 80%, due to—at least in part—the significant variation in the HER2 testing

Correspondence: Dr N Buza, MD, Department of Pathology, Yale University School of Medicine, 310 Cedar Street LH 108, PO Box 208023, New Haven, CT 06520-8023, USA.
E-mail: natalia.buza@yale.edu
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methods, interpretation and scoring criteria used[8–24] (Buza *et al*, in press).

Serous endometrial carcinoma is the most biologically aggressive variant of endometrial cancer with a high recurrence rate and relative resistance to conventional chemotherapy, presenting a significant therapeutic challenge to oncologists.[25–27] In the era of precision cancer therapy, there is a pressing need to identify more effective, targeted treatment for patients with serous carcinoma to reduce the mortality and potentially reduce the morbidity associated with traditional therapies. The reported high rates of HER2 overexpression in endometrial serous carcinoma and the clinical success of HER2-based therapies in other malignancies would make HER2 a promising drug target.

Although *in vivo* clinical activity of trastuzumab (monoclonal antibody against HER2/neu) has been demonstrated in case reports of recurrent endometrial carcinoma,[12,28,29] to date no significant benefit was observed in clinical trials.[30] The results of these trials, however, have been criticized primarily on the basis of patient selection.[31] The importance of appropriate pre-selection of patients in clinical trials evaluating targeted therapies have been emphasized by experts, arguing that using a therapeutic agent in an unselected patient population may potentially lead to incorrect classification of a drug as inactive.[32] A randomized phase II study is currently enrolling patients at multiple institutions in the United States evaluating carboplatin/paclitaxel with or without trastuzumab in patients with advanced or recurrent HER2-positive—3+ immunohistochemistry or FISH amplified—endometrial serous carcinoma (*NCT01367002*). Pathologists have a pivotal role in the patient selection process by evaluating the HER2 status of tumors; therefore, standardized endometrial cancer-specific testing, scoring and reporting guidelines need to be developed.

This study was designed to systemically evaluate HER2 overexpression and amplification based on our 4-year experience of HER2 testing of endometrial serous carcinoma and to develop specific recommendations for assessing the HER2 status in endometrial cancers.

Materials and methods

Text search for endometrial serous carcinoma cases with known HER2 status between July 2008 and August 2012 was performed in our departmental archives. All H&E and immunohistochemical slides were reviewed by two gynecologic pathologists (NB and PH). Tumor stage (per FIGO 2009) and HER2 FISH results were obtained from surgical pathology reports. Herceptest Kit (DAKO, Carpinteria, CA, USA) was used for HER2 immunohistochemical stain in all cases, and PathVysion (Abbott) Kit was used for HER2 FISH evaluation of all cases with an original immunohistochemical score of 2+ and in a small number of immunohistochemical scores of 0, 1+ and 3+ cases.

HER2 immunohistochemical slides were systematically reviewed—blinded to the originally reported HER2 scores and FISH results—to assess the percentage of tumor cells with complete and incomplete membrane staining, staining intensity (weak, moderate, strong) and staining heterogeneity. Immunohistochemical staining heterogeneity was defined by the presence of at least two-degree difference in staining intensity (none to moderate, weak to strong or none to strong) involving at least 5% of tumor cells. HER2 scores were reassigned both per the original United States Food and Drug Administration (FDA) criteria (previous breast scoring criteria)[33] and per the current American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines for breast cancer[4] (Table 1). HER2 amplification by FISH was defined as HER2 to chromosome enumeration probe 17 (CEP17) ratio of $\geq$2.0. Clinical information was retrieved from the patients' medical records. Chi-square test was used for statistical analysis. A P value of <0.05 was considered statistically significant.

Results

A total of 108 endometrial carcinomas were included in the final study cohort, including 85 pure serous carcinomas and 23 mixed endometrial carcinomas with a serous component. Fifty-two of these cases (48%) had available HER2 FISH results, including all HER2 immunohistochemistry 2+ cases.

Thirty-eight cases (35%; 38/108) had HER2 overexpression either by the FDA or ASCO/CAP 2007 scoring criteria—and/or gene amplification by FISH (Table 2, Figure 1). Five of these 38 cases (13%) showed discrepant immunohistochemical scores when using the FDA *versus* the ASCO/CAP scoring criteria: 30 were scored as 3+ per the FDA, whereas only 25 cases fell in that category based on the new ASCO/CAP guidelines and the remaining 5 cases were scored as 2+, as the percentage of tumor cells with intense membrane staining fell between 10 and 30%.

The overall concordance rate between HER2 immunohistochemistry and FISH was 75% (39 of 52 cases) when using the FDA criteria, compared with 81% (42 of 52 cases) for the ASCO/CAP scoring system (Tables 2 and 3). However, when the HER2 immunohistochemistry 2+ cases were excluded, the concordance rates increased to 78% (28/36) using the FDA criteria and 86% (25/29) using the ASCO/CAP criteria. Tumors with equivocal (2+) HER2 immunohistochemical scores showed HER2 gene amplification in 31% (5/16) and 26% (6/23) of cases when using the FDA and ASCO/CAP scoring criteria, respectively.

Table 3 provides detailed information on cases with discordant HER2 immunohistochemistry and FISH results. Three cases (case nos. 1, 2 and 3) with an immunohistochemical score of 1+ (by both scoring criteria) were amplified by FISH (HER2/CEP17 ratios ranging from 2 to 2.6). Five cases with a HER2 3+ immunohistochemical score per the FDA criteria and one 3+ immunohistochemistry case per the ASCO/CAP criteria had no HER2 gene amplification by FISH (HER2/CEP17 ratios ranging from 0.85 to 1.7). Four of these cases (case nos. 9, 11, 12 and 13) showed significant heterogeneity of HER2 protein expression by immunohistochemistry, which may have contributed to the discordant FISH results. Similarly, three of the 2+ immunohistochemistry (by both scoring criteria) cases (case nos. 4, 5 and 7) with HER2 gene amplification demonstrated heterogeneous HER2 protein expression.

Heterogeneous HER2 protein expression by immunohistochemistry was present in 33 cases (31%), including 20 HER2-positive cases (20/38; 53%) and 13 HER2-negative cases (13/70; 19%) (Table 4). The percentage of cells with strong, complete membrane staining in cases with heterogeneity ranged between 5 and 80%. Lateral or basolateral ('U-shaped') membranous protein expression pattern—resulting from lack of apical staining—was encountered (at least focally) in majority of the HER2-positive cases (74%; 28/38 cases) where the tumor demonstrated a glandular (or 'pseudoglandular') architecture (Figure 2). By contrast, cases with a complete membranous staining pattern generally had a solid growth pattern.

Mixed endometrial carcinomas with a serous component were found to be less frequently HER2 positive than pure serous carcinoma (22 *versus* 39%, respectively), although the difference did not reach statistical significance ($P=0.064$). Four of the five HER2-positive mixed endometrial tumors had HER2 immunostaining available on the non-serous components. One of these cases showed a 3+ immunohistochemical score (by both scoring criteria) in the non-serous (endometrioid and undifferentiated carcinoma) component, similar to the serous component. Two cases showed decreased immunostaining

Table 2 HER2 immunohistochemical score distribution and correlation with HER2 FISH results

HER2 IHC score	*FDA scoring criteria,* n	*FISH amplified,* n	*FISH not amplified,* n	*FISH not done,* n
0	19	0	4	15
1+	43	3	14	26
2+	16	5	11	0
3+	30	10	5	15

HER2 IHC score	*ASCO/CAP scoring criteria,* n	*FISH amplified,* n	*FISH not amplified,* n	*FISH not done,* n
0	17	0	3	14
1+	43	3	13	27
2+	23	6	17	0
3+	25	9	1	15

Abbreviations: ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; FDA, Food and Drug Administration; FISH, fluorescent *in situ* hybridization; IHC, immunohistochemistry.

Table 1 Comparison of HER2 immunohistochemistry scoring criteria in breast and gastric cancer

HER2 IHC score	*Breast Ca (FDA; Herceptest package insert)*	*Breast Ca (ASCO/CAP 2007)*	*Gastric/GEJ Ca (ToGA trial)*
0	No staining is observed, or membrane staining is observed in <10% of tumor cells	No staining is observed in invasive tumor cells	No reactivity, or membranous reactivity in <10% of cells (resections) or in <5 clustered tumor cells (biopsies)
1+	Faint/barely perceptible membrane staining in >10% of tumor cells. The cells exhibit incomplete membrane staining	Weak, incomplete membrane staining in any proportion of invasive tumor cells or weak, complete membrane staining in <10% of cells	Faint/barely perceptible membrane staining in ≥10% of tumor cells; cells are only reactive in part of their membrane (resections). Tumor cell cluster (≥5 cells) with a faint/barely perceptible membranous reactivity (biopsies)
2+	Weak-to-moderate complete membrane staining in >10% of tumor cells	Complete membrane staining that is non-uniform or weak but with obvious circumferential distribution in at least 10% of cells or intense complete membrane staining in ≤30% of tumor cells	Weak-to-moderate complete, basolateral or lateral membranous reactivity in ≥10% of tumor cells (resections) or in a tumor cell cluster (≥5 cells) (biopsies)
3+	Strong, complete membrane staining in >10% of tumor cells	Uniform intense membrane staining in >30% of invasive tumor cells	Strong complete, basolateral or lateral membranous reactivity in ≥10% of tumor cells (resections). Tumor cell cluster (≥5 cells) with a strong complete, basolateral or lateral membranous staining, irrespective of the percentage of tumor cells stained (biopsies)

Abbreviations: ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; FDA, Food and Drug Administration; GEJ, gastro-esophageal junction; IHC, immunohistochemistry; ToGA, Trastuzumab for gastric cancer.

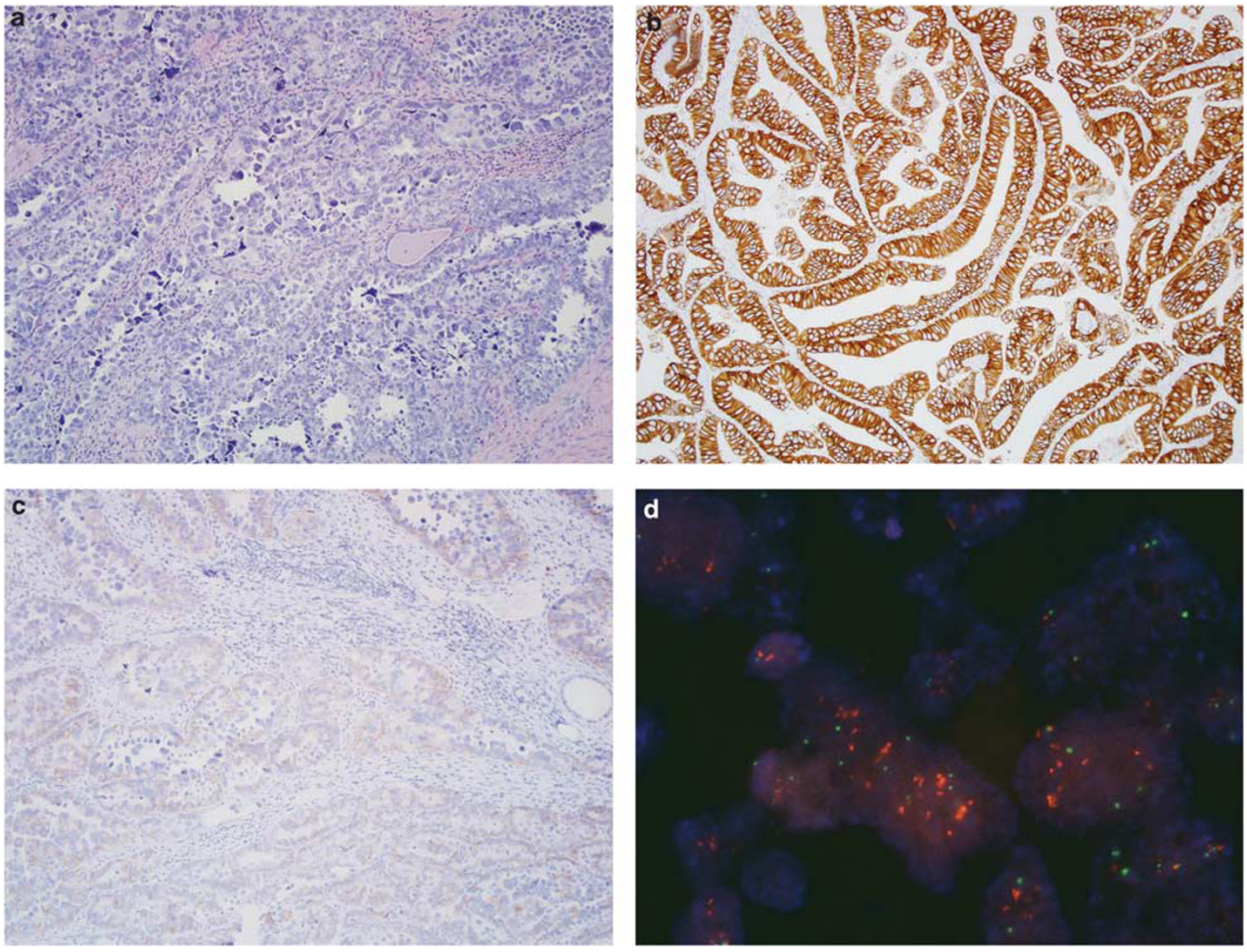

Figure 1 Histomorphology, HER2 protein expression and HER2 gene amplification in endometrial serous carcinoma. (**a**) Characteristic morphology of serous carcinoma, displaying predominantly glandular architecture with high grade nuclei (H&E stain). (**b**) Diffuse complete and basolateral membranous HER2 immunostaining (3+). (**c**) Weak, incomplete membranous HER2 immunostaining in >10% of tumor cells (1+). (**d**) HER2 gene amplification by FISH; HER2:CEP17 ratio = 3.4.

(2+ by both scoring criteria) in the non-serous (undifferentiated and clear cell) components compared with the serous carcinoma (3+). One tumor had a HER2-positive endometrioid (3+) component and decreased staining of the undifferentiated component (2+ by both scoring criteria). No significant difference was observed in the HER2 staining intensity between the different components of the HER2-negative mixed tumors.

No significant statistical correlation was observed between the patients' ethnic origin or tumor stage and HER2 status ($P = 0.098$ and 0.384, respectively; Tables 5 and 6).

Discussion

The current study constitutes the largest series of comprehensive, systematic evaluation of HER2 status in endometrial serous carcinoma to date, with two major findings of clinical relevance: (1) an overall 35% of cases had HER2 protein overexpression and/or gene amplification; and (2) heterogeneity of HER2 protein expression was observed in 53% of immunohistochemistry-positive cases, which has not been previously reported in the literature.

Previous studies evaluating the HER2 status in endometrial carcinomas suffered from several limitations, including inappropriate case selection and lack of standardized HER2 testing and scoring criteria, leading to a wide range—14–80%—of reported HER2 positivity in endometrial serous carcinoma[8–24] (Buza *et al*, in press). One of the largest studies—a phase II clinical trial of single-agent trastuzumab in advanced or recurrent endometrial carcinoma—for example, has been previously criticized for including a large number of type I and mixed endometrial carcinomas, and the histological subtype was not specified in nearly half of the cases.[30,31] Many other studies also included various histological subtypes and grades of endometrial carcinoma, and even malignant mixed mullerian tumors (carcinosarcomas) in the

Table 3 HER2-positive cases with discrepant HER2 immunohistochemistry and FISH results

					HER2 IHC						*HER2 FISH*	
Case No.	*Race*	*Age (year)*	*Histology*	*FIGO stage*	*% Complete membrane staining*	*% Incomplete staining*	*Staining intensity*	*Heterogeneity*	*HER2 score (FDA)*	*HER2 score (ASCO/CAP)*	*Amplification*	*HER2/CEP17 ratio*
1	C	81	SC	1	0	10%	W	No	1+	1+	Yes	2.6
2	C	71	SC	4	0%	80%	W	No	1+	1+	Yes	2.5
3	C	50	SC	1	0	60%	W	No	1+	1+	Yes	2.0
4	O	68	SC	3	10%	0	M	Yes	2+	2+	Yes	3.3
5	C	70	SC	4	20%	30%	W-M	Yes	2+	2+	Yes	2.0
6	C	75	SC	1	90%	10%	W-M	No	2+	2+	Yes	2.7
7	C	78	SC	4	30%	50%	M	Yes	2+	2+	Yes	3.4
8	C	84	SC	3	20%	80%	W-M	No	2+	2+	Yes	2.1
9	C	76	SC	1	20% S, 70% W-M	10%	W-S	Yes	3+	2+	No	1.7
10	C	61	SC	3	50% (20% S, 30% M)	50%	W-S	Yes	3+	2+	Yes	2.5
11	C	75	SC	4	15%	85%	W-S	Yes	3+	2+	No	0.85
12	AA	62	SC	3	15%	85%	W-S	Yes	3+	2+	No	1.7
13	C	74	SC	1	30%	70%	M-S	Yes	3+	2+	No	1.2
14	C	62	SC	1	40%	60%	S	No	3+	3+	No	1.4

Abbreviations: AA, African-American; ASCO, American Society of Clinical Oncology; C, Caucasian; CAP, College of American Pathologists; FDA, Food and Drug Administration; FISH, fluorescent *in situ* hybridization; IHC, immunohistochemistry; M, moderate; O, other; S, strong; SC, serous carcinoma; W, weak.

Table 4 HER2 immunohistochemistry heterogeneity

Total cases	*Positive cases*	*Negative cases*
33/108 (31%)	20/38 (53%)	13/70 (19%)

same analysis, making the interpretation of data difficult.[14–16,34,35] Several previous studies used non-FDA-approved HER2 antibodies, ie, c-erbB-2 clone A0485 (DAKO),[24,35,36] c-erbB-2/Her-2/neu Ab17 (monoclonal antibodies e2-4001 and 3B5; Neomarkers)[8] and clone TAB250 (Zymed).[16,35] Although most studies used the FDA scoring criteria for HER2 immunohistochemistry,[8,9,13–15,18,22,24,30,35,36] many of them considered both the 2+ and 3+ immunohistochemical scores positive for HER2 overexpression.[8,13,15,30,35,36] Others used self-developed semiquantitative immunohistochemical scoring systems, not currently approved for any other tumor types.[10,12,16,20] Application of the updated breast HER2 immunohistochemical scoring system (ASCO/CAP 2007) has not been previously assessed in endometrial cancer.

In this study we used standard FDA-approved test kits—Herceptest and PathVysion—for systematic evaluation of the HER2 status on whole tumor tissue sections and compared the original (FDA approved) and the new (ASCO/CAP 2007) breast cancer HER2 scoring criteria, in addition to assessing the membrane staining pattern and tumor heterogeneity of HER2 expression. Overall, more than one-third (35%) of endometrial serous carcinomas were HER2 positive scored by immunohistochemistry and/or FISH. Concordance rates between HER2 immunohistochemistry and FISH have been calculated based on the limited FISH data available. The highest concordance rate—excluding the immunohistochemistry 2+ equivocal cases—86% was observed between HER2 immunohistochemistry and FISH when using the 2007 ASCO/CAP criteria, compared with a 78% concordance rate when the FDA guidelines were applied. Although using the ASCO/CAP guidelines appears to provide better concordance between immunohistochemistry and FISH than the FDA criteria, it falls below the reported concordance rates of breast cancer.[4]

Characteristics of HER2 amplification/overexpression may vary significantly according to the tumor type and primary site. HER2 overexpression and amplification are relatively uniform throughout the tumor tissue in breast cancer, and tumor heterogeneity of the HER2 status is considered a rare event.[37,38] Gastric and gastro-esophageal junction carcinomas, on the other hand, show significant intratumoral heterogeneity of HER2 status, reported in nearly half of the cases.[39,40] This phenomenon led to specific HER2 scoring guidelines in gastric biopsy specimens, allowing for a 3+ HER2 immunohistochemical score if any tumor cell clusters show strong membranous staining, regardless of the percentage of positive cells.[7] Similar to gastric cancer, endometrial serous carcinoma in our series demonstrated significant heterogeneity of HER2 expression by immunohistochemistry in 53%

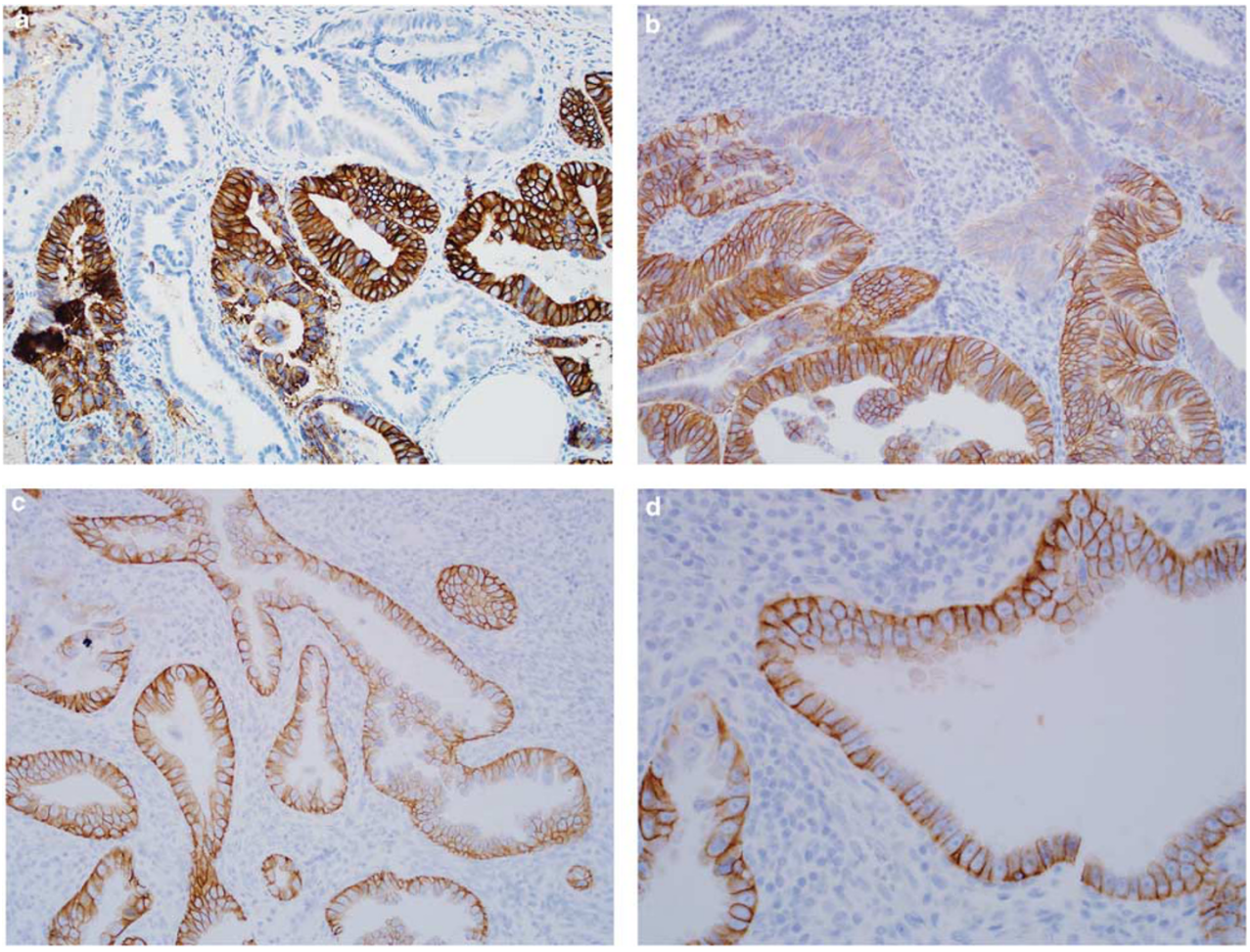

Figure 2 Tumor heterogeneity and membrane staining pattern of HER2 immunohistochemistry in endometrial serous carcinoma. (**a**, **b**) Heterogeneous HER2 expression in serous carcinoma. (**c–d**) Lack of apical HER2 immunostaining results in a lateral or basolateral ('U-shaped') staining pattern.

Table 5 Correlation between HER2 status and patient ethnic origin

	White/Caucasian, n *(%)*	*African-American,* n *(%)*	*Hispanic,* n *(%)*	*Asian,* n *(%)*	*Other,* n *(%)*	*NA,* n *(%)*
HER2 positive (n = 38)	31 (81.5%)	4 (10.5%)	0 (0%)	1 (2.6%)	1 (2.6%)	1 (2.6%)
HER2 negative (n = 70)	50 (71.4%)	14 (20%)	3 (4.3%)	0 (0)	3 (4.3%)	0 (0)

Abbreviation: NA, not available.

Table 6 Correlation between HER2 status and tumor stage

	Stage I, n *(%)*	*Stage II,* n *(%)*	*Stage III,* n *(%)*	*Stage IV,* n *(%)*	*Stage NA,* n *(%)*
HER2 positive (n = 38)	24 (63.2%)	1 (2.6%)	6 (15.8%)	7 (18.4%)	0 (0)
HER2 negative (n = 70)	44 (62.8%)	4 (5.7%)	12 (17.1%)	8 (11.4%)	2 (2.8%)

Abbreviation: NA, not available.

of the HER2-positive cases. Our data on heterogeneity could potentially argue for similar recommendations in endometrial carcinoma, although the tissue sampling tends to be more multifocal in endometrial biopsies and curettings than in typical gastric biopsies. The presence of significant tumor heterogeneity in endometrial serous carcinoma requires selection of larger tumor tissue samples for HER2 testing. Previous investigations analyzing small areas of tumor cells—including a tissue

microarray study[11]—were unable to sufficiently address issues, such as tumor heterogeneity.

Assignments of the FDA and/or ASCO/CAP immunohistochemical scores were problematic in a few of our cases, mainly due to the presence of incomplete moderate or strong membranous staining, which is not specified under either scoring category in breast cancer. When assessing the membrane staining pattern in endometrial serous carcinoma, we found that in contrast to the complete membranous staining of breast carcinomas, large proportion (74%) of HER2-positive serous endometrial tumors lacked staining (at least focally) on their apical membrane surfaces, resulting in a lateral or basolateral ('U-shaped') pattern, much like that of previously observed in gastric/gastro-esophageal junction carcinomas.[7] Tumors with this type of lateral/basolateral staining pattern had a predominantly glandular (or 'pseudo-glandular') architecture, while those with a more solid growth displayed complete membranous HER2 immunostaining.

In conclusion, the current ASCO/CAP breast scoring criteria provide better concordance between immunohistochemistry and FISH than the FDA scoring criteria in serous endometrial carcinoma. Assessment of HER2 immunohistochemistry preferably on multiple different tumor sections or sections with large tumor areas is recommended, due to the presence of significant heterogeneity of HER2 protein expression observed in this study. Tumors with loss of apical HER2 immunostaining and strong lateral/basolateral membranous staining pattern should also be allowed for a 3+ HER2 immunohistochemical score. Evaluation of larger tumor area ($\sim 1\,cm^2$) for HER2 FISH is preferred, and correlation with the highest HER2 protein expression by immunohistochemistry is recommended.

Future studies—including a current multi-institutional randomized phase II clinical trial (*NCT01367002*)—are necessary to correlate the HER2 expression/amplification results with therapeutic response.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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