# A scan of studies on HER2-low breast cancer scoring

## Sherrie Rice

January 2024—Much has been said and written about scoring HER2-low breast cancer, and it has its difficulties. But there are steps and tools to support scoring, and Savitri Krishnamurthy, MD, last fall shined a light on them and several HER2-low breast cancer-related studies.

Dr. Krishnamurthy, professor in the Department of Pathology, Division of Pathology and Laboratory Medicine, University of Texas MD Anderson Cancer Center, spoke on the continuum of HER2 expression in a CAP TODAY webinar sponsored by Daiichi Sankyo and AstraZeneca.

About 85 percent of breast cancers are HER2 negative and 15 percent are HER2 positive. About 60 percent of the HER2-negative cases are HER2-low. Of all breast cancers, about 50 percent can be categorized as HER2-low, defined as those breast cancers that are scored as HER2 IHC 1+ or 2+/in situ hybridization negative.

The 2023 ASCO-CAP guideline update for HER2 testing in breast cancer provides recommendations for HER2-low assessment, one of which is to pay close attention to preanalytic conditions and follow the guidelines for optimal tissue handling. Another is to use controls with a range of HER2 expression, including cases of HER2 IHC 1+, and to examine the HER2 IHC at 40× magnification when distinguishing HER2 IHC 0 from HER2 IHC 1+ staining. Second pathologist review is recommended when results are close to the IHC 0 versus 1+ interpretive threshold.

Dr. Krishnamurthy

On the reporting of HER2 IHC results, the recommendation is to always report semiquantitative (discrete) HER2 IHC scores: 0, 1+, 2+, 3+. Scores cannot be reported as 0 to 1+, 1+ to 2+, or 2+ to 3+. A footnote in the pathology report on the potential therapeutic implications of the result is recommended (Wolff AC, et al. *Arch Pathol Lab Med.* 2023;147[9]:993–1000).

“There’s a lot of research going on to look into the lower boundary of HER2 expression that will be important for clinical decision-making,” Dr. Krishnamurthy said.

Peiffer, et al., investigated whether HER2-low breast cancer is a clinically distinct subtype in terms of prognosis (Peiffer DS, et al. *JAMA Oncol.* 2023;9[4]:500–510). In their study of more than 1 million patients, they found minimal prognostic differences between HER2-low and HER2-negative breast cancer and no support for the classification of HER2-low breast cancer as a unique disease entity.

In the triple-negative breast cancer group, a very minimal survival advantage was seen in the stage II and stage IV cases, Dr. Krishnamurthy said, whereas in the hormone-receptor–positive group it was between stage II and stage IV. “So there is very minimal survival benefit of HER2-low breast cancers in both triple-negative and hormone-receptor–positive breast cancer,” she said.

## The concordance rates when distinguishing between HER2-positive and

## HER2-negative status have improved significantly over the years, Dr. Krishnamurthy noted.

Now the question is: "How can we achieve concordance also in this new category of HER2-low breast cancers?"

Karakas, et al., studied the interobserver and interantibody reproducibility of HER2 IHC scoring among six breast pathologists independently using current HER2 guidelines and reported "notable" variation, especially in cases with scores of 0 to 1+ (Karakas C, et al. *Am J Clin Pathol.* 2023;159[5]:484–491). "Identifying the 10 percent cutoff between IHC 0 and 1+ scores can be difficult," Dr. Krishnamurthy said of the findings.

"It is subjective," she said. "Terms such as 'barely perceptible' used in the ASCO-CAP guidelines can pose challenges to pathologists in real practice. This can result in differences in the interpretation of HER2 IHC scores at the lower end of the spectrum, particularly in borderline cases where it is between 0 and 1+ and you are debating what to do."

But studies have shown that pathologists can reproducibly detect HER2-low tumors with HER2 IHC. A poster presented at the San Antonio Breast Cancer Symposium in 2022 found that overall concordance for classifying HER2 status using the new three-tiered classification scheme was numerically higher than previously reported, and with training it improved further (Rüschoff J, et al. Poster HER2-13 presented at: San Antonio Breast Cancer Symposium; Dec. 6–10, 2022).

"The pathologist concordance for the three-tiered classification—HER2 0, HER2 1+, and HER2 positive—even without any training was about 83 to 84 percent," Dr. Krishnamurthy said. There was improvement with training to up to 85 percent. For the binary classification it was very high—close to 100 percent—and changed only marginally. "This was both for the Dako HercepTest and the Ventana Pathway 4B5 assay. The performance of pathologists is not that bad for the distinction of 0 and 1+ with an intent to separate the two categories," she said.

Another study presented as a poster at the 2022 symposium found the positive agreement between re-scored and historical HER2 scores was 81.2 percent. It was greater (87.3 percent) in HER2-low concordance than in HER2 IHC 0 (70.1 percent) (Viale G, et al. Poster HER2-15 presented at: San Antonio Breast Cancer Symposium; Dec. 6–10, 2022). "So HER2-low is better than HER2 0. Overall, it is clear that if you take all the published reports, the agreement among pathologists for a distinction of 0 and 1+ is around 70 to 80 percent," Dr. Krishnamurthy said.

Finkelman, et al., presented a study at the 2023 USCAP 112th annual meeting showing that 24 percent of patients with breast cancer presented with discordant HER2 IHC scores between core needle biopsy and excision specimens (Finkelman B, et al. Abstract 144 presented at: USCAP 112th annual meeting; March 11–16, 2023; New Orleans). Thirty-eight percent of tumors that were scored as HER2 IHC 0 on excision were HER2-low on core needle biopsy, and 16 percent that were HER2-low on excision were IHC 0 on core needle biopsy.

"These are the challenges for identification of HER2-low breast cancer," Dr. Krishnamurthy said.

## The subjectivity in scoring can be minimized by following standardized procedures and participating in educational programs, she said.

The time to and duration of fixation, antibody clone type, and other preanalytic factors have been shown to affect HER2 IHC interpretation. "We do not want the cold ischemic time to be prolonged as we know the HER2 IHC staining can be compromised in around 24 percent of cases. It is good to put that tissue immediately in formalin and not to leave the sample beyond 24 hours in formalin to get optimal HER2 IHC staining results," Dr. Krishnamurthy noted.

Initial validation with 20 negative and 20 positive cases for FDA-approved assays and 40 negative and 40 positive for laboratory-developed tests is key. If procedures change, revalidation of the tests is required. Torlakovic, et al., "showed the need for revalidation if we change our purpose for the testing results. Now that HER2-low is important as a classification for identifying patients for targeted therapy, it will be useful to consider revalidation of the

laboratory testing to suit the optimal identification of HER2-low breast cancers," Dr. Krishnamurthy said (Torlakovic EE, et al. *Appl Immunohistochem Mol Morphol*. 2017;25[3]:151–159).

A range of controls should be used for optimal identification of HER2-low breast cancers, she said, to include HER2 IHC 1+ cases in addition to 0, 2+, and 3+. And 40× magnification should be used for optimal distinction of HER2 IHC 0 from HER2 IHC 1+ staining.

The previously cited study by Rüschoff, et al., found that training pathologists can improve classification of HER2 IHC 0 and HER2-low cases. In the Rüschoff analysis, training increased the positive percent agreement by 14.6 percent (74.6 percent versus 89.2 percent) for HER2 0 cases and negative percent agreement by 10.5 percent (80.6 percent versus 91.1 percent) for HER2-low cases.

## Computational image analysis technologies, too, can be useful in overcoming the subjectivity, she said.

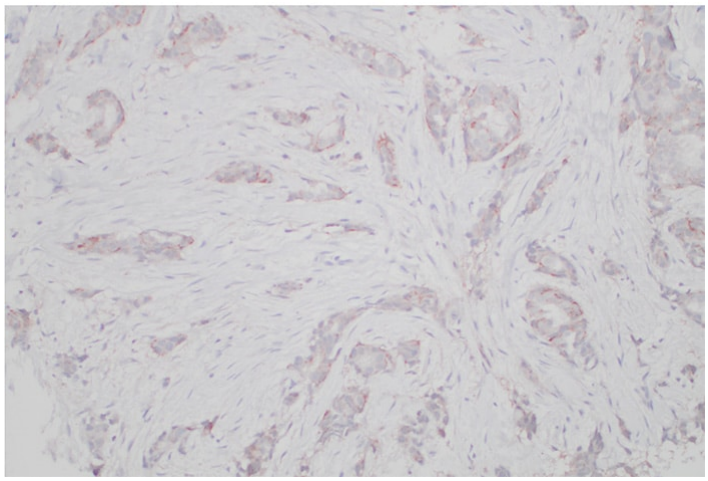
"It is not that computational image analysis is completely going to read the cases, bypassing the pathologist. It is an aid to what the pathologist is already doing. So it's not here to replace that expertise but rather to add value."

Computational image analysis can support the pathologist in assessing HER2 IHC in breast cancer in both single-task and multitask functions. Single-task functions include quantifying the histological parameters or the structural changes and aiding pathologists in HER2 IHC scoring or disease diagnosis. Multitask functions include coupling the histopathological images with transcriptomic results and correlating morphology with molecular profiles and response to therapy to predict progression. "It is limitless," Dr. Krishnamurthy said of the potential applications.

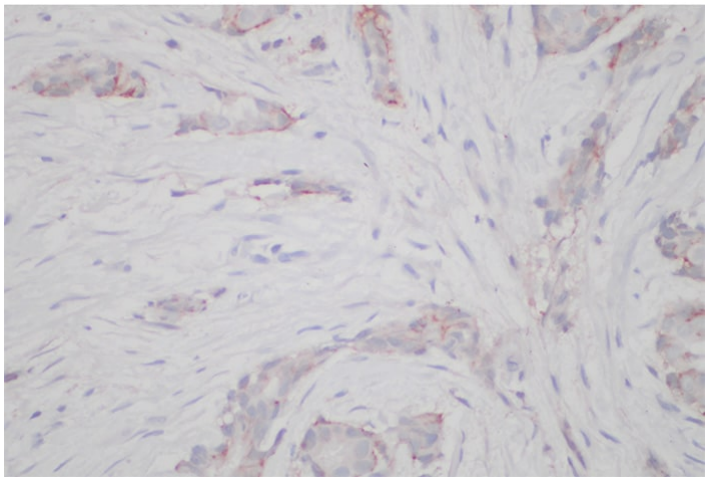
None of the tools is FDA approved now, she noted, but several AI-based computational image analysis tools are being developed for AI-based algorithmic interpretations of digitized IHC slides that can aid the pathologist in assessing HER2 status.

Applying computational image analysis to HER2 assessment using fully automated solutions has shown strong results, she said. Globerson, et al., presented a poster showing the results of a fully automated AI system (Globerson Y, et al. Poster P6-04-05 presented at: San Antonio Breast Cancer Symposium; Dec. 6–10, 2022). "The system first evaluates the invasive tumor, and in this invasive component it can quantitate the HER2 expression and give the output as a HER2 IHC score based on the current ASCO-CAP guideline."

**Case 1 HER2-low immuno ×20**

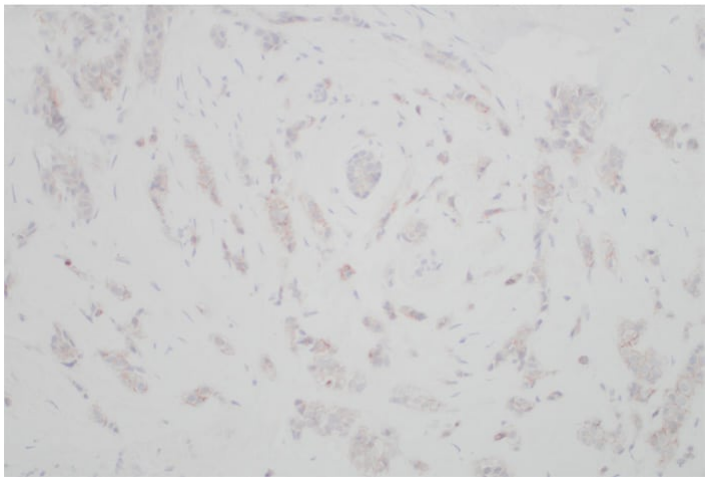


**Case 1 HER2-low immuno ×40**

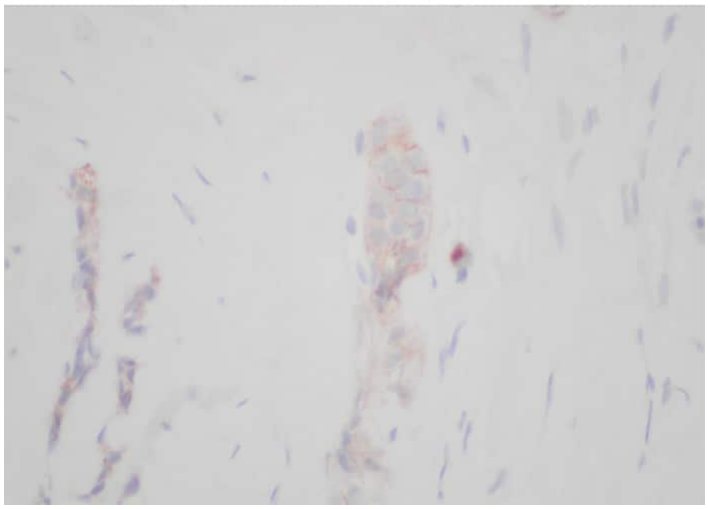


Dr. Krishnamurthy last year presented her own study, which "showed good concordance of pathologists using these AI tools." Concordance improved in the consensus scores of breast pathologists, she said (Krishnamurthy S, et al. Abstract 172 presented at: USCAP 112th annual meeting, March 11–16, 2023; New Orleans). The interobserver agreement between pathologists for identification of HER2-low breast cancer improved from 69.7 percent to 77.2 percent with the aid of an AI tool. Similarly, the agreement of the general pathologists with five breast experts improved from 81.9 percent to 88.8 percent with the help of the AI tool. "So it looks like these fully automated AI solutions can be useful ancillary tools for pathologists," Dr. Krishnamurthy said.

**Case 2 HER2-low immuno ×20**



**Case 2 HER2-low immuno ×40**



Wu, et al., found in their study that pathologists, with the help of AI, improved their accuracy in distinguishing between HER2 IHC 0 and 1+ in cases with heterogeneity, from 0.68 to 0.89 in the AUC results (Wu S, et al. *Mod Pathol.* 2023;36[3]:100054). "They also clearly showed results that it benefits junior pathologists tremendously in achieving better concordance with senior pathologists," she said.

Spitzmüller, et al., studied the role of computational image analysis for HER2 quantification, particularly for HER2 IHC 0 and 1+, and their study showed very good concordance with manual scoring—R = 0.993 (Spitzmüller A, et al. Poster P6-04-03 presented at: San Antonio Breast Cancer Symposium; Dec. 6–10, 2022). This study also shows the value of an AI algorithm in identifying expression patterns in tumors using spatial metrics.

Similarly, Chow, et al., reported on the utility of an AI algorithm for distinguishing 0 and 1+ (Chow A, et al. Abstract 127 presented at: USCAP 112th annual meeting; March 11–16, 2023; New Orleans). Computational image analysis interpretations, they found, were concordant with pathologists in 89 percent of cases when assessing HER2 IHC 0 and HER2-low.

More interesting, Dr. Krishnamurthy said, is applying computational image analysis directly on H&E-stained whole slide images to predict HER2 status, including HER2-low. Conde-Sousa, et al., conducted the HEROHE (HER2 on H&E) challenge, in which 21 groups participated, each with an AI-based image analysis algorithm (Conde-Sousa E, et al. *J Imaging.* 2022;8[8]:213). The ROC curves for the six best-performing algorithms were equal to or greater than 0.84, "which indicates very good specificity in predicting HER2 status directly from H&E slides," she said.

Marra, et al., studied 1,479 H&E-stained whole slide images from 417 primary breast cancers that were categorized according to HER2 IHC, FISH, and HER2 copy number amplification (Marra A, et al. *Ann Oncol.* 2022;33[suppl 7]:S581). "They could categorize HER2-low from HER2 amplified cases very well using just whole slide images of the tumors." The sensitivity for distinguishing HER2-low and amplified cases from HER2 IHC 0 was 76 percent, and the specificity was 73 percent. "You can see the promise of directly predicting HER2-low or HER2-positive status from whole slide images of the H&E slide," Dr. Krishnamurthy said.

Thus, using computational image analysis for assessing HER2-low breast cancer can be a tool to overcome the subjectivity inherent in manual interpretation of HER2 IHC stains, whether on the microscope or on a digital modality, she said. "These modalities can be useful to help us solve this problem of interobserver variability when assessing HER2 IHC at the low end of the spectrum." They can also provide decision-making support pre-read or post-read, she added.

They come with challenges, however, among them that the models are still being validated, there is a need for standardization, the scanners vary, and there are inconsistencies in sample preparation, staining, and image digitization. "The College of American Pathologists is working to bring about uniform, standardized, digitized images across labs that will facilitate the application of these computational image analysis tools," Dr.

Krishnamurthy said.

“We have to iron out those problems,” she added, “before we can consider the utilization of these in standard-of-care practice.” 

*Sherrie Rice is editor of* CAP TODAY.