ORIGINAL ARTICLE

HER2 overexpression of breast cancers in Hong Kong: prevalence and concordance between immunohistochemistry and in-situ hybridisation assays

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Objectives To evaluate the prevalence of human epidermal growth factor receptor 2 (*HER2*) gene overexpression in breast cancer patients encountered in Hong Kong and the concordance of *HER2* findings from primary immunohistochemistry assays and confirmatory in-situ hybridisation assays.

Design Retrospective study.

Setting Department of Clinical Oncology in a public hospital in Hong Kong.

Patients All patient referrals between July 2006 and June 2007 with newly diagnosed invasive breast cancer (for prevalence evaluation), and all patients treated at our unit with confirmatory in-situ hybridisation tests performed within the study period (for concordance evaluation).

Results There were 272 consecutive breast cancer patients eligible for prevalence evaluation. The distribution for immunohistochemistry staining in 249 cases for scores 0, 1+, 2+, and 3+ were 99 (40%), 40 (16%), 58 (23%), and 52 (21%) respectively. In the remaining 23 patients, four and 19 breast cancers were unscored and reported by immunohistochemistry to be *HER2*-positive and -negative, respectively. The overall *HER2* overexpression rate (3+ or reported as positive) was 21%. *HER2* overexpression was associated with grade 3 histology (P<0.001) and negative hormonal receptor status (P<0.001). However, it was not associated with age (P=0.525), T-classification (P=0.740), N-classification (P=0.691), nor group stages (P=0.433). Of the 37 patients with confirmatory in-situ hybridisation tests performed, 10 (71%) of 14 with immunohistochemistry staining of 3+ and 1 (4%) of 23 with immunohistochemistry staining of 2+ were found to have *HER2* gene amplification.

Conclusions More than 25% of *HER2* overexpression identified by immunohistochemistry assays in this Hong Kong cohort could not be verified by confirmatory in-situ hybridisation assays. Compliance with the latest guidelines for *HER2* testing should improve the future accuracy and concordance.

Key words
Breast neoplasms; Gene amplification; Immunohistochemistry; In situ hybridization, fluorescence; Receptor, epidermal growth factor

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Introduction

The human epidermal growth factor receptor 2 (*HER2*) gene has emerged as a very important predictor of outcome among patients having invasive breast cancers; its amplification is associated with more aggressive disease and a worse prognosis.[1,2] *HER2* positivity is also associated with relative resistance to endocrine therapies[3] and non-anthracycline, non-taxane containing chemotherapy regimens.[4] Most importantly, therapies, such as trastuzumab (Herceptin) targeting the HER2 oncoproteins, have recently been established as the standard component in the treatment of *HER2* overexpressed breast cancers, in both adjuvant and metastatic settings.[5-7] Accordingly, starting from 2005, *HER2* status has been incorporated into the risk allocation of the St Gallen consensus to guide the use of systemic adjuvant therapies.[8]

In Hong Kong, since the late 1990s *HER2* testing has been routinely performed for all

newly diagnosed invasive breast cancers. Although in-situ hybridisation (ISH) assay (for *HER2* gene amplification) is often considered the more desirable method of testing for it, the immunohistochemistry (IHC) assay (for HER2 protein overexpression) is cheaper, more widely available, and has long been the primary screening test in all laboratories. In-situ hybridisation tests are often reserved for equivocal cases only, but are not currently available in public hospitals. Despite the critical importance of *HER2* status in prognosis and treatment decisions, the accuracy of the *HER2* test has recently become a matter of much concern.[9-11] Because of the expense and potential life-threatening cardiotoxicity of anti-HER2 therapies,[12,13] the importance of accurate *HER2* testing cannot be overemphasised.

Our study therefore aimed to assess the prevalence of *HER2* overexpression among patients with invasive breast cancers in Hong Kong, and evaluate how well the *HER2* status determined by IHC and the confirmatory ISH assay agreed.

香港乳癌的*HER2*受體基因過度表現：以免疫組織化學分析和原位雜交技術檢視比較現患率和一致性

目的 評估香港乳癌患者*HER2*受體基因過度表現的現患率，和以免疫組織化學分析和確定性原位雜交技術比較*HER2*受體結果的一致性。

設計 回顧研究。

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患者 2006年7月至2007年6月期間獲轉介的侵犯性乳癌新症患者（作現患率評估），以及所有在上述醫院接受治療並進行原位雜交技術的患者（作一致性評估）。

結果 連續272名乳癌患者符合現患率評估資格。在249個進行免疫組織化學染色分析中，有99 (40%)、40 (16%)、58 (23%)和52 (21%)個個案分別得分為0、1+、2+ 和3+；其餘23名沒有評分的患者，當中4名和19名分別對*HER2*受體基因呈陽性和陰性反應。*HER2*受體基因過度整體表現率（即得分為3+和結果呈陽性）為21%。*HER2*受體基因過度表現與第3級組織學(P<0.001)和荷爾蒙受體陰性狀態(P<0.001)有關聯；但與年齡(P=0.525)，T分類(P=0.740)、N分類(P=0.691)以及小組階段(P=0.433)皆不相關。進行確定性原位雜交技術共37名病人，當中進行免疫組織化學染色分析得分為3+的14名患者，以及23名得分為2+的患者當中，分別有10名(71%)和1名(4%)被發現有*HER2*受體基因擴增跡象。

結論 超過25%利用免疫組織化學分析確定*HER2*受體基因過度表現的患者，並不能以原位雜交技術核實病情。遵照*HER2*受體基因測試的最新指引有助改善將來測試的準確和一致性。

Patients and methods

To assess the prevalence of *HER2* overexpression, we retrospectively analysed the records of all newly diagnosed female breast cancer patients referred to the Department of Clinical Oncology, Pamela Youde Nethersole Eastern Hospital, Hong Kong between the period July 2006 and June 2007. Patients with pure in-situ carcinomas or inadequate tissue diagnoses were excluded from analysis. Patients presenting with metastatic disease were included so as to avoid selection bias.

For evaluation of concordance between IHC and ISH results, findings from all ISH tests performed within the study period were compared against the findings of primary IHC testing. Patients, who first presented before the study period, were included as long as they had confirmatory ISH tests within the study period, so that they could be considered for target therapies in either the metastatic or adjuvant setting.

Immunohistochemistry assay

Various commercially available anti-HER2 antibodies (eg CB11 monoclonal antibodies, Novocastra Laboratories, Newcastle-on-Tyne, UK) were used in different local laboratories to detect HER2 protein in IHC assays. The stained slides were scored on a 0 to 3+ scale—0 was given if there was no membrane staining or if staining was present in less than 10% of tumour cells; 1+ for faint, or barely perceptible membrane staining in more than 10% of cells with stain involving only part of the cell membrane; 2+ for weak-to-moderate complete staining in more than 10% of tumour cells; and 3+ for strong complete membrane staining in more than 10% of tumour cells. Respective scores of 0 and 1+ were considered *HER2*-negative, 2+ as *HER2* equivocal, and 3+ as *HER2*-positive.

In-situ hybridisation assay

Although the ISH assay is not yet available in public laboratories, it can be performed in some private or university laboratories, mainly for confirmation of *HER2* status in patients with IHC scores of 2+ or 3+. There were two main types of ISH tests used in our patients; fluorescence in-situ hybridisation (FISH) and chromogenic in-situ hybridisation (CISH). The latter tests were performed in some private laboratories for IHC 2+ patients before referral. For IHC 3+ patients, confirmatory ISH tests were considered unnecessary, even in laboratories with ISH facilities. However, as we preferred to have confirmatory ISH tests for IHC 3+ patients before starting them on anti-HER2 therapy, we arranged FISH tests in the University of Hong Kong for those who agreed to self-finance the confirmatory tests.

Fluorescence in-situ hybridisation tests were performed in the Molecular Pathology Laboratory, Department of Pathology, University of Hong Kong. It involved using the Food and Drug Administration (FDA)–approved PathVysion HER-2 DNA Probe Kit (Abbott Molecular Inc, Des Plaines [IL], US) for dual signal evaluation. *HER2* gene amplification was defined as *HER2* probe/control probe ratio of 2.2 or more for at least 40 nuclei of invasive cancer cells. An additional 20 nuclei were counted, whenever a borderline ratio (1.8-2.2) was initially found.

Chromogenic in-situ hybridisation has emerged as an alternative to FISH, since the latter requires additional analytic equipment not widely available in pathology laboratories (such as dark-field fluorescence microscopy and multiband fluorescence filters). The CISH test uses a digoxigenin-labelled DNA probe (SPPT-Light [Zymed] HER2 CISH kit) to *HER2* and the hybridised *HER2* probe is detected by a chromogenic reaction using mouse antidigoxigenin antibody, followed by antimouse-peroxidase and diaminobenzidine as chromogen. *HER2* amplification is defined by an average of more than 10 dots or large clusters or a mixture of multiple dots and large clusters of the *HER2* gene per nucleus in more than 50% of tumour cells. Although CISH has not yet been approved by the US FDA, a number of studies have shown good (>90 to 100%) concordance between CISH and FISH results.[14,15]

Statistical methods

The Chi squared test was used to test the association between *HER2* overexpression and other clinicopathological characteristics. All statistical tests were two-sided and performed at the 0.05 level of significance (P value). The Statistical Package for the Social Sciences (Windows version 12.0; SPSS Inc, Chicago [IL], US) was used for all analyses.

Results

There were 272 consecutive patients with invasive breast cancers referred to our department between July 2006 and June 2007, who were eligible for review. The median age of the patients was 55 (range, 33-92) years. Their clinical and pathological characteristics are shown in Table 1.

Of the primary IHC tests, 39%, 21%, and 40% were performed in our own laboratory, other public hospital laboratories, and private laboratories respectively. There were 4 and 19 breast cancers reported to be *HER2*-positive and -negative respectively, without detailed IHC scoring. Of the 249 breast cancers with IHC scoring, the distribution for IHC staining of 0, 1+, 2+ and 3+ were 99 (40%), 40 (16%), 58 (23%), and 52 (21%) respectively. The overall *HER2* overexpression rate (IHC staining of 3+

TABLE 1. Demographic, clinical, and pathological characteristics of the 272 patients with invasive breast cancers

Characteristic	Patients No. (%)
Race	
Chinese	261 (96)
Other Asians or Caucasians	11 (4)
Reporting pathology laboratories	
Our own hospital	105 (39)
Other public hospital	57 (21)
Private/others	110 (40)
Pathological T-classification*	
T1	113 (42)
T2	115 (42)
T3	11 (4)
T4	13 (5)
Uncertain	20 (7)
Pathological N-classification*	
N0	128 (47)
N1	70 (26)
N2	36 (13)
N3	15 (6)
Uncertain	23 (8)
Group stage*	
I	77 (28)
II	116 (43)
III	61 (22)
IV	6 (2)
Uncertain	12 (4)
Histological type	
Ductal	248 (91)
Lobular	4 (1)
Others	20 (7)
Histological grade†	
Grade 1	42 (15)
Grade 2	121 (44)
Grade 3	68 (25)
Not applicable or missing	41 (15)
Lymphovascular invasion	
Positive	87 (32)
Negative	129 (47)
Missing	56 (21)
Hormonal receptor status‡	
Positive	212 (78)
Negative	60 (22)
HER2 immunohistochemistry score§	
0	99 (40)
1+	40 (16)
2+	58 (23)
3+	52 (21)

* American Joint Committee on Cancer Staging, 6th edition

† For infiltrating ductal carcinomas only

‡ Tumours with either oestrogen receptor–positive or progesterone receptor–positive would be considered as hormonal receptor–positive

§ For 249 patients with detailed immunohistochemistry (IHC) scores only; there were 4 and 19 breast cancers reported to be *HER2*-positive and -negative respectively without detailed IHC scoring

TABLE 2. Correlation of *HER2* overexpression with clinicopathological characteristics

Variable	No. of patients	*HER2* overexpression		P value
		Positive (%)	Negative (%)	
Age (years)				0.525
≤45	50	9 (18)	41 (82)	
>45	222	49 (22)	173 (78)	
T-classification				0.740
T1	113	20 (18)	93 (82)	
T2	115	25 (22)	90 (78)	
T3-4	24	5 (21)	19 (79)	
N-classification				0.691
N0	128	22 (17)	106 (83)	
N1	70	15 (21)	55 (79)	
N2-3	51	11 (22)	40 (78)	
Group staging				0.433
I	77	11 (14)	66 (86)	
II	116	25 (22)	91 (78)	
III	61	14 (23)	47 (77)	
IV	6	2 (33)	4 (67)	
Histological grade (ductal carcinoma only)				<0.001
1-2	166	21 (13)	145 (87)	
3	68	25 (37)	43 (63)	
Hormonal receptor status				<0.001
Positive	212	34 (16)	178 (84)	
Negative	60	24 (40)	36 (60)	

or reported as positive) was 21%.

Table 2 shows the association of *HER2* overexpression with common clinicopathological characteristics. In our study population, *HER2* overexpression was significantly associated with grade 3 histology (for invasive ductal cancer) [P<0.001], and negative hormonal receptor status (P<0.001). However, there was no association with age (P=0.525), T-classification (P=0.740), N-classification (P=0.691), or group stages (P=0.433).

As many patients were reluctant to self-finance additional ISH tests, only 37 patients had confirmatory ISH tests performed between July 2006 and June 2007. Table 3 shows the results of concordance between their ISH and IHC tests. Overall, 10 (71%) of 14 tumours with IHC staining of 3+ and 1 (4%) of 23 with IHC staining of 2+ were found to have *HER2* gene amplification by ISH assays.

TABLE 3. Degree of concordance between immunohistochemistry (IHC) scores and in-situ hybridisation (ISH) tests in 37 patients

IHC score	No. of patients	ISH tests for *HER2* gene	
		Amplified	Non-amplified
3+ (positive)	14*	10 (71%)	4 (29%)
2+ (equivocal)	23†	1 (4%)	22 (96%)

* All ISH tests for IHC 3+ cases were fluorescence in-situ hybridisation (FISH) tests done in the University of Hong Kong

† For IHC 2+ cases, 21 ISH tests were chromogenic in-situ hybridisation tests performed in private laboratories and 2 were FISH tests done in the University of Hong Kong

Discussion

Although some early studies[16,17] reported *HER2* overexpression in as many as 30% of breast cancers, more recent findings[18,19] suggest the true frequency of *HER2*-positive breast cancer to be only around 15 to 20%. In our prior study of 902 patients with known *HER2* status presenting before 2003, we also found a relatively high *HER2* overexpression rate of 30%.[20] It was unclear whether this was related to the accuracy of *HER2* tests in our community setting or due to ethnic variation. Moreover, in that study population, the adverse impact of *HER2* overexpression was only evident in patients with four or more positive nodes, but not in patients with negative nodes or less than four positive nodes.[20]

The *HER2* overexpression rate of 21% in this more recent study is closer to the findings reported overseas, which is probably related to improved quality assurance of local laboratories in recent years. Similar to other studies,[21,22] *HER2* overexpression in our population was strongly associated with higher histological grades and negative hormonal receptor status. There were no apparent associations of *HER2* overexpression with tumour size, nodal status, age or group stages, but our sample may not have been large enough to detect these associations.

In private laboratories where ISH tests are available, confirmatory ISH tests are often performed if tumours have IHC scores of 2+ (equivocal), as some patients may still have *HER2* gene amplification and hence stand to benefit from target therapies.[23] Although others have reported a 24% chance of detecting *HER2* gene amplification by ISH assays for patients having tumours with IHC 2+ scores,[23] in our series the yield was much lower (only 4%).

Outside a study setting, an IHC score of 3+ is usually accepted as proof of *HER2* overexpression and no confirmatory tests are considered necessary. However, prospective substudies from two of the adjuvant randomised trials of trastuzumab versus nil else[9,10] demonstrated that approximately 20% of *HER2* assays performed at the primary treatment site's pathology department were deemed incorrect, when the same specimen was re-evaluated in a high volume, central laboratory. Although ISH tests were not available in public hospitals, from late 2006, our patients with IHC-determined *HER2* overexpression were offered the option of confirmatory FISH testing by the Molecular Pathology Laboratory of the University of Hong Kong. The concordance rate in these patients was only 71%, much lower than the 95% rate currently recognised as the international standard.[24] However, as only a small proportion of our patients had self-financed confirmatory ISH testing and selection bias could not be excluded, our data should be interpreted with caution. Moreover, the concordance rate of assays does not necessarily assure accuracy, because there is no gold-standard *HER2* testing that can accurately identify all patients who may or may not benefit from anti-HER2 therapy. For patients with discordant *HER2* results (IHC 3+/ISH-negative or IHC 2+/ISH-positive), some of them may nevertheless benefit from anti-HER2 therapies,[25] although mature clinical outcome data are not yet available.

Available information so far does not clearly demonstrate the superiority of either IHC or ISH assays as predictors of benefit from anti-HER2 therapy.[26,27] The original IHC interpretation criteria are now considered to have less-than-desired specificity. To decrease the incidence of false positives, experts have specified that a threshold of more than 30% of tumour cells (rather than 10% as originally specified) should show strong circumferential membrane staining for a positive (3+) result.[24] Strong circumferential staining in 30% or less of cells would then be considered equivocal and be subjected to confirmatory ISH tests (not yet available in public hospitals). Since April 2007, our own pathology laboratory has also adopted this new criterion, although the *HER2* results reported here were analysed according to original criteria, which are still being used in some local laboratories.

Considering the very high cost of anti-HER2 drug therapy (often up to HK $200 000), not to mention the requirement for 12 months of intravenous therapy in an adjuvant setting and the need for regular cardiac monitoring, measures to enhance the accuracy and reproducibility of *HER2* tests could well reduce medical expenses substantially. Adoption of the latest guideline[24] and steps to reduce assay variation (specimen handling, assay exclusion, and reporting criteria) could facilitate better selection of patients for anti-HER2 therapies.

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