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Human epidermal growth receptor-2 overexpressing early operable primary breast cancers in older (≥70 years) women: biology and clinical outcome in comparison with younger (<70 years) patients

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Introduction: There is dearth of literature reporting the prevalence and biological characteristics as well as the long-term clinical outcome of human epidermal growth factor receptor-2 (HER2) overexpressing tumours in older women. Currently, research involving trastuzumab at large focuses on the younger population. This study aimed to analyse their biological characteristics and to compare them with their younger counterparts from a single centre with a long-term clinical follow-up.

Methods: Over 37 years (1973–2010), 1758 older (≥70 years) women with early operable (<5 cm) primary breast cancer were managed in a dedicated clinic and have complete clinical information available. Of these, 813 patients underwent primary surgery and 575 had good quality tumour samples available for tissue microarray analysis using indirect immunohistochemistry. Comparison was made with data from a well-characterised younger (<70 years) series ($N = 1711$) treated between 1986 and 1998 (before adjuvant trastuzumab became standard) in our institution. Forty five (7.6%) and 140 (8.2%) patients from the older and younger series, respectively, had HER2-positive tumours.

Results: HER2 overexpression was seen in 45 (7.6%) older women and 140 (8.2%) in younger patients ($P = 0.56$). HER2 overexpressing tumours in older women when compared with that in their younger counterparts were associated with low Ki67 and high bcl2 expression ($P < 0.05$). Only 26% of the younger patients and none of the older patients received adjuvant chemotherapy, and no patients at the time received trastuzumab. However, there was no significant difference in the outcome of the two age groups (5-year breast cancer-specific survival rate: <70 years = 65% versus >70 years = 70%, $P = 0.51$).

Conclusion: HER2 overexpressing tumours in older women showed relatively a less aggressive phenotype and did not show any inferior long-term clinical outcome despite not having received chemotherapy when compared with the younger patients. The precise role of different adjuvant systemic therapies in this population needs to be delineated.

Key words: HER2 overexpressing, breast cancer, older women

introduction

About one-third of breast cancers occur in women over 70 years of age [1]. With increasing age, there appears to be a change in biology with more oestrogen receptor (ER) positivity, low human epidermal growth factor receptor-2 (HER2) positivity and proliferation [2]. Given the negative correlation between ER and HER2, a low incidence of HER2-positive tumours can be expected [3]. However, there is still a considerable proportion of them who have ER-negative tumours (~20%) [4]. Therefore, alongside the small number of ER-positive cases, older women with HER2-positive disease constitute a significant number in absolute terms.

Overall one-fifth of breast cancers are reported to overexpress HER2 protein or show gene amplification [5, 6]. They are reported to have poor prognosis [5]. However, since the introduction of anti-HER2 therapy, that is trastuzumab in clinical practice, the outcome has improved [7]. On the other hand, randomised controlled trials were mainly focused on younger patients, with exclusion or in-appropriate representation of older women [3]. Thus, due to the limited data reported on the biology and clinical outcome of the HER2-positive tumours in older women, this phenotype remains a challenge for clinicians managing with these patients.

This study aimed to analyse biological characteristics and long-term clinical outcome of HER2 overexpressing tumours in older women and to compare with those of younger patients.

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patients and methods

study patients

Between 1973 and 2010, 1758 older (≥70 years) women with early operable primary breast cancer (T0–2, N0–1 and M0) were managed in a dedicated clinic with clinical information available from diagnosis till death/last follow-up. Eight hundred and thirteen patients underwent primary surgery (with standard adjuvant therapy as per unit policy based on Nottingham Prognostic Index (NPI) criteria and ER status, where those with a NPI score of <3 did not receive any systemic therapy, >3 received adjuvant systemic therapy including endocrine therapy in patients with ER-positive tumours and chemotherapy with ER-negative tumours [8]). Of which 238 tumour blocks were overused or only small number of cells was available which was not sufficient for analysis, thus 575 good quality formalin-fixed, paraffin-embedded surgical specimens were available for tissue microarray (TMA) construction.

For comparison, a previously characterised disease stage-matched series of younger (<70 years) patients ($N = 1711$) was retrieved from the unit's database (Nottingham Tenovus series) [9]. The series comprised clinical and biological data of younger (<70 years) patients with early operable primary breast cancer established in 1980s and tumours were prospectively analysed using immunohistochemistry (IHC) on TMAs, constructed using surgical specimens ($N = 1809$). As it was planned at the outset of the project to compare the older with the younger series, hence variables including biomarkers collected and the methods for tumour analyses were the same for both series. All patients were managed following the same clinical guidelines at the time. None of them received adjuvant trastuzumab, as the younger series covered a period before trastuzumab was in use as a standard adjuvant therapy and its use in older patients remains controversial.

Conventional pathological parameters in both groups, including pathological size, grade and axillary stage (according to the number of positive nodes, 1 = 0 positive, 2 = 1–3 positive, 3 = ≥4 positive), were retrieved from the standard reporting performed at the time of surgery [10].

TMA construction

Construction was made from formalin-fixed, paraffin-embedded tumour sections as previously described [11]. Briefly, 0.6 mm-diameter cores of the representative part of the tumour blocks were implanted in the recipient TMA blocks using Beecher's manual tissue microarrayer (MP06 Beecher Instruments, Inc., USA).

immunohistochemistry

Indirect IHC was the method used for biomarker analyses. A total of 14 markers were analysed in two age groups by StreptAvidin Biotin Complex and EnVision methods as described previously [9]. The markers analysed in the study for comparison include ER, Progesterone receptor (PgR), HER2, p53, Ki67, Bcl2, Muc1, E-Cadherin, basal and luminal cytokeratins. Details of antibodies are summarised in Table 1.

scoring

Immunohistochemical staining of the biomarkers was assessed by the percentage of cells stained as well as McCarty's IHC scoring (H-score) (range 0–300) [12]. Cut-offs to define biomarker positivity were determined by using the X-tile software (Yale University) [13] based on the percentage of cells stained (Table 1). For HER2 scoring, the HercepTest scoring system was used, defined according to the staining of the membrane in 0 = negative (no membrane staining), 1+ = faint part staining in >10% of cells, 2+ = weak–moderate membrane staining in >10% of cells and 3+ = strong complete staining in >10% cells. Those which showed 2+ staining were then analysed on Chromogen *In Situ* Hybridisation (CISH) as below. HER2-positive tumours were defined by IHC 3+ or as gene amplification on CISH.

CISH

Slides were dewaxed in xylene and dehydrated in alcohol followed by washing in wash buffer provided in the Dako, FISH kit. Slides were then pre-treated in the pre-treatment solution by heating them at 99°C for 10 min followed by cooling down at room temperature for 15 min. Slides were soaked into two baths of wash buffer for 3 min each, then wiped and treated with pepsin for exactly 6 min at 37°C followed by washing in wash buffer and dehydration in alcohol. Ten microlitres of probe mix were applied on dry slides and sealed with coverslip sealant gel, and incubated for 20 h at 45°C, beginning with 5 min at 80°C using Dako programme hybridiser. Afterwards, slides were placed in stringent wash buffer at room temperature to allow gentle separation of the coverslip. Slides were then treated by stringent wash buffer again for 10 min at 65°C, followed by washing in the wash buffer. Two hundred microlitres of hydrogen peroxide were applied for 6 min to block

Table 1. A summary of the antibodies source, methods and cut-offs to define positivity of the markers used to analyse biology of early operable primary breast cancer in older women

Biomarker	Antibody reference	Dilution	Incubation time (min)	Antigen retrieval method	Cut-off to define positivity
ER	RM-9101-SP1/NeoMarkers	1:100	45	Citrate buffer	0
PgR	PgR 636/Dako	1:200	45	Citrate buffer	0
HER2	Hercep test Kit- Dako	Pre-diluted	30	Not required	3+
Ki67	M 7240/Dako	1:100	45	Citrate buffer	10
P53	DO-7/Novocastra	1:100	45	Citrate buffer	5
CK5/6	M7237/Dako	1:100	45	Citrate buffer	10
CK7/8	34779/BioSciences	1:100	45	Citrate buffer	10
CK14	LL002/Vector Laboratories	1:100	45	Citrate buffer	10
CK17	E3/ Vector Laboratories	1:100	45	Citrate buffer	10
CK18	M7010/Dako	1:100	45	Citrate buffer	10
CK19	CM 242A/Biocare Medical	1:100	45	Citrate buffer	10
Bcl2	M0887/Dako	1:100	45	Citrate buffer	10
E-Cadherin	M3612/Dako	1:100	45	Citrate buffer	30
Muc1	NCL/Novocastra	1:300	45	Citrate buffer	20

endogenous peroxidase activity. Slides were then immersed in two baths of wash buffer. The CISH antibody was applied for 30 min in a humid chamber, and then washed into two baths of CISH wash buffer. The red chromogen was applied for 10 min in a humid chamber followed by washing in wash buffer. The blue chromogen was then applied in the humid chamber for 10 min followed by washing in wash buffer. The haematoxylin was applied for 5 min, and the slides were then rinsed with wash buffer and placed in the wash buffer bath for 5 min. Slides were cleaned with distilled water and air dried at room temperature, then mounted permanently after drying.

In terms of CISH scoring, the green and red dots were counted in the nuclei by using a fluorescent microscope (×100). At least 20 cells were counted and the ratio of green and red dots was taken. The ratio >2 was considered positive, whereas <2 was negative.

statistical methods

Data were analysed using the Statistical Package for Social Sciences (SPSS version 17.0, Chicago, IL, USA). Within the older cohort, the comparisons were made between HER2-positive and -negative tumours, and then comparisons of biology as well as clinical outcome were made with those of the younger patients. Comparisons were carried out using the χ^2 test, and time-dependent variables were analysed on Kaplan-Meier plots with application of log-rank and Wilcoxon tests as appropriate. The study intended to analyse the relationship between biology and clinical outcome. As overall survival, notably in the older population, would be influenced by competing causes of death, it was decided that breast cancer-specific survival and metastasis-free survival rates were measured and analysed as they were deemed appropriate surrogates for effects of tumour biology and treatments. Data on co-morbidities were not collected as part of this study. A Multinomial regression model was used to analyse the association of age and tumour characteristics (pathological size, grade and axillary lymph node stage) for the selection of adjuvant therapy.

ethical approval

The study was approved by the local Institutional Ethical Committee.

results

rate of HER2 positivity

Immunohistochemical score showed that 81.8% ($N = 439$) were clearly negative with 0 score, 7.3% ($N = 39$) were 1+, 3.4% ($N = 18$), 165 were 2+ and 7.6% ($N = 45$) were scored 3+. All 2+ cases ($N = 18$) were then analysed on CISH and none showed the amplified gene. Thus, 7.6% were considered HER2-positive in our older cohort. From the younger cohort, 140 (8.2%) of 1711 patients had HER2-positive tumours ($P = 0.56$).

For whole series, median age of the patients was 60 years (<70 = 53 years and ≥70 = 75 years). Median pathological size of the tumours was 2.0 cm (<70 = 1.9 cm and ≥70 = 2.3 cm).

treatment pattern

All patients in both groups underwent surgery without any prior intervention. Adjuvant chemotherapy was received by 28% ($N = 36$) younger patients and none of the older women while 26% ($N = 12$) of older patients and over 42% ($N = 57$) of younger patients received adjuvant endocrine therapy. None of the patient in both groups received adjuvant trastuzumab. Multinomial logistic regression analysis did not show any significant influence of patient age and tumour characteristics on the choice of adjuvant therapy.

age stratified pattern of HER2 positivity

There appears a gradual decline in HER2 positivity with advancing age (Figure 1A); however, there was a rapid rise in women <40 years. HER2 positivity in ER-positive and -negative disease appeared as mirror image, where there was a decline in the ER-positive group, and a consistent rise followed by a slight decline in ER-negative tumours (Figure 1B). The decline in HER2 positivity was pronounced in ER-positive tumours, whereas there was a slight fall in ER-negative tumours, which still remained higher.

biology of HER2 overexpressing tumours in older women

HER2 overexpressing tumours were associated with higher grade ($P < 0.001$) and stage ($P = 0.009$), negative expressions of ER ($P < 0.001$), PgR ($P < 0.001$) and Bcl2 ($P < 0.001$), and higher expression of p53 ($P < 0.001$), CK14 ($P = 0.03$) and CK17 ($P = 0.003$) (Table 2).

clinical outcome of HER2 overexpressing tumours in older women

Older women with overexpressing HER2 showed significantly poorer breast cancer-specific and metastasis-free survival when compared with those with HER2-negative tumours (Figure 2A and B). At median follow-up of 61 months, 90% of HER2-negative and 78% of HER2-positive patients were still alive without

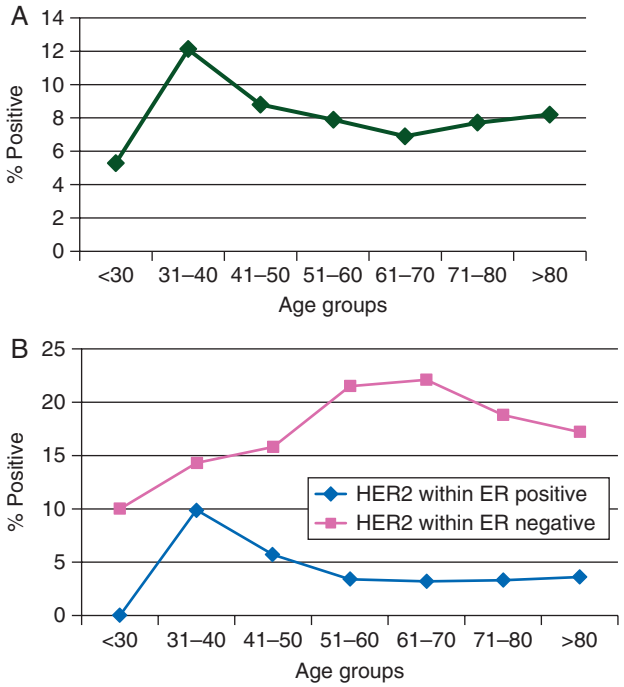

Figure 1. (A) Age standardised rate of HER2 overexpression in women with early operable primary breast cancer. (B) Age standardised rate of HER2 overexpression in women with early operable primary breast cancer within ER-positive and -negative tumour groups.

Table 2. Biological characteristics of HER2 overexpressing tumours in older women with early operable primary breast cancer

Character	HER2 negative	HER2 positive	*P*-value
Grade (*N* = 448)			
1 and 2	226 (54.5%)	7 (21.2%)	<0.001
3	189 (45.5%)	26 (78.8%)	
Stage (*N* = 333)			
1 and 2	279 (90.0%)	16 (69.6%)	0.009
3	31 (10.0%)	7 (30.4%)	
ER (*N* = 518)			
Negative	124 (26.0%)	29 (70.7%)	<0.001
Positive	353 (74.0%)	12 (29.3%)	
PgR (*N* = 517)			
Negative	190 (39.8%)	34 (85.0%)	<0.001
Positive	287 (60.2%)	6 (15.0%)	
P53 (*N* = 479)			
Negative	278 (62.9%)	13 (35.1%)	<0.001
Positive	164 (37.1%)	24 (64.9%)	
CK5/6 (*N* = 468)			
Negative	227 (52.8%)	23 (60.5%)	0.22
Positive	203 (47.2%)	15 (39.5%)	
CK14 (*N* = 471)			
Negative	335 (77.2%)	23 (62.2%)	0.03
Positive	99 (22.8%)	14 (37.8%)	
CK17 (*N* = 506)			
Negative	380 (81.7%)	25 (61.0%)	0.003
Positive	85 (18.3%)	16 (39.0%)	
CK7/8 (*N* = 515)			
Negative	15 (3.2%)	0 (0%)	0.302
Positive	461 (96.8%)	39 (100%)	
CK18 (*N* = 498)			
Negative	19 (4.2%)	0 (0%)	0.189
Positive	438 (95.8%)	41 (100%)	
CK19 (*N* = 511)			
Negative	21 (4.5%)	2 (4.9%)	0.566
Positive	449 (95.5%)	39 (95.1%)	
Ki67 (*N* = 537)			
Negative	333 (67.1%)	23 (56.1%)	0.10
Positive	163 (32.9%)	18 (43.9%)	
Bcl2 (*N* = 501)			
Negative	65 (14.1%)	14 (35.0%)	<0.001
Positive	396 (85.9%)	26 (65.0%)	
E-Cadherin (*N* = 504)			
Negative	186 (40.2%)	7 (17.1%)	0.002
Positive	277 (59.8%)	34 (82.9%)	
MUC1 (*N* = 515)			
Negative	65 (13.7%)	5 (12.5%)	0.532
Positive	410 (86.3%)	35 (87.5%)	

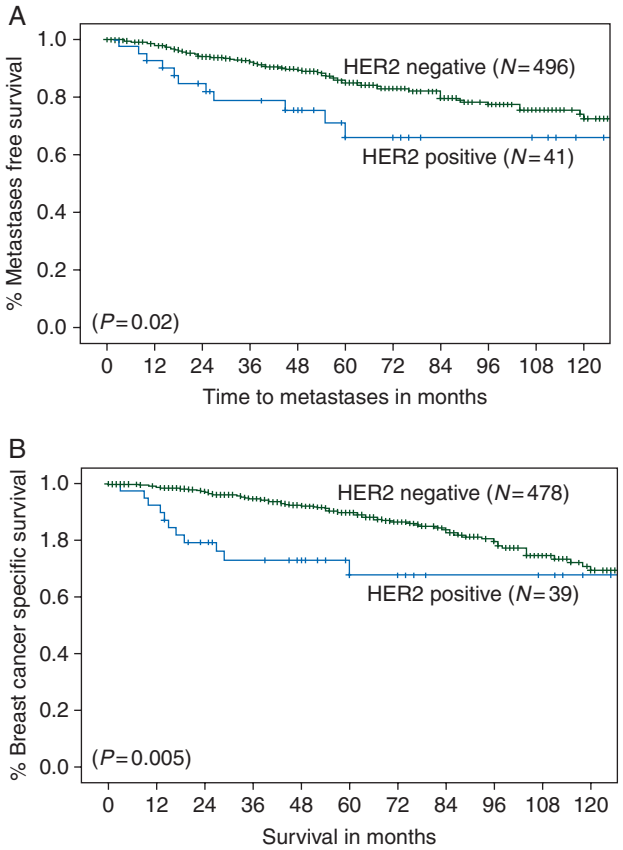

Figure 2. (A) Time to metastases in older women with early operable primary breast cancer according to HER2 overexpression—with and without adjuvant systemic therapy [after median follow-up of 61 (longest 250) months, 17% (*N* = 88) developed metastases (HER2 positive = 28.2% versus HER2 negative = 16.1%)] (log-rank: 4.881). (B) Breast cancer-specific survival in older women with early operable primary breast cancer according to HER2 overexpression—with and without adjuvant systemic therapy [after median follow-up of 61 (longest 250) months, 16.4% (*N* = 85) died of breast cancer (HER2 positive = 28.2% versus HER2 negative = 15.5%)] (log-rank: 7.739).

metastases. None of the biomarkers showed significant association with the clinical outcome due to very small number of events in univariate analysis.

biology and clinical outcome of HER2 overexpressing tumours—younger versus older women

HER2-positive tumours in older women, when compared with their younger counterparts, showed significantly less Ki67 positivity ($P < 0.001$) and higher positivity for bcl2 ($P < 0.001$) and CK14 ($P = 0.01$) (Table 3). Breast cancer-specific survival (5-year rate: <70 years = 65% versus >70 years = 70%, $P = 0.51$) in the two groups did not show any significant difference (Figure 3A).

discussion

The results of the data showed that HER2 was overexpressed in ~7% of early operable primary breast cancers in older women. There appears to be a consistent decline in the expression of HER2 with advancing age. HER2 positivity showed a positive correlation with higher grade, high stage disease, positive expression of p53, CK14, CK17 and E-Cadherin, and negative expression of ER, PgR and Bcl2. HER2 overexpressing tumours showed poor breast cancer-specific survival within the older cohort. When compared with their younger counterparts, they showed less proliferative activity and there appears no significant difference in survival rates in the two age groups, although a considerable proportion of younger patients received chemotherapy. A possible

Table 3. Biology of HER2 overexpressing tumours—younger (<70 years) versus older (≥70 years) of age

Biomarker	<70 years *N* (%) (*N* = 140)	≥70 years *N* (%) (*N* = 45)	*P*-value
Median age (years)	53	75	
Pathological size (median, cm)	1.9	2.3	0.16
<3.0	118 (84.3)	37 (82.2)	
3.1–5	18 (12.9)	8 (17.7)	
>5	4 (2.9)	0 (0)	
Grade			
≤2	22 (15.7)	7 (18.9)	0.401
3	118 (84.3)	30 (81.1)	
Axillary stage			
Node negative	73 (52.5)	12 (44.4)	0.27
1–3 nodes positive	43 (30.9)	8 (29.6)	
≥4 nodes positive	23 (16.5)	7 (25.9)	
ER positive	54 (38.6)	13 (28.9)	0.156
PgR positive	34 (24.3)	8 (18.2)	0.267
Ki67 positive	89 (78.8)	20 (45.5)	<0.001
p53 positive	68 (48.6)	25 (58.1)	0.178
E-Cadherin positive	132 (95.0)	40 (88.9)	0.139
Bcl2 positive	15 (28.3)	30 (73.2)	<0.001
Muc1 positive	103 (92.0)	41 (95.3)	0.366
CK5/6 positive	25 (18.0)	14 (31.8)	0.04
CK7/8 positive	140 (100)	44 (100)	NC
CK14 positive	24 (17.6)	14 (35.0)	0.01
CK17 positive	13 (21.0)	6 (15.4)	0.335
CK18 positive	125 (95.4)	45 (100)	0.165
CK19 positive	133 (95.7)	43 (95.6)	0.624
Treatment pattern			
Surgery	140 (100)	45 (100)	
Pattern of adjuvant therapy			
Radiotherapy	86 (61.4)	9 (20.0)	
Endocrine therapy	57 (42)	12 (26.7)	
Chemotherapy	36 (28)	0	
Trastuzumab	0	0	

NC, not computed.

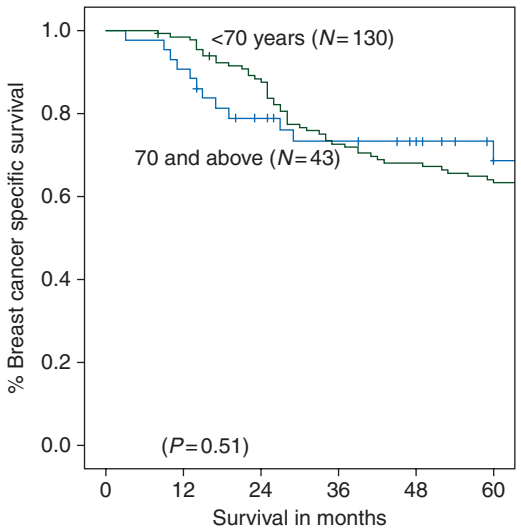

Figure 3. Breast cancer-specific survival of HER2-positive early operable primary breast cancer—young versus older women.

explanation is the low expression of Ki67 (a poor prognostic factor) and high expression of bcl2 (a good prognostic factor) seen in the older cohort.

HER2-positive tumours comprise ∼20% of all breast cancers regardless of age; however with advancing age, there appears to be a decline, as previously reported [14, 15]. In women over 70 years, the rate of HER2 positivity was reported at 5%–15% [3]. The rate of HER2 positivity in our cohort was consistent with that reported in the literature. The study by Durbecq et al. showed the same rate in older women as seen in our study. The study included 14.6% of older women and used IHC, and those with 2+ score were then confirmed on fluorescent ISH [14]. Other reported studies showed inconsistent methods such as using only IHC or difference in the definitions of HER2 positivity. In our study, there was an interesting pattern showing high HER2 positivity in a clearly pre-menopausal group (i.e. <40 years) followed by a decline when the majority reached menopause. This may suggest an association of HER2 status with menopausal status.

HER2 overexpressing tumours tend to have aggressive biological characteristics associated with high grade, ER negative expression and mutant p53 [15]. In addition, our study showed overall high basal cytokeratins and E-Cadherin and low Bcl2. Little is known about the biological associations of HER2 overexpressing tumours with cytokeratins, although the generally high expression of luminal cytokeratins in older women might have led to the less obvious distinction across the two age groups.

Regardless of age, HER2 positivity has been reported to have inferior survival outcome and increased risk of recurrences [16–18]. HER2 has shown a prognostic significance in older women similar to the pattern seen in younger patients, although it is interesting that there was no significant difference in the survival of younger and older patients with HER2 overexpressing tumours. No patients in either group received trastuzumab, because the data of the younger series were collected during 1980–1990s, when trastuzumab was not available, and older women did not receive it due to the scarcity of robust data showing its use in this age group.

There was a considerable proportion of patients in both groups who received adjuvant endocrine therapy, while adjuvant chemotherapy was only given to the younger patients. The role of adjuvant chemotherapy with or without trastuzumab has been reported in the literature, whereas that of endocrine therapy remains controversial [19]. On the other hand, endocrine therapy has been observed as a well-tolerated therapy in older women, where the role of chemotherapy remains in question. In the given scenario, the use of trastuzumab is not widely adopted and the treatment of older women with HER2-positive tumours remains a challenge. Therefore, it is strongly recommended that the role of trastuzumab as monotherapy as well as in combination with endocrine therapy in older women with early operable primary breast cancer should be investigated. Studies are ongoing to investiage the use of trastuzumab, for example 'SafeHer' [A Safety and Tolerability Study of Assisted- and Self-Administered Subcutaneous Herceptin (Trastuzumab) as Adjuvant Therapy in Patients With Early HER2-Positive

Breast Cancer], which would be of clinical relevance in older women.

While the current study is limited by its retrospective nature and potential bias in terms of treatment selection in older women (e.g., due to co-morbidites), it possesses strengths in having both biological information and long-term clinical follow-up data from a single centre. The key finding appears to suggest that at least some older women with HER2-positive tumours may be spared from chemotherapy, with no adverse impact on survival. This is partly in contrast to current belief and available literature, and should be further explored.

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disclosure

The authors have declared no conflict of interest.

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