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CORRESPONDENCE

High concordance rate of HER2 status assessed via silver *in situ* hybridisation (SISH) between core biopsy and excision specimens: a 4 year retrospective review from a single institution

Sir,

HER2 amplification occurs in about 15% of breast carcinomas and these tumours have an aggressive behaviour. Currently, there is no consensus as to whether HER2 *in situ* hybridisation (ISH) is best performed on core biopsy (CBx) or excision specimens. In our department, we prefer to perform testing on excision specimens partly because the initial Roche-funded HER2 testing program was largely limited to excision specimens. We present a 4 year experience of HER2 SISH testing on CBx of invasive breast carcinomas between 2008 and 2012 from the Department of Tissue Pathology and Diagnostic Oncology at Westmead Hospital, Sydney. The overall aim of the study was to assess the suitability of CBx for HER2 ISH testing and to determine the concordance rate between the CBx and the excision specimens.

HER2 SISH testing was performed on a total of 194 breast CBx from 185 patients using either Inform HER2 SISH or INFORM HER2 dual SISH stains (Ventana, USA) in accordance with the protocol from Ventana. The reporting was based on the American Society of Clinical Oncology (ASCO) guidelines.[1] A tumour was HER2 amplified if the HER2 copy was >6 on the single HER2 SISH probe, or if the HER2:Cep 17 ratio was >2.2. Negative tumours would have HER2 copy of <4 or HER2:Cep17 ratio of <1.8. Tumours with HER2 copies between 4 and 6 or HER2:Cep17 ratio of between 1.8 and 2.2 would be considered positive if the average ratio of at least 40 cells was >2. Equivocal HER2 status was only diagnosed if HER2:Cep17 was 2. Insufficient cells would be diagnosed only in cases with low absolute number (<20) of tumour cells with adequate signals. This was more common in biopsies where the interpretation was complicated by other confounding factors such as overlapping cells or crush artefact.

Of the 194 core biopsy specimens, 191 were primary tumours and three were chest wall recurrences. Of these, there were 177 invasive ductal carcinomas, 13 invasive lobular carcinomas, one squamous cell carcinoma, one intracystic papillary carcinoma and two biopsies containing only intralymphatic tumour. HER2 results were reached in 188 cases (96.9% of the total) and 41 cases (21.8% of all) were HER2 amplified. Six cases were excluded because of insufficient tumour (3 cases), repeated stain failure (2 cases) or equivocal result (1 case). Thirty-four CBx (17.5% of total) needed repeat staining including one of the cases that was considered uninterpretable despite repeat testing (see Table 1).

HER2 SISH results from surgical excision were available for comparison for 55 tumours from 54 patients. Of these, 14 patients had neoadjuvant chemotherapy and another was treated with aromatase inhibitor pre-operatively. Overall, HER2 was amplified in 11 CBx but only eight surgical specimens and 52 of 55 tumours (94.5%) had a concordant HER2 SISH result (kappa value=0.85). All three discordant cases had low level HER2 amplification (HER2 gene copy <10) on CBx and negative in the excision specimen. The single case that was reported as equivocal on CBx had retesting performed on the excision, which was reported as HER2 non-amplified.

HER2 ISH guidelines vary in different countries. HER2 ISH testing on CBx may be necessary in patients who are not suitable for surgery, have metastatic disease or when neoadjuvant chemotherapy is required. Our results demonstrate a high concordance rate for HER2 SISH testing between CBx and excision specimens. Our study is limited by the small cohort where only 55 of the tumours were tested on both CBx and excision specimens. Neverthless, the finding is consistent with the evidence so far in the current literature, where various groups have reported concordance rates of 86.5–99% for IHC[2–4] and 87–99% for ISH.[2,3,5] Another study by Lee *et al*. found concordance of 98% based on a combination of IHC and ISH, where ISH was performed only on cases with equivocal 2+ IHC.[6]

ISH is currently considered to be the gold standard in HER2 testing. In Australia, it is mandatory to have ISH testing for HER2 in patients with early breast carcinoma in order to access the government-funded rebate for Herceptin. Our study is one of the only few studies to examine concordance of HER2 ISH testing between CBx and excision specimens.

ISH may be a superior method of HER2 testing compared with IHC for a number of reasons. Firstly, about 15% of breast cancers have an equivocal 2+ result with IHC, although this can vary significantly between laboratories.[1] These tumours with equivocal IHC result require further ISH testing for a definitive result.

In addition, the reliability of HER2 IHC depends significantly on the processing protocol in individual laboratories. Concerns regarding the reproducibility of HER2 IHC testing were first raised by the results from the N9831 Adjuvant Trial, where non-Hercep and Hercep IHC performed at local laboratories had a concordance of 75.0% and 81.6% compared with the central laboratory, respectively.[7] Despite this, there are also studies that were able to find reliable and reproducible HER2 IHC results. The same trial found high concordance of 94.3% between the central and reference laboratories for a subset of tumours that were specifically tested, while other studies also demonstrated that it was possible to perform HER2 IHC reliably such that the IHC results had a high concordance rate with ISH.[2,8] One problem that may complicate the reliability of HER2 IHC is the

Table 1 Summary of HER2 SISH results

Total number of core biopsies ($n = 194$)	
HER2 postive samples	41 (21.2%)
HER2 negative samples	147 (75.8%)
Equivocal biopsies	1 (0.5%)
Uninterpretable	2 (1.0%)
Insufficient cells	3 (1.5%)

Tumours with HER2 results on both core biopsies and excision ($n = 55$)		
	HER2+ on CBx	HER2− on CBx
HER2+ on excision	8	0
HER2− on excision	3	44

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number of commercial HER2 IHC antibodies available. It is likely that not all antibodies are as reliable as others. For example, two papers suggested that 4B5 may be a superior HER2 antibody compared with CB11 because it produces more crisp staining.[9,10] However, the difference in sensitivity, specificity or predictive value did not reach statistical significance in one study.[9] In the other study, the more superior sensitivity for 4B5 was only apparent in the multicentre group and this might have been due to stringency of the processing and staining in some peripheral laboratories rather than the antibody itself.[10] On the other hand, multiple studies have shown fluorescent, silver and chromogenic ISH to be fairly equivalent to each other.[2,9,11,12] These findings suggest that the reliability of HER2 IHC testing depends more on the individual laboratory and pre-analytical procedures rather than the actual antibodies, and perhaps testing should ideally be limited to selected referral laboratories that perform large volume HER2 testing. While reliability of HER2 IHC is less of a problem in Australia because almost all invasive breast carcinomas will have ISH testing (at selected laboratories), the same cannot be said about other countries, since IHC is still cheaper, more widely available, easier to perform and the recommended first line testing modality for HER2 testing as per ASCO/CAP guidelines.[1]

Of our CBx cases, 17.5% required repeat staining. This figure was calculated since we suspected that SISH testing on CBx could be more problematic, and potential issues would include crushed, overlapping cells, precipitation around the edges and scant cells. These problems are expected to be more common in CBx because of the method of sampling. Our figure compares unfavourably with the data from an Australian multi-centre study. This paper indicated that 13.7% of SISH cases in the first year of testing (>90% cases were surgical excision specimens) required repeat testing, and the figure subsequently dropped to 7.2% by the third year of testing.[8] The repeat rate was thought to be higher than expected in the first year due to global silver wash contamination issues.[8] Despite the lower figure from this study, it would be prudent to point out that there was a large range of SISH repeat rates from different laboratories (0.4–26.8%). Ideally there should have been a head to head comparison of repeat staining rates between excision and CBx specimens. However, this is beyond the scope of this study due to many variables such as interobserver variability and different processing protocols from a variety of referring laboratories. Nevertheless, a repeat rate of 17.5% is too high in any case given the cost of the SISH testing, and further study may be helpful to further evaluate this.

In summary, we found a high concordance rate of 96.9% for HER2 SISH results on CBx and surgical excisions performed on the same tumours within a period of 4 years. A total of 17.5% CBx needed repeat staining, a percentage that is higher than desirable given the cost of the SISH testing. Overall, we conclude that HER2 SISH can be reliably performed on CBx and this would be an appropriate choice of testing if required. Testing on excision specimens may be preferable, however, in view of the high repeat test rates largely due to technical and interpretative difficulties on core biopsy samples.

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1. Wolff AC, Hammond EH, Schwartz JN, *et al*. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor 2 receptor testing in breast cancer. *J Clin Oncol* 2007; 25: 118–45.
2. Arnould L, Roger P, MacGrogan G, *et al*. on behalf of the CRITHER participant pathologistsAccuracy of HER2 status determination on breast core-needle biopsies (immunohistochemistry, FISH, CISH and SISH vs FISH). *Mod Pathol* 2012; 25: 675–82.
3. D'Alfonso T, Liu YF, Monni S, *et al*. Accurately Assessing HER-2/neu status in needle core biopsies of breast cancer patients in the era of neoadjuvant therapy: Emerging questions and considerations addressed. *Am J Surg Pathol* 2010; 34: 575–81.
4. Park SY, Kim KS, Lee TG, *et al*. The accuracy of preoperative core biopsy in determining histologic grade, hormone receptors, and human epidermal growth factor receptor 2 status in invasive breast cancer. *Am J Surg* 2009; 197: 266–9.
5. Shousha S, Peston D, Amo-Takyi B, *et al*. Evaluation of automated silver-enhanced in situ hybridization (SISH) for detection of HER2 gene amplification in breast carcinoma excision and core biopsy specimens. *Histopathology* 2009; 54: 248–53.
6. Lee AH, Key HP, Bell JA, *et al*. Concordance of HER2 status assessed on needle core biopsy and surgical specimens of invasive carcinoma of the breast. *Histopathology* 2012; 60: 880–4.
7. Perez EA, Suman VJ, Davidson NE, *et al*. HER2 testing by local, central, and reference laboratories in specimens from the North Central Cancer Treatment Group N9831 Intergroup Adjuvant Trial. *J Clin Oncol* 2006; 24: 3032–8.
8. Bilous M, Morey AL, Armes JE, *et al*. Asssessing HER2 amplification in breast cancer: findings from the Australian in situ hybridization program. *Breast Cancer Res Treat* 2012; 134: 617–24.
9. van der Vegt B, de Bock GH, Bart J, *et al*. Validation of the 4B5 rabbit monoclonal antibody in determining HER2/neu status in breast cancer. *Mod Pathol* 2009; 22: 879–86.
10. Powell WC, Hicks DG, Prescott N, *et al*. A new rabbit monoclonal antibody (4B5) for the immunohistochemical (IHC) determination of the HER2 status in breast cancer: comparison with CB11, fluorescence in situ hybridization (FISH), and interlaboratory reproducibility. *Appl Immunohistochem Mol Morphol* 2007; 15: 94–102.
11. Dietel M, Ellis IO, Hofler H, *et al*. Comparison of automated silver enhanced in situ hybridisation (SISH) and fluorescence ISH (FISH) for the validation of HER2 gene status in breast carcinoma according to the guidelines of the American Society of Clinical Oncology and the College of American Pathologists. *Virchows Arch* 2007; 451: 19–25.
12. Mayr D, Heim S, Weyrauch K, *et al*. Chromogenic in situ hybridization for HER-2/neu-oncogene in breast cancer: comparison of a new dual-colour chromogenic in situ hybridization with immunohistochemistry and fluorescence in situ hybridization. *Histopathology* 2009; 55: 716–23.

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Intravascular large B-cell lymphoma presenting as cauda equina syndrome and showing aberrant cytokeratin expression: a diagnostic challenge

Sir,
A 51-year-old male, without prior medical history, was referred to our hospital for a painful right-side S1 radiculopathy that had started 4 weeks earlier rapidly followed by weakness in the