Axillary lymph node status of operable breast cancers by combined steroid receptor and HER-2 status: triple positive tumours are more likely lymph node positive

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Axillary lymph node status of operable breast cancers by combined steroid receptor and HER-2 status: triple positive tumours are more likely lymph node positive

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Abstract *Aims* To examine the frequency of axillary lymph node (ALN) invasion of operable breast cancers by their combined oestrogen receptor (ER), progesterone receptor (PR) and HER-2 status. *Methods* 2227 recently operated cases in one centre were retrieved from the Multidisciplinary Breast Centre database and stratified according to their combined immunohistochemical (IHC) expression of ER/PR/HER-2 status. An equivocal HER-2 status was further analysed by Fluorescence in situ Hybridisation (FISH). The following 6 groups were considered: $ER^-PR^-HER\text{-}2^-$ (NNN; triple negative), $ER^-PR^-HER\text{-}2^+$ (NNP), $ER^+PR^-HER\text{-}2^-$ (PNN), $ER^+PR^-HER\text{-}2^+$ (PNP), $ER^+PR^+HER\text{-}2^-$ (PPN), $ER^+PR^{+-}HER\text{-}2^+$ (PPP; triple positive). For ALN, the following variables were tested in uni- and multivariate models: age at diagnosis (years), tumour size (mm), tumour grade, ER, PR, HER-2 and the combined steroid receptor and HER-2 status. Likelihood ratio χ^2-tests were used for univariate analysis and logistic regression for multivariate analysis. *Results* Triple positive tumours had a higher likelihood of being ALN positive than others (56.2% versus 35.7%; $P < 0.0001$). Univariate logistic regression also withheld age, size, grade and HER-2 as predictors of ALN involvement. Final multivariate logistic regression revealed age, size, grade and PPP versus non-PPP to be independent predictors of ALN involvement; the odds ratio (OR) and 95% CI for PPP versus non-PPP tumours was 2.169 (1.490–3.156). *Conclusion* Our data provide insight into the natural history of triple positive breast carcinomas. Such tumours are more likely ALN positive than those with another steroid receptor and HER-2 status. How these findings correlate with breast cancer prognosis remains to be investigated.

Keywords HER-2 · Steroid receptors · Lymph node · Breast cancer

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Introduction

In breast cancer, the three predictive markers ER, PR and HER-2 have an independent prognostic value [1]. HER-2 is over-expressed in about 15–20% of cases [2–4]. Its prognostic significance has been obscured by an association with other poor prognostic markers like tumour grade, S phase fraction and a negative steroid receptor status [3–6]. Furthermore, differences in HER-2 detection methods and cut-off values, the small numbers of patients in the $HER\text{-}2^+$ cohorts, confounding effects of treatment and short length of follow-up made the interpretation of HER-2 as a prognostic marker difficult. However, with short follow-up it became clear that HER-2 is prognostic for disease free and overall survival in node positive and with longer follow-up also in node negative breast cancers [6]. ER is expressed in 80–90% of all breast cancers [7]. The ER is prognostic but annual recurrence of breast cancer by ER status varies over time [1, 7, 8]. The PR is expressed in 70–80% of all breast cancers [7] and also PR is considered as a time-dependent prognostic factor in ER^+ breast cancers [1, 9]. Steroid and HER-2 receptors are strongly associated [11–16]. The joint expression of steroid receptors has a greater predictive value than the expression of each receptor on its own. Furthermore, the

joint IHC expression of ER, PR and HER-2 is prognostic as it reflects the prognostic value of breast cancer phenotypes defined by micro-array studies [17–20]. Breast cancers are therefore better presented by their combined receptor expression than by each receptor status alone.

The ALN status is one of the best independent prognostic factors for disease free and overall survival of breast cancer but some tumours are already systemic even if ALN are not involved [1, 21]. Women with an ER^-, ER^+PR^- or $HER\text{-}2^+$ breast cancer experience a shorter disease free period than women with an $ER^+PR^+HER\text{-}2^-$ breast cancer despite the fact that these tumours are not necessarily more likely ALN positive [22]. Rates of ALN involvement in the different prognostic breast cancer subgroups based on their combined IHC expression of steroid receptors and HER-2 status have not been systematically described. We examined the frequency of ALN involvement for these different prognostic breast cancer subtypes. Such differences may not necessarily reflect differences in breast cancer outcome as variables like treatment are important but may differentiate breast cancers on the base of their steroid receptor and HER status for lymphatic spread and local aggressiveness.

Materials and methods

Charts from 2227 cases with an operable breast cancer, treated since January 2000 at Leuven University Hospital, Belgium, were evaluated. All women had a classical ALN dissection, mostly level I–II. After June 2003, patients with a cT1 tumour had the sentinel lymph node procedure. A classical ALN dissection was only performed if the sentinel node was involved. Tumour grading was performed according to the Ellis and Elston grading system [23]. Lymph nodes were examined by H&E staining using 3 sections per node; sentinel lymph nodes and those from lobular breast cancers classified as negative on H&E were additionally stained with epithelial markers. Cases were examined by IHC for the expression of ER, PR and HER2 (NLC-ER-6F11, NCL-PR-312 and CB11 respectively, Novocastra Laboratories, Newcastle-on-Tyne, UK). Since 2005, highly sensitive rabbit monoclonal antibodies are used for the assessment of ER and PR expression (SP1 and SP2 respectively, Labvision Corporation, Fremont, CA, USA). IHC staining was performed according to standard procedures for clinical purposes. Briefly, 4 μm thick paraffin sections were cut. Heat-induced epitope retrieval was carried out in a calibrated water bath (95–99°C) and antibody complexes were visualized by EnVision+ (DakoCytomation, Glostrup, Denmark) and diaminobenzidine. For ER and PR, any nuclear staining of invasive tumor cells was considered as positive. HER-2 immunostaining was scored according to the guidelines for HercepTest® and an equivocal HER-2 status was further investigated by Fluorescence in situ Hybridisation or FISH (PathVision, Vysis, Downers Grove, IL, USA). All clinico-pathological data of the patient were entered in a breast cancer database.

The following factors predicting the axillary lymph node involvement were examined: patient's age at diagnosis ($\leq$ versus >50 years), maximal microscopic tumour size ($\leq$ versus >20 mm), tumour grade (grade 1 to 3), ER, PR and HER-2 status (negative versus positive). The receptor status variables were combined with NNN, NNP, PNN, PNP, PPN and PPP as possible values where PPP stands for triple positive or $ER^+PR^+HER\text{-}2^+$ breast cancers.

Likelihood ratio χ^2-tests were used for univariate analysis and logistic regression for multivariate analysis. We considered the following variables to predict lymph node involvement in all tumours: patient's age at diagnosis, largest microscopic tumour size, tumour grade, ER-status, PR-status, HER-2 status and the combined receptor status with 6 predefined possible values NNN, NNP, PNN, PNP, PPN and PPP. For the multivariate logistic regression model, we considered patient's age, tumour size, tumour grade, and the combined steroid and HER-2 receptor status as possible independent predictors. The presence of multicollinearity was investigated and the assumption of linearity in the logit was checked [24]. It was also checked whether any strong interaction would affect the possible independent effect of group. Finally, regression diagnostics were investigated as explained by Hosmer and Lemeshow [24]. One should always be careful with *P*-values, therefore more attention was paid to odds ratios and their confidence intervals [25]. In particular, in large datasets *p*-values can become very small while the effect is actually small and not highly significant in a clinical sense.

Results

Table 1 showed the clinicopathological features of all 2227 cases as well as univariate results. In univariate analysis a large tumour size (*OR* per cm increase in size = 1.580) and a high tumour grade (compared with grade 1; *OR* for grade 2 = 1.896 and for grade 3 = 2.900) were strongly related to positive ALN status. A positive HER-2 status was also related to a positive ALN status [35.8% $HER\text{-}2^-$ vs 45.7% $HER\text{-}2^+$; *OR* = 1.508] but this effect was mainly due to the relationship between triple positive tumours and positive ALN status [35.7% non-PPP vs 56.2% PPP; *OR* = 2.309]. ER and PR expression did not predict the ALN status. Finally, there appeared to be only a small negative effect of age on ALN status (*OR* per 10 year increase in age = 0.914).

Table 1 Descriptive statistics

Variable	Statistic	Lymph node status			OR (95% CI)	*P*
		Negative $n = 1404$, 63%	Positive $n = 823$, 37%	All		
Age (years)	Median (range)	58 (27–91)	55 (26–95)	57 (26–95)	Per 10 year increase: 0.914 (0.852–0.980)	0.0109
Size (mm)	Median (range)	19 (1–160)	30 (4–160)	20 (1–160)	Per 1 cm increase: 1.580 (1.489–1.676)	<0.0001
Grade						<0.0001
1	N (%)	249 (78.1)	70 (21.9)	319 (14.3)	Versus grade 1:	
2	N (%)	668 (65.2)	356 (34.8)	1024 (46.0)	1.896 (1.412–2.545)	
3	N (%)	487 (55.1)	397 (44.9)	884 (39.7)	2.900 (2.156–3.900)	
ER						0.5206
Negative	N (%)	182 (64.8)	99 (35.2)	281 (12.6)	Positive vs negative:	
Positive	N (%)	1222 (62.8)	724 (37.2)	1946 (87.4)	1.089 (0.839–1.415)	
PR						0.2148
Negative	N (%)	334 (65.4)	177 (34.6)	511 (22.9)	Positive vs negative:	
Positive	N (%)	1070 (62.4)	646 (37.6)	1716 (77.1)	1.139 (0.927–1.401)	
Her-2						0.0023
Negative	N (%)	1265 (64.2)	706 (35.8)	1971 (88.5)	Positive vs negative:	
Positive	N (%)	139 (54.3)	117 (45.7)	256 (11.5)	1.508 (1.160–1.961)	
Group						0.0002
PPP	N (%)	60 (43.8)	77 (56.2)	137 (6.2)	PPP vs group:	
PPN	N (%)	1010 (64.0)	568 (36.0)	1579 (70.9)	2.278 (1.600–3.236)	
PNP	N (%)	23 (74.2)	8 (25.8)	31 (1.4)	3.690 (1.541–8.850)	
PNN	N (%)	129 (64.8)	71 (35.2)	199 (8.9)	2.364 (1.515–3.690)	
NNP	N (%)	56 (63.6)	32 (36.4)	88 (4.0)	2.247 (1.295–3.891)	
NNN	N (%)	126 (65.3)	67 (34.7)	193 (8.7)	2.415 (1.541–3.788)	
Triple positive						<0.0001
No	N (%)	1344 (64.3)	746 (35.7)	2090 (93.8)	Yes vs No:	
Yes	N (%)	60 (43.8)	77 (56.2)	137 (6.2)	2.309 (1.631–3.279)	

P: *P*-value for the likelihood ratio χ^2-test

In multivariate logistic regression analysis, high tumour size (*OR* per cm increase in size = 1.540), high tumour grade (*OR* per grade increase = 1.384) and being PPP were predictive for a positive ALN status. Table 2 presents the final logistic regression analysis: PPP tumours were more strongly related to positive ALN status than any other combined phenotype. The OR ranged from 1.883 (PPP versus PNN) to 3.471 (PPP versus PNP). Age was also related to ALN status, albeit not in a straightforward way: age was related to negative ALN status for women up to 70 years while it was related to positive ALN for women older than 70 years. The Hosmer–Lemeshow test for goodness-of-fit gave a *P*-value of 0.2136. No interactions affected the effect of being PPP on ALN status.

Table 3 illustrated the proportion of grade 3 tumours, median tumour size and patient's age at diagnosis in different ER/PR/HER-2 phenotypes. Women with a triple positive breast cancer were more likely to be diagnosed at a younger age than tumours of any other phenotype. Tumours in this group were often high grade and larger. The difference between triple positive tumours on the one hand, and triple negative tumours and tumours overexpressing HER-2 with a negative PR-status ($ER^+PR^-HER\text{-}2^+$ and $ER^-PR^-HER\text{-}2^+$) on the other hand is less clear.

Finally, Table 4 represented the ALN status comparing PPP cases with non-PPP cases adjusting for tumour grade. When only considering grade 3 lesions, the sample proportion of ALN positive tumours was 43% higher for PPP cases than for non-PPP cases (relative risk = 61.4/42.8 = 1.43).

Discussion

We found that triple positive breast cancers—$ER^+PR^{+-}HER\text{-}2^+$—had an odds of ALN involvement of 130.9% higher than breast cancers of any other ERPRHER-2 phenotype (OR = 2.309). Triple positive breast tumours were diagnosed at a younger age and tumour characteristics like tumour size and grade could have been the main reason for this higher probability of ALN involvement [26–32]. However, the multivariate logistic regression analysis identified "being triple positive" as an independent predictor for lymph node involvement. Our observation is another proof of the heterogeneity of the natural history of breast carcinomas acquiring new insights in breast cancer biology and tumour cells dissemination.

HER-2 over-expression in operable breast cancer has only in 8 out of 23 studies been associated with a positive ALN-status [15, 33]. Reasons why a predictive role of HER-2 for ALN involvement has not been reported are that subgroup analysis by combined steroid receptor expression was not done, $ER^+PR^+HER\text{-}2^+$ tumours are rare (in this series only 6.2% of all cases) and HER-2 as a predictor for the ALN status is overlapping with other poor prognostic factors (high tumour grade, a large tumour size, younger age at diagnosis) already predicting ALN involvement [32]. Therefore, our results need being cross checked with other large series. Bartlett et al. recently also identified significant interactions between HER-2, ER-expression and lymph node involvement [34]. They found a different probability of HER-2 over-expression with lymph node involvement by ER-status in a very high risk group of early-stage breast cancers selected for adjuvant chemotherapy. Our confirmatory findings of this interaction in a non-selected patient group of consecutive cases is therefore interesting. Controversy also remains regarding the value of steroid receptor expression as a reliable predictor for the ALN status [32]. Some studies reported no value for both ER and PR [27, 30] whereas others pointed to a lower risk of ALN metastases for tumours negative for either receptor

Table 2 Results from the final logistic regression model

Variable	Level	Parameter estimate (SE)	OR (95% CI)	LR chi-square	Df	*P*
Age (≤70 years)		−0.0147 (0.0051)	0.864[a] (0.782–0.954)	8.29	1	0.0040
Age (>70 years)		0.0303 (0.0166)	1.353[a] (0.977–1.875)	3.28	1	0.0701
Size (mm)		0.0432 (0.0030)	1.540[b] (1.451–1.634)	281.52	1	<0.0001
Grade [1–3]		0.3247 (0.0802)	1.384[c] (1.182–1.619)	16.62	1	<0.0001
Group	PPP vs PPN	0.6429 (0.1977)	1.902 (1.290–2.801)	21.32	5	0.0007
	PPP vs PNP	1.2445 (0.4694)	3.471 (1.383–8.696)			
	PPP vs PNN	0.6328 (0.2465)	1.883 (1.161–3.049)			
	PPP vs NNP	0.9577 (0.3039)	2.606 (1.437–4.717)			
	PPP vs NNN	1.0108 (0.2447)	2.748 (1.701–4.444)			

SE, standard error; OR, odds ratio; CI, confidence interval; LR, likelihood ratio; Df, degrees of freedom; *P*, *P*-value

[a] Odds ratio is computed for each 10-year increase in age

[b] Odds ratio is computed for each centimeter increase in size

[c] Odds ratio is computed for each increase in grade

Table 3 Proportion being grade 3, median tumour size and median age by ER, PR and HER-2 status

Prognostic factor	[a]PPP 56.2% ALN$^+$ $N = 137$	[b]PPN 36.0% ALN$^+$ $N = 1579$	[c]PNN 35.2% ALN$^+$ $N = 199$	[d]NNN 34.7% ALN$^+$ $N = 193$	[e]PNP and NNP 33.6% ALN$^+$ $N = 119$
Grade 3, %	73.7%	27.4%	40.2%	90.2%	81.5%
Size, median (range)	25 (4–130)	20 (1–160)	20 (2–80)	24 (1–160)	23 (1–100)
Age, median (range)	51 (27–86)	57 (26–95)	62 (27–91)	55 (26–90)	57 (32–88)

ALN: Axillary lymph node status

($^+$): Positive

($^-$): Negative

[a] PPP: ER$^+$PR$^+$HER-2$^+$

[b] PPN: ER$^+$PR$^+$HER-2$^-$

[c] PNN: ER$^+$PR$^-$HER-2$^-$

[d] NNN: ER$^-$PR$^-$HER-2$^-$

[e] PNP and NNP: ER$^+$PR$^-$HER-2$^+$ and ER$^-$PR$^-$HER-2$^+$

Table 4 ALN-status by tumour grade comparing PPP with non-PPP lesions

Tumor grade	[a]PPP $N = 137$			[b]Non-PPP $N = 2090$		
	ALN$^+$	ALN$^-$	All	ALN$^+$	ALN$^-$	All
1	0 (0.0%)	3 (100%)	3	70 (22.2%)	246 (87.8%)	316
2	15 (45.5%)	18 (54.5%)	33	341 (34.4%)	650 (65.6%)	991
3	62 (61.4%)	39 (38.6%)	101	335 (42.8%)	448 (57.2%)	783

ALN: Axillary lymph node status

($^+$): Positive

($^-$): Negative

[a] PPP: ER$^+$PR$^+$HER-2$^+$

[b] Non-PPP: ER$^+$PR$^+$HER-2$^-$, ER$^+$PR$^-$HER-2$^-$, ER$^-$PR$^-$HER-2$^-$, ER$^+$PR$^-$HER-2$^+$, ER$^-$PR$^-$HER-2$^+$

[28] or for PR only [26, 29, 31]. Our findings are in line with these last reports. ALN invasion was least likely in PNP (25.8%) and most likely in PPP (56.2%) breast cancers referring to an interaction between PR and HER-2 for tumour cell migration and lymph node invasion.

It remains unclear whether the small subgroup of triple positive breast carcinomas might deserve a specific treatment if our observation of increased cell migration or motility is confirmed. Nonetheless, it is worth reporting that triple positive breast cancers are more likely lymph node positive. Our findings are probably also yet another reason to test HER-2 in a breast cancer population which by definition has a low risk to be HER-2$^+$ [35, 36]. The predictive role of HER-2 for endocrine agents, chemotherapeutic regimen and trastuzumab (Herceptin®) in ER$^+$ breast cancers has already been proven [36–38].

Why HER-2 over-expression favours axillary metastases more in ER$^+$PR$^+$ cases than in breast cancers with another joint ERPR status is also puzzling. Lange et al. suggested already in 1998 that progestins sensitize breast cancer cells for growth factor and cytokine signalling [39]. Furthermore, the role of plasminogen activator inhibitor (PAI-1), another predictor for ALN metastases, is limited to tumours expressing PR [40]. Also, a recent study performed on endometrial stromal cells has shown that epithelial growth factors and PR are needed for maximal PAI-I overexpression [41].

Furthermore, cross talk between ER and the growth-factor-signalling pathways suggests a growth advantage if HER-2 is expressed in ER$^+$ breast cancer cells [5, 13, 37, 42–49]. Saal et al. recently described PI3K/AKT signalling through *PIK3CA* mutations that correlate with hormone receptors, HER-2 and lymph node metastases. The mutation was present in 58% among ER$^+$HER-2$^+$ node positive cases but in less than 7% of ER$^-$HER-2$^-$ tumours [50]. *PIK3CA* mutations may explain enhanced invasion of breast cancer cells to lymph nodes. ER$^-$ tumours and especially the basal like phenotype might posses a distinct mechanism of metastatic spread [51].

Whether triple positive breast cancers have a worse outcome than other ER$^+$ breast cancers in the luminal B group may be suggested by our findings but absence of information about the different subgroup's outcome is a major weakness of this report. However, such data are

currently not mature enough to report and will also reflect the predictive value of different treatment modalities in different receptor subgroups. Another potential weakness of our report may be that we based the ALN status on results from a mixture of complete axillary node dissection and sentinel lymph node procedure. However, it has previously been shown that predictors for ALN status are independent of how the lymph node resection was performed although the metastatic detection rate in lymph nodes may be higher using the sentinel node procedure as more thorough histologic examination of the sentinel lymph node results in a higher number of metastases detected [31]. Another weakness may be that we did not consider other predictors for ALN status as tumour localisation within the breast, tumour vascularisation, lymphangiogenesis, protein and genetic markers for ALN involvement [32]. Another important information which is missing is whether triple positive breast cancers, apart from being more likely lymph node positive, also involved a higher number of lymph nodes as compared to breast cancers with a non-triple positive phenotype. Such information may strengthen our findings. A strength of our study is that cases were unselected and consecutively treated in one centre. Also, analyses for ER, PR and HER-2 were confirmed by one pathologist. Successful quality control and quality assurance programs were guaranteed in our laboratory according to requested guidelines. Another strength of our study is that we defined HER-2^+ on the base of membrane staining when they either had a DAKO score 3^+ or 2^+ with a positive FISH test.

Although our study does contrast substantially with gene micro-array studies showing that gene expression profiles of node positive and node negative tumours do not differ, the present study highlights the association between the combined steroid receptor expression, the HER-2 status and qualitative ALN involvement. HER-2 defined by a DAKO score 3^+ or by FISH for those with a DAKO score 2^+ is a predictor for ALN involvement in consecutive women with an ER^+PR^+ operable breast cancer. Whether other pathways are involved in breast cancer cell migration in conjunction with the steroid and HER-2 receptors through *PIK3CA* mutations or the PAI-I system is a working hypothesis.

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