ORIGINAL ARTICLE

Frequency and reliability of oestrogen receptor, progesterone receptor and HER2 in breast carcinoma determined by immunohistochemistry in Australasia: results of the RCPA Quality Assurance Program

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Background and Aims: Immunohistochemistry (IHC) has replaced radioligand binding assay for the determination of oestrogen receptor (ER) status in breast carcinoma. IHC is also used for assessment of progesterone receptor (PR) and HER2. The Royal College of Pathologists of Australasia (RCPA) Quality Assurance Program (QAP) introduced a breast markers module in 2003 to evaluate the performance of laboratories with IHC for ER, PR and HER2.

Methods: An audit of laboratories reporting breast carcinomas was performed in 2005 and 2006 to evaluate in-house results. Laboratories were asked to submit the hormone receptor and HER2 status on each invasive breast carcinoma for the previous 6 month period up to a maximum of 100 cases. The time periods were 1 July 2004 to 31 December 2004, and 1 July 2005 to 31 December 2005. A total of 55 laboratories returned information for 2004 and 67 for 2005.

Results: Complete data on 8128 patients was returned for both surveys, 3353 cases for 2004 and 4775 for 2005. The results were similar for both surveys. Of the 8128 cases, 59.0% were ER+/PR+, 15.9% ER+/PR−, 2.4% ER−/PR+ and 22.7% ER−/PR−. HER2 data were submitted for a total of 6512 patients (excludes 52 patients with incomplete data sets); 17.1% were reported as 3+ positive on IHC, 12.5% as 2+ and 70.4% as negative.

Conclusions: A laboratory audit was introduced into the RCPA QAP for breast markers due to concerns raised by participating laboratories about technical differences in supplied tissues for testing. This audit indicates that overall the results for ER, PR and HER2 fall inside established parameters. However, a number of individual laboratories do not meet the target values and variation in results would impact on patient treatment decisions.

In Australia there are approximately 12 000 new cases of breast carcinoma diagnosed each year. The dependence of some breast carcinomas on hormone receptors was identified more than 100 years ago with tumour regression in two of six patients after oophorectomy.[1] Assessment of hormone receptor status is utilised as a predictive marker to determine patient treatment and is performed on all breast carcinomas. Current assessment of hormone receptor status is based on immunohistochemistry (IHC). This has supplanted the radioligand binding assay that previously used frozen tissue.

The Royal College of Pathologists of Australasia (RCPA) Quality Assurance Program (QAP) introduced a breast markers module in 2003 because of the critical importance of hormone receptor determination. It is a requirement of Australian laboratories that they participate in a quality assurance programme if this is available. While the majority of participating laboratories are from Australia, there are a number of laboratories from New Zealand and international locations. This module comprises two exercises each year consisting of a number of patient samples in a ring trial format. The original material was a composite block, but this has been changed to a tissue microarray construct for the past two years. The specimens consist of routine formalin fixed paraffin embedded material that has been supplied to the QAP by participants. Participating laboratories are sent these slides and asked to perform IHC for oestrogen receptor (ER), progesterone receptor (PR) and HER2 on the sections, and return the slides for assessment as per their usual staining protocol. Homogeneity slides are stained by H&E; IHC and stability testing has been performed on the test slides. The participant's slides are reviewed by a panel of scientists and pathologists and are scored individually from 0 to 5. Average scores are returned to participants. A score of 2 indicates that the result would affect patient treatment or management. Scores of 3 and above are considered satisfactory. This evaluation of the laboratories' performance is included in the report as satisfactory, borderline or unsatisfactory.

At any one episode approximately 30% of participating laboratories will have an unsatisfactory result in at least one of the three IHC markers. Using this system, a number of participating laboratories that achieved unsatisfactory results raised concerns about the supplied material. This included differences in fixation protocols, processing times and transit time to the laboratories. The laboratories indicated that the results on in-house material were acceptable and therefore the ring studies were not a true reflection of the laboratory's performance.

To address these issues, laboratories were asked to perform an audit in 2005 and 2006 for the previous 6 month period. The aim was to truly indicate the reported results from each laboratory; this would overcome issues associated with external

Abbreviations: ER, oestrogen receptor; FISH, fluorescence in-situ hybridisation; HR, hormone receptor; IHC, immunohistochemistry; ISH, in-situ hybridisation; PR, progesterone receptor; QAP, Quality Assurance Program; RCPA, Royal college of Pathologists of Australasia

Table 1 Number of laboratories using cut-off values for reporting positive/negative status in the 2006 audit

Test	Yes	No	Unsure	No answer
ER	56	10	3	13
PR	58	10	3	11
HER2	53	6	5	18

ER, oestrogen receptor; PR, progesterone receptor.

material and indicate if the in-house results were achieving acceptable results.

MATERIALS AND METHODS

Participants were requested to indicate each invasive breast carcinoma case and return ER, PR and HER2 status for each case. This was retrieved from reports to a maximum of 100 patients in each 6 month period. The time periods were 1 July 2004 to 31 December 2004 and 1 July 2005 to 31 December 2005. Participating laboratories used their own cut-off values for hormone positivity; HER2 status was defined as for the HercepTest (Dako, CA, USA) scoring system. For this scoring system 3+ is positive, 2+ is equivocal and 1+ and 0 are scored as negative. Duplicate cases were to be excluded.

The first exercise required the number of cases in each category (hormone receptor and HER2) to be returned. This was modified for the second exercise to an Excel spreadsheet to facilitate data collection. The results were analysed using SPSS V.14.0 for Windows (SPSS Inc, Chicago, IL, USA).

From the ring studies for ER and PR approximately 37% of laboratories use a manual method, with the remaining laboratories utilising various forms of automation. For ER, primary antibodies include 6F11, 1D5 and SP1; for PR, PgR636, 16, SP2 and 1A6; and for HER2, AO485, SP3, CB11, 10A7, 4B5 and TAB250 from a variety of suppliers. There are at least 20 different retrieval systems and detection systems in use.

A number of participants reported that they used cut-off values for reporting positive results (table 1) and the cut-offs used are indicated in table 2.

RESULTS

A total of 55 laboratories returned information for the time periods in 2004 and 67 for 2005. For the 2005 series there were 42 Australian sites, 9 New Zealand sites and 16 international laboratories. The results are very similar for both audits (table 3).

For the IHBR06 audit, data were returned for a total of 4807 patients for ER/PR and 3980 patients for HER2. However, 19 patients were reported as ER+ with no PR status, 13 patients were reported as ER− with no PR status (28 had HER2 data) and 20 patients had HER2 status reported with no ER/PR data.

Overall 59.0% of cases were ER+/PR+, 15.9% were ER+/PR−, 2.4% were ER−/PR+ and 22.7% were ER−/PR− for patients with complete data. Similarly there was little difference for the two years with respect to HER2 status and for the hormone receptor subgroups within HER2 (tables 4–6).

Table 2 Cut-off values used by participating laboratories for the 2006 audit

Test	1–4%	5–9%	10%	Other	No answer
ER	15	7	22	13	22
PR	14	7	22	13	23
HER2	3	2	42	9	26

ER, oestrogen receptor; PR, progesterone receptor.

Table 3 Results for hormone receptor status from the two audits (IHBR05 and IHBR06)

Receptor status	IHBR05	IHBR06	Total
ER+/PR+	1969 (58.7%)	2826 (59.2%)	4795 (59.0%)
ER+/PR−	560 (16.7%)	731 (15.3%)	1291 (15.9%)
ER−/PR+	72 (2.1%)	123 (2.6%)	195 (2.4%)
ER−/PR−	752 (22.4%)	1095 (22.8%)	1847 (22.7%)
Total	3353	4775	8128

ER, oestrogen receptor; PR, progesterone receptor.

HER2 status was provided on 6512 patients (80.1%) with complete data sets. While it is recommended that laboratories perform HER2 testing at the time of diagnosis, this is not a requirement. Discussions with laboratories indicated that some use a triage system and exclude patients based on clinical decisions (eg, patients who are ineligible for chemotherapy) or pathological decisions (eg, if the tumour is unlikely to be positive, such as classic lobular carcinoma or tubular carcinoma). Overall 17.1% of patients were reported as HER2 positive on IHC with a rate of 16% for Australian cases. Collectively the results are similar to those reported from other studies,[2 3] however individual laboratories showed variation in the percentages within each subgroup.

In the 2006 audit, ER positive rates varied from 26% to 100%, PR positive rates from 23% to 96%, and HER2 positive rates from 0% to 66% (figs 1–3, table 7).

Table 7 shows individual laboratory results for participating laboratories. There was still a large variation in positive rates, even in laboratories reporting 100 cases in the 6 month time frame. Differences in rates were also observed across states within Australia and between Australian, New Zealand and international laboratories (tables 8 and 9).

DISCUSSION

In breast carcinomas, immunohistochemistry plays an essential role in determining patient treatment. The decision to use selective oestrogen receptor modulators or aromatase inhibitors in adjuvant therapy is determined by the reported hormone receptor status. Similarly with the recent approval of trastuzumab for therapy in HER2 positive breast carcinomas in Australia, IHC for HER2 has assumed equal importance.

In the current technical breast markers module for IHC that uses supplied material in a ring study format, participants have argued that the results do not truly reflect the in-house results and that the IHC performed by the laboratories is optimised for in-house material. This has been used as an argument for unsatisfactory results in the technical slides. The audit was introduced in 2005 to assess the reported cases that should therefore indicate the in-house results. Participants indicated some confusion for the requirements for this audit and as a response the reporting format was changed. The results for both audits are virtually identical, indicating little bias between the two time frames and data collection methods. For both audits, participants complained about the time taken to complete the survey. It should be remembered that for the audits these cases have had reports generated and therefore action has already

Table 4 HER2 status reported by laboratories

Audit	HER2+	HER2 equivocal	HER2−	Total
IHBR05	420 (16.3%)	280 (10.9%)	1880 (72.9%)	2580
IHBR06	692 (17.6%)	536 (13.6%)	2704 (68.8%)	3932*
Total	1112 (17.1%)	816 (12.5%)	4584 (70.4%)	6512

*Excludes patients with incomplete data sets and those with only HER2 data.

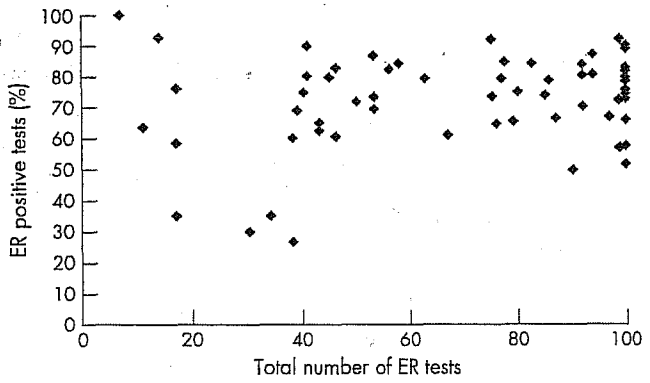

Figure 1 Positive oestrogen receptor (ER) rates for reporting laboratories plotted against number of reported cases for the 2006 audit.

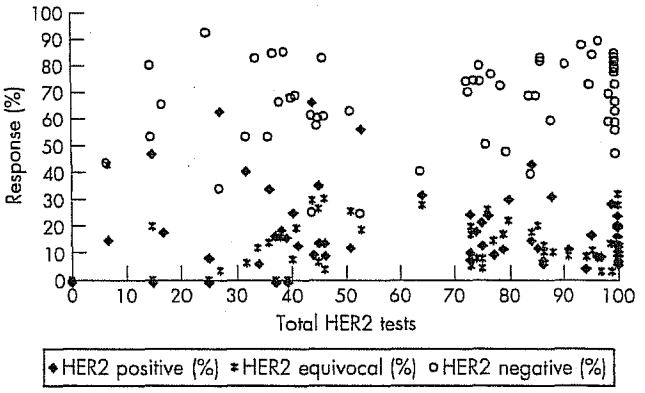

Figure 3 HER2 subgroup rates for reporting laboratories plotted against number of reported cases for the 2006 audit.

been taken based on these results. This has the potential to impact significantly on patient treatment and outcome.

It was anticipated that the results of the audits would indicate satisfactory performance for the majority of laboratories. While a number of laboratories do achieve excellent results, there are a number that have reported hormone receptor and HER2 status positivity rates that fall outside expected results.

Arpino *et al*[4] reported on 54 865 patients from a database of patients diagnosed with breast cancer between 1970 and 1998. ER was evaluated using the dextran-coated charcoal method and PR was evaluated using sucrose density gradient (table 10).

Rhodes *et al*[5] reported on hormone status of 4053 breast carcinomas using IHC, Francis *et al*[8] reported on 591 patients, Killeen *et al*[6] reported 667 patients and Huang *et al*[7] reported on 1362 patients (table 10).

HER2 status in 669 patients was also assessed by Killeen *et al*[6] using image analysis; 69.5% were HER2 negative, 15.8% were borderline and 14.6% were HER2 positive. Francis *et al*[8] reported HER2 positivity in 15.4% of patients, HER2 was equivocal in 18.4% and negative in 66.2%. Slamon *et al*[9] reported HER2 overexpression in 15–25% of breast carcinomas. Huang *et al*[7 10] reported an HER2 positive rate of 10.9% in 1362 patients as defined by IHC 3+ staining. Taucher *et al*[11] reported an HER2 positive rate of 17.3% in 923 patients, but this was defined as 2+ or 3+ staining. Gancberg *et al*[12] reported an HER2 positive rate of 23.1% in 160 tumours, and Yaziji *et al*[13] an HER 2 positive rate of 18.6% in 2913 tumours using fluorescence in-situ hybridisation (FISH). Lal *et al*[14] tested 561 tumours for HER2 using IHC and FISH; 10.3% were positive with IHC and 24.1% showed gene amplification with FISH. Gusterson *et al*[15] used IHC to test HER2 status in 1506 patients; overall the positive rate was 17.5% (16% for node negative patients and 19% for node positive patients), and a further 613 node negative patients had a 14.3% HER2 positive rate.[16] In a large study by Owens *et al*,[17] 3+ staining was seen in 10.9%, and 2+ staining in 9.1% of 116 736 samples tested by HER2 IHC and 22.7% of 6556 specimens tested by FISH.

In the four hormone receptor (HR) IHC studies, the average HR positive rate was 80.9% (SD 2.3%). The average HR negative rate was 19.0% (SD 2.2%). Therefore 95% of laboratories should have an HR positive rate between 76.3% and 85.5%, and an HR negative rate between 14.6% and 23.4%. Similarly ER+ rates should fall between 73% and 84.6% and PR+ rates between 53.1% and 75.9%. For the 2006 audit, 22 laboratories had ER positivity rates below 70%, and 8 laboratories had PR positivity rates below 50%. Of the laboratories reporting 100 patients in the 6 month period for the 2006 audit, four had ER+ rates below 70% and 2 had PR+ rates below 50%.

For the IHC studies with HER2 data,[6 8 10 12 14 15 17] the average HER2 3+ positive rate was 14.7% (SD 4.6%). In the 2006 audit, 15 laboratories reported HER2 positive rates below 10%, and 17

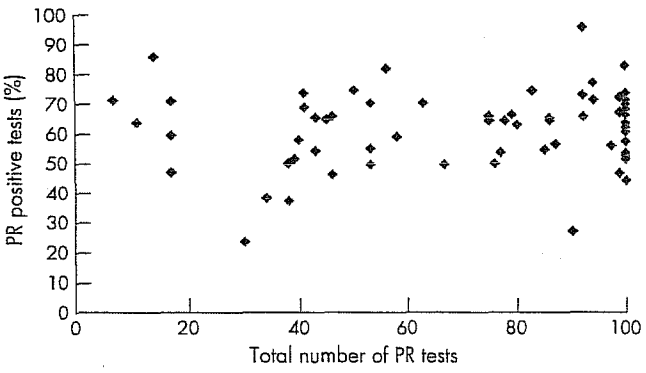

Figure 2 Positive progesterone receptor (PR) rates for reporting laboratories plotted against number of reported cases for the 2006 audit.

Table 5 Subgroups of hormone receptor status and HER2 status for the 2005 audit

Receptor status	HER2+	HER2 equivocal	HER2−	Total
ER+/PR+	152 (10%)	163 (10.8%)	1199 (79.2%)	1514 (58.7%)
ER+/PR−	76 (17.6%)	55 (12.8%)	300 (69.6%)	431 (16.7%)
ER−/PR+	12 (19.7%)	1 (1.6%)	48 (78.7%)	61 (2.4%)
ER−/PR−	180 (31.4%)	61 (10.6%)	333 (58%)	574 (22.2%)
Total	420 (16.3%)	280 (10.9%)	1880 (72.9%)	2580

ER, oestrogen receptor; PR, progesterone receptor.

Table 6 Subgroups of hormone receptor status and HER2 status for the 2006 audit

Receptor status	HER2 +	HER2 equivocal	HER2−	Total
ER+/PR+	230 (9.7%)	326 (13.8%)	1803 (76.4%)	2359 (60%)
ER+/PR−	142 (23.2%)	105 (17.2%)	363 (59.5%)	610 (15.5%)
ER−/PR+	20 (23.2%)	9 (10.5%)	57 (66.3%)	86 (2.2%)
ER−/PR−	300 (34.2%)	96 (10.9%)	481 (54.8%)	877 (22.3%)
Total	692 (17.6%)	536 (13.6%)	2704 (68.8%)	3932*

ER, oestrogen receptor; PR, progesterone receptor.
*Excludes patients with incomplete data sets and those with only HER2 data.

Table 7 Individual laboratory results for the 2006 audit (excludes those cases with HER2 only results)

Laboratory	% ER positive	% PR positive	% HER2 positive	% HER2 2+	% HER2 negative	Count ER	Count PR	Count HER2
1	66	66	21	4	75	79	79	75
2	89	66	21	32	47	100	100	100
3	80	73	13	4	83	100	100	46
4	79	64	6	13	81	86	86	86
5	82	82	8	0	92	56	56	25
6	73	67	11	9	80	99	99	90
7	84	96	7	19	74	92	92	73
8	79	70	12	20	68	100	100	85
9	80	73	12	20	68	41	41	41
10	87	70	12	25	63	53	53	51
11	75	58	25	8	68	40	40	40
12	74	53	12	10	78	100	100	100
13	73	64	12	8	80	75	75	75
14	74	54	14	18	68	85	85	84
15	80	52	16	5	79	100	100	100
16	79	62	24	18	58	100	100	100
17	80	64	36	7	58	45	45	45
18	80	53	9	14	77	79	77	77
19	90	68				41	41	
20	76	53	13	10	77	100	100	100
21	69	51	18	16	66	39	39	38
22	92	65	10	16	74	75	75	73
23	79	65	7	10	83	86	86	86
24	90	83	8	3	89	100	100	97
25	73	60	21	6	73	100	100	100
26	81	77	16	28	56	94	94	94
27	75	71	6	13	81	100	100	100
28	66	51	20	14	66	100	100	100
29	87	71	9	30	61	94	94	46
30	79	70	0	20	80	63	63	15
31	72	74	33	14	53	50	50	36
32	82	68	8	8	84	100	100	100
33	74	55	57	19	25	53	53	53
34	82	63	28	3	69	100	100	99
35	70	49				53	53	
36	57	46	28	13	59	100	99	99
37	82	68	9	28	63	100	100	100
38	94	86				16	14	
39	35	47				17	17	
40	76	57	10	8	82	100	100	100
41	61	46	15	0	85	46	46	39
42	84	75	17	7	76	83	83	70
43	100	71	14	43	43	7	7	7
44	85	64	12	17	72	78	78	78
45	83	68	17	11	73	100	100	95
46	61	49	31	28	41	67	67	64
47	83	65	9	30	61	46	46	44
48	61	50	6	12	82	38	38	34
49	30	23				30	30	
50	52	51				100	100	
51	50	27				90	90	
52	44	71	63	4	33	27	17	27
53	26	37	16	0	84	38	38	37
54	84	59	13	27	60	58	58	45
55	64	50	24	26	50	76	76	76
56	92	72	4	9	87	100	99	94
57	71	65	41	6	53	94	92	32
58	80	73	20	6	74	92	92	65
59	75	63	30	23	48	80	80	80
60	58	44	31	10	59	100	100	88
61	67	56	8	8	83	97	97	96
62	76	59	18	18	65	17	17	17
63	65	53				43	43	
64	67	64	47	0	53	15	11	15
65	43	38	66	9	25	44	34	44
66	63	65				43	43	
67	67	56	43	18	39	87	87	84
Total						4807	4775	3960

ER, oestrogen receptor; PR, progesterone receptor.

laboratories reported rates above 20%. Five laboratories reporting 100 patients in the time period had HER2 positive rates outside the range of 10–20%.

The data from the 2006 audit were analysed in conjunction with the technical performance of each laboratory. The results were evaluated using the technical results obtained from 2003–05. An unsatisfactory performance was regarded if a laboratory had received this evaluation for any of the modules in that time frame. There was no correlation between number of exercises performed or unsatisfactory performance in the technical

Table 8 Cases and percentages for IHC HER2 status reported by Australian state and international laboratories for the 2006 audit (excludes those cases with HER2 only results)

	HER2 positive No. (%)	HER2 equivocal No. (%)	HER2 negative No. (%)	Total no.
NSW	81 (13.1%)	87 (14.1%)	451 (72.9%)	619
Vic	164 (16.0%)	167 (16.3%)	691 (67.6%)	1022
Qld	56 (11.6%)	48 (10%)	377 (78.4%	481
SA	49 (18.9%)	36 (13.9%)	174 (67.2%)	259
WA	60 (15.2%)	34 (8.6%)	300 (76.1%)	394
Tas	20 (20.0%)	14 (14.0%)	66 (66.0%)	100
NZ	53 (13.1%)	56 (13.9%)	295 (73.0%)	404
International	222 (32.6%)	98 (14.4%)	361 (53.0%)	681
Total	705 (17.8%)	540 (13.6%)	2715 (68.6%)	3960

exercises and percentages of ER positive, PR positive or HER2 positive cases by state (table 9). There was no correlation between the audit results and number of unsatisfactory results in the 2003–05 time frame (data not shown). There was variation in results in different states in Australia; international participants had lower rates of ER and PR positivity and higher rates of HER2 positivity compared to other locations (table 9).

As already mentioned, each laboratory used their own specified cut-off value for ER and PR status, and while it is possible that changes to the cut points would have an impact on the percentage positivity rates for ER and PR, Layfield *et al*[18] showed no difference in concordance for ER when cut-points were standardised. Only approximately 3% of breast carcinomas have 1–4% of cells staining, and approximately 7% have 1–19% of cells staining.[8] This would be unlikely to account for the wide variation of results in the two audits. It would also not account for differences in reporting of HER2 for which there are well defined criteria. Since patients in these audits were non-selected and routinely reported, patient bias is unlikely to have a major impact for those laboratories reporting large numbers of cases. There was no correlation between methodology and positivity rates; however, the wide variation in methods and reagents made statistical comparisons impossible (data not shown).

Rhodes *et al*[19] assessed the performance of 200 laboratories to perform ER by IHC. Their study showed a false negative rate for ER status of 30–60%. The interlaboratory variation in results persisted with the laboratories' chosen threshold for determination of ER status; however, a cut-off of 1% would have resulted in a higher number of laboratories reporting ER positivity. Considerable variability was also identified by Layfield *et al*[18] with ER results in different laboratories; this would result in clinically significant differences in therapy.

In the technical component of the RCPA QAP breast markers module similar results are observed, with an increased proportion of laboratories achieving unsatisfactory results with low ER expressing tumours.

The issues are similar for HER2. Press *et al*[20] evaluated HER2 status in central and outside laboratories and concluded that FISH for assessment of HER2 status was superior when performed in a central laboratory.

Despite analysis of methodology, antibodies and detection systems, no single issue has been identified as contributing to the decreased performance; and conversely, no optimal method has been identified to achieve consistently good results.

CONCLUSIONS

The variation in results reported by laboratories has the potential to impact significantly on patients. Despite the search for predictive and prognostic markers in breast carcinoma and the description of hundreds of potential markers, only hormone receptor and HER2 status have been translated into clinical practice.[21] IHC is at best a semiquantitative method, and while cell lines and image analysis can be used to establish defined staining intensities and scoring systems, this does not enable transition to solid tissues where the vagaries of fixation and processing impact significantly on staining quality. It is interesting that HER2 gene amplification more accurately reflects response to trastuzumab, when logically it should be protein expression.

IHC is limited by variability in tissue quality and methodology. While some of this can be overcome by meticulous attention to assay performance, correlation with patient outcome and treatment response, the majority of laboratories do not have the resources or access to patient information to closely control or monitor these factors. Over the last decade, IHC has moved from a qualitative test (brown or not brown) to a quantitative test that determines selection of patient therapy and treatment outcome. With the development of new targeted therapy, the imperative to identify predictive tests will increase. IHC may be able to fulfil this role, but the data from this and other studies indicate it is fraught with difficulty. The difficulties with predicting response to epidermal growth factor receptor by tyrosine kinase inhibitors or monoclonal antibodies based on IHC graphically illustrates this point.[22 23]

In the case of HER2, the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) have released guideline recommendations for HER2 testing in breast

Table 9 ER+, PR+ and HER2 subgroup rates for the 2006 audit according to location of laboratory and rates of unsatisfactory performance in one or more technical exercises

Location	Location of labs		ER positive (%)	PR positive (%)	HER2 positive (%)	ER technical unsatisfactory (%)	PR technical unsatisfactory (%)	HER2 technical unsatisfactory (%)
Australia	NSW		73	66	13	51	40	71
	Vic		80	62	16	56	47	43
	Qld		82	65	12	40	34	58
	SA		78	70	19	26	17	37
	WA		79	65	15	57	35	38
	Tas		66	51	20	50	33	50
NZ			77	67	13	46	39	55
International			57	50	33	50	28	41
Total laboratories	67	Average	74	62	18	47	34	49
		SD	8	7	7	10	9	12

ER, oestrogen receptor; PR, progesterone receptor.

Table 10 Studies reporting cohorts of patients with hormone receptor status

Reference	No. of cases	ER+/PR+	ER+/PR−	ER−/PR+	ER−/PR−
Arpino *et al*[4]	54865	31415 (57%)	13404 (25%)	1621 (3%)	8425 (15%)
Rhodes *et al*[5]	4053	2222 (54.8%)	804 (19.8%)	131 (3.2%)	896 (22.1%)
Killeen *et al*[6]	667	416 (62.4%)	113 (16.9%)	9 (1.3%)	129 (19.3%)
Huang *et al*[7]	1362	852 (62.6%)	252 (18.5%)	22 (1.6%)	236 (17.3%)
Francis *et al*[8]	591	410 (69.4%)	63 (10.7%)	15 (2.5%)	103 (17.4%)
Total	61538	35315 (57.4%)	14636 (23.8%)	1798 (2.9%)	9789 (15.9%)

ER, oestrogen receptor; PR, progesterone receptor.

Take-home messages

- Individual pathology laboratories show a wide variation in reported immunohistochemistry results for oestrogen receptor, progesterone receptor and HER2 in invasive breast cancer.
- These differences would have an impact on patient treatment.
- No methodology or specific factors have been identified to account for these differences.
- It is critical that laboratories monitor the performance of immunohistochemistry testing for breast markers to ensure optimum patient care.

cancer.[24] These recommendations include testing on all invasive breast cancers, validation of laboratory assays, use of standardised operating procedures and compliance with stringent proficiency and accreditation requirements.

As from 1 October 2006 in Australia, in-situ hybridisation (ISH) is mandatory to be eligible for the government subsidised drug trastuzumab for adjuvant treatment in early breast cancer. It is the aim to test all patients with early breast cancer with ISH for HER2, with a transition phase of 12 months for continued IHC testing. ISH testing for HER2 has been introduced with stringent training and reporting requirements to avoid similar issues identified with IHC testing.

While the guidelines for the introduction of ISH testing in Australia have been developed independently to the ASCO/CAP guidelines, they are virtually identical. These guidelines include an (equivocal) borderline category for chromogenic ISH testing that requires confirmatory FISH testing. They also include minimum numbers to be performed by participating laboratories and reporting pathologists. There are both training and accreditation standards with mandatory performance of ongoing on-line training and evaluation. The audit will be repeated in 2007 and will include results for ISH testing on the patient specimens.

However, for ER and PR, no alternative methodology to IHC is available. Therefore it is essential that the clinician and laboratory are at all times aware of the potential impact reporting of IHC has on patient treatment and outcome. Internal audits should be performed in the laboratories to ensure results are as accurate as possible and referral laboratories should be considered for laboratories performing small numbers of cases.

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REFERENCES

1 **Beatson G.** On the treatment of inoperable cases of carcinoma of the mammary gland: suggestions for a new method of treatment with illustrative cases. *Lancet* 1896;ii:104–7.
2 **Rhodes A,** Jasani B, Balaton AJ, *et al.* Immunohistochemical demonstration of oestrogen and progesterone receptors: correlation of standards achieved on in house tumours with that achieved on external quality assessment material in over 150 laboratories from 26 countries. *J Clin Pathol* 2000;**53**:292–301.
3 **Rhodes A,** Jasani B, Balaton A, *et al.* Frequency of estrogen and progesterone receptor positivity by immunohistochemical analysis in 7016 breast carcinomas: correlation with patient age, assay sensitivity, threshold value and mammographic screening. *J Clin Pathol* 2000;**53**:699–96.
4 **Arpino G,** Weiss H, Lee AV, *et al.* Estrogen receptor-positive, progesterone receptor-negative breast cancer: association with growth factor receptor expression and tamoxifen resistance. *J Natl Cancer Inst* 2005;**97**:1254–61.
5 **Rhodes A,** Jasani B, Balaton AJ, *et al.* Frequency of oestrogen and progesterone receptor positivity by immunohistochemical analysis in 7016 breast carcinomas: correlation with patient age, assay sensitivity, threshold value, and mammographic screening. *J Clin Pathol* 2000;**53**:688–96.
6 **Killeen J,** Ortega-Lopez A, Shaha J, *et al.* Pathology of borderline HER-2/neu breast carcinoma: a biologically distinct phenotype. *Breast Cancer Res Treat* 2006;**98**:99–108.
7 **Huang HJ,** Neven P, Drijkoningen M, *et al.* Association between tumour characteristics and HER-2/neu by immunohistochemistry in 1362 women with primary operable breast cancer. *J Clin Pathol* 2005;**58**:611–16.
8 **Francis G,** Beadle G, Thomas S, *et al.* Evaluation of oestrogen and progesterone receptor status in HER-2 positive breast carcinomas and correlation with outcome. *Pathology* 2006;**38**:391–8.
9 **Slamon D,** Godolphin W, Jones L, *et al.* Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* 1989;**244**:707–12.
10 **Huang HJ,** Neven P, Drijkoningen M, *et al.* Hormone receptors do not predict the HER2/neu status in all age groups of women with an operable breast cancer. *Ann Oncol* 2005;**16**:1755–61.
11 **Taucher S,** Rudas M, Mader RM, *et al.* Do we need HER-2/neu testing for all patients with primary breast carcinoma? *Cancer* 2003;**98**:2547–53.
12 **Gancberg D,** Lespagnard L, Rouas G, *et al.* Sensitivity of HER-2/neu antibodies in archival tissue samples of invasive breast carcinomas. Correlation with oncogene amplification in 160 cases. *Am J Clin Pathol* 2000;**113**:675–82.
13 **Yaziji H,** Goldstein LC, Barry TS, *et al.* HER-2 testing in breast cancer using parallel tissue-based methods. *JAMA* 2004;**291**:1972–7.
14 **Lal P,** Salazar PA, Ladanyi M, *et al.* Impact of polysomy 17 on HER-2/neu immunohistochemistry in breast carcinomas without HER-2/neu gene amplification. *J Mol Diagn* 2003;**5**:155–9.
15 **Gusterson BA,** Gelber RD, Goldhirsch A. Prognostic importance of c-erbB-2 expression in breast cancer. *J Clin Oncol* 1992;**10**:1049–56.
16 **Pegram MD,** Pauletti G, Slamon DJ. HER-2/neu as a predictive marker of response to breast cancer therapy. *Breast Cancer Res Treat* 1998;**52**:65–77.
17 **Owens MA,** Horten BC, Da Silva MM. HER2 amplification ratios by fluorescence in situ hybridization and correlation with immunohistochemistry in a cohort of 6556 breast cancer tissues. *Clin Breast Cancer* 2004;**5**:63–9.
18 **Layfield LJ,** Goldstein N, Perkinson KR, *et al.* Interlaboratory variation in results from immunohistochemical assessment of estrogen receptor status. *Breast J* 2003;**9**:257–9.
19 **Rhodes A,** Jasani B, Barnes DM, *et al.* Reliability of immunohistochemical demonstration of oestrogen receptors in routine practice: interlaboratory variance in the sensitivity of detection and evaluation of scoring systems. *J Clin Pathol* 2000;**53**:125–30.
20 **Press MF,** Sauter G, Bernstein L, *et al.* Diagnostic evaluation of HER-2 as a molecular target: an assessment of accuracy and reproducibility of laboratory

testing in large, prospective, randomized clinical trials. *Clin Cancer Res* 2005;**11**:6598–607.
21 **Reis-Filho JS**, Westbury C, Pierga JY. The impact of expression profiling on prognostic and predictive testing in breast cancer. *J Clin Pathol* 2006;**59**:225–31.
22 **Younes M**, Brown RW, Mody DR, *et al.* GLUT1 expression in human breast carcinoma: correlation wiht known prognostic markers. *Anticancer Res* 1995;**15**:2895–8.
23 **Saltz LB**, Meropol NJ, Loehrer PJ. Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. *J Clin Oncol* 2004;**22**:1201–8.
24 **Wolff AC**, Hammond ME, Schwartz JN, *et al.* American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *J Clin Oncol* 2007;**25**:118–45.