# ER, PgR, HER2 expression rates seen in Q-Probes

## Anne Paxton

June 2020—With release of the latest Q-Probes study, titled “Expression Rates in Invasive Breast Carcinoma,” the CAP Quality Practices Committee fills a gap by providing data collected from a diverse set of 21 U.S. laboratories on the average frequency of various ER, PgR, and HER2 expression results.

Because the CAP Laboratory Accreditation Program requires that laboratories make annual comparisons of how their results line up with published benchmarks, the committee wished to find out if data from multiple laboratories would support the accreditation program checklist recommendations. The resulting Q-Probes study is part of an effort “to help laboratories make sure their results are in the right ballpark,” says study coauthor and former committee member Daniel David Mais, MD, director of surgical pathology, University of Texas Health Science Center, San Antonio.

“With ER, PgR, and HER2 testing, there’s tremendous concern at all times that your antibodies and your IHC system are working correctly. So we have multiple checks built into the system, and one of them is the guidelines published by the CAP and American Society of Clinical Oncology to annually compare frequencies of expression to benchmarks,” Dr. Mais says. Few data were available on what those benchmarks were, however. “So, here, we assessed the results people were getting in their real-life, day-to-day practice settings.”

The Q-Probes data from 687 breast carcinoma cases generally support accreditation program checklist recommendations (ANP.22970), which are as follows: The overall proportion of ER-negative breast cancers should not exceed 30 percent, somewhat lower in postmenopausal patients; the proportion of ER-negative cases is considerably lower in well-differentiated carcinomas (less than 10 percent); the proportion of PgR-negative cases is 10 percent to 15 percent higher than for ER-negative; for HER2 studies, the overall proportion of HER2-positive breast cancers is 10 to 25 percent; and well-differentiated tumors and lobular carcinomas are almost uniformly ER-positive.

(In the 2020 checklists edition, released June 4, reference to PgR studies was removed because PgR is now considered a prognostic marker rather than predictive.)

In the study, the overall ER-negative rate was 14.4 percent; for PgR it was 24.9 percent. Well-differentiated tumors (97.4 percent) and lobular carcinomas (98.7 percent) were almost uniformly ER-positive.

“The Q-Probes report provides another way to fine-tune the benchmarks that laboratories look at on a day-to-day basis,” says former Quality Practices Committee member and study coauthor Anthony J. Guidi, MD, chair of pathology at Newton-Wellesley Hospital in Massachusetts. “The checklist recommendations have a few specific criteria, but this study provides more benchmark data the CAP can use to potentially refine the checklist criteria. The diverse group of 21 labs that submitted information are all CAP accredited, so we know they use CAP criteria in setting up their assays. And when you can use multi-institutional data, that’s a better way to set up benchmark data than relying on the mostly single-institutional data that’s in the published literature.”

Dr. Brown

While a number of studies address positivity rates, they tend to be from single institutions, says committee chair

and study coauthor Richard W. Brown, MD, medical director for system laboratory services, Memorial Hermann Health System, Houston. “The power of this study is that there was a broad range of laboratory size and institution type, so the study provides a robust data set that laboratories can use for comparisons.”

The study found fairly uniform expression rates among the participating laboratories, Dr. Mais says. “That would suggest to me that testing across laboratories is fairly reproducible and reliable. The accreditation program recommends that the overall portion of ER-negative breast cancer not exceed 30 percent. So if you find you’re falling outside of the recommendation, if more than 30 percent of your patients are ER-negative, that would be somewhat biologically improbable. It would suggest that maybe there is something wrong with either your test system or your interpretations.”

Included in this study is the aggregate data, he says. “Our look at the data is agnostic as to what antibody clone and test systems people are using in their particular lab.” The authors plan, in a separate project, to break down the data by antibody clone to see if anything can be gleaned from that analysis.

One slightly surprising finding of the study was the HER2 positivity rate of nine percent. “That’s lower than most published studies and obviously important because this data helps you compare to real-world test results,” Dr. Mais says. Similarly, a relatively high proportion of low-grade tumors tested as HER2-positive. “We found a 3.2 percent HER2-positive rate in grade one tumors in the study”—also a surprising percentage because grade one tumors are generally HER2-negative. “So potentially some participating institutions could be undergrading tumors or overinterpreting the HER2, or their HER2 system is staining them too darkly.”

Because HER2 expression is strongly associated with high-grade tumors, Dr. Brown says, “the finding of a well-differentiated carcinoma with HER2 expression, while not impossible, raises concern for an immunostain that is not providing an appropriate level of sensitivity.”

Dr. Guidi

If laboratories are outliers and starting to see too many grade one cases be HER2 3+ positive, Dr. Guidi suggests, “That should spur them to look at their assays in more detail and make sure everything is correct in terms of total fixation time and cold ischemic time, and make sure their positive and negative controls are appropriate.” Some of the 3+ positive grade one tumors may reflect undergrading the core due to sampling issues, he notes. “Sometimes it’s just that you are looking at a relatively small amount of tumor on a core; however, on the excised specimen it might not be grade one anymore. You might see mitotically active areas in the tumor and you might bump it up to a grade two.”

But because such cases are a little unusual, his lab decided to verify rare HER2 3+ grade one tumors with a FISH test. “In fact we perform confirmatory HER2 FISH testing in grade one invasive ductal cancers and in grade one and grade two invasive lobular cancers with 3+ HER2 staining because the percentage of true HER2-positive tumors in this cohort should be very low.”

The study found that aggregate frequency distributions of ER and PgR results were of particular interest. They highlight the relative homogeneity of ER expression (85.2 percent of cases showed strong average intensity of ER staining and 83.9 percent of cases with 91 to 100 percent of cells with nuclear positivity) and the more heterogeneous expression of PgR in contrast (60 percent showed strong average intensity of staining and 30.7 percent moderate expression, and 61 percent of PgR-tested cases showed 91 to 100 percent of cells with nuclear positivity). Says Dr. Brown: “Those of us who perform large numbers of these studies have conversationally noted

for some time that there is a basic difference in receptor expression, with ER typically either completely positive, low positive, or negative. There is little in between. In contrast, there is sizable variation in PgR expression from case to case."

This anecdotal experience is supported by the CAP proficiency testing program for ER and PgR, he says, in which "there is a high level of agreement for ER but, in many of the challenges, sufficient variation in interpretation of the PgR cores to preclude grading by 80 percent consensus of at least one tissue core."

"The data from this study provide additional evidence of the inherent variation in PgR expression."

Dr. Mais

The largest percentage of tumors—58.5 percent—were ER/PgR-positive and HER2-negative, an expected rate. "That would be fairly typical," Dr. Mais says. "That's how the majority of our cases tend to stain." Triple-negative tumors represented 8.2 percent of cases and triple-positive tumors represented 3.6 percent of cases. "We all know the triple-positive group exists and that it's a small number of patients, but it's an under-studied group. We've looked long and hard at triple-negative patients, but not much work has been done on the triple-positive group. And it would be an interesting group to look at."

Laboratories will want to assess the reason why their rates might differ from those in the Q-Probes study. Differing expression results for these predictive markers could occur because the patient population is unique in some way, Dr. Mais says. "You may have a particular ethnic group or age group overrepresented. Or you may have a grade of tumor overrepresented."

One can never be certain, Dr. Brown says. "But in general, the results, if deviating from the mean significantly, should be explainable by known associations. For example, one would expect high rates of ER and PR expression if the patient population were predominantly women who are postmenopausal with well-differentiated tumors. A population in which young women with aggressive tumors were overrepresented would be expected to have lower ER and PR expression rates."

But there could be analytic factors that labs would want to check out. "The major place to look would be your test systems," Dr. Mais says. "There could be something anomalous with the reagent antibody you are using or the hardware of the testing system. Or you may be overinterpreting those slides. Things that other people would call 2+ for HER2, you are calling 3+, for example. You'd even want to evaluate whether you are picking the right blocks for staining. We find that we get more reliable results when we choose blocks that have an internal control we can use."

If a laboratory thinks it might have a problem with HER2 staining, the best thing to do may be to retest those cases by FISH or retest in another laboratory and compare the results, Dr. Mais says. "But also reassess the H&E slides in those cases and make sure your grade is appropriate—that you haven't undergraded cases. In my lab, for example, if I have a HER2-positive tumor and I see that it was graded as one, that would cause me to hesitate before reporting those results."

Laboratories that lie above the 90th or below the 10th percentiles in this Q-Probes study should examine their procedures to ensure that the level of sensitivity is appropriate, Dr. Brown recommends. "This involves careful evaluation of internal controls—normal breast tissue—and external positive and negative controls, as well as recent results on proficiency testing challenges. In some cases, a revalidation against tissues with known receptor expression may be warranted."

ER, PgR, and HER2 testing points clinicians to the right way to treat patients, Dr. Guidi notes, and adds, “We don’t want to get that wrong. To make sure we are reporting correctly, we have to participate in proficiency testing, which I think most laboratories do, and also look at benchmark data and follow it over time to make sure nothing drifts.”

He’s a strong believer in using benchmark data to maintain quality and gives an example of the reason based on his laboratory’s experience. “It’s fairly unusual to get patients who are negative for ER and positive for PgR. In the last Q-Probes study of ER and PgR, that combination occurred just over two percent of the time.”

His laboratory saw that combination less than one percent of the time. “But a year or two ago we noticed, because we looked at our data, that this rate was rising in our laboratory. It was approaching the four percent range. This wasn’t picked up through proficiency testing, but by trending data and looking at it.” The laboratory realized it needed to retool its PgR immunostain process, it made modifications, and the rate is once again less than one percent a year. This experience confirms the value of benchmarking, he says. “We need to get it right, and benchmarking is one way, one tool in our tool belt, to do that.”

The key takeaway of this study for Dr. Brown is that ongoing attention to quality is essential.

“We know from clinical trials in which central review is performed that there can be significant variation in the way in which these predictive markers are performed and reported. Recent data from CAP Surveys suggest that in that cohort of participating laboratories, concordance is excellent. However, we must all remain vigilant, as the immunostains currently in use require significant antigen retrieval, which is known to be a cause of both false-positive and false-negative results, depending on the integrity of tissue fixation and processing.” So comparison of positivity rates and patterns of expression against those of other laboratories and against prior performance within the laboratory, he says, should remain an important tool for quality management. 

*Anne Paxton is a writer and attorney in Seattle. Full results of the study will be submitted for publication in the Archives of Pathology & Laboratory Medicine.*