Table 1 Clinicopathological factors of patients with SCCHN

Variables	Number		
Gender			
Male	135		
Female	70		
Age			
Range	36–85		
Mean	62		
Median	61		
Tumor site			
Larynx	51		
Oropharynx	71		
Hypopharynx	83		
TNM classification	Larynx	Oropharynx	Hypopharynx
pT[a]			
T1	5	7	10
T2	21	32	28
T3	17	21	23
T4	8	11	22
pN[a]			
N0	31	22	20
N1	7	12	20
N2	13	36	43
N3	0	1	0
pStage[a]			
I	3	2	6
II	12	10	8
III	18	15	16
IV	18	44	53
Post-operative therapy			
Yes		19	15 (RT[b]) 4 (CT[c])
No		186	
Alcohol consumption			
Yes		139	
No		55	
Unknown		11	
Smoking			
Yes		159	
No		38	
Unknown		8	
Multiple cancers			
Yes		69	
No		136	

[a] UICC classification 7th edition

[b] Radiotherapy

[c] Chemotherapy

Table 2 KRAS gene status and EGFR protein expression by immunohistochemistry

KRAS gene status		EGFR protein expression	
Wild cases (%)	Mutant cases (%)	Positive cases (%)	Negative cases (%)
183 (100 %)	0 (0 %)	200 (97.6 %)	5 (2.4 %)

(100 %) (Table 2). Wild-type *KRAS* was found in all patients examined (100 %). Two representative cases are shown in Fig. 1.

EGFR protein expression

EGFR positivity

All 205 patients underwent immunohistochemical analysis of EGFR protein expression. Negative controls did not show any EGFR staining. Five male patients (2.4 %) had EGFR-negative SCCHN, while 200 patients (97.6 %) had EGFR-positive tumors (Table 2). Two representative cases are shown in Fig. 1. All major clinicopathological characteristics were similar among different levels of positivity or intensity of EGFR expression. Although all EGFR-negative patients were male, no difference was noted for the age at diagnosis (mean age 65.5 years). Four EGFR-negative tumors were located in the hypopharynx and one was in the oropharynx.

Heterogeneity of EGFR expression and clinicopathological factors

There was marked intratumoral heterogeneity of EGFR positivity (%) and staining intensity. Staining pattern was qualified as 'diffuse' when intensity was alike in more than 99 % of carcinoma cells, and termed as 'mosaic' in the case with heterogeneous intensity of staining or positivity less than 99 % of carcinoma cells. As shown in Table 3, there was no significant association between EGFR positivity and intensity. We used Kaplan–Meier analysis to detect differences in the survival of patients with higher EGFR positivity or more intense EGFR staining versus those with normal or low EGFR expression. We found that increased positivity of EGFR protein expression was not associated with overall survival (OS) (Fig. 2a), although it was significantly associated with better DFS ($P = 0.0471$) (Fig. 2b). In contrast, there was no significant association between the intensity of EGFR expression and either OS or DFS (Fig. 2c, d).

Next, we explored the relationship between the positivity or intensity of EGFR protein expression and various clinicopathological factors. The relationship between the

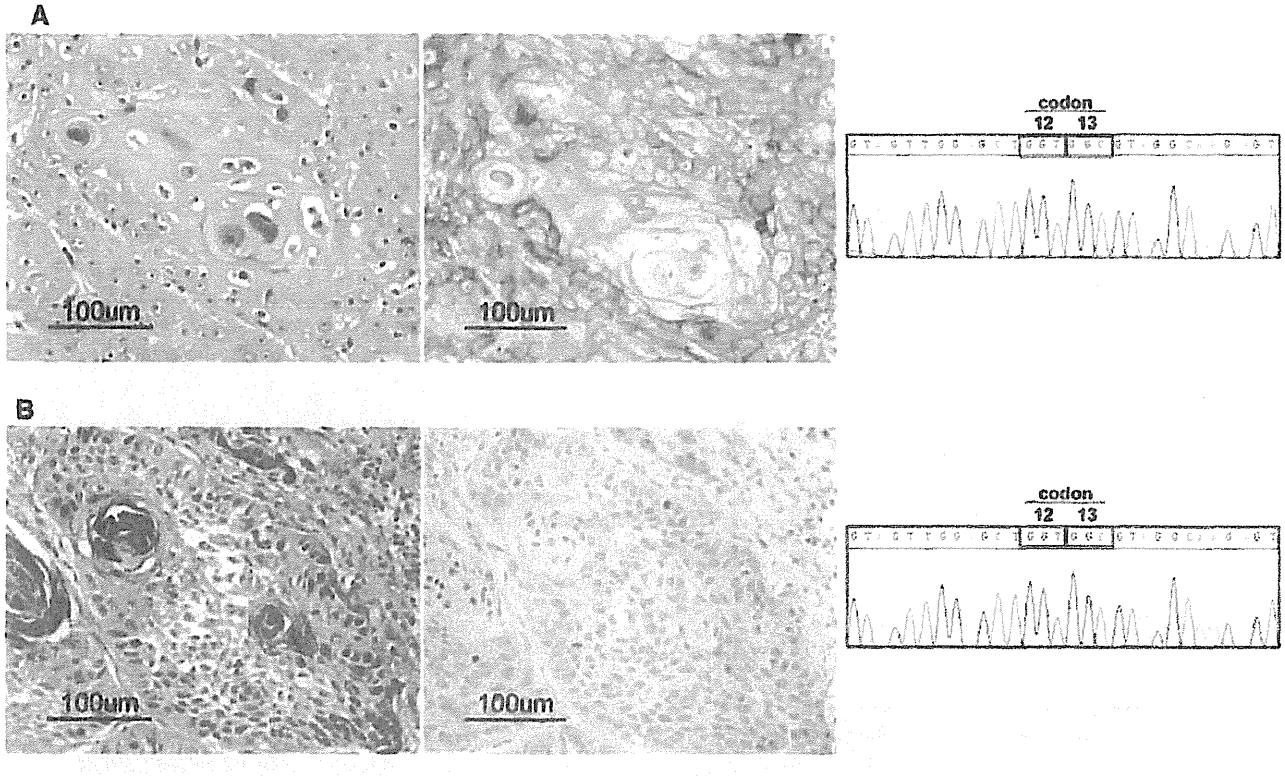

Fig. 1 Representative cases with and without EGFR expression. a Case no. 50 (55 years old, male, tumor site: larynx) shows EGFR expression and *KRAS* wild type. b The case no. 155 (69 years old, male, tumor site: hypopharynx) showed no EGFR expression and *KRAS* wild type

Table 3 Relationship between positivity and intensity of EGFR protein expression

Intensity	Positivity			
	0–10 % (grade 0)	11–50 % (grade 1)	51–100 % (grade 2)	Total
Negative	5	0	0	5
Weak	1	3	0	44
Moderate	14	22	5	41
Strong	16	77	62	155
Total	36	102	67	205

positivity of EGFR protein expression and the intensity of EGFR protein expression was shown in Table 3. However, there was no significant association among these two factors. Higher positivity and intensity of EGFR expression were significantly associated with well differentiated cancer ($P = 0.0003$ and 0.0007, respectively) (Fig. 3a, b). Laryngeal SCC is reported to have a better prognosis than oropharyngeal or hypopharyngeal SCC [25], as confirmed by the OS and DFS data in this study (Fig. 4a, b). However, we found no significant difference in the prevalence of well differentiated SCC between laryngeal, oropharyngeal, and hypopharyngeal cancer (Fig. 4c). There was no significant correlation between tumor differentiation and either OS or DFS for cancer of the hypopharynx and larynx, unlike cancer of the oropharynx (Supplementary Fig. 1). These findings suggested that EGFR positivity may predict DFS independently of tumor location.

EGFR expression and gender

We also examined whether gender was related to the positivity and intensity of EGFR protein expression. There was a weak correlation between gender and both EGFR positivity and intensity in the 205 patients ($P = 0.0630$ and 0.0699, respectively) (Fig. 5a, b). Male patients with hypopharyngeal SCC had significantly higher positivity for EGFR protein expression than female patients ($P = 0.0169$) (Fig. 5c), but there was no significant difference in the intensity of EGFR expression ($P = 0.0828$) (Fig. 5d).

EGFR expression and clinicopathological factors in hypopharyngeal SCC

As shown in Fig. 4a, patients with hypopharyngeal SCC had the worst prognosis among hypopharyngeal, oropharyngeal, and laryngeal cancer. When we examined the relationship between EGFR protein expression and various clinicopathological factors, patients with lower EGFR positivity had the worst prognosis (Fig. 6a, b), but there was no significant correlation between the positivity or intensity of EGFR

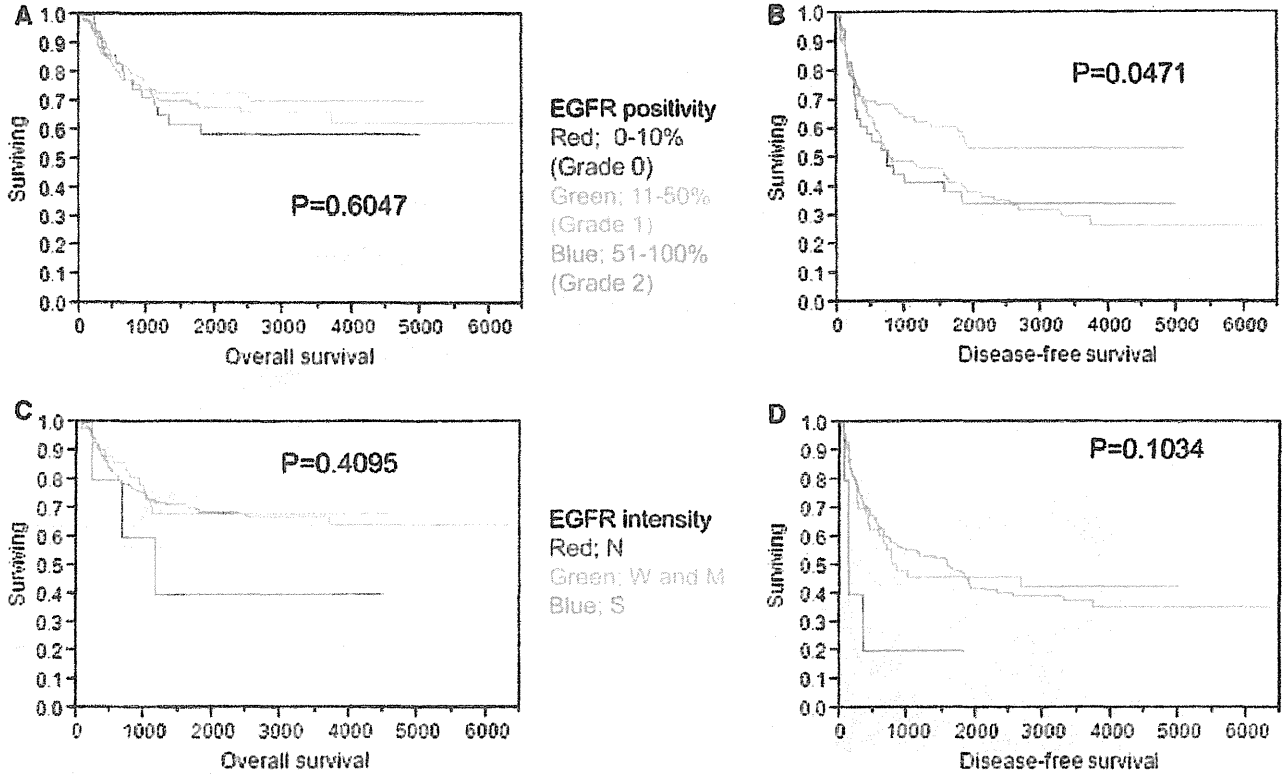

Fig. 2 Relationship between positive rate or intensity of EGFR protein expression and OS or DFS. Immunohistochemical analysis of SCCHNs using epitope-specific antibody for EGFR: **a** positive rate versus OS, **b** positive rate versus DFS, **c** intensity versus OS, **d** intensity versus DFS. *N* negative, *W* weak, *M* moderate, *S* strong

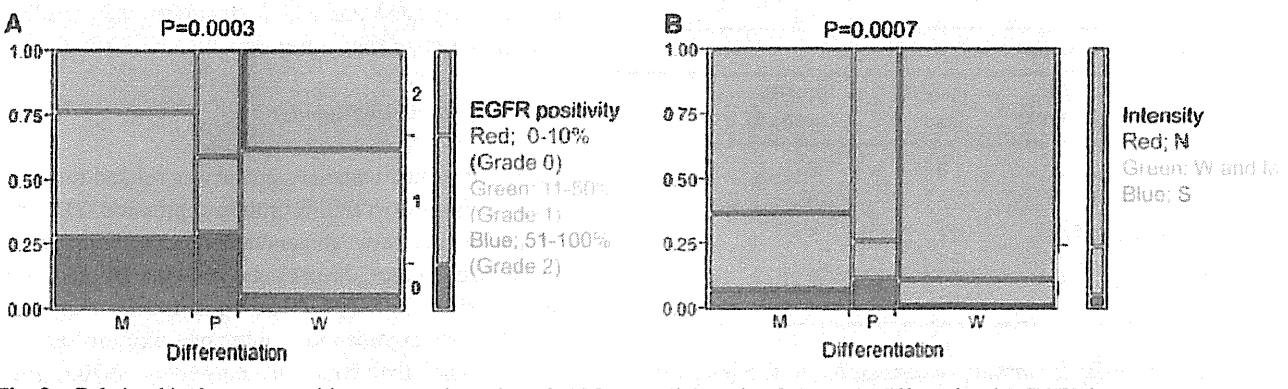

Fig. 3 Relationship between positive rate or intensity of EGFR protein expression and differentiation of SCCHNs. **a** EGFR positive rate versus tumor differentiation. *W* well differentiated, *M* moderately differentiated, *P* poorly differentiated. **b** EGFR intensity versus tumor differentiation. *N* negative, *W* weak, *M* moderate, *S* strong

expression and either OS or DFS (Fig. 6a–d). Multivariate analysis (EGFR positivity, EGFR intensity, gender, tumor, and nodal status) showed that lymph node metastasis was an independent predictor of OS and DFS (Tables 4, 5).

Discussion

The association between members of the EGFR expression or *KRAS* gene mutation-driven pathway and the clinical outcome has been extensively investigated mainly as a predictor of prognoses and sensitivity to cetuximab in CRC. However, the results obtained have been often controversial and not easily applicable in clinical practice. Data obtained for CRC have suggested the association between a worse prognosis of patients and abnormal expression of EGFR, but the findings have not shown reproducible results across different studies [26]. The omission into the analysis of other known factors with an established prognostic role, the small number of patients

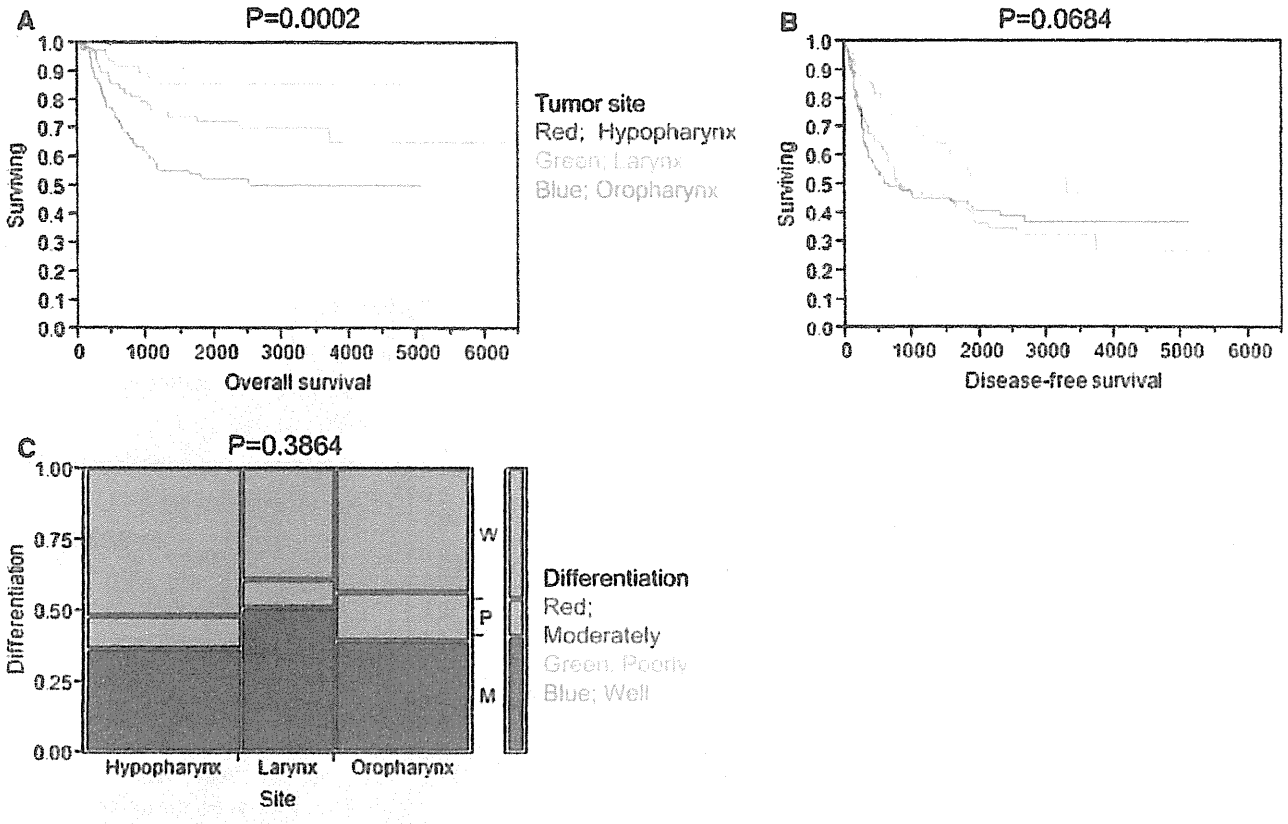

Fig. 4 Relationship between tumor site and OS or DFS (a, b), and relationship between tumor differentiation and sites (c)

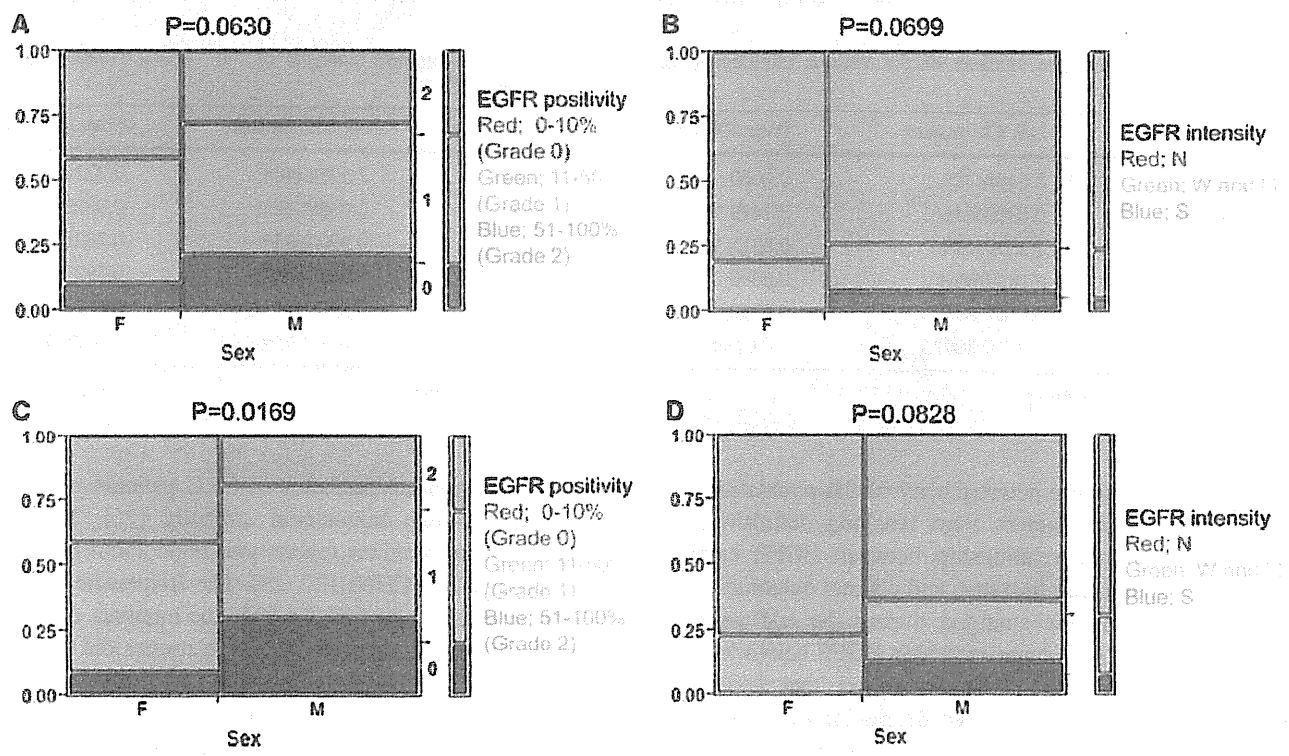

Fig. 5 Relationship between positive rate or intensity of EGFR protein expression and gender. a, b All cases c, d hypopharyngeal cases. *N* negative, *W* weak, *M* moderate, *S* strong

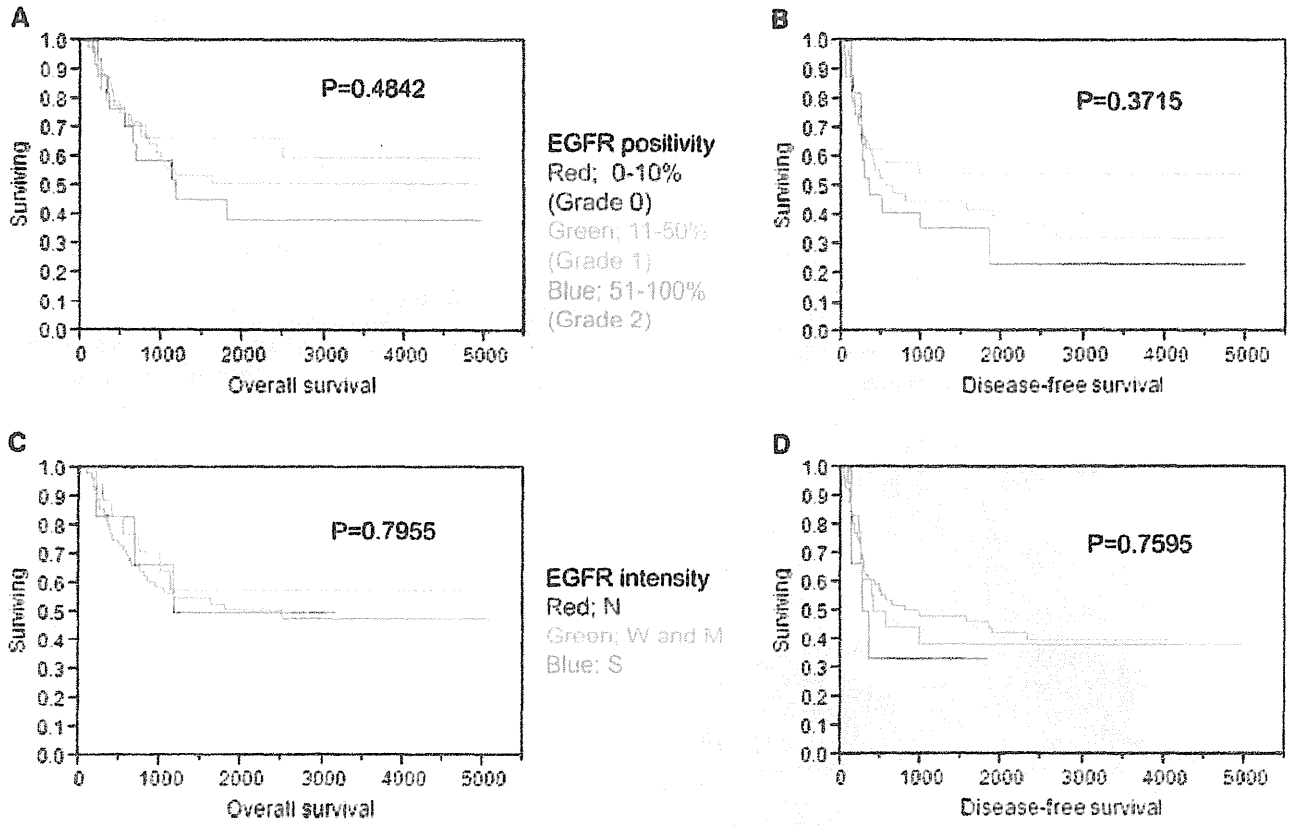

Fig. 6 Relationship between positive rate or intensity of EGFR protein expression and OS or DFS in hypopharyngeal cases. **a** Positive rate versus OS, **b** positive rate versus DFS, **c** intensity versus OS, **d** intensity versus DFS. *N* negative, *W* weak, *M* moderate, *S* strong

Table 4 Multivariate analysis of the various clinicopathological factors for OS

Source	L-R Chi square	Prob > Chi square
Positivity	2.64814728	0.2660
Intensity	0.58104816	0.7479
Diff	1.26395599	0.5315
Sex	0.18296970	0.6688
T	0.85633419	0.8360
N	8.54583035	0.0139*

Diff differentiation

* $P < 0.05$

Table 5 Multivariate analysis of the various clinicopathological factors for DFS

Source	L-R Chi square	Prob > Chi square
Positivity	2.59842484	0.2727
Intensity	0.03598025	0.9822
Diff	0.49056834	0.7825
Sex	0.06567847	0.7977
T	0.79049711	0.8517
N	14.0217666	0.0009*

Diff differentiation

* $P < 0.01$

examined, and the heterogeneity of the population studied prevented investigators from reaching definitive conclusions about the relationship between EGFR expression, *KRAS* gene mutation status, and clinical outcome. A strong scientific rationale emerged during the last years for a position of *KRAS* mutation within EGFR networks in CRC. Preclinical findings have suggested that blocking the EGFR-activated downstream signaling network in presence of *KRAS* gene mutation may result in an ineffective therapeutic outcome. This scientific rationale has been postulated for CRC. *KRAS* gene status has largely been known to be an appropriate predictor of CRC patients treated with EGFR-targeting monoclonal antibody [26]. There have been several previous reports published about KRAS gene mutation of SCCHNs [27, 28]. The frequencies of KRAS gene mutation was 6 and 4.5 %, the numbers of SCCHNs were 89 and 22, respectively. Both studies did not include Japanese SCCHNs, the tumor site was limited to tonsil in the assay of Van Damme et al. [28]. In Japanese cohort study, 2 % of SCCHNs showed KRAS gene mutation (codon 12) [29]. However, the number of case was 102 and the sites of SCCHNs were unknown in detail [29]. Thus,

data about the prevalance of *KRAS* mutation has not been published in a large number of SCCHN patients, there has been no report about the data in more than 200 of Japanese SCCHNs like our study. For the ethnic difference, further studies using a huge number of cases are necessary. In present study, we found that all 205 SCCHNs had *KRAS* wild type and may be identified as candidates for responders to EGFR-targeting monoclonal antibody.

The value of EGFR expression for predicting the response to EGFR-targeting monoclonal antibody such as cetuximab has remained unclear. A recent study showed that EGFR expression [fluorescence in situ hybridization (FISH) and immunohistochemistry] was useful for determining resistance to anti-EGFR therapies [30]. The author stated that FISH and CISH *EGFR* gene copy number may both represent effective tools for a further patients' selection in *KRAS* wild-type CRC treated with cetuximab [22]. EGFR amplification detected by FISH and EGFR protein expression detected by immunohistochemistry have also been observed in SCCHN [31]. However, their relationship with clinical response to treatment with anti-EGFR monoclonal anti-bodies has not been examined for Japanese SCCHN patients. EGFR protein expression by immunohistochemistry was heterogeneous in almost all of the SCCHNs investigated in the current study. It remains unclear if all SCCHNs have an aberrant stimulation of EGFR pathway deriving from heterogeneous expression of EGFR. A study published has suggested that there is no association between the EGFR expression and clinical response to EGFR inhibitor in SCCHN [32]. Further analyses are necessary to find a key regulator for the responder or non-responder of EGFR targeted therapy.

It has also been remained unclear whether heterogeneous EGFR expression observed in SCCHNs represents a stronger determinant for the biological behavior of the tumor. There have been no reports about the prognostic significance of heterogeneous EGFR expression detected by immunohistochemistry in a large series of SCCHNs, except for the present study involving tumors in the larynx, oropharynx and hypopharynx. In the two previous studies on the relationship between EGFR expression and the prognosis of SCCHN [22, 28], only 65 and 71 patients were examined, respectively. Also SCC of oral cavity and metastatic SCC were included and the small number of SCC of the larynx, oropharynx and hypopharynx were examined in these studies. One of the two reports indicated that EGFR expression was not significantly related to DFS or OS [22], while the other study showed that EGFR protein expression with high intensity was associated with a significantly worse survival, although interestingly increased expression of phospho-EGFR protein (activated EGFR expression) showed a tendency for better survival compared with patients with normal or decreased EGFR activation [28]. It has also been reported that the OS and DFS rates of patients with high EGFR-expressing SCCHNs were highly significant lower and local–regional relapse rate was highly significantly higher compared with those of patients with low EGFR-expressing HNSCCs in the most of cases with stage III or IV [33]. However, none of these reports examined the relationship between the percentages of positive area of EGFR protein expression which reflects heterogeneity of tumor, and OS or DFS such as performed in the present study. We found that a higher positive rate of EGFR protein expression was significantly associated with better DFS ($P = 0.0471$), and more intense EGFR protein expression tended to be associated with better DFS ($P = 0.1034$) (Fig. 2b, d). Both higher positive rate and stronger intensity of EGFR protein expression were significantly associated with well differentiated SCC ($P = 0.0003$ and $P = 0.0007$, respectively) (Fig. 3a, b). These results suggest that well differentiated SCCHN with higher positive rate and stronger intensity EGFR protein expression may be a good target population for anti-EGFR antibody therapies. However, further studies are necessary to investigate the efficacy of EGFR targeted therapy for these patients. On the other hand, the patients with lower positive rate of EGFR protein expression showed a significantly poorer DFS ($P = 0.0471$) and the cases with weaker intensity of EGFR protein expression showed a tendency for poorer DFS ($P = 0.1034$) (Fig. 2b, d). Both lower positive rate and weaker intensity of EGFR protein expression were associated with a significantly poorly differentiated SCC ($P = 0.0003$ and $P = 0.0007$, respectively) (Fig. 3a, b). However, these findings should be considered with caution and further studies including novel anti-cancer therapies to confirm them are needed in the future.

This study included 70 women with SCCHN. Interestingly, females with SCCHN tended to higher positive rate or stronger intensity of EGFR protein expression compared with those of males ($P = 0.0630$ and 0.0699, respectively) (Fig. 5a, b).

In conclusion, this is the first investigation of *KRAS* gene mutation and EGFR protein expression in more than 200 Japanese patients with SCCHN. No *KRAS* gene mutation was detected and only five of the 205 patients (2.4 %) were negativity for EGFR protein expression. This investigation is necessary and primary work before treatment with anti-EGFR antibody therapies to SCCHN in Japan. On the basis of our findings, most Japanese patients with SCCHN will be a good target for anti-EGFR antibody therapies such as cetuximab.

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References

1. Ferlay J, Shin HR, Bray F et al (2010) Estimates of worldwide burden of cancer in 2008. GLOBOCAN 2008. Int J Cancer 127:2893–2917
2. Diaz Jr EM, Sturges EM, Laramore GE (2003) Neoplasms of the head and neck. Holland JF, Frei E (eds) Cancer Medicine. BC Decker Inc., London, pp 1325–1371
3. Ferlay J, Parkin DM, Steliarova-Foucher E (2010) Estimates of cancer incidence and mortality in Europe in 2008. Eur J Cancer 46:765–781
4. Matsuda T, Marugame T, Kamo KI et al (2011) The Japan Cancer Surveillance Research Group. Cancer incidence and incidence rates in Japan in 2005: Based on data from 12 population-based cancer registries in the Monitoring of Cancer Incidence in Japan (MCIJ) Project. Jpn J Clin Oncol 41:139–147
5. The Research Group for Population-based Cancer Registration in Japan (2004) Cancer incidence and incidence rates in Japan in 1999: estimates based on data from 11 population-based cancer registries. Jpn J Clin Oncol 34(6):352
6. Ohshima A et al (2004) Cancer statistics morbidity/death/prognosis 2004. Shinohara Shuppan Shinsha, Tokyo
7. Iro H, Waldfahrer F (1998) Evaluation of the newly updated TNM classification of head and neck carcinoma with data from 3247 patients. Cancer 83:2201–2207
8. Vokes E, Weichselbaum R, Lippman S et al (1993) Head and neck cancer. New Engl J Med 328:184–194
9. Argiris A, Bruce E (2004) Competing causes of death and second primary tumors in patients with locoregionally advanced head and neck cancer treated with chemotherapy. Clin Cancer Res 10:1956–1962
10. Garrett CR, Eng C (2011) Cetuximab in the treatment of patients with colorectal cancer. Expert Opin Biol Ther 11(7):937–949
11. Jonker DJ, O'Callaghan CJ, Karapetis CS et al (2007) Cetuximab for the treatment of colorectal cancer. N Engl J Med 357(20): 2040–2048
12. Karapetis CS, Khambata-Ford S, Jonker DJ et al (2008) K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med 359(17):1757–1765
13. Carpenter G (1983) The biochemistry and physiology of the receptor-kinase for epidermal growth factor. Mol Cell Endocrinol 31:1–19
14. Salomon DS, Brandt R, Ciardiello F et al (1995) Epidermal growth factor-related peptides and their receptors in human malignancies. Crit Rev Oncol Hematol 19:183–232
15. Mendelsohn J, Baselga J (2003) Status of epidermal growth factor receptor antagonists in the biology and treatment of cancer. J Clin Oncol 21:2787–2799
16. Mendelsohn J, Baselga J (2006) Epidermal growth factor receptor targeting in cancer. Semin Oncol 33(4):369–385
17. Lockhart AC, Berlin JD (2005) The epidermal growth factor receptor as a target for colorectal cancer therapy. Semin Oncol 32(1):52–60
18. Steele RJ, Kelly P, Ellul B et al (1990) Epidermal growth factor receptor expression in colorectal cancer. Br J Surg 77(12):1352–1354
19. Kluftinger AM, Robinson BW, Quenville NF et al (1992) Correlation of epidermal growth factor receptor and c-erbB2 oncogene product to known prognostic indicators of colorectal cancer. Surg Oncol 1(1):97–105
20. Chung CH, Ely K, McGavran L et al (2006) Increased epidermal growth factor receptor gene copy number is associated with poor prognosis in head and neck squamous cell carcinomas. J Clin Oncol 24:4170–4176
21. Cunningham D, Humblet Y, Siena S et al (2004) Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 351(4): 337–345
22. Sheikh Ali MA, Gunduz M, Nagatsuka H et al (2008) Expression and mutation analysis of epidermal growth factor receptor in head and neck squamous cell carcinoma. Cancer Sci 99(8):1589–1594
23. Karapetis CS, Khambata-Ford S, Jonker DJ et al (2004) K-ras mutations and benefit from cetuximab in advanced colorectal cancer. J Histochem Cytochem 52(7):893–901
24. Atkins D, Reiffen KA, Tegtmeier CL et al (2008) Immunohistochemical detection of EGFR in paraffin-embedded tumor tissues: variation in staining intensity due to choice of fixative and storage time of tissue sections. J Histochem Cytochem 52(7):893–901
25. Japanese Society of Medical Oncology (2009) Clinical oncology update-essentials for medical oncologists, 2nd edn. Nankodo, Tokyo
26. Dahabreh IJ, Terasawa T, Castaldi PJ et al (2011) Systematic review: anti-epidermal growth factor receptor treatment effect. Ann Intern Med 154(1):37–49
27. Weber A, Langhanki L, Sommerer F et al (2003) Mutations of the *BRAF* gene in squamous cell carcinoma of the head and neck. Oncogene 22(30):4757–4759
28. Van Damme N, Deron P, Van Roy N et al (2010) Epidermal growth factor receptor and K-RAS status in two cohorts of squamous cell carcinomas 10:189
29. Hama T, Kato T (2010) Molecular targeted therapy for EGFR and VEGF in head and neck squamous cell carcinomas. Toukeibu-Gan 36(4):436–441
30. Scartozzi M, Bearzi I, Mandolesi A et al (2009) Epidermal growth factor receptor (EGFR) gene copy number (GCN) correlates with clinical activity of irinotecan-cetuximab in K-RAS wild-type colorectal cancer: a fluorescence in situ (FISH) and chromogenic in situ hybridization (CISH) analysis. BMC Cancer 9:303
31. Szabó B, Nelhűbel GA, Kárpáti A et al (2011) Clinical significance of genetic alterations and expression of epidermal growth factor receptor (EGFR) in head and neck squamous cell carcinomas. Oral Oncol 47(6):487–496
32. Sharafinski ME, Ferris RL, Ferrone S et al (2010) Epidermal growth factor receptor targeted therapy of squamous cell carcinoma of the head and neck. Head Neck 32(10):1412–1421
33. Ang KK, Berkey BA, Tu X et al (2002) Impact of epidermal growth factor receptor expression on survival and pattern of relapse in patients with advanced head and neck carcinoma. Cancer Res 62(24):7350–7356

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CLINICAL TRIAL

International multicenter tool to predict the risk of four or more tumor-positive axillary lymph nodes in breast cancer patients with sentinel node macrometastases

Tuomo J. Meretoja · R. A. Audisio · P. S. Heikkilä · R. Bori · I. Sejben · P. Regitnig · G. Luschin-Ebengreuth · J. Zgajnar · A. Perhavec · B. Gazic · G. Lázár · T. Takács · B. Kővári · Z. A. Saidan · R. M. Nadeem · I. Castellano · A. Sapino · S. Bianchi · V. Vezzosi · E. Barranger · R. Lousquy · R. Arisio · M. P. Foschini · S. Imoto · H. Kamma · T. F. Tvedskov · M.-B. Jensen · G. Cserni · M. H. K. Leidenius

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Abstract Recently, many centers have omitted routine axillary lymph node dissection (ALND) after metastatic sentinel node biopsy in breast cancer due to a growing body of literature. However, existing guidelines of adjuvant treatment planning are strongly based on axillary nodal stage. In this study, we aim to develop a novel international multicenter predictive tool to estimate a patient-specific risk of having four or more tumor-positive axillary lymph nodes (ALN) in patients with macrometastatic sentinel node(s) (SN). A series of 675 patients with macrometastatic SN and completion ALND from five European centers were analyzed by logistic regression analysis. A multivariate predictive model was created and validated internally by 367 additional patients and then externally by 760 additional patients from eight different centers. All statistical tests were two-sided. Prevalence of four or more tumor-positive ALN in each center's series ($P = 0.010$), number of metastatic SNs ($P < 0.0001$), number of negative SNs ($P = 0.003$), histological size of the primary tumor ($P = 0.020$), and extra-capsular extension of

T. J. Meretoja (✉) · M. H. K. Leidenius
Breast Surgery Unit, Helsinki University Central Hospital, P.O. Box 140, 00029 HUS Helsinki, Finland
e-mail: tuomo.meretoja@fimnet.fi

R. A. Audisio
Department of Surgery, St Helens Teaching Hospital, St Helens, UK

P. S. Heikkilä
Department of Pathology, Helsinki University Central Hospital, Helsinki, Finland

R. Bori · I. Sejben · G. Cserni
Department of Pathology, Bács-Kiskun County Teaching Hospital, Kecskemet, Hungary

P. Regitnig
Department of Pathology, Medical University of Graz, Graz, Austria

G. Luschin-Ebengreuth
Department of Obstetrics and Gynecology, Medical University of Graz, Graz, Austria

J. Zgajnar · A. Perhavec
Department of Surgical Oncology, Institute of Oncology, Ljubljana , Slovenia

B. Gazic
Department of Pathology, Institute of Oncology, Ljubljana, Slovenia

G. Lázár · T. Takács
Department of Surgery, University of Szeged, Szeged, Hungary

B. Kővári · G. Cserni
Department of Pathology, University of Szeged, Szeged, Hungary

Z. A. Saidan · R. M. Nadeem
Department of Breast Surgery, Lancashire Teaching Hospitals, Chorley, UK

I. Castellano · A. Sapino
Breast Unit Azienda Ospedaliera Città della Salute e della Scienza of Torino, Department of Medical Sciences, University of Torino, Turin, Italy

S. Bianchi · V. Vezzosi
Section of Pathological Anatomy, Department of Medical and Surgical Critical Care, University of Florence, Florence, Italy

E. Barranger · R. Lousquy
Department of Gynecology and Obstetrics, Lariboisiere Hospital, Paris , France

SN metastasis ($P < 0.0001$) were included in the predictive model. The model's area under the receiver operating characteristics curve was 0.766 in the internal validation and 0.774 in external validation. Our novel international multicenter-based predictive tool reliably estimates the risk of four or more axillary metastases after identifying macrometastatic SN(s) in breast cancer. Our tool performs well in internal and external validation, but needs to be further validated in each center before application to clinical use.

Keywords Breast cancer · Sentinel node biopsy · Axillary lymph node dissection · Tumor staging

Introduction

Completion axillary lymph node dissection (ALND) has been the standard treatment in the management of breast cancer patients with a tumor-positive sentinel node (SN). Completion ALND reveals the number of metastatic as well as tumor-free axillary lymph nodes (ALNs) and thus provides prognostic information beyond the SN status [1, 2]. However, patients receiving ALND are exposed to an increased morbidity when compared with sole SN biopsy (SNB). [3, 4]

During recent years, many centers have modified their axillary treatment protocols due to a growing body of literature indicating that completion ALND after the identification of a metastatic SN might neither provide survival benefit nor reduce axillary recurrence rates [5–8]. Although the debate generated by the ACOSOG Z0011 trial is still ongoing, many centers have opted for the omission of ALND after the detection of metastatic SNs [9]. Nonetheless, omitting completion ALND after positive SNB leads to less accurate axillary staging, as it remains unknown whether and how many additional ALN metastases remain in the axilla.

The existing guidelines of adjuvant treatment planning of breast cancer are largely based on axillary nodal stage as the main prognostic factor. The American Society of Clinical Oncology (ASCO) and the European Society for Medical Oncology (ESMO) both recommend postmastectomy radiotherapy (PMRT) in the presence of four or more metastatic ALNs (pN2 disease) [10, 11]. This has also implications on the choice of immediate breast reconstruction, since several surgeons and radiation oncologists do not recommend immediate reconstruction if PMRT is likely to be required. Furthermore, the ESMO guidelines recommend the inclusion of supraclavicular lymph nodes within the target radiotherapy field in the presence of pN2 disease, after mastectomy as well as breast-conserving surgery.

Therefore, the knowledge of both the total axillary tumor burden and the extent of residual axillary disease are important in case of one or more metastatic SN. To our knowledge, only three centers have developed predictive tools to estimate the risk of having four or more metastatic ALNs in the constellation of a tumor-positive SN. These were developed from relatively small series and were scarcely validated. Furthermore, these models included patients with micrometastatic disease in their SNs although these patients very rarely had four or more tumor-positive ALNs [12–15].

In this study, we aim to develop a novel international multicenter predictive tool to estimate a patient-specific risk of having four or more tumor-positive ALN in patients with macrometastatic SN. We thus aim to examine whether it is possible to recognize patients with macrometastatic SN(s) who are at risk of a substantial axillary tumor burden and who could therefore benefit from completion ALND. We further aim to validate the predictive tool both internally and externally.

Patients and methods

Original patient series

Retrospective data was collected in five European centers, each on 200 consecutive women with invasive breast cancer and one or more tumor-positive SNs and a completion ALND. Altogether a series of 1,000 patients was surgically treated between January 2004 and January 2011. Patients who received neoadjuvant treatment or previous axillary surgery were excluded. This data was originally collected in order to assess how the differences in SNB procedures and

R. Arisio
Department of Pathology, O.I.R.M.-Sant'Anna Hospital, Turin, Italy

M. P. Foschini
Section of Anatomic Pathology at Bellaria Hospital, Department of Biomedical Sciences and Neuromotory Disorders, University of Bologna, Bologna, Italy

S. Imoto
Department of Breast Surgery, Kyorin University School of Medicine, Tokyo, Japan

H. Kamma
Department of Pathology, Kyorin University School of Medicine, Tokyo, Japan

T. F. Tvedskov
Department of Breast Surgery, Copenhagen University Hospital, Copenhagen, Denmark

M.-B. Jensen
Danish Breast Cancer Cooperative Group, Copenhagen University Hospital, Copenhagen, Denmark

pathology practices would impact the performance of existing predictive models for nonsentinel node involvement [16, 17].

Twelve patients had four tumor-positive SNs and were excluded from analysis. Patients with isolated tumor cells (ITC) or micrometastasis as the largest tumor-positive SN finding [18] were also excluded from the original 1,000 patient as their probability of having four or more tumor-positive ALNs was very low. 68 patients had ITC as their largest SN finding and only one (1.5 %) of them had four tumor-positive ALNs. Similarly 245 patients had micrometastatic SN finding and only seven (2.9 %) of them had four or more tumor-positive ALNs. The remaining 675 patients with macrometastases in their SNs were included in the analysis.

The centers who contributed to the collection of the original series are: Bács-Kiskun County Teaching Hospital, Kecskemét, Hungary; Helsinki University Central Hospital, Finland; Medical University of Graz, Austria; Institute of Oncology, Ljubljana, Slovenia and University of Szeged, Hungary.

The data gathered was based on known risk factors for additional axillary metastases in patients with tumor-positive SNs [19–33]. The collected data included both primary tumor and SN-specific variables (Table 1). The detection method of the SN metastases was categorized as: intraoperative (frozen section/imprint cytology), paraffin standard staining, serial sectioning or immunohistochemistry. Patient, tumor and lymph node characteristics of the original patient series are given in Table 1.

Breast and axillary surgery as well as pathological work-up of the primary tumors and the axillary specimen were conducted according to each center's own protocols [16].

Internal validation patients

Internal validation series included additional series of consecutive patients treated at the same centers with similar inclusion and exclusion criteria. Consequently, the surgical technique and the methods of pathological assessment were the same as in the original series. The internal validation series included 367 additional patients with macrometastatic SNs operated between 2003 and 2011.

External validation patients

The external validation was performed on consecutive patients from eight European centers plus one unit from Japan. Each center in the external validation provided consecutive series with similar inclusion and exclusion criteria to the original series. The performance of the predictive tool was examined separately for each external validation center in addition to pooled data; the number of patients included from each center was hence unrestricted. A total of 760 patients with macrometastatic SNs were collected to form the series for external validation. These patients received surgery between 2003 and 2011.

The centers contributing to the external validation were: Lariboisiere Hospital, Paris, France; Lancashire Teaching Hospitals, Chorley, UK; AziendaOspedalieraCittàdella Salute e dellaScienza of Torino, Italy; Careggi Hospital and University of Florence, Florence, Italy; Sant'Anna Hospital, Turin, Italy; Bellaria Hospital, University of Bologna, Bologna, Italy; Kyorin University Hospital, Tokyo, Japan; Copenhagen University Hospital, Copenhagen, Denmark.

Statistical analyses

The individual risk factors for four or more tumor-positive ALNs associated with a macrometastatic SN were examined by univariate analysis of the original series. Distribution of continuous variables was analyzed with the Mann–Whitney *U*-test and the Chi-squared test was used for categorical variables.

Variables with a P value of less than 0.05 in the univariate analysis were included into a binary logistic regression analysis using a backward stepwise likelihood ratio method. Variables with a P value of less than 0.05 were included in the final predictive model.

The subsequent multivariate predictive model was then validated first internally and then externally by the independent patient series. Discrimination of the model was evaluated by area under the receiver operating characteristic curve (AUC) and the calibration of the model by Hosmer–Lemeshow goodness-of-fit test. A cutoff value of less than 20 % risk of four or more metastatic ALNs was considered as low-risk. Sensitivity and specificity were determined for various risk estimates. All statistical tests were two-sided.

IBM® SPSS® Statistics Version 20 (SPSS Inc., Chicago, IL) software was used to conduct the statistical analyses.

Ethical considerations

The patients' treatment was not influenced by our study as the data was collected retrospectively and anonymously. Institutional review boards and ethical committees were consulted as required in each center with no ethical objections raised.

Results

130 (19.1 %) of the 675 patients had four or more tumor-positive ALNs after SNB and completion ALND. The proportion of patients with four or more tumor-positive ALNs varied between centers (from 11.4 to 25.0 %).

Table 1 Patient, tumor and lymph node characteristics in the original patient series of 675 patients

	Center A (Bács-Kiskun) $n = 135$	Center B (Helsinki) $n = 113$	Center C (Graz) $n = 124$	Center D (Ljubljana) $n = 137$	Center E (Szeged) $n = 166$
Patient age (years)					
Mean	58	58	58	57	56
Standard deviation	13	12	13	10	11
Histological size of the primary tumor (mm)					
Mean	20	21	18	22	22
Standard deviation	14	18	9	11	11
Multifocal primary tumor	43 (31.9 %)	30 (26.5 %)	14 (11.3 %)	38 (27.7 %)	18 (10.8 %)
Lymphovascular invasion in the primary tumor	56 (41.5 %)	34 (30.1 %)	35 (28.2 %)	57 (41.6 %)	46 (27.7 %)
Estrogen receptor positive	121 (89.6 %)	107 (94.7 %)	98 (79.0 %)	122 (89.1 %)	124 (74.7 %)
Progesterone receptor positive	108 (80.0 %)	85 (75.2 %)	92 (74.2 %)	107 (78.1 %)	120 (72.3 %)
HER-2 positive	13 (9.6 %)	10 (8.8 %)	15 (12.1 %)	11 (8.0 %)	29 (17.5 %)
Nuclear grade of the primary tumor					
I	10 (7.4 %)	9 (8.0 %)	25 (20.2 %)	4 (2.9 %)	10 (6.0 %)
II	52 (38.5 %)	66 (58.4 %)	50 (40.3 %)	84 (61.3 %)	64 (38.6 %)
III	73 (54.1 %)	38 (33.6 %)	49 (39.5 %)	49 (35.8 %)	92 (55.4 %)
Histological grade of the primary tumor					
I	34 (25.2 %)	25 (22.1 %)	14 (11.3 %)	18 (13.1 %)	18 (10.8 %)
II	55 (40.7 %)	56 (49.6 %)	46 (37.1 %)	73 (53.3 %)	83 (50.0 %)
III	46 (34.1 %)	32 (28.3 %)	64 (51.6 %)	46 (33.6 %)	65 (39.2 %)
Histology of the primary tumor					
Ductal	98 (72.6 %)	82 (72.6 %)	100 (80.6 %)	111 (81.0 %)	140 (84.3 %)
Lobular	12 (8.9 %)	22 (19.5 %)	6 (4.8 %)	17 (12.4 %)	12 (7.2 %)
Mixed	8 (5.9 %)	4 (3.5 %)	14 (11.3 %)	7 (5.1 %)	3 (1.8 %)
Other	17 (12.6 %)	5 (4.4 %)	4 (3.2 %)	2 (1.5 %)	11 (6.6 %)
Detection method of the sentinel node metastasis					
Intraoperative analysis	88 (65.2 %)	113 (100 %)	95 (76.6 %)	51 (37.2 %)	Not done
Paraffin standard staining	45 (33.3 %)	0	2 (1.6 %)	49 (35.8 %)	166 (100 %)
Paraffin immunohistochemistry	2 (1.5 %)	0	4 (3.2 %)	28 (20.4 %)	0
Serial sectioning	0	Not done	23 (18.5 %)	9 (6.6 %)	Not done
ECE of sentinel node metastasis present	77 (57.0 %)	52 (46.0 %)	36 (29.0 %)	46 (33.6 %)	34 (20.5 %)
Sentinel nodes harvested					
Mean	1.8	2.5	1.7	1.8	1.9
Standard deviation	0.9	1.4	1.0	0.9	1.0
Nonsentinel nodes harvested					
Mean	11.7	19.8	15.2	17.4	10.0
Standard deviation	4.8	6.3	6.0	6.3	4.6
Patients with four or more tumor-positive axillary lymph nodes	33 (24.4 %)	28 (24.8 %)	31 (25.0 %)	19 (13.9 %)	19 (11.4 %)

HER-2 human epidermal growth factor receptor 2, *ECE* extra-capsular extension

Univariate analysis comparing the two patient groups with less than four versus four or more tumor-positive ALNs is given in Table 2. Prevalence of four or more tumor-positive ALNs in each center's series, histological tumor size, multifocality of the primary tumor, lymphovascular invasion of the primary tumor, histological type of the primary tumor, SN metastasis detection method, extracapsular extension (ECE) of the SN metastasis and number of positive and negative SNs all had a P value of less than 0.05 and were included in the multivariate analysis.

The prevalence of four or more tumor-positive ALNs in each center ($P = 0.010$), the number of tumor-positive SNs

Table 2 Univariate analysis comparing the patient groups with four or more tumor-positive axillary lymph nodes to those with less than four in the original patient series

	Less than four positive nodes ($n = 545$)	Four or more positive nodes ($n = 130$)	All patients ($n = 675$)	P
Prevalence of four or more positive nodes in each center				
Mean (range)	18.8 % (11.4–25.0)	21.2 % (11.4–25.0)	19.3 % (11.4–25.0)	<0.0001
Standard deviation	6.2	5.6	6.1	
Patient age (years)				0.382
Mean (range)	57.2 (26–87)	58.0 (27–80)	57.4 (26–87)	
Standard deviation	11.6	11.5	11.6	
Histological size of the primary tumor (mm)				0.003
Mean (range)	20.5 (2–160)	25.0 (4–200)	21.4 (2–200)	
Standard deviation	11.7	11.5	13.7	
Multifocality of the primary tumor				0.043
No	438	94	532	
Yes	107	36	143	
Lymphovascular invasion in the primary tumor				0.004
No	375	72	447	
Yes	170	58	228	
Estrogen receptor status				0.189
Negative	88	15	103	
Positive	457	115	572	
Progesterone receptor status				0.751
Negative	133	30	163	
Positive	412	100	512	
HER-2 status				0.356
Negative	479	118	597	
Positive	66	12	78	
Nuclear grade of the primary tumor				0.250
Grade I	51	7	58	
Grade II	257	59	316	
Grade III	237	64	301	
Histological grade of the primary tumor				0.210
Grade I	94	15	109	
Grade II	253	60	313	
Grade III	198	55	253	
Histology of the primary tumor				0.040
Ductal carcinoma	436	95	531	
Lobular carcinoma	51	18	69	
Mixed	24	12	36	
Other	34	5	39	
Detection method of the sentinel node metastasis				<0.0001
Frozen section analysis	256	91	347	
Paraffin standard staining	231	31	262	
Paraffin immunohistochemistry	30	4	34	
Serial sectioning	28	4	32	
Extra-capsular extension				<0.0001
No	381	49	430	
Yes	164	81	245	
Number of negative sentinel nodes harvested				<0.0001
Mean (range)	0.7 (0–8)	0.3 (0–3)	0.6 (0–8)	

Table 2 continued

	Less than four positive nodes ($n = 545$)	Four or more positive nodes ($n = 130$)	All patients ($n = 675$)	P
Standard deviation	1.0	0.7	0.9	
Number of positive sentinel nodes harvested				<0.0001
Mean (range)	1.2 (1–3)	1.6 (1–3)	1.3 (1–3)	
Standard deviation	0.5	0.7	0.6	
Sentinel node ratio				<0.0001
Mean (range)	0.8 (0.1–1.0)	0.9 (0.3–1.0)	0.8 (0.1–1.0)	
Standard deviation	0.3	0.2	0.3	

HER-2 human epidermal growth factor receptor 2, *sentinel node ratio* ratio of tumor-positive sentinel nodes to all harvested sentinel nodes

Table 3 Binary logistic regression analysis using backward stepwise likelihood ratio method in the original patient series

	Coefficient	Standard error	Wald	P	Odds ratio	95 % CI for odds ratio	
						Lower	Upper
Prevalence of four or more tumor-positive nodes	0.049	0.019	6.637	0.010	1.050	1.012	1.090
Number of positive SNs	0.943	0.166	32.147	<0.0001	2.567	1.853	3.556
Number of negative SNs	−0.443	0.150	8.751	0.003	0.642	0.479	0.861
Histological tumor size (mm)	0.018	0.008	5.391	0.020	1.018	1.003	1.034
ECE of SN metastasis	1.036	0.218	22.539	<0.0001	2.818	1.837	4.321
Constant	−4.392	0.516	72.389	<0.0001	0.012		

CI confidence interval, *SN* sentinel node, *ECE* extra-capsular extension

($P < 0.0001$), number of tumor-negative SNs ($P = 0.003$), histological size of the primary tumor ($P = 0.020$) and ECE of SN metastasis ($P < 0.0001$) had statistical significance in the binary logistic regression model and were included in the predictive model (Table 3). The Hosmer–Lemeshow test produced a P value of 0.101 indicating that the model fits and calibrates well for the patient population.

The AUC for the original patient population was 0.769 (95 % confidence interval: 0.721–0.816) suggesting a very good discrimination. The multivariate model predicted 455 (67.4 %) of the 675 patients in the original patient series to have less than four metastatic ALN with a sensitivity of 66.9 % and specificity of 75.6 % at the <20 % cutoff threshold.

A mathematical equation was deduced from the logistic regression analysis to predict a patient specific risk of having four or more tumor-positive ALNs, with p denoting the probability of this risk:

$$\text{logit}(p) = -4.392 + 0.049 \times a + 0.943 \times b - 0.443 \times c + 0.018 \times d + 1.036 \times e.$$

The letters in the equation denote the following variables: a = prevalence of four or more tumor-positive ALNs in patient series (percentage of patients), b = number of tumor-positive SNs, c = number of tumor-negative SNs, d = histological size of primary tumor (mm), e = ECE of SN metastasis (0 if not present, 1 if present). The predictive model is also provided as an excel-based calculator in the online only version of this article and at the website of the Breast Surgery Unit of Helsinki University Central Hospital (www.hus.fi/breastsurgery/predictivemodel).

The predictive model was then validated by insertion of each patient's data from the internal and external validation series into the equation. AUC, sensitivity and specificity for each internal and external validation center are given in Table 4. Receiver operating characteristic curves for the original series and validation series are presented in Fig. 1.

Sensitivity and specificity are given for <20 % risk in the internal and external validation series in Table 4. Sensitivity and specificity of our model in the external validation series for other predicted probabilities are: <5 % risk (sensitivity 99.5 %, specificity 3.4 %), <10 % risk (sensitivity 95.7 %, specificity 22.1 %), <20 % risk (sensitivity 76.1 %, specificity 61.5 %), <30 % risk (sensitivity 54.3 %, specificity 84.6 %), <40 % risk (sensitivity 44.0 %, specificity 91.8 %), <50 % risk (sensitivity 27.3 %, specificity 96.9 %), <60 % risk (sensitivity 23.0 %, specificity 98.7 %). Our model identifies 389 (51.2 %) of the 760 patients in the external

Table 4 Performance of the predictive model in internal and external validation; sensitivity and specificity for <20 % risk of additional metastases

	N	Four or more metastatic ALNs	AUC (95 % CI)	Sensitivity (%)	Specificity (%)
Original patient series	675	130 (19.1 %)	0.769 (0.721–0.816)	66.9	75.6
Internal validation series	367	67 (18.3 %)	0.766 (0.698–0.834)	68.7	70.3
Center A	72	12 (16.7 %)	0.726 (0.559–0.893)	58.3	55.0
Center B	91	19 (20.9 %)	0.767 (0.637–0.897)	68.4	69.4
Center C	33	8 (24.2 %)	0.893 (0.778–1.000)	75.0	76.0
Center D	149	27 (18.1 %)	0.759 (0.647–0.871)	74.1	72.1
Center E	22	1 (4.5 %)	0.667 (0.465–0.868)	–	100
External validation series	760	209 (27.5 %)	0.774 (0.736–0.812)	76.1	61.5
Center F	77	30 (39.0 %)	0.868 (0.782–0.954)	96.7	25.5
Center G	88	20 (22.7 %)	0.775 (0.661–0.889)	75.0	64.7
Center H	42	8 (19.0 %)	0.741 (0.536–0.946)	37.5	85.3
Center I	132	40 (30.3 %)	0.778 (0.693–0.862)	75.0	60.9
Center J	17	3 (17.6 %)	0.750 (0.405–1.000)	66.7	42.9
Center K	79	18 (22.8 %)	0.638 (0.477–0.800)	38.9	85.2
Center L	141	27 (19.1 %)	0.845 (0.772–0.918)	88.9	64.9
Center M	184	63 (34.2 %)	0.736 (0.653–0.818)	77.8	54.5

ALN axillary lymph node, *AUC* area under the receiver operating characteristic curve, *CI* confidence interval

validation series to have a less than 20 % risk of having more than four ALN metastases. Similarly the model identified 73.9 % patients to have a less than 30 % risk and 82.0 % to have a less than 40 % risk. The present model predicts only 20 (2.6 %) patients in the external validation series to have a less than 5 % risk of four or more ALN metastases.

Calibration of the predictive model was examined by grouping patients in each series into quartiles according to the predicted probabilities of four or more tumor-positive ALNs. A calibration plot was acquired by plotting the mean predicted probability of each quartile against the actual proportion of patients with four or more tumor-positive ALNs in each quartile (Fig. 2).

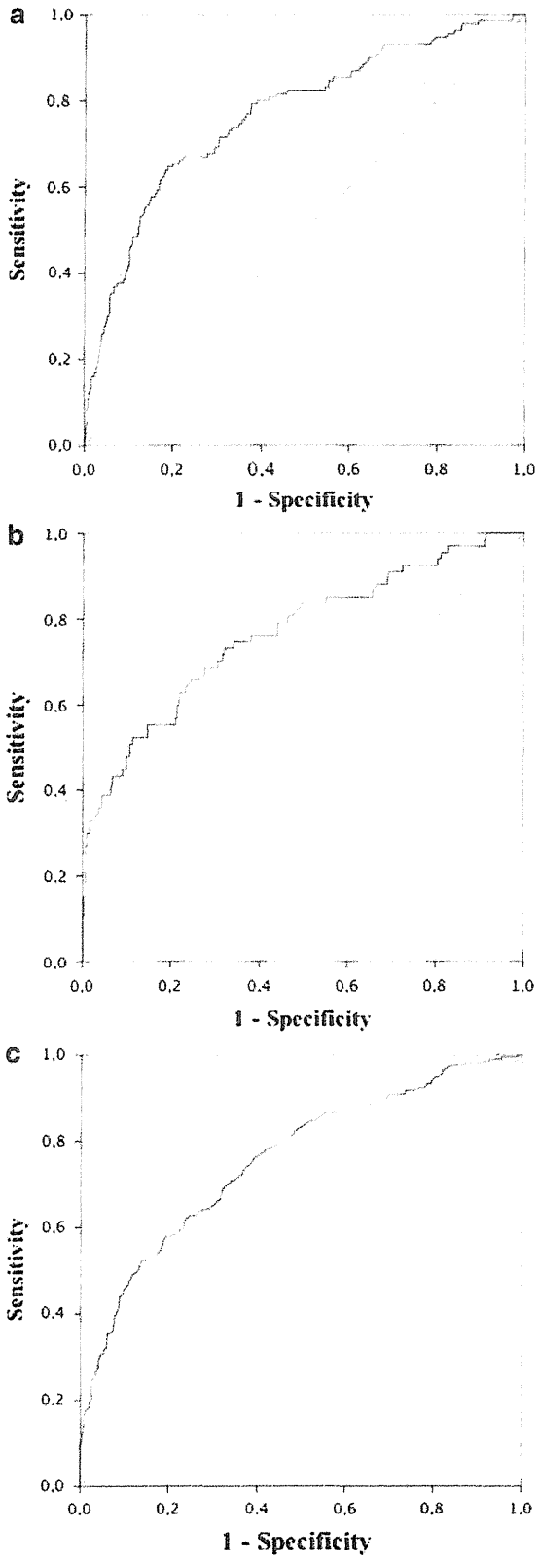
Discussion

The present predictive model

Our predictive model performs very well in the internal and external validation, both having AUC values of >0.75. Our model is also well calibrated as shown by the Hosmer–Lemeshow test and the calibration lines in Fig. 1. As expected, the performance of our model varies between validation centers. The centers at the extremities in terms of AUC values have contributed relatively small numbers of patients and this variation may well be caused by statistical deviation due to the small sample size. The case mix might also differ between centers, for example, in terms of tumor sizes. Growing tumor size increases the prevalence of ALN metastases. The use and sensitivity of preoperative axillary ultrasound affects the prevalence of four or more positive ALNs as well. Patients with a significant axillary tumor burden are more likely to be preoperatively detected by axillary ultrasound compared to patients with only a single ALN metastasis.

We present our predictive model as an equation predicting the probability of an individual patient with macrometastatic SN having four or more tumor-positive ALNs. The three previously published predictive tools are presented in forms of a scoring system [12], a table [13], and a nomogram [14], all of which are approximations of the original logistic regression model. Presenting the predictive model in the form of a nomogram or scoring system allows an easy risk prediction, but such tools work ideally in paper format. At present, most patient records and medical applications display data in electronic format, therefore, entering information into a software-based calculator seems the easiest and certainly most accurate way to proceed with the estimation of patient-specific risks.

Furthermore, by producing a continuous risk percentage our model is not tied up to specific cutoff thresholds as clinical decision-making thresholds may well change in the future. The model's sensitivity and specificity were tested in Table 4 for a cutoff value of less than 20 % risk of having four or more metastatic ALNs. This threshold was chosen as an example and is considerably higher than the <5 % threshold given by the previous predictive models [12–14]. Our model predicts over 50 % of the patients in the external validation series to have a less than 20 % risk



◀ **Fig. 1** Receiver operating characteristic curves for the original patient series (**a**), the internal validation patient series (**b**), and the external validation patient series (**c**)

for four or more ALNs metastases but the proportion of patients understandably changes with the risk level. Because other risk levels may be relevant in various clinical circumstances, we calculated our model's sensitivity and specificity for risk levels ranging from less than 5 % to less than 60 % in the external validation series. At 5 and 10 % cutoff levels, the sensitivity of our model was extremely high, that is over 95 %, but at the cost of the specificity. However, such low cutoff levels will be used when it is important to minimize the risk of missing the diagnosis of pN2–pN3 disease, and then the high sensitivity can be considered as an advantage. Although the risk cutoff level can be altered with changing settings of clinical decision-making, the model needs to be validated for each center's patient population, and sensitivity and specificity need to be calculated for each threshold.

Factors in our model

Our predictive model is based on heterogeneous patient data from five European centers. The exclusion criteria were intentionally kept minimal in order to produce a series closely resembling the normal clinical setting. It is noteworthy that the prevalence of patients with four or more tumor-positive ALNs has not been previously incorporated into any predictive model. This adds an important factor into the model as it facilitates calibration of the model in different centers with varying patient material and surgical and pathological methodology. The importance of such institutional calibrations and validations has been highlighted by

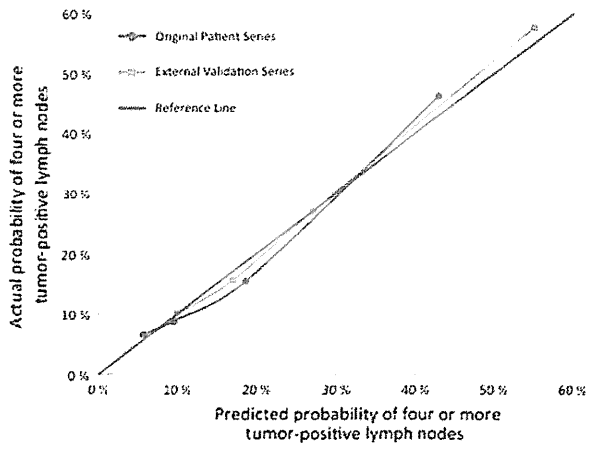


Fig. 2 Calibration plot for the predictive model applied to the original patient series and external validation series split to quartiles

our previous studies demonstrating relevant inter-institutional differences in both the variables used by the predictive models [16] and the best performing predictive tools for patients with low [16] and high [16, 17] risk of NSN involvement [34].

In addition to the prevalence of four or more positive ALNs, four tumor and SN-related variables were found to be statistically significant in the binary logistic regression analysis and were included in our predictive model. Tumor size, number of positive SNs, and SN ratio or number of negative SNs were found to be significant factors also in all of the previous models [12–14]. ECE of SN metastasis was also a significant factor in two of the previous models [13, 14]. Other factors that were found to be significant in previous studies are: lymphovascular invasion of the primary tumor [13, 14], detection method of SN metastasis [12] and tumor histology [14]. As a whole, all of the published predictive models are surprisingly similar regarding factors influencing the prevalence of four or more metastatic ALNs with tumor-positive SNs.

Implications of our predictive model—towards tailored axillary treatment

Completion ALND after tumor-positive SNs has been the gold standard of distinguishing pN1 patients from pN2–pN3 breast cancer patients. Accurate evaluation of the lymph node tumor burden in the axilla has been considered an important prognostic factor influencing adjuvant treatment planning. ALND also reveals the lymph node ratio, i.e., the proportion of metastatic ALNs among all examined nodes. The prognostic value of the lymph node ratio has been considered superior to the number of involved axillary nodes [1, 2].

The results of ACOSOG Z0011 trial were groundbreaking suggesting that omitting ALND in patients with limited number of metastatic SNs may not increase regional recurrence rate nor decrease survival in general [5, 6]. The Z0011 trial had, however, many limitations that reduce its generalizability. The Z0011 trial only included breast cancer patients undergoing breast-conserving surgery followed by whole-breast radiotherapy. The study therefore excluded patients undergoing mastectomy who may benefit from accurate discrimination between pN1 and pN2–pN3 disease in the planning of adjuvant radiotherapy. Moreover, the Z0011 trial included only patients with one or two tumor-positive SNs and a substantial proportion of the patients had micrometastasis as their SN finding (44.8 % in the SNB only arm) [5, 6]. Therefore, the ACOSOG Z0011 trial results do not indicate that every patient with SN metastases can safely avoid ALND.

The forthcoming results of the AMAROS-trial may further increase the need for predictive tools. The AMAROS-trial compares axillary radiotherapy to completion ALND in a randomized setup [35]. If ALND were to be replaced by axillary radiotherapy, the exact extent of the axillary tumor burden would remain unknown. Even if axillary radiotherapy proves equal to completion ALND, a subgroup of patients with extensive axillary tumor burden might benefit from completion ALND either alone or together with axillary radiotherapy.

The trend of omitting routine ALND after tumor-positive SNs increases the importance of statistical models in predicting patients with a risk of significant tumor burden left behind in their axilla. Such patients may benefit from either radiotherapy to regional nodal basins, completion ALND, or even both. The treatment of ALN positive breast cancer patients is undergoing a dramatic change. In the future, these patients may be offered tailored axillary treatment options based on patient-specific risk and need analyses. As clinicians' decisions may be inferior to nomogram-based decisions [36], predictive tools such as the present one will be of major importance in this development.

Conclusion

In this study, we present a novel international multicenter predictive tool to assess patient-specific risk of having four or more tumor-positive ALNs in patients with SN macrometastases. Our model performs very well both in internal and external validation patient series, but needs further validation in each center before application to clinical practice.

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Conflict of interest The authors declare that they have no conflicts of interest.

Ethical standard This study complies with the current law of all the countries in which the patients were treated.

References

1. Truong PT, Vinh-Hung V, Cserni G, Woodward WA, Tai P, Vlastos G, Group MotINRW (2008) The number of positive nodes and the ratio of positive to excised nodes are significant predictors of survival in women with micrometastatic node-positive breast cancer. Eur J Cancer 44(12):1670–1677
2. Vinh-Hung V, Verkooijen HM, Fioretta G, Neyroud-Caspar I, Rapiti E, Vlastos G, Deglise C, Usel M, Lutz JM, Bouchardy C (2009) Lymph node ratio as an alternative to pN staging in node-positive breast cancer. J Clin Oncol 27(7):1062–1068
3. Kim T, Giuliano AE, Lyman GH (2006) Lymphatic mapping and sentinel lymph node biopsy in early-stage breast carcinoma: a metaanalysis. Cancer 106(1):4–16

4. Gärtner R, Jensen MB, Nielsen J, Ewertz M, Kroman N, Kehlet H (2009) Prevalence of and factors associated with persistent pain following breast cancer surgery. JAMA 302(18):1985–1992
5. Giuliano AE, McCall L, Beitsch P, Whitworth PW, Blumencranz P, Leitch AM, Saha S, Hunt KK, Morrow M, Ballman K (2010) Locoregional recurrence after sentinel lymph node dissection with or without axillary dissection in patients with sentinel lymph node metastases: the American College of Surgeons Oncology Group Z0011 randomized trial. Ann Surg 252(3):426–432 discussion 432–433
6. Giuliano AE, Hunt KK, Ballman KV, Beitsch PD, Whitworth PW, Blumencranz PW, Leitch AM, Saha S, McCall LM, Morrow M (2011) Axillary dissection vs. no axillary dissection in women with invasive breast cancer and sentinel node metastasis: a randomized clinical trial. JAMA 305(6):569–575
7. Wasif N, Ye X, Giuliano AE (2009) Survey of ASCO members on management of sentinel node micrometastases in breast cancer: variation in treatment recommendations according to specialty. Ann Surg Oncol 16(9):2442–2449
8. Bilimoria K, Bentrem D, Hansen N, Bethke K, Rademaker A, Ko C, Winchester D, Winchester D (2009) Comparison of sentinel lymph node biopsy alone and completion axillary lymph node dissection for node-positive breast cancer. J Clin Oncol 27(18):2946–2953
9. Pepels MJ, Vestjens JH, de Boer M, Smidt M, van Diest PJ, Borm GF, Tjan-Heijnen VC (2011) Safety of avoiding routine use of axillary dissection in early stage breast cancer: a systematic review. Breast Cancer Res Treat 125(2):301–313
10. Recht A, Edge SB, Solin LJ, Robinson DS, Estabrook A, Fine RE, Fleming GF, Formenti S, Hudis C, Kirshner JJ et al (2001) Postmastectomy radiotherapy: clinical practice guidelines of the American Society of Clinical Oncology. J Clin Oncol 19(5):1539–1569
11. Aebi S, Davidson T, Gruber G, Cardoso F, Group EGW (2011) Primary breast cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 22(Suppl 6):vi12–vi24
12. Chagpar AB, Scoggins CR, Martin RC, Cook EF, McCurry T, Mizuguchi N, Paris KJ, Carlson DJ, Laidley AL, El-Eid SE et al (2007) Predicting patients at low probability of requiring postmastectomy radiation therapy. Ann Surg Oncol 14(2):670–677
13. Rivers AK, Griffith KA, Hunt KK, Degnim AC, Sabel MS, Diehl KM, Cimmino VM, Chang AE, Lucas PC, Newman LA (2006) Clinicopathologic features associated with having four or more metastatic axillary nodes in breast cancer patients with a positive sentinel lymph node. Ann Surg Oncol 13(1):36–44
14. Katz A, Smith BL, Golshan M, Niemierko A, Kobayashi W, Raad RA, Kelada A, Rizk L, Wong JS, Bellon JR et al (2008) Nomogram for the prediction of having four or more involved nodes for sentinel lymph node-positive breast cancer. J Clin Oncol 26(13):2093–2098
15. Leidenius M, Vaalavirta L, Heikkilä P, von Smitten K, Salmenkivi K (2008) The prevalence of and risk factors for four or more metastatic axillary lymph nodes in breast cancer patients undergoing sentinel node biopsy. J Surg Oncol 98(1):21–26
16. Cserni G, Boross G, Maráz R, Leidenius MH, Meretoja TJ, Heikkila PS, Regitnig P, Luschin-Ebengreuth G, Zgajnar J, Perhavec A et al (2012) Multicentre validation of different predictive tools of non-sentinel lymph node involvement in breast cancer. Surg Oncol 21(2):59–65
17. Cserni G, Bori R, Maráz R, Leidenius MH, Meretoja TJ, Heikkila PS, Regitnig P, Luschin-Ebengreuth G, Zgajnar J, Perhavec A et al (2012) Multi-institutional comparison of non-sentinel lymph node predictive tools in breast cancer patients with high predicted risk of further axillary metastasis. Pathol Oncol Res. doi: 10.1007/s12253-012-9553-5
18. Sobin LH, Wittekind Ch (eds) (2002) TNM classification of malignant tumours. Wiley-Liss, New York
19. Van Zee KJ, Manasseh DM, Bevilacqua JL, Boolbol SK, Fey JV, Tan LK, Borgen PI, Cody HS, Kattan MW (2003) A nomogram for predicting the likelihood of additional nodal metastases in breast cancer patients with a positive sentinel node biopsy. Ann Surg Oncol 10(10):1140–1151
20. Hwang RF, Krishnamurthy S, Hunt KK, Mirza N, Ames FC, Feig B, Kuerer HM, Singletary SE, Babiera G, Meric F et al (2003) Clinicopathologic factors predicting involvement of nonsentinel axillary nodes in women with breast cancer. Ann Surg Oncol 10(3):248–254
21. Farshid G, Pradhan M, Kollias J, Gill PG (2004) A decision aid for predicting non-sentinel node involvement in women with breast cancer and at least one positive sentinel node. Breast 13(6):494–501
22. Saidi RF, Dudrick PS, Remine SG, Mittal VK (2004) Nonsentinel lymph node status after positive sentinel lymph node biopsy in early breast cancer. Am Surg 70(2):101–105 discussion 105
23. Barranger E, Coutant C, Flahault A, Delpech Y, Darai E, Uzan S (2005) An axilla scoring system to predict non-sentinel lymph node status in breast cancer patients with sentinel lymph node involvement. Breast Cancer Res Treat 91(2):113–119
24. Degnim AC, Reynolds C, Pantvaidya G, Zakaria S, Hoskin T, Barnes S, Roberts MV, Lucas PC, Oh K, Koker M et al (2005) Nonsentinel node metastasis in breast cancer patients: assessment of an existing and a new predictive nomogram. Am J Surg 190(4):543–550
25. Chagpar AB, Scoggins CR, Martin RC, Carlson DJ, Laidley AL, El-Eid SE, McGlothin TQ, McMasters KM, Study UoLBSLN (2006) Prediction of sentinel lymph node-only disease in women with invasive breast cancer. Am J Surg 192(6):882–887
26. Pal A, Provenzano E, Duffy SW, Pinder SE, Purushotham AD (2008) A model for predicting non-sentinel lymph node metastatic disease when the sentinel lymph node is positive. Br J Surg 95(3):302–309
27. Houvenaeghel G, Nos C, Giard S, Mignotte H, Esterni B, Jacquemier J, Buttarelli M, Classe JM, Cohen M, Rouanet P et al (2009) A nomogram predictive of non-sentinel lymph node involvement in breast cancer patients with a sentinel lymph node micrometastasis. Eur J Surg Oncol 35(7):690–695
28. Kohrt HE, Olshen RA, Bermas HR, Goodson WH, Wood DJ, Henry S, Rouse RV, Bailey L, Philben VJ, Dirbas FM et al (2008) New models and online calculator for predicting non-sentinel lymph node status in sentinel lymph node positive breast cancer patients. BMC Cancer 8:66
29. Cho J, Han W, Lee JW, Ko E, Kang SY, Jung SY, Kim EK, Moon WK, Cho N, Park IA et al (2008) A scoring system to predict nonsentinel lymph node status in breast cancer patients with metastatic sentinel lymph nodes: a comparison with other scoring systems. Ann Surg Oncol 15(8):2278–2286
30. Gur AS, Unal B, Ozbek U, Ozmen V, Aydogan F, Gokgoz S, Gulluoglu BM, Aksaz E, Ozbas S, Baskan S et al (2010) Validation of breast cancer nomograms for predicting the non-sentinel lymph node metastases after a positive sentinel lymph node biopsy in a multi-center study. Eur J Surg Oncol 36(1):30–35
31. Perhavec A, Perme MP, Hocevar M, Besić N, Zgajnar J (2010) Ljubljana nomograms for predicting the likelihood of non-sentinel lymph node metastases in breast cancer patients with a positive sentinel lymph node. Breast Cancer Res Treat 119(2):357–366
32. Coufal O, Pavlík T, Fabian P, Bori R, Boross G, Sejben I, Maráz R, Koca J, Krejcí E, Horáková I et al (2009) Predicting non-sentinel lymph node status after positive sentinel biopsy in breast cancer: what model performs the best in a Czech population? Pathol Oncol Res 15(4):733–740

33. Meretoja TJ, Strien L, Heikkilä PS, Leidenius MH (2012) A simple nomogram to evaluate the risk of nonsentinel node metastases in breast cancer patients with minimal sentinel node involvement. Ann Surg Oncol 19(2):567–576
34. Meretoja TJ, Leidenius MHK, Heikkilä PS et al (2012) International multicenter tool to predict the risk of nonsentinel node metastases in breast cancer. J Natl Cancer Inst 104:1888–1896
35. Straver ME, Meijnen P, van Tienhoven G, van de Velde CJ, Mansel RE, Bogaerts J, Demonty G, Duez N, Cataliotti L, Klinkenbijl J et al (2010) Role of axillary clearance after a tumor-positive sentinel node in the administration of adjuvant therapy in early breast cancer. J Clin Oncol 28(5):731–737
36. Specht MC, Kattan MW, Gonen M, Fey J, Van Zee KJ (2005) Predicting nonsentinel node status after positive sentinel lymph biopsy for breast cancer: clinicians versus nomogram. Ann Surg Oncol 12(8):654–659

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Original article

Use of the neo-adjuvant exemestane in post-menopausal estrogen receptor-positive breast cancer: A randomized phase II trial (PTEX46) to investigate the optimal duration of preoperative endocrine therapy

Takashi Hojo [a,*], Takayuki Kinoshita [a], Shigeru Imoto [b], Chikako Shimizu [c], Hirotsugu Isaka [b], Hiroki Ito [b], Kentaro Imi [b], Noriaki Wada [d], Masashi Ando [c], Yasuhiro Fujiwara [c]

[a] Department of Breast Surgery, National Cancer Center Hospital, 1-1, Tsukiji 5-chome, Chuo-ku, Tokyo, Japan
[b] Department of Breast Surgery, Kyorin University, Tokyo, Japan
[c] Department of Breast and Medical Oncology, National Cancer Center Hospital, Tokyo, Japan
[d] Department of Breast Surgery, National Cancer Center Hospital East, Chiba, Japan

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ABSTRACT

Purpose: The optimal treatment duration time and the causal relationship between neoadjuvant endocrine therapy and clinical response are not clear. Therefore, we conducted the present study to investigate the potential benefits of neoadjuvant exemestane therapy with the goal of identifying the optimal treatment duration.

Methods: This study was conducted at three hospitals, as a multicenter, randomized phase II trial(UMIN000005668) of pre-operative exemestane treatment in post-menopausal women with untreated primary breast cancer. Fifty-one post-menopausal women with ER-positive and/or PgR-positive invasive breast cancer were randomly assigned to exemestane for 4 months or 6 months. Clinical response, pathological response, and decisions regarding breast-conserving surgery were the main outcome measures.

Results: Of the 52 patients that enrolled, 51 patients underwent surgery. Of those, 26 and 25 patients had been treated with exemestane for 4 and 6 months, respectively. Treatments were performedat 3 hospitals in Japan between April 2008 and August 2010. The response rates as assessed by clinical examination were 42.3% and 48.0% for 4 and 6 months of treatment, respectively. Pathological responses (minimal response or better) were observed in 19.2% and 32.0% of patients, and breast-conserving surgery was performed on 50.0% and 48.0% of patients from the 4 and 6 month treatment groups, respectively.

Conclusion: The results of this study demonstrate that responses were equal to 4 or 6 months of exemestane treatment. Therefore, we propose that the rates of breast-conserving surgery could be maximized by 4 months of treatment. Furthermore, in addition to using exemestane as a preoperative treatment in post-menopausal women with ER-positive breast cancer, we envision administering the drug over the long term under careful clinical supervision.

Introduction

Since the 1990s, primary endocrine therapy has been considered the gold standard in the adjuvant and metastatic treatment settings for estrogen (ER) and/or progesterone (PR) receptor-positive breast cancer. The NSABP B-18 clinical trial[1] in 1988 demonstrated that neoadjuvant chemotherapy yielded the same survival rate as adjuvant chemotherapy, with an improved rate of breast-conserving surgery, indicating that neoadjuvant therapy could have important clinical ramifications. With that in mind, neoadjuvant endocrine therapy for hormone receptor-positive breast cancer was also assessed, and was shown to be effective in a number of clinical trials (Table 1). Recently, clinical interest has shifted from tamoxifen to third-generation aromatase inhibitors. A few trials[2–8] have indicated that anastrozole led to improved response rates as compared to tamoxifen, but the results were not statistically significant. The PROACT trial reported that anastrozole treatment allowed for breast-conserving surgery in significantly

* Corresponding author. Tel.: +81 3 3542 2511; fax: +81 3 3542 3815.
E-mail address: tahojo@ncc.go.jp (T. Hojo).

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Table 1
Neoadjuvant endocrine trials.

Author or trial name	Number of patients	Design	Duration (month)	Clinical ORR[e]
IMPACT[2]	330	ANA[a] vs TAM[b] vs ANA + TAM	3	37%, 36%, 39%
PROACT[3]	451	ANA vs TAM	3	49.7%, 39.7%
PO24 Trial[4]	337	LET[c] vs TAM	4	55%, 36%
GENARI Trial[5]	29	EXE[d]	4	37.0%
French study[6]	45	EXE	14–27 weeks	70.6%
Gil Gil (Spain)[7]	55	EXE	6	50%
Mustacchi[8]	44	EXE	6	66%

[a] ANA = Anastrozole.
[b] TAM = Tamoxifen.
[c] LET = Letrozole.
[d] EXE = Exemestane.
[e] ORR = objective response rates.

more patients than did tamoxifen. The neoadjuvant drug, exemestane, has been evaluated in several small studies. The results have been promising and warrant further evaluation to determine the optimal therapeutic conditions for hormone receptor-positive patients. Specifically, the optimal treatment duration time and the causal relationship between neoadjuvant endocrine therapy and clinical response are not clear (Table 1). In addition, there are studies that have reviewed the optimal duration time of hormone treatments. Here, we investigated the benefits of 4 and 6 month long neoadjuvant exemestane therapy.

Materials and methods

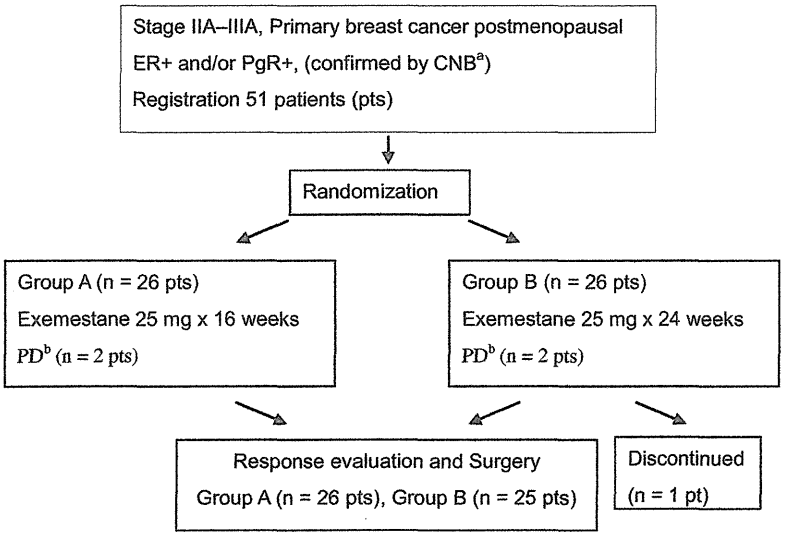
Patients

We enrolled ≥55-year-old post-menopausal women (defined as: no spontaneous menses for > 1 year; LH levels > 30 IU/L; or bilateral oophorectomy prior to breast cancer diagnosis) with stage IIA–IIIA invasive ER- and/or PgR-positive breast carcinoma, as confirmed by immunohistochemical examination of core-needle biopsies (defined as: >10% endocrine receptor + nuclear staining). We further required that tumors be measurable by clinical palpation. Written informed consent was obtained from each patient.

Patients were ineligible if they had any severe coincident medical disease that would prevent them from receiving surgery, place them at unusual risk, or confound the study results; were unwilling or unable to discontinue using drugs affecting sex hormones (including hormone replacement therapy); had suffered from any invasive malignancy within the previous 5 years (other than carcinoma of the skin or carcinoma in situ of the cervix, adequately cone biopsied); had received any previous breast cancer treatment or tamoxifen as part of a breast cancer prevention study; or, had received treatment with non-approved drugs during the 3 months prior to randomization. Criteria for withdrawal from the study included patients who had completed the 5-year treatment course; did not begin randomized therapy; withdrew informed consent; had confirmed clinically significant disease before surgery or confirmed recurrence after surgery; had an adverse event; or, were withdrawn at the investigator's discretion.

Study design and setting

This study was conducted at three hospitals in Japan as a multicenter, open-label, double-arm, randomized, phase II clinical trial of pre-operative exemestane treatment in post-menopausal women with primary breast cancer. In order to optimally balance the patients in the two treatment arms with respect to prognostic factors, the patients were stratified by tumor factor, node factor, and age. The neoadjuvant endocrine treatment regimen consisted of one 25 mg exemestane tablet daily for 4 or 6 months. Fifty-one post-menopausal women with ER-positive and/or PgR-positive invasive breast cancer were randomly assigned to exemestane (25 mg/day) for 4 months (Group A) or exemestane



Setting: Multicenter study involving 3 hospitals in Japan

[a]CNB = core needle biopsy

[b]PD = Progressive Disease

The patient with PD canceled treatment and underwent immediate surgery.

Fig. 1. Study design.