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Current Laboratory Testing Practices for Assessment of *ERBB2*/HER2 in Endometrial Serous Carcinoma and Colorectal Carcinoma

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Abstract

Context.——Therapy targeted at human epidermal growth factor receptor 2 (HER2; also known as *ERBB2*) was used initially for breast and gastroesophageal carcinoma and has more recently been adopted for endometrial serous carcinoma (ESC) and colorectal carcinoma (CRC). There is evidence that predictive biomarker testing algorithms for HER2 must be tumor type specific and that an algorithm validated for one tumor type cannot be applied to another.

Objective.——To describe current laboratory practices for HER2 assessment in ESC and CRC.

Design.——We surveyed laboratories participating in the 2021 College of American Pathologists (CAP) HER2 immunohistochemistry proficiency testing program.

Results.——The survey was distributed to 1548 laboratories and returned by 1195, of which 83.5% (998) were in the United States. For ESC, 24.0% (287) of laboratories reported performing in-house testing for HER2 by immunohistochemical staining and/or in situ hybridization; of these, 44.3% (127) performed it reflexively on all cases of ESC. The most common criterion for evaluating HER2 was the American Society of Clinical Oncology/CAP 2018 guideline for breast carcinoma (69.0%; 194 of 281), whereas only 16.0% (45) of laboratories used guidelines specific to ESC. For CRC, 20.2% (239 of 1185) of laboratories performed in-house HER2 testing, and 82.0% of these (196) did the test only at the clinician's request. A plurality (49.4%; 115 of 233) used gastroesophageal cancer guidelines when scoring CRC, 30.0% (70) used the CRC scoring system from the HERACLES trial, and 16.3% (38) used the American Society of Clinical Oncology/CAP 2018 guideline for breast carcinoma.

Conclusions.——Laboratories vary in their approach to HER2 testing in ESC and CRC. Most laboratories did not report using tumor type–specific recommendations for HER2 interpretation. The lack of standardization could present a challenge to evidence-based practice when considering targeted therapy for these diseases.

As targeted therapy has become available for an increasing number of tumor types, it has become important to assess predictive biomarkers in the pathology laboratory. For example, the monoclonal antibody trastuzumab (Herceptin) was developed as a treatment for breast cancer overexpressing *HER2*, also known as *ERBB2*. It functions by binding to the extracellular domain of the HER2 (human epidermal growth factor receptor 2) receptor and preventing its dimerization and downstream signaling. Second-generation agents such as pertuzumab (Perjeta), lapatinib (Tykerb), and neratinib (Nerlynx) have a similar mechanism of action. A key insight in the implementation of HER2-targeted therapy was that the drug is effective only in the subset of breast cancers that overexpresses the protein. Moreover, most cases of HER2 overexpression are caused by *HER2* gene amplification and can be detected either by immunohistochemical staining (IHC) for the gene product or by in situ hybridization (ISH) to determine the gene copy number.[1]

Specific guidelines have been developed to promote the analytic and clinical sensitivity and specificity of HER2 testing in breast cancer. Since the original 1999 Consensus Statement,[2] the HER2 scoring guidelines have undergone significant evolution, with several updates being published as clinical response data were accumulated.[1,3] The guideline was revised most recently in 2018 and promulgated jointly by the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP).[1]

Currently for breast cancer, a 3+/positive HER2 IHC test result is defined as strong circumferential membranous staining in more than 10% of tumor cells. Weak to moderate complete membrane staining observed in more than 10% of tumor cells is defined as 2+/equivocal and requires reflex to ISH for final classification.[1] Most ISH-positive cases have an HER2/CEP17 ratio 2.0 or higher and average HER2 signals/cell 4.0 or higher; other, less common combinations of ratio and copy number can also be classified as positive.[1]

Although these criteria are currently widely accepted for breast cancer, they have evolved over time as data on therapeutically relevant thresholds have become available. Successive iterations of the guidelines have lowered the threshold for a 3+ IHC result from 30% of cells to 10%[4] and made adjustments to the definition of a 2+result,[3,4] reducing the number of cases that require reflex ISH testing. Furthermore, earlier guidelines included an ISH-equivocal category that has now been eliminated.[3]

Given the successful implementation of anti-HER2 therapy for breast cancer, *HER2* gene amplification has been identified in several other tumor types as a predictor of response to targeted therapy. It has also been reported that IHC patterns, protein expression, and gene amplification metrics (levels) relevant for the therapeutic response may be different in different tumor types.

In gastric and gastroesophageal adenocarcinoma (GC), HER2 is positive in 6.8% to 42.5% of cases, depending on the tumor site, assay, and definition of positivity.[5] Based on the results of the Trastuzumab for Gastric Cancer (ToGA) study, trastuzumab in combination with chemotherapy is approved in the first-line setting for these cases.[6] Specific guidelines for performing HER2 testing in GC have been promulgated.[7] In contrast to the IHC staining pattern seen in breast cancer, GC tumors often show not only circumferential membranous staining but also basolateral membranous staining (lack of apical membrane expression),[8,9] with both patterns predicting response to targeted therapy. ToGA defined the positivity (3+) threshold in GC as staining in 10% or more of neoplastic cells for resection specimens, in contrast to the 30% threshold at the time for breast cancer. This distinction was important because GC is more likely to have heterogeneous staining and because half of the ToGA specimens exhibited 10% to 30% positively stained cells.[5,8] In a biopsy, the guidelines allow a positive result to be called on as few as 5 cells.[8]

More recently, endometrial serous carcinoma (ESC) has been shown to harbor *ERBB2* (*HER2*) amplification.[10–13] ESC is a rare but highly aggressive type of endometrial cancer with relatively limited therapeutic options.[14,15] Early single-institution experience showed that heterogeneous HER2 expression is present in the majority of ESCs with either protein overexpression or gene amplification; most of these cases show basolateral expression rather

than a complete circumferential pattern.[16] A multi-institutional phase 2 trial demonstrated that addition of trastuzumab to standard therapy increased progression-free[17] and overall[18] survival in advanced-stage or recurrent ESC with HER2 overexpression/amplification. This targeted therapy was rapidly adopted in clinical practice and has been incorporated into National Comprehensive Cancer Network[19] and Society of Gynecologic Oncology[20,21] guidelines, leading to an increase in requests for clinical testing for this tumor type.

The initial clinical trial report[17] gave limited detail on the protocol used for HER2 assessment because of space constraints but stated that the 2007 ASCO/CAP guideline was used. The reference cited was, however, for the 2013 ASCO/CAP guideline, leading to a lack of clarity as to which specific criteria were used for patient eligibility; these 2 versions of the guideline differ significantly in that they set the cutoff for a 3+ HER2 IHC score at 30% versus 10% of cells, respectively. Moreover, because the trial results were published in March 2018 and an ASCO/CAP breast guideline update was published online shortly thereafter (May 2018), laboratories may have assumed this new guideline was applicable to ESC. The CAP template for endometrial biomarker reporting, last updated in 2019, states that in the absence of guidelines for reporting HER2 status in endometrial cancer, the 2018 breast cancer guideline[22] should be followed. More specific guidance was subsequently published in a separate expert opinion/review manuscript, clarifying the 30% threshold and the inclusion of the basolateral staining pattern,[23] but this guidance has not yet been incorporated into the CAP template.

In colorectal carcinoma (CRC), several trials have been reported. First, the HERACLES trial provided proof of concept for combination therapy with trastuzumab and the EGFR/HER2 inhibitor lapatinib[24] in patients with HER2-positive, metastatic, *KRAS* wild-type disease. The trial used a validated CRC-specific HER2 testing algorithm using the Ventana 4B5 IHC assay and a 50% proportion cutoff by IHC, after finding that this assay showed better concordance with ISH as compared with the Dako HercepTest method.[25] Second, the MyPathway[26] basket trials provided evidence for combination trastuzumab and pertuzumab therapy using a combination of methods for HER2 assessment: local HER2 determinations by IHC, ISH, or next-generation sequencing (NGS) were accepted, and single-slide chromogenic ISH (CISH)/IHC and NGS were repeated centrally when material was available. NGS and fluorescence ISH (FISH)/CISH were only 81% concordant. It is not clear how discrepancies were resolved, but analysis of a subgroup that was positive by FoundationOne (Foundation Medicine, Cambridge, Massachusetts) testing showed an overall response rate similar to that of the full cohort. Third, DESTINY-CRC01[27] reported on T-DXd, a HER2-directed antibody conjugated to a topoisomerase inhibitor, using criteria of centrally determined IHC 3+ or IHC 2+ with ISH amplification, not further specified. Thus, in CRC, data supporting anti-HER2 therapy are based on trials with differing criteria and in which the assays used have not necessarily been explicitly stated. Resulting issues in implementation include (1) lack of clarity as to the interchangeability of the assays or the preferred technical approaches for clinical practice, (2) increased cost to the health care system, and (3) challenges to reimbursement if multiple tests are attempted.

There are additional differences among the various HER2 testing guidelines in terms of test selection. In breast cancer the primary HER2 testing modality can be either IHC or ISH

(FISH/CISH/etc), as both methods have been shown to be predictive of response to targeted therapy. In GC, ESC, and CRC, the established or proposed testing algorithms start with IHC, followed by reflex ISH only if the score is 2+.

CAP recognized the need to gather data on current practices in HER2 testing for these 2 diseases, ESC and CRC. CAP offers proficiency testing programs for HER2 IHC and ISH that are mailed to subscribing laboratories, typically consisting of several specimens for analysis and a result form that includes supplemental questions. These questions provide the opportunity to obtain data on current clinical practices in numerous accredited laboratories. Herein, the results of supplemental questions about HER2 testing that were distributed to laboratories in early 2021 are reported.

MATERIALS AND METHODS

Survey

A list of 14 questions was developed by the CAP Molecular Oncology Committee and included as a supplemental questionnaire (SQ) in the 2021-A HER2 Immunohistochemistry Program distributed to laboratories subscribing to this CAP proficiency testing program (mailed March 15, 2021, and due back April 6, 2021). The full questionnaire is available in the supplemental digital content at https://meridian.allenpress.com/aplm in the October 2023 table of contents.

To annotate the SQ data, we extracted respondent institution type from CAP's demographics database. For 69 laboratories not in the database, the missing type was determined by online institution searches. "Physician office laboratory/clinic" and "nonhospital laboratories" were combined into an "other, nonhospital laboratory" group.

Statistics

Data validity adjustments were applied to responses that did not follow the skip-sequence directions in the SQ. Missingness checks were conducted to address incomplete and nonrandom response patterns. Responses relating to testing practices were excluded for laboratories that indicated that they performed the test at a reference laboratory. The "other" response code was populated for laboratories that wrote text in the open comment field without choosing any specific item.

Multivariate logistic regression models were used to test for laboratory characteristics associated with HER2 testing practices. The models were fit with 2 factors: institution location and institution type. Institution location was defined as a 2-level factor that classified laboratories as domestic (United States) or international. Institution type included 4 levels: independent/commercial reference laboratory, academic hospital/medical center laboratory, nonacademic hospital/medical center laboratory, and other, nonhospital laboratory. For any model that did not meet the convergence criterion, a second model was fit with the location factor. Multiple pairwise testing for the institution type differences was adjusted with a Bonferroni correction, and the adjusted P values are reported. Analyses were performed with SAS 9.4 (SAS Institute, Cary, North Carolina). A significance level of .05 was used for the statistical testing.

RESULTS

Respondent Demographics

The survey was completed by 1229 of 1548 laboratories that received it (79.4%). Thirty-four (2.2%) of these were excluded because of survey duplication (3) or missing responses (31), resulting in the inclusion of 1195 questionnaires (77.2%) for analysis. Responding laboratories included 998 United States facilities (83.5%) and 197 international facilities (16.5%) from 37 countries. In the total group, 506 (42.3%) were nonacademic hospitals/medical centers, 399 (33.4%) were academic, and 192 (16.1%) were independent/commercial reference aboratories.

HER2 Testing in ESC

Among the 1195 included laboratories, 287 (24.0%) reported that they performed testing for HER2 in ESC, 94 (7.9%) planned to start offering the testing in 2021, 95 (7.9%) planned to start offering it after 2021, and the remaining 719 (60.2%) had no plans to start offering it (Figure 1). Domestic laboratories were more likely to perform in-house HER2 testing for ESC compared with international laboratories (25.9% [258 of 998] versus 14.7% [29 of 197]; $P = .01$) (Figure 2, A). Academic laboratories were also significantly more likely to perform testing (35.6%; 142 of 399) than the other institution types: nonacademic hospital laboratories (20.2%; 102 of 506; $P < .001$), commercial reference laboratories (20.8%; 40 of 192; $P = .006$), and other, nonhospital laboratories (3.1%; 3 of 98; $P <. 001$).

Almost half of laboratories (141 of 287; 49.1%) reported performing this test on fewer than 10 specimens in 2020, 61 (21.3%) had a volume of 10 to 20 tests, and 32 (11.1%) had a volume of more than 20 specimens (Table 1).

With regard to indications for testing, 135 of 287 laboratories (47.0%) reported routinely performing the test only at the clinician's request, 127 (44.3%) reported performing it reflexively in all cases, and 22 (7.7%) performed it reflexively for advanced-stage cases (International Federation of Gynecology and Obstetrics stages III and IV) (Table 1). Interestingly, international laboratories were statistically more likely to perform HER2 testing only at the clinician's request (82.8% of international laboratories [24 of 29] versus 43.0% of domestic laboratories [111 of 258], $P = .001$).

Laboratories were asked which type of specimen was routinely selected if more than one was available. The most frequent response was primary tumor from the hysterectomy (117 of 286; 40.9%). The next most frequent response was "no selection made; our laboratory routinely tests any available tumor material" (77; 26.9%). Other laboratories routinely tested the biopsy/curettage or metastatic tumor (Table 1).

The most common testing method was IHC with reflex to ISH for equivocal results (254 of 286; 88.8%). Twenty laboratories (7.0%) reported simultaneous IHC and ISH testing. Three laboratories performed initial ISH with or without reflex to IHC (1.0%). Of laboratories that reported performing ISH in-house, 187 of 213 (87.8%) used FISH and 20 (9.4%) used CISH (Table 2).

The most commonly reported scoring criteria was the ASCO/CAP 2018 guideline for breast carcinoma[1] (195 of 281; 69.4%). Nine laboratories reported using the 2013 version[3] or the 2007 version[4] of the guideline for breast carcinoma (total of 3.2%); 45 (16.0%) reported using the Fader et al[17] 2018 clinical trial guidelines, but the survey did not ask respondents to clarify their understanding of what those guidelines were. Ten laboratories (3.6%) stated that they gave an "overall" assessment of positive versus negative result, 14 (5.0%) used the original US Food and Drug Administration scoring criteria (HercepTest package insert) for breast carcinoma, and 8 (2.9%) reported "other," including the gastric cancer criteria[7] (4 respondents) and an in-house guideline (Table 2).

HER2 Testing in CRC

Similar to the results for ESC, 239 of 1185 respondents (20.2%) reported performing HER2 testing in this setting. Among the remaining 946 laboratories, 100 (8.4%) planned to start testing in 2021, 85 (7.2%) planned to start after 2021, and 761 (64.2%) had no plans to start testing (Figure 1). Testing was not more common in domestic versus international laboratories, but academic hospital laboratories (25.9%; 102 of 394) were more likely to offer it than either nonacademic hospitals (90 of 501; 18.0%; $P = .02$), commercial reference laboratories (33 of 192; 17.2%; $P = .02$), or other, nonhospital sites (14 of 98; 14.3%; $P = .04$) (Figure 2, B).

Among laboratories performing the testing, most (111 of 239; 46.4%) reported a volume of fewer than 10 specimens in 2020, and 41 (17.2%) reported more than 20 (Table 3). Some respondents (39) did not know their laboratory's specimen volume.

Of the 239 laboratories that reported information about clinical indications for testing, 196 (82.0%) stated that the test was performed only at the clinician's request, 20 (8.4%) indicated that they performed it for all metastatic cases, and 8 (3.3%) performed it in all cases (Table 3).

A question about specimen type (primary tumor from colectomy, primary tumor from a biopsy, metastatic tumor, etc) revealed no strong pattern of preference (Table 3).

The most common testing method was IHC with reflex to ISH for equivocal results (195 of 237; 82.3%) (Table 4); 18 laboratories (7.6%) reported performing both tests, 5 (2.1%) performed ISH with reflex to IHC, 7 (2.9%) performed IHC only with no reflex, and 4 (1.7%) performed ISH only. Most respondents (211 of 234; 90.2%) stated that they did not require the tumor to be *RAS* wild-type to obtain HER2 testing, 17 (7.3%) required the tumor to be *RAS* wild-type only if *RAS* status was available, and 6 (2.6%) absolutely required known RAS wild-type status before performing HER2 testing.

Half of the laboratories (115 of 233; 49.4%) indicated that they applied the CAP/American Society for Clinical Pathology/ASCO guideline for GC[7] when scoring colorectal cancer, 70 (30.0%) used the scoring system validated for the HERACLES trial,[25] and 38 (16.3%) reported using the 2018 ASCO/CAP guideline for breast cancer.[1]

Cross-tabulation of laboratories performing testing in ESC and CRC showed that 162 of 1195 respondents (13.6%) performed both types of testing and 824 (69.0%) performed

neither type, whereas some offered HER2 testing in only one or the other tumor type. Our data do not indicate the reasons for these practices.

DISCUSSION

This study provides the first systematic survey of laboratory testing practices for HER2 in 2 tumor types, ESC and CRC, since the clinical adoption of HER2-targeted testing/therapies in these entities. Findings include that a minority of laboratories are performing the testing, and most of the rest do not plan to initiate testing in 2022. We assume that all respondents have the technical capability to offer HER2 IHC, because they subscribe to CAP HER2 IHC proficiency testing. It is not possible to determine from these data if laboratories with no plans to offer testing intend to obtain it via send-out to a reference laboratory or if, instead, they do not anticipate clinician requests for such testing.

Given that a minority of respondents perform the test or plan to do so, it might be expected that testing would be concentrated in a few reference laboratories; however, most laboratories performing this testing reported a low volume (<10 cases/y). This finding has implications for dissemination of guidelines for HER2 testing, because it indicates that both large and small stakeholders must be reached.

Our survey results show that most laboratories are using IHC as the primary test for both ESC (88.8%) and CRC (82.3%), with reflex to ISH for equivocal results. Therefore, the criteria used for IHC scoring are of great relevance to test performance. Our survey was designed to determine which criteria are in current use. In drafting the survey questions and responses, we assumed that most laboratories performing HER2 testing in ESC would either use criteria previously adopted for breast cancer or attempt to implement criteria specific for ESC. Similarly, we assumed that laboratories performing HER2 testing in CRC would use either breast cancer criteria or guidelines published for other gastrointestinal sites. The survey results support this assumption, as only 4 laboratories stated that they used GC criteria for ESC, and no laboratory reported using ESC criteria for CRC.

A recently published study demonstrated reasonable interobserver agreement rates among gynecologic pathologists when clear criteria for scoring HER2 IHC are used.[28] However, a barrier to consistent and widespread adoption of HER2 testing for ESC and CRC in clinical practice is the lack of consensus (or, rather, lack of clear guidance) on the optimal scoring criteria.

Comparing the published guidelines used for HER2 IHC interpretation in ESC shows the potential for both overcalling and undercalling *HER2* overexpression by using breast carcinoma guidelines (Table 5) compared with the criteria that were used in the phase 2 trial[17] but only comprehensively articulated in a separate and subsequent publication by Buza.[23] Most laboratories (69.4%) reported using ASCO 2018 criteria, which will lead to overcalling HER2 overexpression relative to the Buza criteria because cases with 10% to 29% strong circumferential membranous staining will be scored as positive in the former but not the latter. Using breast criteria (any version) will also lead to undercalling HER2 overexpression by not accepting basolateral staining as positive, whereas this pattern is

accepted in Buza et al.[28] It should be noted that there is limited evidence to determine an actual gold standard set of criteria because there has not, to our knowledge, been a systematic study of borderline cases that would be assigned differently under different guidelines. This will be exceedingly difficult in ESC because these patterns are rare and ESC is itself a rare entity. Even in breast cancer, uncommon and unusual patterns of HER2 ISH results are interpreted based on expert opinion in the face of limited evidence.[1]

Further guidance is needed to address HER2 interpretation when intratumoral heterogeneity is present, which is rare in breast cancer[29] and CRC,[25] but common in ESC[28,30] and GC.[7,31] The 2018 ASCO/CAP breast criteria[1] specify that staining for a 3+ score has to be "within a homogeneous and contiguous invasive cell population." In tumor types where HER2 heterogeneity is common, this requirement would tend to move cases into the HER2-equivocal group. It will be helpful to obtain data (ideally with a clinical endpoint) to indicate whether HER2-heterogeneous ESC can be considered positive by IHC. When cases with intrasample heterogeneity do undergo reflex ISH testing, ESC[23] and GC[7] guidelines recommend correlating with the IHC so that the area with highest HER2 expression can be analyzed.

With regard to intersample heterogeneity in ESC, our data showed a lack of agreement among laboratories as to whether the endometrial biopsy or hysterectomy should be tested (Table 1). This issue is important because recently published data showed that only 84% of ESCs had concordant HER2 on an endometrial biopsy/curettage and paired hysterectomy.[32] The discrepancies were attributed to both heterogeneity of HER2 expression and sampling issues.

Along similar lines, more than half of the laboratories (56.6%) reported that they tested the primary tumor from either biopsy or hysterectomy specimens (pooled data from Table 1), whereas 34.9% of laboratories tested either metastatic or primary tumor without specific preference, and only 5.6% tested metastatic tumor specifically, if available. We conclude that laboratories do not have a consistent approach to addressing differences between primary and metastatic sites. Change in HER2 status between the primary tumor and metastasis has been reported for breast and gastric/gastroesophageal cancer[33–36] and has been reported in ESC as well. In one report, only 45.0% of ESCs with HER2 overexpression at the primary site (3+ by HercepTest) were also 3+ at any metastatic site, and the metastatic sites sometimes were divergent from one another.[37] Given the clinical applicability of HER2 testing in advanced-stage and/or recurrent ESC,[19] it would be advisable for future studies and guidelines to acknowledge spatiotemporal heterogeneity and provide clear guidance on how to address it.

Like those performing HER2 testing in ESC, laboratories performing HER2 testing in CRC reported using a variety of guidelines (Table 4). The HERACLES investigators found that CRC HER2 results depended on the IHC assay that was used, with virtually all positive specimens (by gold standard ISH) having 50% or higher positivity with the Ventana 4B5 clone, whereas more than half had 50% or lower positivity by HercepTest.[25] The Ventana clone was ultimately incorporated into the HERACLES diagnostic criteria.

Applying HERACLES criteria to tests performed with other antibodies could result in false-negative results (Table 6).

Responses also indicated that only 2.6% of laboratories required proof of *RAS* wild-type status before performing HER2 testing in CRC. *RAS* activation is common in CRC and predicts lack of response to receptor tyrosine kinase inhibition, presumably because it signals downstream from *EGFR* family members.[38] Although National Comprehensive Cancer Network guidelines recommend that all CRC be tested for *RAS* mutations,[39] there can be challenges in integrating results that may be obtained at different times, on different test platforms, and often in different laboratories without full information interchange.

Similar to IHC, there are also significant differences in criteria for HER2 ISH among different tumor types. Most breast cases are assigned based on the HER2/CEP17 ratio when performing dual-probe ISH, but the ASCO/CAP breast guidelines have additional clauses to address chromosome 17 aneuploidy,[1,3] which is not addressed in the Buza[23] guidelines for ESC or the HERACLES guidelines for CRC.[25] Further, the HERACLES criteria require CRC to have 50% of cells or more with HER2/CEP17 2.0 or higher for a positive result, whereas ASCO/CAP breast criteria are indifferent to the proportion of cells with amplification, but rather consider the average ratio across scored cells. Thus, for ISH as for IHC, guidance is needed to promote uniform HER2 interpretation for a given tumor type and to validate the recommended criteria.

Strengths of the current study include that it samples a large number and wide variety of laboratories, including those that, because they already offer HER2 IHC testing for breast cancer, are likely to have the technical resources to offer it for other tumor types. The high response rate of 77.2% means that the results are likely to be generalizable. Limitations are that the survey did not reach laboratories that do not participate in CAP IHC proficiency testing for HER2 because either they do not offer the test or they perform another type of proficiency testing. We did not gather data on the specific antibody clone or immunostaining platform used for IHC or the probe set used for ISH. These parameters affect the performance of a test system and must be specified as part of any clinical assay validation.

Prompt adoption of new clinical practices as trial data become available has been improving the care of cancer patients. In the rapidly evolving field of clinical oncology, timely communication among those involved in clinical trials and the pathology community is increasingly important to ensure adequate biomarker testing support. Practical implementation of biomarker assessment based on the knowledge of the practices in the field should be considered early in clinical trial design. Moreover, the diversity of testing platforms, instrumentation, and reagents calls for efforts to harmonize biomarker testing and reporting algorithms.

Laboratories performing HER2 testing and seeking to maintain CAP accreditation are required to perform annual proficiency testing, which is monitored as part of the CAP Laboratory Accreditation Program. Specific requirements pertaining to HER2 testing are described in the CAP Anatomic Pathology Checklist and Molecular Pathology Checklist.

Based on the results of this survey study, it may be advisable to implement proficiency testing programs for ESC and CRC to promote quality and standardization of clinical testing for these disease types.

CONCLUSIONS

To gain a better understanding of current clinical HER2 testing practices for ESC and CRC, we surveyed laboratories participating in the CAP HER2 IHC proficiency testing. The survey revealed a lack of consensus as to the criteria to be used for HER2 interpretation in these disease types. Moreover, most laboratories report using interpretive criteria that are not specific to the disease type. Stakeholders, including CAP, should consider providing clear guidance for standardization of HER2 testing and interpretation in these diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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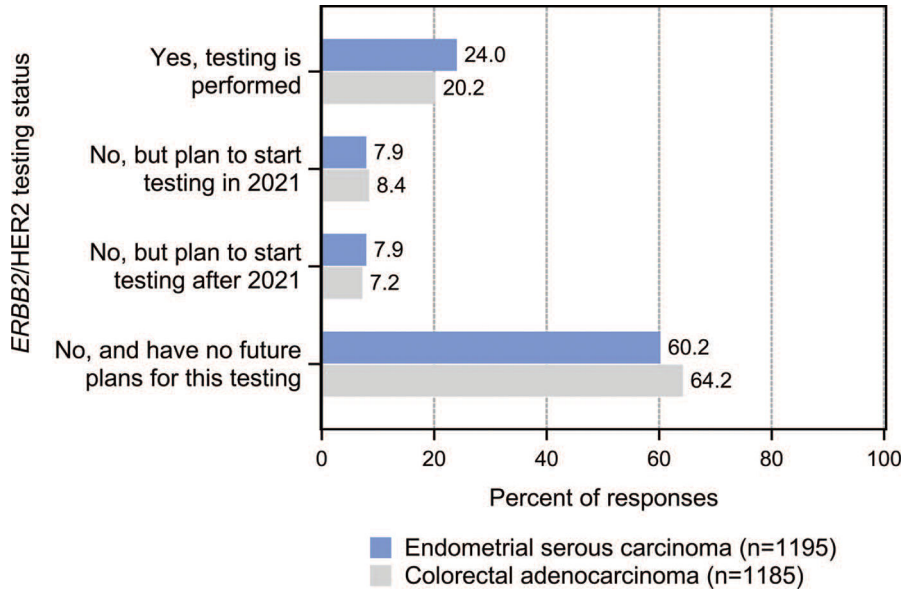

Figure 1.

Percentage of laboratories performing in-house ERBB2/HER2 (human epidermal growth factor receptor 2) testing in endometrial serous carcinoma and colorectal carcinoma.

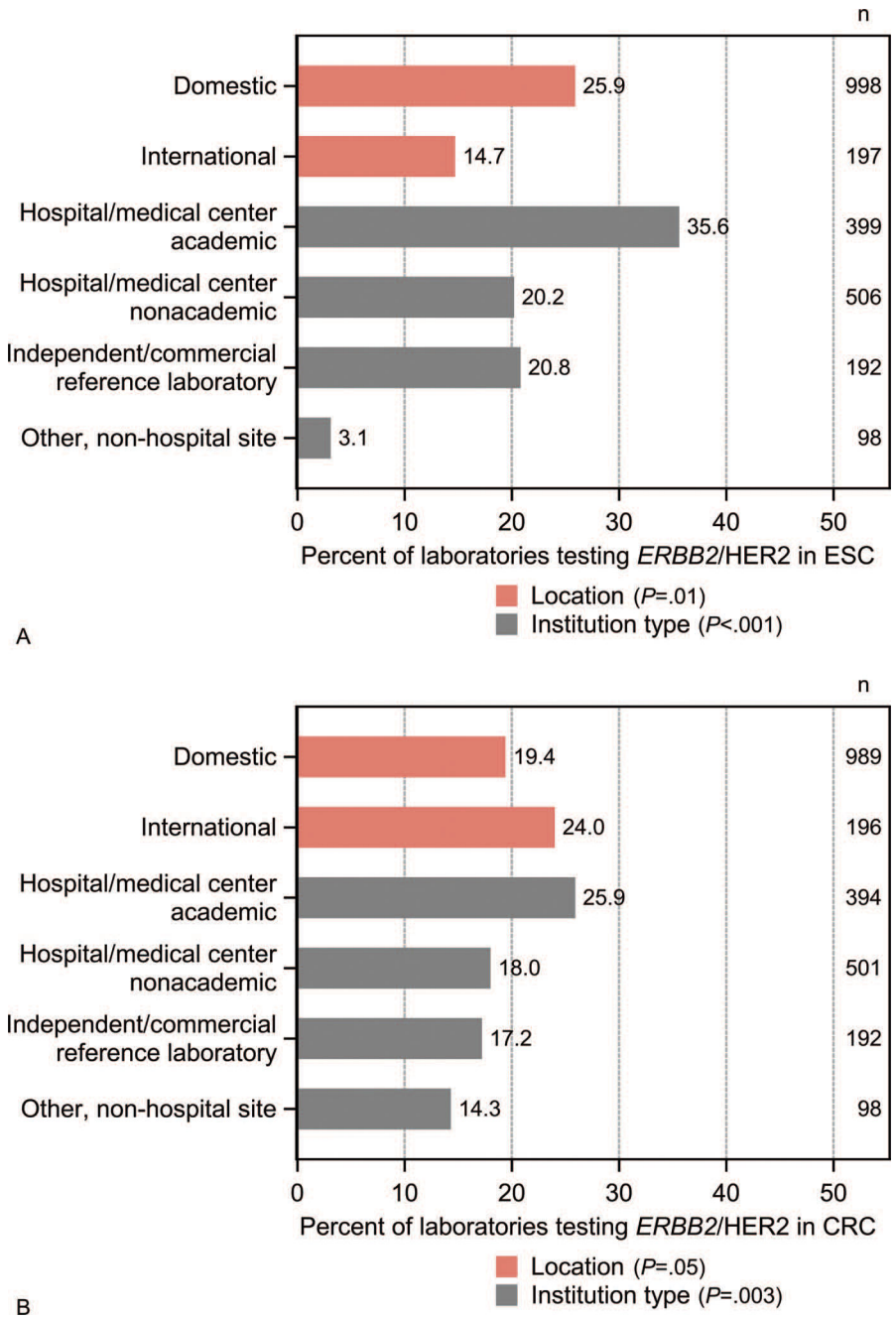

Figure 2.

Percentage of laboratories performing ERBB2/HER2 testing in (A) endometrial serous carcinoma (ESC) and (B) colorectal carcinoma (CRC) as a function of location and institution type. The numbers of laboratories responding to the survey are shown at the right of each panel.

Table 1.

Case Volume and Testing Practices for *ERBB2*/HER2 (Human Epidermal Growth Factor Receptor 2) Testing in Endometrial Serous Carcinoma (ESC)

	No. (%)
Approximate No. of ESC cases evaluated for *ERBB2*/HER2 amplification/overexpression in 2020	287
<10	141 (49.1)
10–20	61 (21.3)
>20	32 (11.1)
Unknown	53 (18.5)
How *ERBB2*/HER2 testing in ESC is routinely performed in the laboratory	287
Only at the clinician's request	135 (47.0)
Reflexively, in all cases	127 (44.3)
Reflexively, in all advanced-stage cases (FIGO stages III and IV)	22 (7.7)
Other[a]	3 (1.0)
If multiple specimens are available for *ERBB2*/HER2 testing in ESC, which types are routinely selected for testing	286
Primary tumor from hysterectomy	117 (40.9)
No selection made;our laboratory routinely tests any available tumor material	77 (26.9)
Primary tumor from biopsy	45 (15.7)
Tumor from metastatic site, if present	16 (5.6)
No preference or routine practice	23 (8.0)
Other[b]	8 (2.8)

Abbreviation: FIGO, International Federation of Gynecology and Obstetrics.

[a]Other responses: validation cohort (1), tissue-based (1), and reflex all FIGO ≥IB (1).

[b]Other responses: clinician request (5), most recent specimen (2), and depends on clinical information and available material (1).

Table 2.

Algorithm, Technique, and Reporting for *ERBB2*/HER2 (Human Epidermal Growth Factor Receptor 2) Testing in Endometrial Serous Carcinoma (ESC)

	No. (%)
Algorithm used for *ERBB2*/HER2 testing in ESC	286
IHC with reflex to ISH for equivocal results	254 (88.8)
Both IHC and ISH testing	20 (7.0)
ISH only testing	2 (0.7)
ISH with reflex to IHC for equivocal results	1 (0.3)
Other[a]	9 (3.1)
ISH technique used for *ERBB2*/HER2 testing in ESC	213
FISH	187 (87.8)
CISH	20 (9.4)
Other[b]	6 (2.8)
Scoring criteria/guidelines used to report *ERBB2*/HER2 IHC and ISH in ESC	281
ASCO/CAP 2018 guideline for breast carcinoma (PMID 29846122)	195 (69.4)
ASCO/CAP 2013 guideline for breast carcinoma (PMID 24101045)	5 (1.8)
ASCO/CAP 2007 guideline for breast carcinoma (PMID 19548375)	4 (1.4)
Fader et al[17] 2018 clinical trial guidelines requiring >30% strong complete or basolateral/lateral IHC or *HER2*/CEP17 ratio ≥2.0 (PMID 32649220)	45 (16.0)
"Overall" assessment of positive versus negative result	10 (3.6)
Original FDA scoring criteria (HercepTest package insert) for breast carcinoma (PMID 10888772)	14 (5.0)
Other[c]	8 (2.9)

Abbreviations: ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; CISH, chromogenic (bright-field) ISH; FDA, US Food and Drug Administration; FISH, fluorescence ISH; IHC, immunohistochemistry; ISH, in situ hybridization.

[a]Other responses: IHC only (4), clinician request (3), IHC in laboratory with ISH send-out for 2+ and 3+ (1), and no algorithm defined (1).

[b]Other responses: dual ISH (3) and silver ISH (2).

[c]Other responses: CAP/American Society for Clinical Pathology/ASCO gastroesophageal adenocarcinoma (PMID 27841667) (4) and in-house guideline (1).

Table 3.

Case Volume and Testing Practices for *ERBB2*/HER2 (Human Epidermal Growth Factor Receptor 2) Testing in Colorectal Carcinoma (CRC)

	No. (%)
Approximate number of CRC cases evaluated for *ERBB2*/HER2 amplification/overexpression in 2020	239
<10	111 (46.4)
10–20	48 (20.1)
21–50	25 (10.5)
>50	16 (6.7)
Unknown	39 (16.3)
How *ERBB2*/HER2 testing in CRC is routinely performed in the laboratory	239
Only at the clinician's request	196 (82.0)
Reflexively, in certain cases[a]	32 (13.4)
Other[b]	11 (4.6)
If multiple specimens are available for *ERBB2*/HER2 testing in CRC, which types are routinely selected for testing	237
Primary tumor from colectomy	74 (31.2)
No selection made; our laboratory routinely tests any available tumor material	69 (29.1)
Tumor from metastatic site, if present	35 (14.8)
Primary tumor from biopsy	24 (10.1)
No preference or routine practice	22 (9.3)
Other[c]	13 (5.5)

[a]Certain cases responses: metastatic cases (17), all cases (4), wild-type *RAS* results (1), and stomach/esophagus source (1).

[b]Other responses: all cases (4), metastatic cases (3), clinical trial (2), and tissue based (1).

[c]Other responses: clinician request (10), clinical trial, most recent specimen (1), and biopsy/colectomy (1).

Table 4.

Algorithm, Technique, and Reporting for ERBB2/HER2 (Human Epidermal Growth Factor Receptor 2) Testing in Colorectal Carcinoma (CRC)

	No. (%)
Laboratory requires the tumor to be *RAS* wild-type for *ERBB2*/HER2 testing in CRC	234
Yes, required for all tumors	6 (2.6)
Yes, required when *RAS* status is available	17 (7.3)
No	211 (90.2)
Algorithm used for *ERBB2*/HER2 testing in CRC	237
IHC with reflex to ISH for equivocal results	195 (82.3)
Both IHC and ISH testing	18 (7.6)
ISH with reflex to IHC for equivocal results	5 (2.1)
ISH only testing	4 (1.7)
Other[a]	15 (6.3)
Scoring criteria/guidelines used to report *ERBB2*/HER2 IHC and ISH in CRC	233
CAP/ASCP/ASCO gastroesophageal adenocarcinoma HER2 guideline (PMID 27841667)	115 (49.4)
The scoring system used in the HERACLES trial (PMID 26449765)	70 (30.0)
ASCO/CAP 2018 guideline for breast carcinoma (PMID 29846122)	38 (16.3)
Other[b]	10 (4.3)

Abbreviations: ASCO, American Society of Clinical Oncology; ASCP, American Society for Clinical Pathology; CAP, College of American Pathologists; IHC, immunohistochemistry; ISH, in situ hybridization.

[a]Other responses: IHC only (7), clinician request (3), clinical trial requirement (1), ad hoc (1), HERACLES (1), and reflexively (1).

[b]Other responses: original US Food and Drug Administration scoring criteria—HercepTest (2), positive versus negative (1), CAP 2013 breast guideline (1), based on ordering physician (1), and MyPathway (1).

Table 5.

Summary of Published *ERBB2*/HER2 (Human Epidermal Growth Factor Receptor 2) Immunohistochemistry (IHC) Guidelines Relevant to Breast Cancer and Endometrial Serous Carcinoma

Guideline	% Positive Cells by IHC for 3+	Staining Pattern Considered Positive	Consequences of Using This Guideline if Buza is Taken as Gold Standard
ASCO/CAP 2018 for breast[1]	10	Circumferential	May overcall 3+ and not do reflex ISH May undercall by not including basolateral pattern May undercall by excluding heterogeneous cases
ASCO/CAP 2013 for breast[3]	10	Circumferential	May overcall 3+ and not do reflex ISH May undercall by not including basolateral pattern
ASCO/CAP 2007 for breast[4]	30	Circumferential	May undercall by not including basolateral pattern
Fader et al,[17] 2018;Buza,[23] 2021	30	Circumferential + basolateral	Not applicable
FDA/HercepTest insert[40]	10	Circumferential	May overcall 3+ and not do reflex ISH May undercall by not including basolateral pattern

Abbreviations: ASCO, American Society of Clinical Oncology; FDA, US Food and Drug Administration; ISH, in situ hybridization.

Table 6.

Summary of Published *ERBB2*/HER2 (Human Epidermal Growth Factor Receptor 2) Immunohistochemistry (IHC) Guidelines Relevant to Breast and Colorectal Carcinoma

Guideline	% Positive Cells by IHC for Positive Without ISH Confirmation	Staining Pattern Considered Positive	Consequences of Using This Guideline if HERACLES is Taken as Gold Standard
ASCO/CAP GC 2016[7]	10.0 by any validated assay	Circumferential + basolateral	May overcall 3+ and not do reflex ISH
ASCO/CAP breast 2018[1]	10.0 by any approved assay	Circumferential	May overcall 3+ and not do reflex ISH May undercall by not including basolateral pattern
HERACLES trial[25]	50.0 by Ventana 4B5	Circumferential, basolateral, or lateral	May undercall 3+ if a different antibody is used

Abbreviations: ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; GC, gastroesophageal adenocarcinoma; ISH, in situ hybridization.