Original article

IHC4 score plus clinical treatment score predicts locoregional recurrence in early breast cancer

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ABSTRACT

Purpose: Immunohistochemical 4 (IHC4) score plus Clinical Treatment Score (CTS) is an inexpensive tool predicting risk of distant recurrence in women with early breast cancer (EBC). IHC4 score is based on ER, PR, HER2 and Ki67 index. This study explores the role of the combined score (IHC4 + CTS) in predicting risk of locoregional recurrence (LRR) in women with EBC who had breast conservation surgery (BCS) without adjuvant radiotherapy (study group). The secondary objective was to evaluate the clinicopathological differences between our study group and women who had adjuvant radiation following BCS (control group).

Methods and materials: Patients were selected from the local database over a 13-year period. IHC testing was done where results were missing. Combined scores were calculated using the appropriate formulae.

Results: Patients in the study group (81 patients) had favorable clinicopathological features compared to the control group (1406 patients).

The Cox regression indicated a statistically significant association between the combined score and the risk of LRR ($p = 0.03$). The incidence of LRR was zero, 20% and 33.3% in the low, intermediate and high risk groups respectively ($p = 0.007$).

Margin status was the only variable not included in the combined score. The Cox regression analysis demonstrated that the combined score ($p = 0.02$) and the ordinal measure of margins ($p = 0.03$) were significant independent predictors of LRR.

Conclusion: This is the first study of its kind. The IHC4 score + CTS can be used to identify low risk women who can potentially avoid adjuvant radiotherapy.

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Introduction

Breast cancer represents a heterogeneous group of diseases. Molecular profiling has helped stratify breast cancer into risk categories [1,2] to optimize systemic options and improve clinical outcomes. More refined assays such as the 21-gene expression profile (Oncotype Dx Recurrence Score) [3,4], the 70-gene expression profile (Mammaprint) [5], and Mammostrat [6] have shown promise for predicting prognostic categories for personalized systemic treatment.

An inexpensive gene expression-profiling tool is the Immuno-histochemical 4 (IHC4) score [7] that measures the levels of four key proteins (estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor-2 (HER2), and human Ki-67). Cuzick et al. [7] developed an overall prognostic score that combines the IHC4 score and the Clinical Treatment Score (CTS), providing prognostic information on the risk of distant recurrence similar to that provided by the 21-gene recurrence score. The CTS is based on clinical parameters such as tumor size, grade, nodal

status, age, and endocrine treatment. This prognostic model was developed using data from the Arimidex, Tamoxifen, Alone or in Combination (ATAC) study [8] and validated on another cohort (the "Nottingham group") [7]. The IHC4 score + CTS is therefore applicable to post-menopausal women with ER positive breast cancer receiving adjuvant endocrine therapy without chemotherapy. The IHC4 score + CTS is more cost effective than the above-mentioned genomic tools, as it can be calculated from parameters available in routine clinical practice. Unlike IHC4 score + CTS, the 21-gene recurrence score does not take into account classical risk factors such as nodal status, tumor size, and grade.

The molecular profiling of breast cancer to assess the risk of local recurrence (LR) risk after breast conservation surgery (BCS) is comparatively underdeveloped. A tool such as IHC4 score + CTS may be useful in identifying a group of women with minimal risk in whom radiotherapy can be omitted.

Given that LRR is a significant predictor of and has a temporal and proportional relationship to the risk of distant recurrence [9–11] we hypothesized that the IHC4 score + CTS also predicts the risk of LRR which is the primary objective of our study. This hypothesis was tested in women who had BCS for early invasive breast cancer but no adjuvant radiotherapy (study group), and within that group, women who were post-menopausal and did not have adjuvant chemotherapy (the validated subgroup).

The secondary objective was to evaluate the clinicopathological and survival differences between our study group and the cohort of women who had adjuvant radiation following BCS during the same period (control group). The purpose of this analysis is to determine if the score can be widely applied to all breast cancers patients or to a predefined subset.

Methods and materials

Patient population

This is a prospective cohort study of women with early, unilateral, invasive breast cancer amenable to and treated with breast conservation surgery over a 13-year period (July 1997 to June 2010) in the Australian Capital Territory and the South East New South Wales (ACT&SENSW) region. The ACT&SENSW Breast Cancer Treatment Group Quality Assurance Project began accruing patients in 1997. Ninety-five percent of all breast cancer patients from ACT and SENSW region have given informed consent for their demographic indicators, disease pathology and treatment details to be recorded in this database and for their disease status to be followed up annually.

The cohort was divided into those who had breast conservation surgery and adjuvant radiotherapy called the "control group", and those that did not have radiotherapy after breast conservation surgery called the "study group". A subgroup of patients from the study group, labeled 'the validated subgroup', was created that had the same inclusion and exclusion criteria as the original study by Cuzick et al. [7]. This group includes women who were post-menopausal and ER-positive and/or PR-positive and excluded patients who were both ER-negative and PR-negative and had received adjuvant chemotherapy. The data were analyzed for: 1) the entire study group and 2) the validated subgroup.

Patients were excluded due to insufficient tumor (<5% of the overall tissue), phyllodes histology, if slides could not be retrieved or there was an inability to do IHC4 tests because of lack of invasive tumor in the section. Women gave their informed consent for their data to be stored with the ACT&SENSW BCTG database and the ACT Human Research Ethics Committee approved both the database and the current study.

Study design and endpoints

Pathology reports of the eligible patients were evaluated, and if results were missing, the tumor blocks were retrieved from the respective laboratories within the ACT and SENSW. The required IHC4 tests were performed at the ACT pathology laboratory. IHC4 score and the CTS were calculated for all eligible patients using the formulae described by Cuzick et al. [7]. These formulae are as follows:

IHC4 score $= 94.7 \times \{ -0.100 \ ER_{10} - 0.079 \ PgR_{10} + 0.586 \ HER2 + 0.240 \ In \ (1 + 10 \times Ki67)\}$ where ER_{10} was obtained by dividing the H score by 30 and PgR was scored as a percentage of cells staining positive for progesterone receptors and PgR_{10} was obtained by dividing this percentage by 10.

CTS $= 100 \times \{0.417 \ N_{1-3} + 1.566 \ N_{4+} + 0.930(0.497 \ T_{1-2} + 0.882 \ T_{2-3} + 1.838 \ T_{>3} + 0.559 \ Gr_2 + 0.970 \ Gr_3 + 0.130 \ Age_{\geq 65} - 0.149 \ Ana)\}$ where N, T, Gr denote categories of nodal status, tumor size, grade respectively and Ana denotes treatment with anastrozole versus tamoxifen.

The IHC4 score plus CTS were added for each patient to get a combine score. Patients were categorized as having low risk (<1.183), intermediate risk (1.183–1.909), or high risk (>1.909) [7]. These cut-offs correlate with a 10-year distant recurrence risk less than 10%, 10–20%, and greater than 20%, respectively in the transATAC group [12].

As margin status is an important predictor of the risk of LRR and it was not included in the IHC4 + CTS, we decided to explore the relationship between margin status and IHC4 + CTS in predicting risk of LRR. We extracted data on margin status for the validated subgroup and categorized it into two groups (margin less than 5 mm and margins greater than 5 mm). The association between margin data relative to the IHC4 + CTS in predicting the risk of LRR was determined.

The pre-specified primary endpoint for this analysis is the time to ipsilateral LRR. LRR was defined as recurrence of the same histological type of invasive breast cancer as the original invasive breast cancer in the ipsilateral treated breast and/or ipsilateral lymph nodes. Time to ipsilateral LRR is the instantaneous probability of the event at 5 years as described in the Kaplan Meier Curve. Patients on this were censored if they died without local recurrence. The Local Regional Recurrence Free Survival includes invasive ipsilateral tumor and/or ipsilateral lymph nodes. The rates of distant recurrence, overall survival, and disease-free survival, the overall median survival time, and the median time to death from breast cancer were also recorded for the study group and the control group. The follow up data was available till 31-7-2012.

Laboratory methods

Formalin-fixed, paraffin-embedded (FFPE) tumor blocks were used for IHC analysis performed on tissue sections cut at 4 microns. All antibodies were pre-diluted by Ventana Medical Systems. Staining was performed on the Ventana Benchmark Ultra using SP1 and 1E2 antibodies for ER and PR staining, respectively. All of the ER-stained slides were reviewed in order to classify the proportion of cells that stained strongly, moderately, and weakly for ER in order to record the "H score" [7] in the IHC4 formula. Ventana anti-HER2/neu (4B5) rabbit monoclonal primary antibody was used for IHC determination of HER2 status. Tumors were considered positive if the IHC staining score was 3+ or 2+. Ki67 analysis was performed using 30-9 stain and manual counting. To avoid inter-observer bias, the "H score" and the Ki67 score were each reported by a single pathologist.

Table 1
Patient demographics and tumor characteristics.

	Study	Control	p Value
Menopausal status			
Perimenopausal	2.5%	9.6%	<0.001
Premenopausal	13.4%	24.5%	
Postmenopausal	84%	65.6%	
Mean age	67.6 years	57.5 years	<0.001
	(43–95 yrs)	(40–82 yrs)	
Postcode			
ACT	55.5	74.8	<0.0001
Non ACT	44.9	25.2	
T stage			
T1	32.8%	26%	0.2
T2	39.5%	50.5%	
T3	27.7%	22.9%	
T4	0%	0.50%	
Nodal status			
N0	43.7	70.1	<0.0001
N1–4	11.8	21.4	
N > 4	1.7	2.6	
NA	42.9	5.2	
Nodal status [7]			
NA plus N0	86.6	75.3	0.02
N1–4	11.8	21.4	
N > 4	1.7	3.3	
Histology			
Invasive ductal	69.7	82.9	<0.0001
Ductal mixed	0	0.9	
Invasive lobular	7.6	6.4	
Special histological[a]	22.7	9.7	
Grade[b]			
I	37	30.7	0.001
II	37	37.4	
III	21.8	30.7	
Unknown	4.2	1.1	
Margin status			
Positive	10.9	1.8	<0.0001
<5 mm	41.2	29.8	
5–10 mm	25.2	38.6	
>10 mm	21.8	28.9	
Unknown	0.8	0.9	
Lymphovascular			
Yes	12.6	15.6	0.17
No	84	78.4	
Unknown	3.4	6.0	
ER status[c]			
Positive	80.7	82	0.01
Negative	16	17.4	
Unknown	3.4	0.6	
PR status[c]			
Positive	69.7	71.3	0.02
Negative	26.9	28	
Unknown	3.4	0.4	
HER2 status[d]			
Positive	15.1	7.1	<0.001
Negative	69.7	60.6	
Unknown	15.1	32.2	
Adjuvant chemotherapy			
Not offered	84.8	53.6	<0.001
Offered but refused	7.6	4.8	
Offered and received	7.6	41.5	
Adjuvant hormones			
Not offered	22.7	21.8	<0.001
Offered but refused	15.1	2.4	
Offered and received	62.2	75.7	

[a] Special histological types included mucinous and intracystic papillary carcinoma.
[b] More patients in the control group had grade III tumors than in the study group ($p = 0.04$), and fewer patients in the control group had unknown tumor grade than in the control group ($p = 0.04$).

[c] For ER and PR status there was no statistically significant difference between the positive and negative subgroups. Women in the study group were more likely to have unknown hormone receptor status. When we excluded the patients with unknown status, the p value for the difference between the groups was not statistically significant ($p = 0.78$ for ER, and $p = 0.98$ for PR).
[d] For HER2 status, when we excluded patients with unknown status, the p value remained statistically significant ($p = 0.03$).

Statistical analysis

In addition to descriptive statistics, Cox proportional hazards models were used to assess the association between the IHC4 score + CTS and the risk of LRR. Kaplan–Meier estimates were used to determine the cumulative proportions of LRR within the patient subgroups. The importance of margin status relative to IHC4 score + CTS in predicting LRR was determined using score tests in a Cox proportional hazard model. A forward likelihood-ratio method was used to enter predictors into the Cox models with an entry probability of 0.05 or less. Overall, two-sided p values of 0.05 or less were considered significant. SPSS v22.0 was used to undertake the analyses.

Results

The study group initially included 119 patients, and the control group included 1406 patients. The following patients were excluded from the study group: 5 patients because of insufficient tissue in the blocks, 2 patients because of phyllodes histology, 9 patients because slides were unavailable, and 22 patients because of a lack of invasive tumor in the section. Of the remaining 81 patients in the study group, 53 were postmenopausal with ER-positive disease and no history of chemotherapy (validated subgroup).

Clinicopathological differences between the study group and control group

Table 1 shows the patient demographics and tumor characteristics in the study group and the control group. Patients in the study group were more likely to be postmenopausal ($p < 0.0001$), older (mean age 67.6 years versus 57.5 years; $p < 0.0001$), and non-urban ($p < 0.0001$) compared with those in the control group. Of the patients in the study group, 42.9% did not have pathological nodal staging and these were included in the node negative group similar to the Cuzick study [7]. Taking this into account, there were more node-positive patients in the control group than in the study group ($p = 0.02$).

Outcome differences between the study group and control group

The median follow-up time was 69 months, the minimum follow up time was 8.8 months and the maximum follow-up time was 184 months for women who had breast conservation surgery during this period. The 10-year overall survival rate was 90% for the control group and 82% for the study group ($p < 0.001$; Fig. 4a). The 10-year disease-free survival rate was 89.6% for the control group and 81.8% for the study group ($p = 0.02$; Fig. 4b). The incidence of local recurrence was 1.8% in the control group and 5.9% in the study group ($p = 0.01$). The incidence of regional recurrence was 1.2% in the control group and 5% in the study group ($p = 0.008$). The incidence of distant recurrence was 5.5% in the control group and 3.4% in the study group ($p = 0.2$). The overall median survival time was 181.7 months (95% CI: 169.1–194.4) in the control group and 117 months (95% CI: 89.6–144.5) in the study group ($p < 0.0001$). The mean time to death from breast cancer was 170.5 months (95%

CI: 167.6–173.4) in the control group and 146.9 months (95% CI: 135.3–163.8) in the study group ($p = 0.02$).

Statistical correlation between IHC4 + CTS and risk of LRR

The mean IHC4 + CTS for the low, intermediate and high risk groups was 0.4, 1.6 and 2.95 respectively in the study group. The LRR rate was 2.7% (1/37), 22.2% (4/18), and 23.7% (6/26) in the low-risk, intermediate-risk, and high-risk groups, respectively ($p = 0.02$). The Cox regression of the study group ($n = 81$) revealed a statistically significant association between the IHC4 score + CTS and the risk of LRR (HR = 1.01, 95% CI 1.00–1.01, $p = 0.01$). There was a significant difference in the LRR rate between the low-risk and high-risk groups ($p = 0.03$) and also low and intermediate risk groups ($p = 0.03$) but not between the intermediate-risk and high-risk groups ($p = 0.9$), suggesting that the IHC4 score + CTS is best for predicting patients with a low LRR risk. The Kaplan Meier curve (Fig. 1) revealed that at 5 years, 97% of the patients in the low-risk group, 79% of the patients in the intermediate-risk group, and 75.2% of the patients in the high-risk group were free from LRR ($p = 0.02$). The mean progression-free survival in the study group was 14.4 years, 8.6 years, and 7.6 years for the low-risk, intermediate-risk, and high-risk groups, respectively.

Similarly, the Cox regression of the validated subgroup ($n = 53$) indicated a statistically significant association between the IHC4 score + CTS and the risk of LRR (HR = 1.01, 95% CI 1.00–1.02, $p = 0.03$). The mean IHC4 + CTS for the low, intermediate and high risk groups was 0.34, 1.62 and 2.7 respectively. The IHC4 score + CTS showed good association with LRR on the Receiver Operating Characteristic (ROC) curve (Fig. 2), with an area under the curve of 83.8%.

When the cutoff points used in the original study [7] were applied to LRR outcome, incidence of LRR was zero (0/32) in the low-risk group, 20% (3/15) in the intermediate-risk group, and 33.3% (2/6) in the high-risk group ($p = 0.007$). The Kaplan–Meier curve (Fig. 3) revealed that at 5 years, 100% of the patients in the low-risk group, 83% of the patients in the intermediate-risk group,
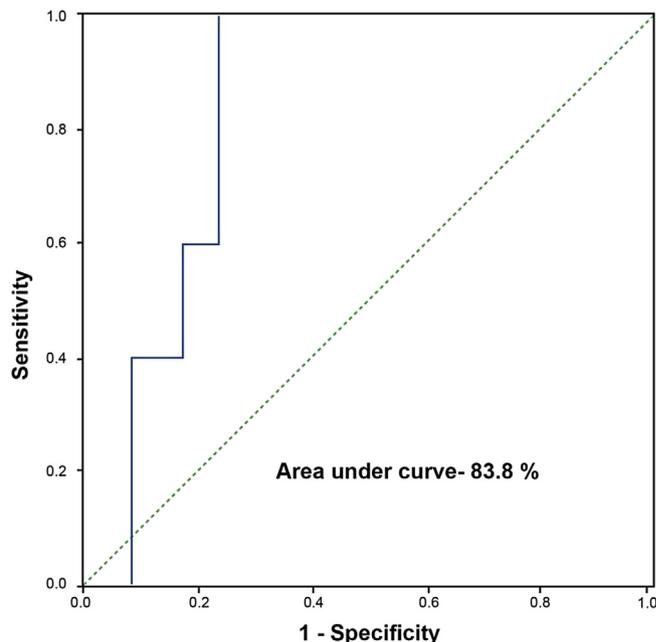

Fig. 2. ROC curve for the validated subgroup ($n = 53$).

and 60% of the patients in the high-risk group were free from LRR ($p = 0.007$). The Cox regression model did not indicate a significant difference in the incidence of LRR between the intermediate-risk and high-risk groups.

Relationship between margin status and IHC4 + CTS in predicting risk of LRR

The margin status was the only important pathological variable with respect to local recurrence that was not included in the IHC4 score + CTS. In the validated subgroup, 5 (15.2%) of the 33 patients
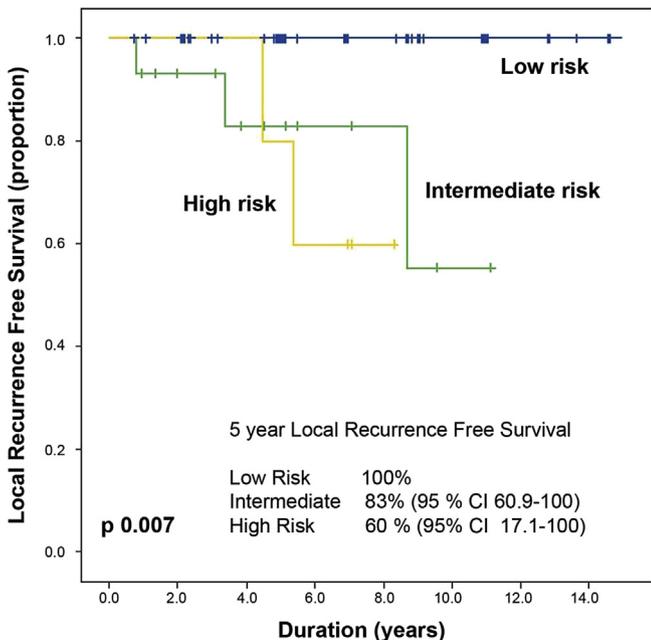

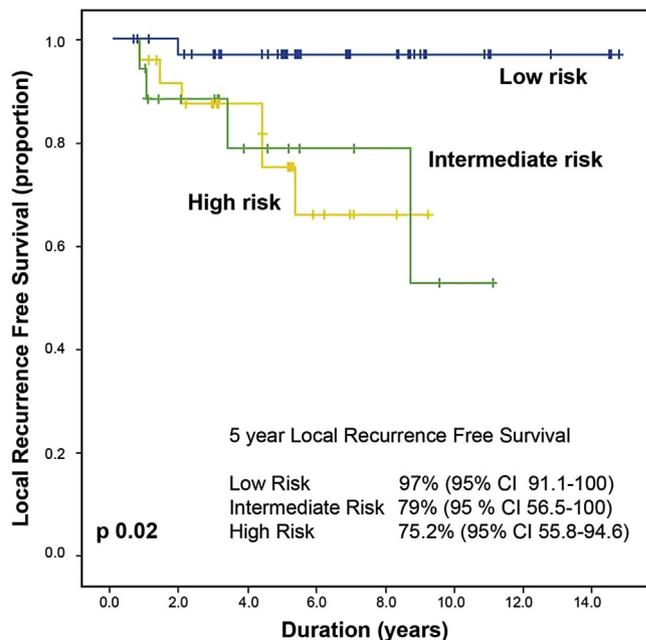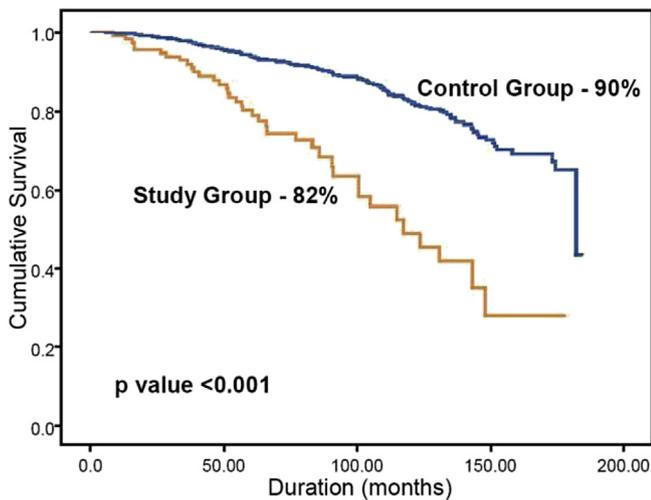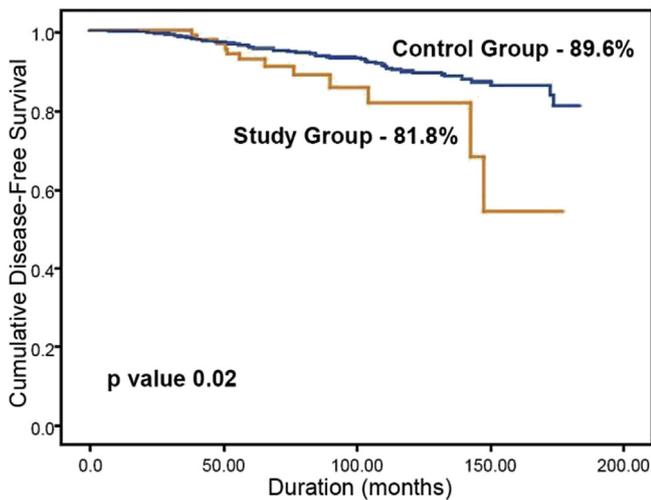**Fig. 1.** Kaplan–Meier curve for locoregional recurrence free survival for the study group ($n = 81$).

Fig. 3. Kaplan–Meier curve for locoregional recurrence free survival for the validated subgroup ($n = 53$).

a- 10 year Overall Survival for the Control and Study group

b- 10 year Disease-Free Survival for the Control and Study group

Fig. 4. Survival curves for the control ($n = 1406$) and study groups ($n = 119$).

with a margin smaller than 5 mm experienced LRR, and none of the 19 patients with a margin larger than 5 mm experienced LRR. The margin data was missing for one patient. The Cox regression analysis demonstrated that the IHC4 score + CTS ($p = 0.02$) and the ordinal measure of margins ($p = 0.03$) were significant independent predictors of LRR. In the final regression model, the IHC4 score + CTS ($p = 0.04$) and the margin status ($p = 0.04$) both significantly contributed to LRR (see Table 2). The risk of LRR increased with an increase in the IHC4 score + CTS (hazard ratio

Table 2
Cox proportional hazards regression model showing predictors of LRR for validated subgroup ($n = 53$).

Predictor	HR (95% CI)	*p* Value[a]
IHC4 score + CTS	1.008 (1.000–1.017)	0.04
Margin status	0.23 (0.045–1.176)	0.04

[a] Derived from the likelihood-ratio method.

[HR] = 1.008, 95% confidence interval [CI]: 1.00–1.01) and decreased with an increase in the margin (HR = 0.23, 95% CI: 0.04–1.17).

Discussion

There was a significant association between the IHC4 score + CTS and the risk of LRR in postmenopausal women with ER-positive early breast cancer who did not receive adjuvant radiotherapy. Women with low IHC4 score + CTS had a negligible risk of LRR. We explored the relationship between the margin status and the IHC4 score + CTS and found that both independently predicted loco-regional recurrence. This study showed that the IHC4 score + CTS predicted LR in a larger group than the validated cohort that includes premenopausal, hormone receptor-negative patients who had received chemotherapy.

Women that did not receive breast-conservation radiotherapy were different to those that did receive it in that they were older, and had lower-grade and HER2-negative tumors; factors associated with a lower LRR risk. However the study group women were more likely to live in rural locations and had tumors with positive and tighter margins. These factors were associated with higher LRR risk. [13,14] The women who did not receive radiotherapy also had less axillary surgery and a high proportion of "unknown" receptor status tumors. These findings may indicate differences in patient co-morbidities and preferences and, perhaps, quality of treatment delivery. The women who did not receive radiotherapy had poorer overall survival rates, suggesting that they had competing risks for death, especially given that the rates of distant metastases in the two groups were not significantly different.

Immunohistochemistry methods used in this study are different to those used in the Cuzick study [7]. Our study used rabbit monoclonal antibodies against the ER and PR receptors, which offer increased sensitivity compared with the mouse monoclonal antibodies used in the original study [15]. In the original study, HER-2 expression was demonstrated using a Dako Hercep Test, which uses a rabbit polyclonal antibody, whereas we used a rabbit monoclonal antibody. Both methods are highly sensitive and specific [16]. Fluorescence in situ hybridization was not done on the 2+ HER-2 samples in our study, which may have led to an overestimation of the number of HER-2 positive cases. Ki67 analysis was done by a manual count by one pathologist, whereas it was done by image analysis in the Cuzick study [7]. One drawback of the IHC4 score is that it requires a "H score" [7], the calculation of which is labor intensive and operator dependent.

A limitation of this study is the small sample size of the study group and the small number of LRR events. However it is important to acknowledge that this is a unique patient group, as adjuvant radiation post BCS is still the standard of care. Despite the low numbers, the statistical significance in identifying the low risk group was striking. The independent relationships of IHC4 score + CTS to LRR and margin status to LRR are also notable. A major strength of this study is that it is population-based and therefore includes sub-groups of women that may be poorly represented in randomized trials for the omission of radiotherapy. Outside of clinical trials, capturing data on women who decline adjuvant therapies is challenging. The ACT and SENSW BCTG Quality Assurance Project is designed to ensure a high proportion of follow-up information is captured [17]. Another limitation is the median follow-up of 69 months which is relatively short-term for ER-positive breast cancer.

Multi-gene profiling assays are used in clinical practice to inform systemic treatment decisions based on tumor biology but these have not been validated for decisions regarding adjuvant breast conservation radiotherapy. Mamounas [18] showed a

significant association between the 21-gene recurrence score and the LRR risk that discriminates better among patients who have had surgery alone than among patients treated with lumpectomy and radiation. On similar principle we decided to test the utility of the IHC4 score + CTS in patients who had BCS but had not received radiation therapy; therefore the confounding effect of radiation on the LRR risk was eliminated.

The identification of a group of women in whom radiotherapy can be safely omitted has been the focus of several randomized trials [19–22]. One breast cancer death is avoided for every four local recurrences prevented by adjuvant radiation. However, the Number Needed To Treat (NNT) [23] to avoid one recurrence increases dramatically for women older than 80 years. The LUMINA study is an ongoing, single-arm prospective study for the omission of radiotherapy in women over 55 with T1 N0 Luminal A breast cancer. It is hypothesized that using a more restrictive inclusion criterion, rates of local recurrence will be so low that omission of radiotherapy will be an option for such women. The advantage of IHC4 score + CTS is that it takes into account and gives relative weighting to the variables associated with LRR. These variables have defined low risk groups in the studies for omission of radiotherapy, that is, age, tumor size, grade, nodal status and the type of endocrine therapy used. The IHC4 score + CTS is an inexpensive tool using information readily available to the clinician for patients that may not have access to more sophisticated genomic tools especially in developing countries. In summary, our findings suggest that with further validation, the IHC4 score + CTS may prove to be a low cost tool to identify low-risk women in whom adjuvant radiation may be omitted. The fact that our study group consisted of older women who had a high risk of death from other causes should be taken into account when IHC4 score + CTS is used in clinical practice.

Ethical approval

The ACT Human Research Ethics Committee approved the current study.

Conflict of interest statement

All authors declare that there is no conflict of interest.

Acknowledgment

References

[1] Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. Nature 2000;406:747–52.
[2] Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A 2001;98:10869–74.
[3] Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med 2004;351:2817–26.
[4] Paik S, Tang G, Shak S, Kim C, Baker J, Kim W, et al. Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. J Clin Oncol Off J Am Soc Clin Oncol 2006;24:3726–34.
[5] Cardoso F, Piccart-Gebhart M, Van't Veer L, Rutgers E. The MINDACT trial: the first prospective clinical validation of a genomic tool. Mol Oncol 2007;1: 246–51.
[6] Bartlett JM, Thomas J, Ross DT, Seitz RS, Ring BZ, Beck RA, et al. Mammostrat as a tool to stratify breast cancer patients at risk of recurrence during endocrine therapy. Breast Cancer Res 2010;12:R47.
[7] Cuzick J, Dowsett M, Pineda S, Wale C, Salter J, Quinn E, et al. Prognostic value of a combined estrogen receptor, progesterone receptor, Ki-67, and human epidermal growth factor receptor 2 immunohistochemical score and comparison with the Genomic Health recurrence score in early breast cancer. J Clin Oncol 2011;29:4273–8.
[8] Cuzick J, Sestak I, Baum M, Buzdar A, Howell A, Dowsett M, et al. Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 10-year analysis of the ATAC trial. Lancet Oncol 2010;11:1135–41.
[9] Early Breast Cancer Trialists' Collaborative Group, Darby S, McGale P, Correa C, Taylor C, Arriagada R, et al. Effect of radiotherapy after breast-conserving surgery on 10-year recurrence and 15-year breast cancer death: meta-analysis of individual patient data for 10,801 women in 17 randomised trials. Lancet 2011;378:1707–16.
[10] Wapnir IL, Anderson SJ, Mamounas EP, Geyer Jr CE, Jeong JH, Tan-Chiu E, et al. Prognosis after ipsilateral breast tumor recurrence and locoregional recurrences in five National Surgical Adjuvant Breast and Bowel Project node-positive adjuvant breast cancer trials. J Clin Oncol 2006;24:2028–37.
[11] Taghian A, Jeong JH, Mamounas E, Anderson S, Bryant J, Deutsch M, et al. Patterns of locoregional failure in patients with operable breast cancer treated by mastectomy and adjuvant chemotherapy with or without tamoxifen and without radiotherapy: results from five National Surgical Adjuvant Breast and Bowel Project randomized clinical trials. J Clin Oncol 2004;22:4247–54.
[12] Dowsett M, Salter J, Zabaglo L, Mallon E, Howell A, Buzdar AU, et al. Predictive algorithms for adjuvant therapy: TransATAC. Steroids 2011;76:777–80.
[13] Craft PS, Buckingham JM, Dahlstrom JE, Beckmann KR, Zhang Y, Stuart-Harris R, et al. Variation in the management of early breast cancer in rural and metropolitan centres: implications for the organisation of rural cancer services. Breast 2010;19:396–401.
[14] Behm EC, Beckmann KR, Dahlstrom JE, Zhang Y, Cho C, Stuart-Harris R, et al. Surgical margins and risk of locoregional recurrence in invasive breast cancer: an analysis of 10-year data from the Breast Cancer Treatment Quality Assurance Project. Breast 2013;22:839–44.
[15] Rocha R, Nunes C, Rocha G, Oliveira F, Sanches F, Gobbi H. Rabbit monoclonal antibodies show higher sensitivity than mouse monoclonals for estrogen and progesterone receptor evaluation in breast cancer by immunohistochemistry. Pathol Res Pract 2008;204:655–62.
[16] Nunes CB, Rocha RM, Buzelin MA, Balabram D, de Souza Foureaux F, Porto SS, et al. False positivity in HER2 testing of breast cancer: novel paths for approaching an old dilemma. J Clin Pathol 2013;66:946–50.
[17] Craft PS, Zhang Y, Brogan J, Tait N, Buckingham JM. Implementing clinical practice guidelines: a community-based audit of breast cancer treatment. Australian Capital Territory and South Eastern New South Wales breast Cancer treatment group. Med J Aust 2000;172:213–6.
[18] Mamounas EP, Tang G, Fisher B, Paik S, Shak S, Costantino JP, et al. Association between the 21-gene recurrence score assay and risk of locoregional recurrence in node-negative, estrogen receptor-positive breast cancer: results from NSABP B-14 and NSABP B-20. J Clin Oncol 2010;28:1677–83.
[19] Fisher B, Bryant J, Dignam JJ, Wickerham DL, Mamounas EP, Fisher ER, et al. Tamoxifen, radiation therapy, or both for prevention of ipsilateral breast tumor recurrence after lumpectomy in women with invasive breast cancers of one centimeter or less. J Clin Oncol 2002;20:4141–9.
[20] Fyles AW, McCready DR, Manchul LA, Trudeau ME, Merante P, Pintilie M, et al. Tamoxifen with or without breast irradiation in women 50 years of age or older with early breast cancer. N Engl J Med 2004;351:963–70.
[21] Hughes KS, Schnaper LA, Bellon JR, Cirrincione CT, Berry DA, McCormick B, et al. Lumpectomy plus tamoxifen with or without irradiation in women age 70 years or older with early breast cancer: long-term follow-up of CALGB 9343. J Clin Oncol 2013;31:2382–7.
[22] Kunkler IH, Williams LJ, Jack WJ, Cameron DA, Dixon JM, Investigators PI. Breast-conserving surgery with or without irradiation in women aged 65 years or older with early breast cancer (PRIME II): a randomised controlled trial. Lancet Oncol 2015;16(3):266–73.
[23] Smith BD, Gross CP, Smith GL, Galusha DH, Bekelman JE, Haffty BG. Effectiveness of radiation therapy for older women with early breast cancer. J Natl Cancer Inst 2006;98:681–90.