



Case report

Human epidermal growth factor receptor 2 (Her2) testing for uterine serous carcinoma: Report of scenarios of unusual overexpression

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ABSTRACT

The human epidermal growth factor receptor 2 (Her2) is tested in many human cancers, including breast, bladder, pancreatic, ovarian and stomach. The American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) have issued Clinical Practice Guidelines for reporting Her2 results for breast carcinomas (Wolf et al., 2018). For the last 1–2 years Her2/neu is tested in endometrial serous carcinoma, especially in recurrent tumors or non-responsive tumors as an option for additional treatment. College of American Pathologists (CAP) offers a template for prognostic marker reporting results for specimens with endometrial carcinomas (Fitzgibbons et al., 2019). Her2/neu testing by immunohistochemistry (IHC) mandates rigorous fixation time control, e.g., fixation time should fall within 6–72 h (Recommendations for Her2 Testing in Breast Cancer, 2013). For that reason, in breast cancers, Her2/neu testing is done on initial core biopsy specimens. The test is however, repeated on excision specimen in high grade tumors where Her2/neu expression was initially negative on core biopsies.

For endometrial serous carcinoma no guidelines have been set or proposed as of yet. The Gynecologic Oncologists request this test because of proven benefit of adding Trastuzumab (Fader et al., 2018) and that is why it is important to documenting the findings in this report in the literature so that an informed request can be made by the treating oncologist when multiple tissue samples from the same patient are available for testing. Similarly pathologists also can decide which would be the best sample to test when no instruction is received.

We report here three separate scenarios of uterine serous carcinomas in which the Her2/neu expressions were unique enough to justify documentation and therefore have implications for determining which specimen is ideal for the Her2 overexpression testing and likely to have highest possibility in identifying the Her2/neu overexpressed clone in the tumor which would expand the therapeutic options for the patients.

1. Case report

1.1. Scenario 1

A 62 year-old postmenopausal woman, complaining of postmenopausal vaginal bleeding had endometrial curettings with Myosure polypectomy and hysterectomy at a later date. Histologic examination of the curettings revealed high grade uterine papillary serous endometrial carcinoma, endometrial polyp fragments, and rare fragments of superficial myometrium with invasive serous carcinoma. Hysterectomy revealed only focal residual tumor. The tumor showed no loss of nuclear expression of MMR proteins. Her2/neu test performed by immunohistochemistry on the initial curettings which included superficial myometrial fragments with invasive tumor, and overexpression

(3+) was only noted in the invasive component of the tumor and not the non-invasive component. Fluorescent In-situ hybridization (FISH) was also done on the invasive tumor and Her2/neu gene amplification was detected in the invasive carcinoma (Ratio: 9.8; Average Copy No. 20) (Fig. 1).

1.2. Scenario 2

A 63 year old woman with a recently diagnosed high grade uterine papillary serous carcinoma of the endometrium underwent laparoscopic total hysterectomy, omentectomy, and sentinel lymph node biopsies. Rare foci of residual serous carcinoma were identified on the surface endometrium. However, the entire myometrium showed extensive lymph vascular space invasion by high grade serous carcinoma.

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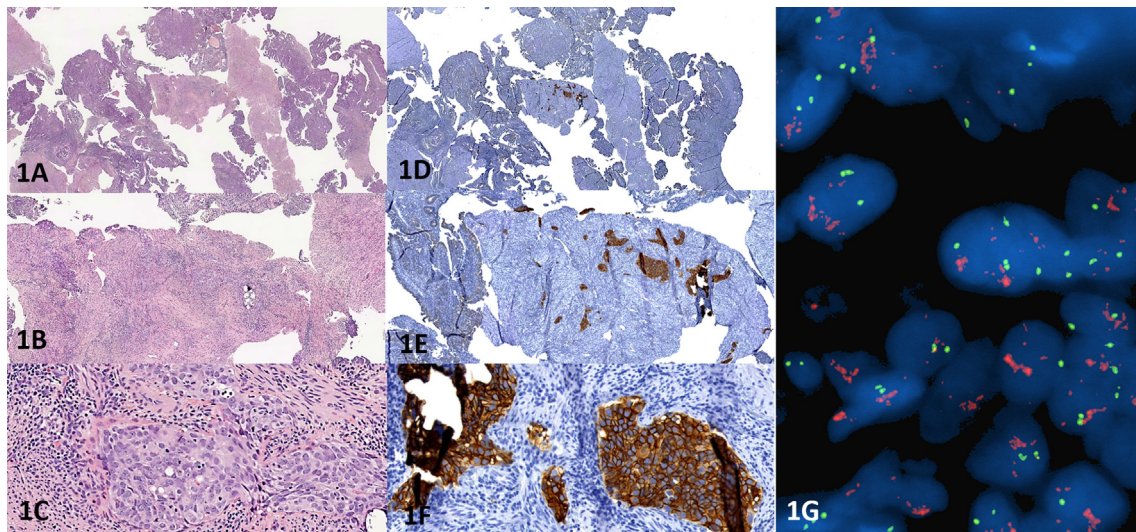


Fig. 1. A. Endometrial curettage with serous carcinoma (HE, 2×); B. Superficial myometrium with invasive serous carcinoma (HE 4×); C. Myoinvasive serous carcinoma (HE 20×); D. Her2 overexpression in myoinvasive tumor (IHC 2×); E and F. Her2 overexpression (IHC 4× and 20× respective); G. Her2 gene amplified (FISH).

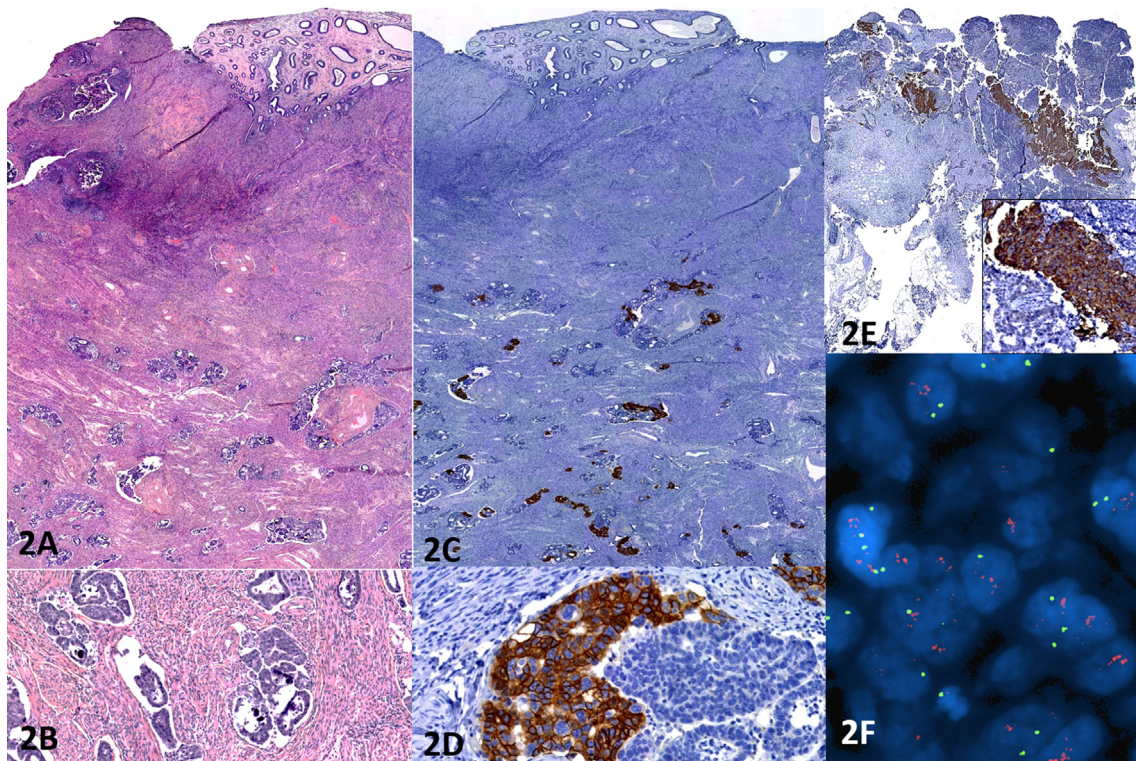


Fig. 2. A. Uterine wall with tumor emboli in myometrium (HE 1×); B. Tumor emboli (HE 10×). C. Her2 overexpressed (IHC 1×); D. Her2 overexpress and negative in same vessel (IHC 20×); E. Her2 overexpressed in metastatic tumor in lymph node (IHC 2×; Inset 20×); F. Her2 gene amplified (FISH).

The final pathological staging was pT1bN1 (FIGO IIIC1) high grade papillary serous adenocarcinoma. The tumor showed no loss of nuclear expression of MMR proteins. Her2/neu overexpression (3+) by immunohistochemistry (IHC), done on hysterectomy sample, was detected in the tumor emboli only. Interestingly, not all tumor emboli were Her2/neu overexpressed. Her2/neu negative and Her2/neu overexpressed tumor emboli were noted in the same vessel on IHC slides (Fig. 2). This represents intratumoral heterogeneity and emphasizes that the testing when done on a sample with largest volume of tumor, preferably where invasive tumor, tumor emboli or tumor with diverse morphology are present, may increase the likelihood of identifying the

Her2/neu amplified clone. The HER2 gene amplification tested by FISH showed gene amplification (Ratio 10.1; Average Copy No. 20) (Fig. 2). The metastatic carcinoma in both sentinel lymph nodes were also tested for Her2/neu overexpression by immunohistochemistry. Her2 was found to be overexpressed (3+) in most of the metastatic carcinoma. However, Her2 negative tumor was noted contiguous to Her2 overexpressed tumor (Fig. 2).

1.3. Scenario 3

A 63-year-old woman with abnormal uterine bleeding was found to

have a high grade endometrial papillary serous carcinoma. During the preoperative work-up she was also found to have a Nottingham grade III, axillary lymph node positive invasive duct carcinoma of the right breast. The breast tumor was ER/PR negative and Her2/neu equivocal (2+) by IHC. However, the Her2/neu gene in breast tumor was found not to be amplified by FISH (Ratio: 1.1; and average copy # 2.15). She was treated with neoadjuvant chemotherapy and subsequently underwent a post neoadjuvant partial mastectomy, axillary dissection, and total abdominal hysterectomy with omentectomy. No residual breast carcinoma was identified and all thirteen lymph nodes were negative as well. The final staging was ypT0N0.

The uterine high grade serous carcinoma had outer half myometrial invasion and microscopic spread of tumor to the right paratubal soft tissue. Macroscopic serous carcinoma was also present in the omentum. The tumor showed no loss of nuclear expression of MMR proteins. However, Her2/neu immunohistochemical staining showed noteworthy overexpression (3+). The Her2/neu overexpressed and Her2 negative tumors were seen in contiguity with a sharp demarcation between them, reflecting intratumoral heterogeneity. Her2 gene amplification (FISH) was studied and the signals of both positive and negative areas were counted separately. The Her2 overexpressed area showed gene amplification whereas the Her2 negative areas were not gene amplified by FISH. (Ratios: 7.7 and 1.1; Average Copy Nos. 20 and 2.2, respectively) (Fig. 3).

The formalin fixation time of all these specimens were within 6–72 h fixation time range and cold ischemia time within one hour.

2. Discussion

Novel therapeutic targets have opened up new horizon in cancer treatment for tumors which are either totally or partially nonresponsive to standard treatment protocols and/or recur. It is a common practice in medicine to apply the effective treatment knowledge/experience of tumors of one body site on tumors of other body sites. Trastuzumab, a known treatment option for Her2 amplified breast tumors, is currently being tested in uterine serous carcinomas. The utility of identifying a

potential therapeutic target, and testing the cases of high grade endometrial for her2/neu overexpression has become more apparent. It has already been shown that addition of Trastuzumab to the traditional regimen of carboplatin-paclitaxel increased the progression-free survival in patients with uterine serous carcinoma (Fader et al., 2018).

Musselman et al. (2019) reported a case of recurrent carcinosarcoma where the epithelial component was serous carcinoma. The recurrent tumor was tested for Her2 overexpression and treated with Trastuzumab, and the complete remission was achieved. This tumor has demonstrated the efficacy of targeted therapy. This report justified Her2/neu testing not only on uterine papillary serous carcinomas, but also uterine carcinosarcomas having a serous carcinoma component.

HER2/neu overexpression has been associated with a variety of cancer types, including breast, ovarian, endometrial, gastric, bladder and cervical cancers (Yan et al., 2014). Overexpression of HER2/neu in uterine serous adenocarcinoma has been examined in prior studies, with reported immunohistochemistry (IHC) positivity rates ranging from 10 to 62% (Buza et al., 2014; Slomovitz et al., 2004; Singh et al., 2008; Mentrikoski and Stoler, 2014), indicating that many of these tumors may be treated by Trastuzumab.

The testing for Her2/neu amplification by immunohistochemistry is guided by strict guidelines in breast carcinoma. It is known that formalin fixation time can alter Her2/neu expression in fixed tissue. The recommended formalin fixation time is between 6 and 72 h and cold ischemia time less than one hour. The sample that is tested is usually a core biopsies where the fixation time can be easily regulated and also because of small and thin nature of the tissue allows fixation to be uniform. However, the testing is repeated in cases of high grade tumor where the initial testing was negative (not amplified).

Testing for Her2/neu overexpression eventually will come into practice and the question of which tissue sample should be tested will have to be determined. Three scenarios reported in this series document some unusual expressions of Her2. All these cases show that testing of the initial biopsy potentially would have missed the Her2 overexpression and, therefore, raises the legitimate question of examining of which specimen should be tested? Unlike breast carcinoma,

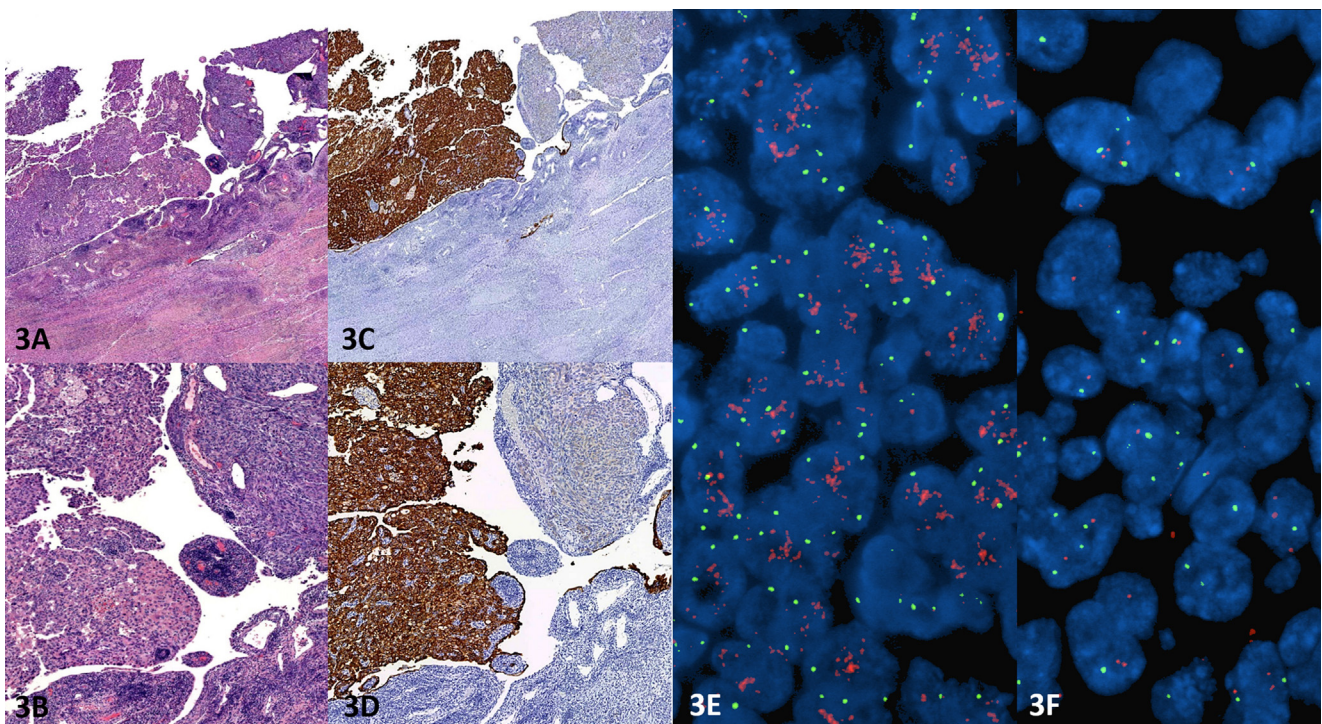


Fig. 3. A. Endometrial serous carcinoma, hysterectomy (HE 4×); B. Higher magnification (HE 10×); C. Her2 overexpressed and negative areas (IHC 4×); Higher magnification (IHC 10×); E. Her2 gene amplified in overexpressed area (FISH); F. Her2 no amplification in negative area (FISH).

endometrial serous carcinomas possibly show more intratumoral heterogeneity and thus testing the larger tissue sample, e.g., hysterectomy specimen, may take precedence over the biopsy because of limited nature of the sample.

From the perspective of controlling formalin fixation time, it would not be unwise to assume that Her2/neu should be tested on the initial endometrial biopsy or curettage. However, one should remember that testing biopsies may miss the overexpressed clone of tumor which may ultimately dictate the treatment choice.

The findings of the current series of three scenarios are vital for the decision making of which tissue sample may be tested for Her2/neu overexpression by immunohistochemistry. Her2/neu testing by IHC in all three cases were 3+ positive. The Her2/neu overexpressed areas were circled and FISH signals were scored in all three samples within those designated circles to confirm gene amplification and not just protein overexpression as we are in the initial phase of Her2/neu testing; traditionally 1+ Her2/neu expression by IHC implies “negative test”, 2+ “equivocal test”, and 3+ “overexpressed”. FISH is done on “2+ or Equivocal” cases to rule in/out gene amplification. The Gynecologic Oncologists request this test because of proven benefit of adding Trastuzumab (Fader et al., 2018) and that is why it is important to document these findings in the literature so that informed request can be made when multiple tissue samples from the same patient are available for testing. Similarly pathologists also can decide which would be the best sample to test when no instruction is received.

It is not necessary to confirm all IHC 3+ overexpression of Her2/neu by FISH for gene amplification. In the current study that was done to validate the correlation between IHC and FISH results, something similar to what was done in breast carcinoma cases at the initial stage. The testing guidelines for uterine serous carcinoma have not been proposed or formulated yet! As it is deemed predictable that more and more tumors will be tested for Her2/neu amplification, it becomes necessary to come up with a guideline. While doing so, one may consider testing the sample preferably where invasive tumor, tumor emboli or tumor with diverse morphology are present, as shown in these three scenarios presented in this report. All these scenarios represent tumor heterogeneity and possibly clonal diversity within the same tumor. Selecting a block with most volume of tumor will possibly include all unusual areas of the tumor which may in turn increase the likelihood of identifying Her2/neu overexpressed clone of the tumor. The fixation and cold ischemia time, as practiced in Breast carcinoma testing, may remain the same (6–72 h and less than one hour respectively). The pathology department should develop protocol to documenting fixation and cold ischemia time as these may also affect testing as shown in

breast carcinomas.

3. Conclusions

All these scenarios elucidate that Her2/neu testing on biopsy only or limited samples would probably have failed to identify the her2/neu overexpressed/amplified clone of the tumor. Therefore, we propose that initial Her2/neu testing for uterine serous carcinomas should be done on hysterectomy specimens when available or on tissue blocks with largest volume of tumor on biopsies or curettings. If the initial testing were done on endometrial biopsies or curettings and were negative for overexpression, it may be retested on a larger specimen showing myoinvasive tumor, lymph vascular space invasion, area with diverse tumor morphology, metastatic tumors sites, or when the oncologists prefer to add Trastuzumab to the regimen.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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