症　　例

側頸部嚢胞性腫瘤として発見された異所性腺腫様甲状腺腫の1例

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　症例は41歳，女性．左側頸部腫大を自覚し近医を受診，頸部エコーで左側頸部嚢胞を認めた．フォロー中に増大傾向を認め，当科紹介となった．頸部エコーで，甲状腺腺外に境界明瞭で内部無エコーの嚢胞性病変を認めた．原発不明の側頸部腫瘍として腫瘍摘出術を施行．腫瘍は，左内頸静脈外側に接するように存在し，容易に剥離できた．咽頭方向には索状物が連続していたが，甲状腺や迷走神経，頸神経ワナとの連続は認めなかった．摘出した嚢胞は5cm大で，表面平滑，暗赤色の液体内容を含んでおり，透光性良好であった．病理組織診断は，嚢胞壁内と壁在結節に異形成に乏しい甲状腺濾胞の集簇を認め，異所性腺腫様甲状腺腫であった．頸部腫瘍には正中・側頸嚢胞や異所性甲状腺，甲状腺乳頭癌のリンパ節転移などがある．治療方針を決める上で，悪性の可能性を十分検討することが重要であり，穿刺では再発することもあることから，外科的摘出が望ましいと考えられる．

索引用語：異所性腺腫様甲状腺腫，側頸部嚢胞性腫瘍

緒　　言

　頸部の腫瘤には正中頸嚢胞や側頸嚢胞，異所性甲状腺，リンパ管腫，脂肪腫などがある．その中で，異所性甲状腺は発生過程からみると正中や舌根部に多く認められる[1]．われわれは，側頸部の嚢胞性腫瘤として発見された異所性腺腫様甲状腺腫の1例を経験したので若干の文献的考察を含め報告する．

症　　例

患者：41歳，女性．

主訴：左頸部腫大．

既往歴：虫垂炎．

家族歴：特記事項なし．

生活歴：喫煙，飲酒なし．

現病歴：2009年頃から左側頸部の腫大を自覚し近医を受診した．頸部エコーを施行したところ左側頸部に嚢胞性腫瘤を認めたが，明らかな悪性所見はなく経過観察となった．2012年12月に増大傾向を認めたため，精査および加療目的に当科紹介となった．

理学所見：左胸鎖乳突筋付近に3cm大の弾性軟な

腫瘤を触知した．甲状腺には異常所見はなく，頸部・鎖骨上に腫大したリンパ節も触知しなかった．

　血液検査：甲状腺機能正常，サイログロブリンの上昇を認めず，可溶性IL-2レセプターは陰性であった．その他特記すべき異常所見は認めなかった．

　頸部エコー：甲状腺は正常位置にあり，異常所見は認めなかった．甲状腺腺外で左内頸静脈より外側に3.5×1.5cm，境界明瞭で内部無エコーの嚢胞性病変を認めた．内部に壁在結節を伴っていた（**Fig. 1**）．

　頸部CT：左頸部声門レベルに境界明瞭で甲状腺と同CT値のやや高いdensityの腫瘤を認めた．内部に低吸収結節を含み，側頸嚢胞および異所性甲状腺内に生じた嚢胞や異所性甲状腺腫が疑われた（**Fig. 2**）．

　頸部MRI：内頸静脈背側に4.5×2.3cmの境界明瞭な嚢胞性腫瘤を認めた．T2強調画像で内部に低信号領域を認めた．嚢胞性リンパ管腫や嚢胞変性の強い神経原性腫瘍の可能性も示唆された（**Fig. 3**）．

　穿刺吸引細胞診：経過中に2回細胞診を行ったが，いずれも血液成分や変性壊死物質と泡沫細胞のみで，異型のある細胞は見られなかった．

　以上より，側頸嚢胞や異所性甲状腺内に生じた嚢胞などが鑑別にあがった．増大傾向にある原発および良悪性不明の側頸部嚢胞に対して，腫瘍摘出術を施行す
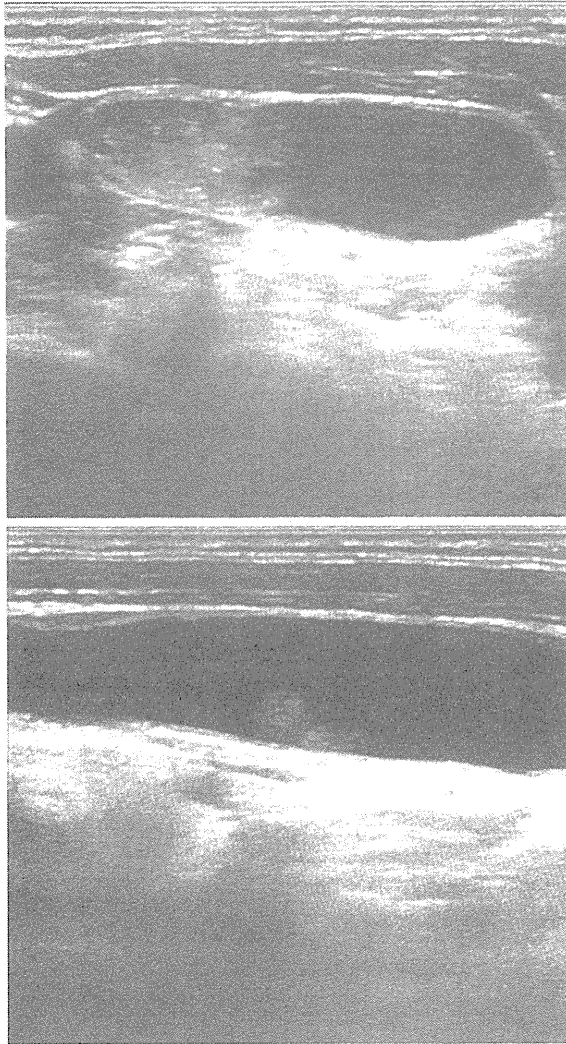
Fig. 1　頸部エコー：甲状腺腺外に境界明瞭で内部に
壁在結節を伴う囊胞性病変を認めた．

a
——
b

Fig. 2　頸部CT：左頸部声門レベルに内部に低
吸収結節を含む腫瘤性病変を認めた．

る方針となった．

　手術所見：全身麻酔下で，腫瘍直上に 5 cm の皮膚切
開をおき，腫瘍摘出術を施行．腫瘍は，左内頸静脈の
外側に接するように存在し，容易に剥離できた．咽頭
方向には索状物が続いていたため可能な限りこれも切
除した．甲状腺や迷走神経，頸神経ワナとの連続は認
めなかった．

　摘出標本所見：表面平滑，暗赤色の液体で充満した
5 cm 大の囊胞で透光性がみられた（**Fig. 4**）．

　病理組織所見：4 cm 大の表面平滑な単房性囊胞で，
内部に壁在結節を認めた（**Fig. 5**）．囊胞壁内と壁在
結節には，異形成に乏しい大小の甲状腺濾胞の集簇を
認め，腺腫様甲状腺腫であった（**Fig. 6**）．明らかな
悪性所見はなく，異所性腺腫様甲状腺腫と診断した．

　経過：術後合併症なく経過し，術後 3 日目に退院さ
れた．術後 1 年経過しているが再発や甲状腺の異常所
見を認めず経過良好である．

考　　察

　頸部に生じる囊胞性病変として，甲状腺の発生過程
で生じる正中頸囊胞や側頸囊胞が鑑別としてあがる．
甲状腺は，胎生 3 週頃に第 1 ・ 2 鰓囊の間（舌盲孔）
で内胚葉から発生し，舌骨と甲状軟骨前方の間を下降，
胎生 7 週頃に甲状腺が気管前面に到達し下降が止ま
る．舌盲孔と甲状腺を結ぶ通り道が甲状舌管であり，
甲状腺が活動を開始する胎生 3 カ月頃に甲状舌管が消
失する[2)3)]．側頸部という部位からまず疑われた側頸
囊胞は，胎生期の鰓裂に由来し，鰓性器官の遺残上皮
（耳下腺，口蓋扁桃，甲状腺，副甲状腺，胸腺，気管支）
がリンパ組織に迷入，もしくはリンパ組織に近接して
囊胞化すると考えられている[4)5)]．病理学的には，重
層扁平上皮や円柱上皮で覆われ，上皮下にリンパ組織
を伴う[4)6)]．側頸囊胞内の異所性甲状腺は発生学的に
ほとんどないと言われている．一方，正中頸囊胞は，
甲状舌管の閉鎖不全や遺残物に由来し，舌骨下部の前
頸筋下に多く認める[7)]．しかし，甲状舌管には稀に側
枝が存在し，その側枝が遺残し正中以外に正中頸囊胞
が生じることがあるとの報告がある[8)]．囊胞壁は円柱
上皮や線毛上皮などが覆い，上皮下に60％程度は正常
甲状腺組織を認める[9)]．本症例も，甲状舌管の側枝の
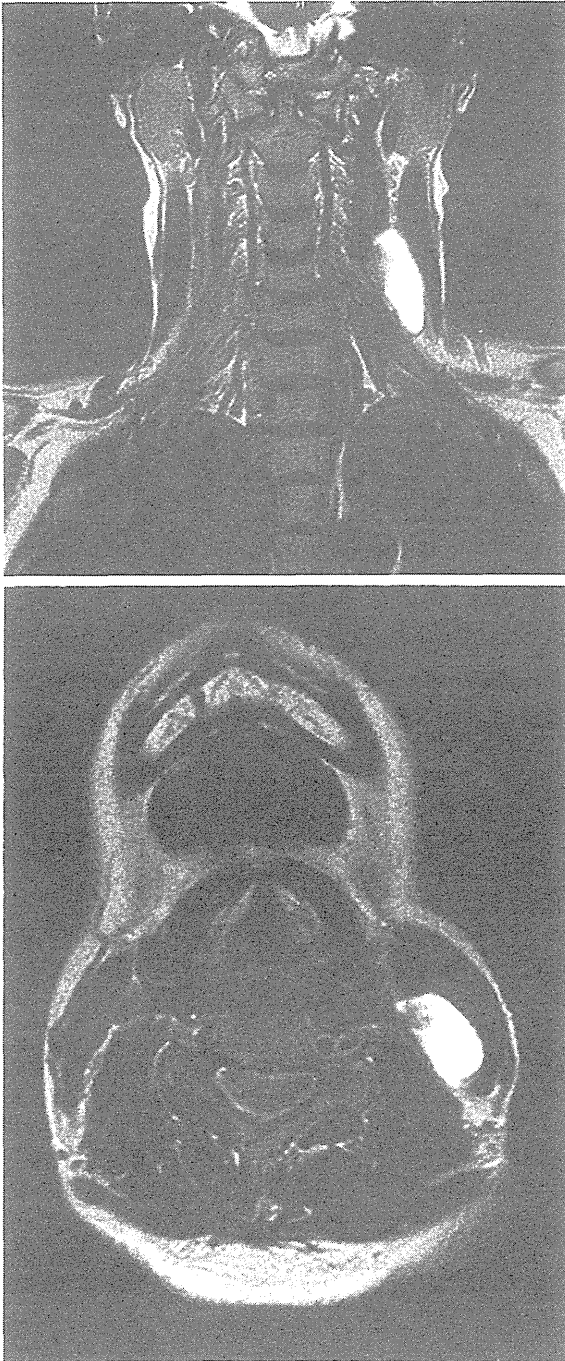
Fig. 3　頸部MRI（T2強調）：内頸静脈背側に境界
　　明瞭な嚢胞性腫瘤を認めた．T2強調画像で内部に
　　低信号領域を認めた．
$$\frac{a}{b}$$

Fig. 4　摘出標本所見：表面平滑，暗赤色の液体で充
　　満した 5 cm の嚢胞であった．
$$\frac{a}{b}$$
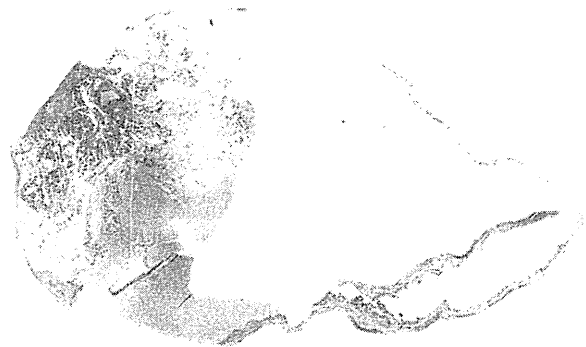
Fig. 5　病理組織所見（ルーペ像）：表面平滑な単房性嚢
　　胞で，内部に壁在結節を認めた．

遺残から生じた正中頸嚢胞の甲状腺組織から腺腫様甲
状腺腫が生じた可能性は否定できない．

　本症例は，術前評価では原発が不明であったが，病
理組織学的に正中頸嚢胞や側頸嚢胞に特異な上皮構造
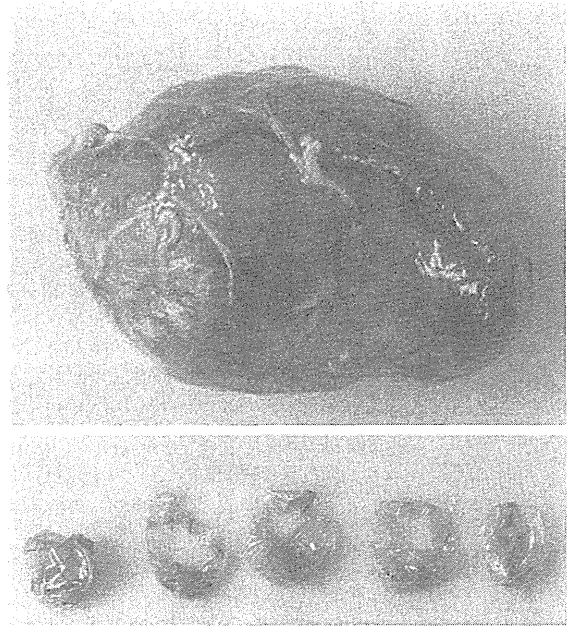はなく，嚢胞壁および壁在結節に甲状腺組織を認めた
ことから，異所性甲状腺に生じた腺腫様甲状腺腫と診

断された．異所性甲状腺は，甲状腺原基の下降障害や
迷入が原因となる．甲状腺原基の下降障害の場合，発
生過程から頸部正中や舌根部に多く認められ，75％は
正常甲状腺を欠くと言われている．迷入性異所性甲状
腺は，甲状腺が固有位置に定着し，甲状腺の被膜形成
をする前に近位組織に迷入することで生じると考えら
れている．迷入部位としては，喉頭・気管・食道・頸
部リンパ節内などがあるが，40％は舌根部という報告
があり，本症例のように，側頸部に認める例は稀であ
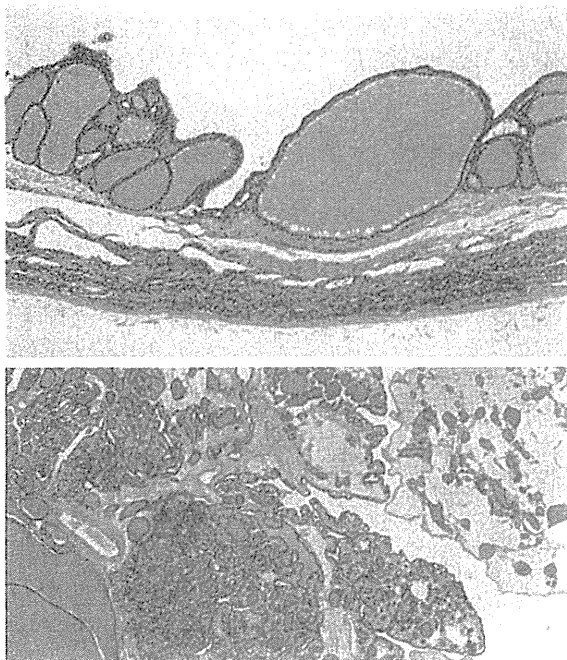る[1)10)]．縦隔内異所性腺腫様甲状腺腫の報告は散見す
るが，側頸部に生じる異所性腺腫様甲状腺腫について
の報告は認めなかった．

－19－

Fig. 6　病理組織所見（拡大）：嚢胞壁（a）および
　　　　壁在結節（b）に異形成に乏しい大小の甲状腺濾
　　　　胞の集簇を認め，腫瘍様甲状腺腫の像であった．

a
―
b

　異所性甲状腺に悪性腫瘍を生じることは稀なうえ，正中から離れた異所性甲状腺に悪性腫瘍を伴うことは，非常に稀と考えられている[10]．しかし，本症例のように嚢胞性病変で，術前に側頸嚢胞を疑い摘出したところ，異所性甲状腺に生じた乳頭癌であったという例も報告されており[10]，稀ではあるが悪性の可能性も少なからずある．また，正中頸嚢胞にも悪性腫瘍を1～2％認めるといった報告もある[11]．さらに，頸部腫瘤には悪性リンパ腫や頭頸部悪性腫瘍のリンパ節転移なども鑑別としてあがる．原発巣は不明だが頸部にリンパ節転移を認める症例は，頭頸部癌の約1～5％を占めていると言われている[12]．また，外側区域リンパ節への転移により発見される甲状腺オカルト癌は0.1～2.2％と稀ではあるが存在する[13]．明らかなリンパ節転移を有するオカルト癌は頸部郭清も含めた甲状腺摘出術が必要と考えられている[13]ため，摘出生検後に転移と診断された場合は，再手術を検討しなければならない．頸部の再手術は癒着により手術困難が予想され，合併症を引き起こすリスクも高い．側頸部腫瘤を認めた場合，画像検査で他の部位にも病変がないかを検索し，できる限り細胞診をするなどして甲状腺癌のリンパ節転移の可能性を十分に検討しておく必要がある．本症例では，甲状腺には異常所見がなく嚢胞性の側頸

部腫瘍であったこと，2回の細胞診で悪性所見を認めなかったことから甲状腺癌のリンパ節転移は積極的には疑わなかった．今回の症例では検討していなかったが，細胞診では診断に至るのが難しい頸部嚢胞性病変に対して，穿刺液中のサイログロブリン値を測定することで診断の補助になるという報告もある[14]．正中頸嚢胞や側頸嚢胞に比べ，甲状腺乳頭癌の嚢胞変性した転移性リンパ節では，嚢胞内液中のサイログロブリン値が高値であることから，この値を測定し画像検査や細胞診に加えて検討することで，より術前診断の精度を向上させる可能性が考えられる．側頸部腫瘍の治療に関しては，保存的治療では，炎症を繰り返し腫瘍が増大傾向を呈することや，疼痛などの症状を引き起こす可能性がある．また，穿刺による内容物の吸引だけでは再発することがあるとの報告もある[15]．

　以上のことから，治療方針としては，悪性を念頭に置き術前に十分精査を行い，外科的摘出が望ましいと考えられる．

結　語

　今回，われわれは側頸部の嚢胞性腫瘍として発見された異所性腺腫様甲状腺腫の稀な1例を経験した．病理組織学的に，腺腫様甲状腺腫が異所性甲状腺に発生したと考えられたが，側頸部に生じた正中頸嚢胞であった可能性もある．

文　献

1) 記本　晃，幸田純治，近藤昭男他：異所性甲状腺に発生したと思われる乳頭癌の1例．耳鼻臨床補冊　1999；101：168-172

2) Moore KL：鰓性器官．MOORE人体発生学，星野一正／訳，医歯薬出版，東京，1977，p178-179

3) Sadler TW：甲状腺．ラングマン人体発生学，沢野十蔵／編，医歯薬出版，東京，1978，p257-258

4) Bhasker SN, Bernier JL：Histogenesis of branchial cysts. A report of 468 cases. Am J Pathol 1959；35：407-423

5) Little JW, Rickles NH：The histogenesis of the branchial cysts. Am J Pathol 1967；50：533-547

6) Paley WG, eddie NC：The aetiology and management of branchial cysts. Br J Surg 1970；57：822-824

7) 平野裕士，篠原正徳，川野芳春他：甲状舌管嚢胞

の臨床的・病理組織学的検索. 日口腔外会誌 1988；34：695−703

8) 坪井佳世子, 神谷祐二, 鈴木晋也他：右側頸部甲状舌管囊胞から発生したと思われる乳頭状腺癌の1例. 日口腔腫瘍会誌　2003；15：51−56

9) Vlaeminck M：Thyroglossal duct carcinoma. Acta Otorhinolaryngol Belg 1977；31：615−621

10) 土屋松実, 関　敦郎, 三澤　清他：側頸部に発生した異所性甲状腺乳頭癌例. 耳鼻臨床　2007；100：919−922

11) 長嶺信夫, 宮城　靖, 正　義之：甲状舌管遺残組織に発生した癌の1例. 癌の臨　1976；22：793−797

12) de Braud F, Heilbrun LK, Ahmed K, et al：Met-astatic squamous cell carcinoma of unknown primary localized to neck. Cancer 1989；64：510−515

13) 河野文彰, 和田俊介, 仙波速見他：転移リンパ節の交感神経鎖浸潤にて術前よりHorner症候群を呈した甲状腺オカルト癌の1例. 日内分泌・甲状腺外会誌　2012；29：71−75

14) 稲垣洋三, 坂本耕二, 井上泰宏他：甲状腺乳頭癌囊胞性頸部リンパ節転移の鑑別診断−穿刺液中サイログロブリン測定の有用性−. 日耳鼻会報 2011；114：912−916

15) 藤村長久, 渡辺正敏, 名倉英明他：鰓囊胞の臨床的, 病理組織学的検討. 日口腔外会誌　1989；35：249−257

A CASE OF ECTOPIC ADENOMATOUS GOITER IN THE LATERAL CERVICAL REGION

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A 41-year-old woman noticed a tumor on the left side of her neck. Cervical ultrasonography showed a cystic mass in the left lateral neck region. The mass was further enlarged at follow-up, and hence, the patient was referred to our hospital. A cystic tumor with an internal anechoic area and clear boundary was seen outside the thyroid gland. No abnormal cells were observed on aspiration cytology. The patient underwent tumorectomy. The tumor was present on the outside of the left internal jugular vein. It could be exfoliated easily. Pathologically, a follicular gland with poor dysplasia in the mural nodule and cyst wall was observed, so a pathological diagnosis of ectopic adenomatous goiter was made. Lateral cervical cystic tumors should be treated by surgical resection because of the possibility of malignancy as well as recurrence after aspiration.

Key words：ectopic adenomatous goiter, lateral cervical cystic tumor

CLINICAL TRIAL

Survival of HER2-positive primary breast cancer patients treated by neoadjuvant chemotherapy plus trastuzumab: a multicenter retrospective observational study (JBCRG-C03 study)

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Abstract We investigated the disease-free survival (DFS) of HER2-positive primary breast cancer patients treated with neoadjuvant chemotherapy plus trastuzumab, as well as predictive factors for DFS and pathologic response. Data from 829 female patients treated between 2001 and 2010 were collected from 38 institutions in Japan. Predictive factors were evaluated using multivariate analyses. The 3-year DFS rate was 87 % [95 % confidence interval (CI) 85–90]. The pathologic complete response (pCR: ypT0/is + ypN0) rate was 51 %. The pCR rate was higher in the ER/PgR-negative patients than in the ER/PgR-positive patients (64 vs. 36 %, $P < 0.001$). Patients with pCR showed a higher DFS rate than patients without pCR (93 vs. 82 %, $P < 0.001$). Multivariate analysis revealed three independent predictors for poorer DFS: advanced nodal stage [hazard ratio (HR) 2.63, 95 % CI 1.36–5.21, $P = 0.004$ for cN2–3 vs. cN0], histological/nuclear grade 3 (HR 1.81, 95 % CI 1.15–2.91, $P = 0.011$), and non-pCR (HR 1.98, 95 % CI 1.22–3.24, $P = 0.005$). In the ER/PgR-negative dataset, non-pCR (HR 2.63, 95 % CI 1.43–4.90, $P = 0.002$) and clinical tumor stage (HR 2.20, 95 % CI 1.16–4.20, $P = 0.017$ for cT3–4 vs. cT1–2) were independent predictors for DFS, and in the ER/PgR-positive dataset, histological grade of 3 (HR 3.09, 95 % CI 1.48–6.62, $P = 0.003$), clinical nodal stage (HR 4.26, 95 % CI 1.53–13.14, $P = 0.005$ for cN2–3 vs. cN0), and young age (HR 2.40, 95 % CI 1.12–4.94, $P = 0.026$ for ≤40 vs. >40) were negative predictors for DFS. Strict pCR (ypT0 + ypN0) was an independent predictor for DFS in

On behalf of the JBCRG-C03 Collaborative Group.

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both the ER/PgR-negative and -positive datasets (HR 2.66, 95 % CI 1.31–5.97, $P = 0.006$ and HR 3.86, 95 % CI 1.13–24.21, $P = 0.029$, respectively). These results may help assure a more accurate prognosis and personalized treatment for HER2-positive breast cancer patients.

Keywords Breast cancer · HER2 · Neoadjuvant chemotherapy · Pathologic complete response · Prognostic factors · Trastuzumab

Introduction

Amplification or overexpression of human epidermal growth factor receptor-2 (HER2) is associated with a high risk of breast cancer recurrence and metastasis [1]. Adjuvant use of cytotoxic chemotherapy and trastuzumab, a recombinant humanized monoclonal antibody that targets HER2, improves the overall survival (OS) and disease-free survival (DFS) of patients with HER2-positive primary breast cancer [2, 3].

Neoadjuvant chemotherapy (NAC) reduces tumor size, which improves the rate of breast-conserving surgery, and provides information about chemosensitivity that helps with the design of postoperative therapy. Several meta-analyses have revealed that patients with a pathologic complete response (pCR) after NAC had higher survival rates than those without pCR, indicating that pCR represents a surrogate prognostic indicator [4–6].

Adding trastuzumab to NAC doubles the rate of pCR in patients with HER2-positive primary breast cancer [7–9]. The NOAH trial showed better 3-year event-free survival for chemotherapy plus trastuzumab versus chemotherapy alone [8]. In the TECHNO trial, patients with pCR after NAC plus trastuzumab showed better 3-year DFS than patients without pCR [10]; however, predictors for pCR and survival after treatment are unknown.

This multicenter retrospective study investigated the survival after NAC with trastuzumab among patients with HER2-positive primary breast cancer in efforts to identify predictive factors.

Patients and methods

Patients

In this multicenter retrospective cohort study, the inclusion criteria were female sex, histologically confirmed HER2-positive invasive breast cancer diagnosed between 2001 and 2010, no distant metastasis, age 20–70 years, and received NAC containing trastuzumab. Eligible patients were identified from the institutional databases. Data were managed by the data center of the Japan Breast Cancer Research Group (JBCRG).

The study protocol was approved by the Institutional Review Board at Kyoto University Hospital and participating institutions. All patient data were anonymized and

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allocated numbers according to Japanese ethics guidelines for epidemiologic research.

Pathological assessment

Pathology specialists at each institution performed the pathological investigation. HER2-positive status was defined as 3+ overexpression by immunohistochemical testing or HER2 amplification by fluorescent in situ hybridization (HER2/CEP17 ratio ≥2.0). At each institution, surgical specimens obtained following NAC were serially sectioned, stained with hematoxylin and eosin (H&E), and diagnosed by experienced pathologists. pCR was defined as the absence of residual invasive cancer cells in the breast and axillary lymph nodes (ypT0/is + ypN0). Strict pCR (spCR), another pCR definition, was defined as no invasive and non-invasive residuals in the breast and axillary nodes (ypT0 + ypN0).

Statistical analysis

All survival outcomes were measured from the date of starting NAC to the date of first event. The primary survival outcome was DFS defined as time to occurrence of recurrence, secondary malignancy (including contralateral breast cancer, hematological malignancy, and sarcoma), or death as a result of any cause. Secondary survival outcomes were OS defined as time to death as a result of any cause, distant recurrence-free survival (DRFS) defined as time to any recurrence except for ipsilateral breast or regional lymph node, and death as a result of any cause.

The Kaplan–Meier method was used to estimate survival outcomes. χ^2 tests for categorical data and log-rank tests for time-to-event endpoints provided two-sided P values, and P values < 0.05 were considered statistically significant. Cox proportional hazards regression analysis was used to estimate hazard ratios (HRs) and 95 % confidence intervals (CIs). Logistic regression was used to estimate odds ratios (ORs) and 95 % CIs. Covariates used in the multivariate model were age, body mass index, clinical tumor stage, clinical nodal stage, estrogen receptor (ER)/progesterone receptor (PgR) status, histological/nuclear grade, pCR/spCR, surgery type, radiation therapy, adjuvant hormonal therapy, adjuvant chemotherapy, and adjuvant trastuzumab. Menopausal status was not included in the model because of collinearity with age. Patients with missing data were excluded from the multivariate analysis (e.g., patients whose adequate pathologic responses were not confirmed due to insufficient local therapy or lack of information regarding local therapy type). All statistical analyses were performed using JMP® (ver. 10.0.2, SAS Institute Inc. Cary, NC, USA). All analyses were supervised by a statistician (SM).

Results

Patient characteristics

Data of 829 patients from 38 institutions in Japan were collected. Among them, 53 did not meet the inclusion criteria and were excluded, leaving a total of 776 patients for analysis (whole dataset). HER2-positive tumors could be subdivided into ER/PgR positive and negative, and we therefore divided the patients into an ER/PgR-positive dataset (N = 334) and ER/PgR-negative dataset (N = 439) and also performed the analyses for each dataset (Fig. 1).
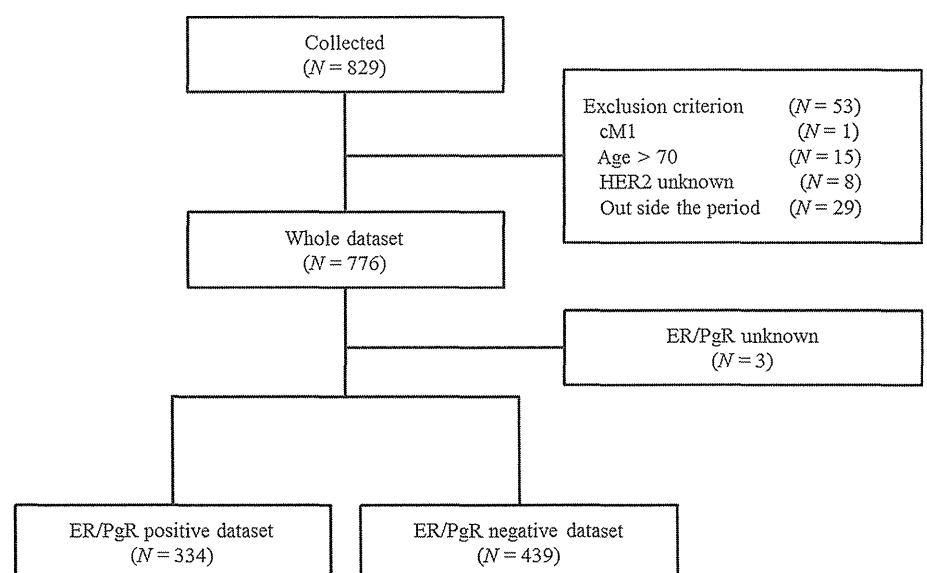
Fig. 1 Flowchart of data collection and analysis

Table 1 Patient, disease, and treatment characteristics

Factors	n	(%)
All cases	776	(100)
Age		
Median (min–max)	53	(25–70)
BMI		
Median (min–max)	22.0	(15.0–47.3)
Unknown	2	(0.3)
Menopausal status		
Pre-menopausal	335	(43.2)
Post-menopausal	422	(54.4)
Unknown	19	(2.4)
Clinical tumor size		
T1b	9	(1.2)
T1c	77	(9.9)
T2	476	(61.3)
T3	122	(15.7)
T4	91	(11.7)
Unknown	1	(0.1)
Clinical nodal status		
N0	252	(32.5)
N1	366	(47.2)
N2	103	(13.3)
N3	54	(7)
Unknown	1	(0.1)
ER/PgR status		
Positive	334	(43)
Negative	439	(56.6)
Unknown	3	(0.4)
Histological/nuclear grade		
1	107	(13.8)
2	184	(23.7)
3	350	(45.1)
Unknown	135	(17.4)
NAC regimen		
Anthracycline and taxane	676	(87.1)
Taxane only	78	(10.1)
Anthracycline only	7	(0.9)
Others	1	(0.1)
Unknown	14	(1.8)
Local therapy		
Mastectomy + XRT	96	(12.4)
Mastectomy alone	181	(23.3)
BCS + XRT	449	(57.9)
BCS alone	44	(5.7)
Needle biopsy + XRT	1	(0.1)
Needle biopsy alone	1	(0.1)
Unknown	4	(0.5)
pCR (ypT0/is + ypN0)		
Yes	399	(51.4)

Table 1 continued

Factors	n	(%)
No	365	(47)
Unknown	12	(1.5)
spCR (ypT0 + ypN0)		
Yes	240	(30.9)
No	525	(67.7)
Unknown	11	(1.4)
Adjuvant hormonal therapy		
Yes	281	(36.2)
No	440	(56.7)
Unknown	55	(7.1)
Adjuvant trastuzumab therapy		
Yes	697	(89.8)
No	65	(8.4)
Unknown	14	(1.8)
Adjuvant chemotherapy		
Yes	45	(5.8)
No	720	(92.8)
Unknown	11	(1.4)

BMI body mass index, *ER/PgR* estrogen receptor/progesterone receptor, *NAC* neoadjuvant chemotherapy, *XRT* radiation therapy, *BCS* breast-conserving surgery, *pCR* pathologic complete response

Baseline characteristics and treatment of the whole dataset are summarized in Table 1. Median age was 53 (range 25–70) years. Most patients had tumor stage T2 (61 %) and were clinically node positive (67 %). ER and PgR were negative in 57 % of the patients. Most patients received anthracycline- and taxane-containing chemotherapy (87 %), and trastuzumab was administered concurrently with taxane (80 %). Breast-conserving surgery was performed in 64 % of the patients, most of whom (91 %) received radiation therapy. Radiation therapy was performed in 35 % of the patients who received mastectomy. Adjuvant hormonal therapy was performed in 86 % of the ER/PgR-positive patients. Most patients received adjuvant trastuzumab (90 %).

Clinical outcomes

The median follow-up period was 42 (interquartile range 30–58) months. For the whole dataset, the 3-year DFS rate was 87 % (95 % CI 85–90) (Fig. 2a). 3-year OS and DRFS were 97 % (95 % CI 96–98) and 91 % (95 % CI 89–93), respectively. pCR was achieved in 399 (51 %) patients and spCR in 240 (31 %) patients.

The 3-year DFS rate was almost the same among patients in the ER/PgR-positive and -negative datasets (87 vs. 88 %, $P = 0.888$) (Fig. 2B). The pCR and spCR rates were higher in the ER/PgR-negative patients than in the ER/PgR-positive
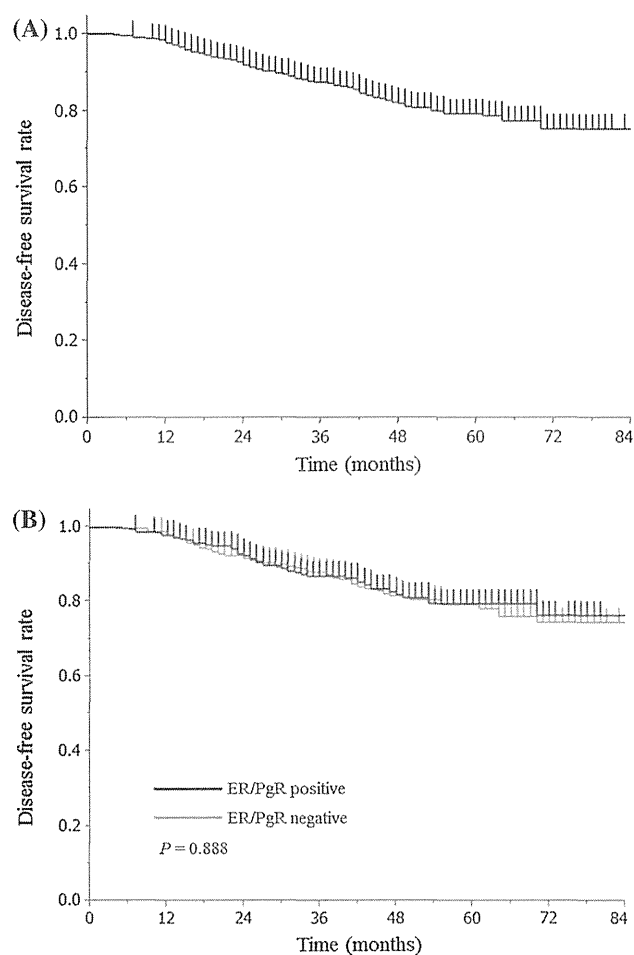
Fig. 2 DFS curves of the a whole dataset and b ER/PgR-positive and -negative datasets

patients (64 vs. 36 % for pCR, $P < 0.001$; 38 vs. 23 % for spCR, $P < 0.001$, respectively).

Prognostic factors for survival outcomes

The results of Cox proportional hazard regression performed to evaluate the prognostic effect of baseline characteristics and pathologic tumor response to NAC with trastuzumab are shown in Table 2. In the whole dataset, independent predictors for poorer DFS were advanced clinical nodal stage (adjusted HR 2.63, 95 % CI 1.36–5.21, $P = 0.004$ for cN2–3 vs. cN0; adjusted HR 1.64, 95 % CI 0.91–3.09, $P = 0.100$ for cN1 vs. cN0), histological/ nuclear grade 3 (adjusted HR 1.81, 95 % CI 1.15–2.91, $P = 0.011$), and failure to achieve pCR (adjusted HR 1.98, 95 % CI 1.22–3.24, $P = 0.005$). Neither age nor ER/PgR status was an independent predictor for DFS. Multivariate analysis including spCR yielded the same results. The DFS rate was higher among patients with pCR than those without pCR (93 vs. 82 %, $P < 0.001$) (Fig. 3a). Patients

who achieved spCR had a higher DFS rate than those who did not (96 vs. 84 %, $P < 0.001$) (Fig. 3b).

In the ER/PgR-positive dataset, independent predictors for poorer DFS were advanced clinical nodal stage, histological/ nuclear grade 3, young age (≤ 40), and not achieving spCR. pCR was not an independent predictor for DFS on multivariate analysis (Table 2; Fig. 3c, d). For the ER/PgR-negative dataset, clinical tumor stage and both pCR and spCR were independent predictors for DFS (Table 2; Fig. 3e, f).

Predictors for other survival outcomes are listed in Supplementary Table S1. Predictors for OS were clinical nodal stage, histological/nuclear grade, and spCR, but pCR was not an independent predictor. Predictors for DRFS were clinical nodal stage, histological/nuclear grade, young age, pCR, and spCR.

Predictive factors for pCR

The association of baseline characteristics with pCR/spCR following NAC plus trastuzumab was evaluated by multivariate logistic regression (Table 3). In the whole dataset, independent predictors for pCR were negative ER/PgR status (adjusted OR 3.42, 95 % CI 2.42–4.86, $P < 0.001$) and clinical tumor stage T1–2 compared with T3–4 (adjusted OR 1.88, 95 % CI 1.27–2.79, $P = 0.002$). Histological/nuclear grade 3 showed a statistically marginal association with pCR (adjusted OR 1.39, 95 % CI 0.99–1.95, $P = 0.060$). The same factors were selected as independent predictors in the multivariate model for spCR.

In the ER/PgR-positive dataset, clinical tumor stage was a predictor for pCR and spCR. In the ER/PgR-negative dataset, clinical tumor stage was an independent predictor for both pCR and spCR. Histological/nuclear grade was marginally predictive of pCR and spCR.

Discussion

In this analysis, we assessed survival after NAC plus trastuzumab among patients with HER2-positive breast cancer. Although clinical nodal status, histological/nuclear grade, and pCR/spCR were independent predictors for DFS, the prognostic impact differed depending on ER/PgR status. pCR was a predictor for DFS particularly in patients with ER/PgR-negative tumor, and spCR—a stricter definition of pCR—was an independent prognostic factor regardless of ER/PgR status.

Our data included more patients with clinical tumor stage T2 or higher (89 %) and clinically node positive (67 %). In this population, a 3-year DFS rate of 87 % was relatively good; however, a considerable number of patients experienced disease relapse during the follow-up period. Risk factors associated with disease relapse need to

| Table 2 Adjusted hazard ratios of factors predicting DFS	Factor	pCR (ypT0/is + ypN0)			spCR (ypT0 + ypN0)		
		HR	95 % CI	P value	HR	95 % CI	P value
	Whole dataset						
	Age						
	≤40 vs. >40	1.67	(0.95–2.81)	0.074	1.63	(0.93–2.75)	0.088
	BMI						
	25≤ vs. <22	1.31	(0.74–2.24)	0.351	1.31	(0.74–2.24)	0.348
	22≤ , <25 vs. <22	0.96	(0.56–1.61)	0.891	1.00	(0.58–1.67)	0.993
	Clinical tumor size						
	T3–4 vs. T1–2	1.53	(0.93–2.49)	0.093	1.42	(0.87–2.32)	0.160
	Clinical nodal status						
	N2–3 vs. N0	2.63	(1.36–5.21)	0.004	2.58	(1.34–5.12)	0.004
	N1 vs. N0	1.64	(0.91–3.09)	0.100	1.73	(0.96–3.26)	0.070
	ER/PgR						
	Negative vs. positive	0.97	(0.47–2.08)	0.933	0.93	(0.46–1.96)	0.842
	Histological/Nuclear grade						
	3 vs. 1&2	1.81	(1.15–2.91)	0.011	1.77	(1.12–2.84)	0.014
	pCR/spCR						
	Non-pCR vs. pCR	1.98	(1.22–3.24)	0.005	2.90	(1.57–5.90)	<0.001
	ER/PgR-positive dataset						
	Age						
	≤40 vs. >40	2.40	(1.12–4.94)	0.026	2.33	(1.08–4.80)	0.031
	BMI						
	25≤ vs. <22	1.49	(0.63–3.38)	0.354	1.54	(0.66–3.45)	0.313
	22≤, <25 vs. <22	0.69	(0.25–1.67)	0.419	0.69	(0.25–1.68)	0.433
	Clinical tumor size						
	T3–4 vs. T1–2	0.83	(0.35–1.88)	0.653	0.69	(0.28–1.62)	0.399
	Clinical nodal status						
	N2–3 vs. N0	4.26	(1.53–13.14)	0.005	4.54	(1.62–14.13)	0.004
	N1 vs. N0	2.55	(0.99–7.43)	0.053	2.83	(1.08–8.39)	0.034
	Histological/Nuclear grade						
	3 vs. 1&2	3.09	(1.48–6.62)	0.003	3.14	(1.49–6.85)	0.003
	pCR/spCR						
	Non-pCR vs. pCR	1.20	(0.57–2.69)	0.634	3.86	(1.13–24.21)	0.029
	ER/PgR-negative dataset						
	Age						
	≤40 vs. >40	0.95	(0.35–2.18)	0.913	1.01	(0.38–2.28)	0.979
	BMI						
	25≤ vs. <22	0.94	(0.39–2.05)	0.886	0.97	(0.40–2.11)	0.942
	22≤, <25 vs. <22	1.10	(0.56–2.08)	0.774	1.10	(0.56–2.08)	0.779
	Clinical tumor size						
	T3–4 vs. T1–2	2.20	(1.16–4.20)	0.017	2.11	(1.11–4.04)	0.024
	Clinical nodal status						
	N2–3 vs. N0	2.04	(0.85–5.07)	0.112	1.73	(0.73–4.27)	0.217
	N1 vs. N0	1.49	(0.70–3.38)	0.306	1.39	(0.66–3.13)	0.398
BMI body mass index, ER/PgR estrogen receptor/progesterone receptor, pCR pathologic complete response, spCR strict pathologic complete response, HR hazard ratio	Histological/Nuclear grade						
	3 vs. 1&2	1.33	(0.74–2.48)	0.354	1.29	(0.72–2.41)	0.393
	pCR/spCR						
	Non-pCR vs. pCR	2.63	(1.43–4.90)	0.002	2.66	(1.31–5.97)	0.006
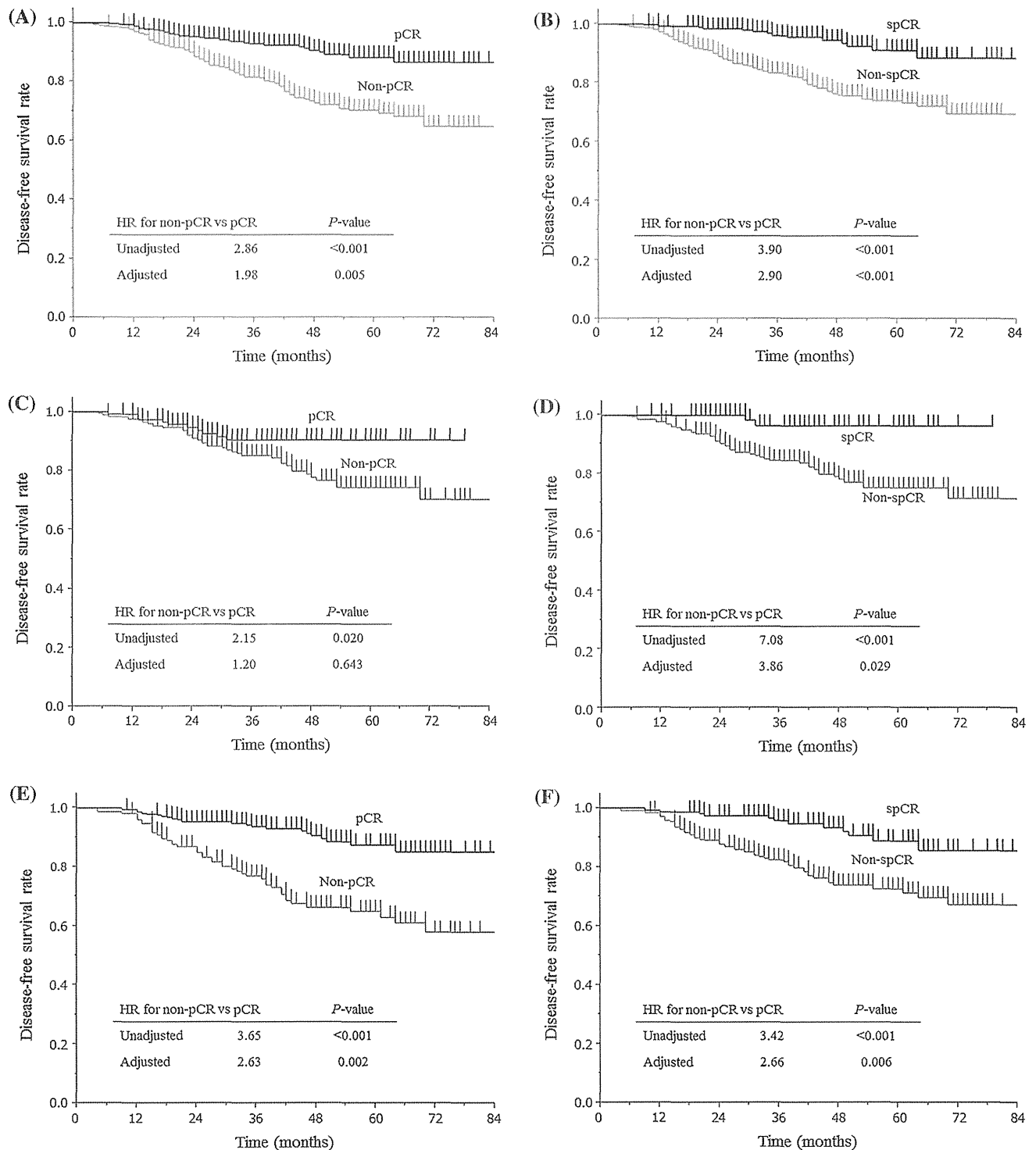
Fig. 3 DFS curves of patients with pCR (ypT0/is + ypN0) versus non-pCR in the **a** whole dataset, **c** ER/PgR-positive dataset, and **e** ER/PgR-negative dataset. DFS curves of patients with spCR (ypT0 + ypN0) versus non-spCR in the **b** whole dataset, **d** ER/PgR-positive dataset, and **f** ER/PgR-negative dataset

be clarified to conduct a clinical trial aimed at improving these patients' prognosis.

In two phase-III trials in which patients with HER2-positive disease were randomly allocated to NAC with trastuzumab or NAC only, the addition of trastuzumab to NAC resulted in a higher pCR rate and improved DFS [8, 11]. The pCR rate in our study (51 %) is comparable to those reported in previous trials of NAC with trastuzumab

Table 3 Adjusted odds ratios of factors predicting pCR

Factor	pCR (ypT0/is + ypN0)			spCR (ypT0 + ypN0)		
	OR	95 % CI	P value	OR	95 % CI	P value
Whole dataset						
Age						
>40 vs. ≤40	0.97	(0.60–1.58)	0.907	1.45	(0.84–2.63)	0.191
BMI						
25≤ vs. <22	1.22	(0.78–1.91)	0.388	1.31	(0.80–2.11)	0.280
22≤, <25 vs. <22	1.38	(0.94–2.04)	0.100	1.47	(0.98–2.21)	0.062
Clinical tumor size						
T1–2 vs. T3–4	1.88	(1.27–2.79)	0.002	2.16	(1.39–3.41)	0.001
Clinical nodal status						
N0 vs. N2–3	0.65	(0.40–1.07)	0.093	0.98	(0.57–1.71)	0.942
N1 vs. N2–3	0.83	(0.53–1.31)	0.435	1.44	(0.88–2.39)	0.152
ER/PgR status						
Negative vs. positive	3.42	(2.42–4.86)	<0.001	2.27	(1.55–3.35)	<0.001
Histological/Nuclear grade						
3 vs. 1&2	1.39	(0.99–1.95)	0.060	1.29	(0.90–1.88)	0.169
ER/PgR-positive dataset						
Age						
>40 vs. ≤40	0.74	(0.40–1.39)	0.343	1.22	(0.56–2.89)	0.622
BMI						
25≤ vs. <22	1.65	(0.85–3.20)	0.140	1.27	(0.56–2.81)	0.559
22≤, <25 vs. <22	1.43	(0.77–2.61)	0.253	1.46	(0.71–2.97)	0.296
Clinical tumor size						
T1–2 vs. T3–4	1.76	(0.94–3.43)	0.078	2.95	(1.28–7.72)	0.010
Clinical nodal status						
N0 vs. N2–3	0.98	(0.46–2.11)	0.954	0.89	(0.36–2.32)	0.810
N1 vs. N2–3	0.80	(0.39–1.67)	0.547	0.93	(0.39–2.35)	0.869
Histological/Nuclear grade						
3 vs. 1&2	1.22	(0.73–2.05)	0.454	1.00	(0.54–1.86)	0.991
ER/PgR-negative dataset						
Age						
>40 vs. ≤40	1.43	(0.68–2.94)	0.344	1.73	(0.80–4.08)	0.170
BMI						
25≤ vs. <22	0.95	(0.52–1.76)	0.871	1.29	(0.69–2.36)	0.422
22≤, <25 vs. <22	1.35	(0.81–2.27)	0.248	1.47	(0.89–2.43)	0.132
Clinical tumor size						
T1–2 vs. T3–4	1.93	(1.17–3.20)	0.010	1.89	(1.13–3.24)	0.016
Clinical nodal status						
N0 vs. N2–3	0.48	(0.24–0.92)	0.027	0.98	(0.49–1.95)	0.943
N1 vs. N2–3	0.89	(0.48–1.61)	0.692	1.75	(0.97–3.26)	0.065
Histological/Nuclear grade						
3 vs. 1&2	1.53	(0.97–2.42)	0.068	1.50	(0.94–2.40)	0.087

BMI body mass index, *ER/PgR* estrogen receptor/progesterone receptor, *pCR* pathologic complete response, *spCR* strict pathologic complete response, *OR* odds ratio

(30–67 %) [7–10, 12–15]. In our study, ER/PgR status was the strongest predictor for pCR or spCR. Our results were consistent with those of two meta-analyses in which the pCR rate of NAC with trastuzumab was about 50 % for patients with ER/PgR-negative disease and 30 % for those with ER/PgR-positive disease [6, 16].

In the TECHNO trial, a phase-II trial of 217 patients with HER2-positive disease who received NAC with trastuzumab, failure to achieve pCR was a significant predictor for DFS in the multivariate analysis [10]. Kim et al. [12] retrospectively investigated the prognostic value of pCR using data from 229 patients with HER2-positive

tumor who were treated with NAC with trastuzumab. They reported that pCR, clinical tumor stage, and lymphovascular invasion were independent predictors for DFS. In our study, pCR and spCR were predictors for DFS; in addition, conventional prognostic factors such as nodal stage and histological/nuclear grade were predictors for DFS.

In this study, the association of age with DFS was not statistically significant in the whole dataset, consistent with the results of the TECHNO trial and Kim et al. Partridge et al. [17] reported that young age was not associated with worse DFS in patients with HER2-positive disease using large cohort data from the HERA trial. When we divided the patients into ER/PgR-positive and -negative groups, multivariate analysis showed that young age (age ≤40) was an independent predictor for poorer DFS in the ER/PgR-positive dataset. Our result was consistent with earlier studies showing that younger age is an independent predictor for worse DFS, especially in patients with ER/PgR-positive disease [18, 19].

After dividing the patients into ER/PgR-positive and -negative datasets, we performed multivariate analysis for DFS using each dataset. About 30–40 % of HER2-enriched subtype tumors are reported to be ER positive [20, 21]. Among clinically HER2-positive tumors, up to 60 % are classified as the HER2-enriched subtype, with the rest classified as luminal B, luminal A, or basal-like [22]. Adjuvant systemic therapy differs according to ER/PgR status [23]. Therefore, it seemed reasonable to perform the analysis based on ER/PgR status; however, the results should be interpreted carefully because of the relatively small event rate in each dataset.

In relation to the two aforementioned meta-analyses, pooled analysis from the German study group [6] indicated that pCR was a prognostic factor for the HER2-positive non-luminal subgroup, but not for those in the HER2-positive luminal subgroup. In the meta-analysis from the Collaborative Trials in Neoadjuvant Breast Cancer (CTNeoBC) [16], there was a stronger association of pCR with event-free survival in the HER2-positive non-luminal subgroup compared with those in the HER2-positive luminal subgroup. In our study, pCR was an independent predictor for DFS in the ER/PgR-negative dataset, but not ER/PgR-positive dataset, and spCR was an independent predictor for DFS regardless of ER/PgR status.

The limitations of this study include its retrospective design. Adjustment using multivariate analysis is mandatory to minimize selection bias. The relatively short observation period may also limit the interpretation of our results. The median follow-up period of our study (42 months) covered the time when recurrence risk is high in HER2-positive disease [24]. Strength of our study was the large number of patients, which allowed us to conduct

multivariate analysis separately according to ER/PgR status.

In conclusion, pCR/spCR, nodal status, and grade were predictors for DFS in patients with HER2-positive disease treated with NAC plus trastuzumab. Response to therapy and prognostic impact of the factors differed according to ER/PgR status. Our results may help identify patients who are not likely to achieve pCR or whose outcome would otherwise be unfavorable. New treatment approaches, such as the incorporation of novel anti-HER2 drugs, are needed for patients with high-risk disease.

References

1. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL (1987) Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 235(4785):177–182
2. Costa RB, Kurra G, Greenberg L, Geyer CE (2010) Efficacy and cardiac safety of adjuvant trastuzumab-based chemotherapy regimens for HER2-positive early breast cancer. Ann Oncol 21(11):2153–2160. doi:10.1093/annonc/mdq096
3. Moja L, Tagliabue L, Balduzzi S, Parmelli E, Pistotti V, Guarneri V, D'Amico R (2012) Trastuzumab containing regimens for early breast cancer. Cochrane Database Syst Rev 4:CD006243. doi:10.1002/14651858.CD006243.pub2

4. Mauri D, Pavlidis N, Ioannidis JP (2005) Neoadjuvant versus adjuvant systemic treatment in breast cancer: a meta-analysis. J Natl Cancer Inst 97(3):188–194. doi:10.1093/jnci/dji021

5. Mieog JS, van der Hage JA, van de Velde CJ (2007) Preoperative chemotherapy for women with operable breast cancer. Cochrane Database Syst Rev 2:CD005002. doi:10.1002/14651858.CD00 5002.pub2

6. von Minckwitz G, Untch M, Blohmer JU, Costa SD, Eidtmann H, Fasching PA, Gerber B, Eiermann W, Hilfrich J, Huober J, Jackisch C, Kaufmann M, Konecny GE, Denkert C, Nekljudova V, Mehta K, Loibl S (2012) Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. J Clin Oncol 30(15):1796–1804. doi:10.1200/jco.2011.38.8595

7. Buzdar AU, Ibrahim NK, Francis D, Booser DJ, Thomas ES, Theriault RL, Pusztai L, Green MC, Arun BK, Giordano SH, Cristofanilli M, Frye DK, Smith TL, Hunt KK, Singletary SE, Sahin AA, Ewer MS, Buchholz TA, Berry D, Hortobagyi GN (2005) Significantly higher pathologic complete remission rate after neoadjuvant therapy with trastuzumab, paclitaxel, and epirubicin chemotherapy: results of a randomized trial in human epidermal growth factor receptor 2-positive operable breast cancer. J Clin Oncol 23(16):3676–3685. doi:10.1200/JCO.2005. 07.032

8. Gianni L, Eiermann W, Semiglazov V, Manikhas A, Lluch A, Tjulandin S, Zambetti M, Vazquez F, Byakhow M, Lichinitser M, Climent MA, Ciruelos E, Ojeda B, Mansutti M, Bozhok A, Baronio R, Feyereislova A, Barton C, Valagussa P, Baselga J (2010) Neoadjuvant chemotherapy with trastuzumab followed by adjuvant trastuzumab versus neoadjuvant chemotherapy alone, in patients with HER2-positive locally advanced breast cancer (the NOAH trial): a randomised controlled superiority trial with a parallel HER2-negative cohort. Lancet 375(9712):377–384. doi:10.1016/s0140-6736(09)61964-4

9. Untch M, Rezai M, Loibl S, Fasching PA, Huober J, Tesch H, Bauerfeind I, Hilfrich J, Eidtmann H, Gerber B, Hanusch C, Kuhn T, du Bois A, Blohmer JU, Thomssen C, Dan Costa S, Jackisch C, Kaufmann M, Mehta K, von Minckwitz G (2010) Neoadjuvant treatment with trastuzumab in HER2-positive breast cancer: results from the GeparQuattro study. J Clin Oncol 28(12):2024–2031. doi:10.1200/JCO.2009.23.8451

10. Untch M, Fasching PA, Konecny GE, Hasmuller S, Lebeau A, Kreienberg R, Camara O, Muller V, du Bois A, Kuhn T, Stickeler E, Harbeck N, Hoss C, Kahlert S, Beck T, Fett W, Mehta KM, von Minckwitz G, Loibl S (2011) Pathologic complete response after neoadjuvant chemotherapy plus trastuzumab predicts favorable survival in human epidermal growth factor receptor 2-overexpressing breast cancer: results from the TECHNO trial of the AGO and GBG study groups. J Clin Oncol 29(25):3351–3357. doi:10.1200/JCO.2010.31.4930

11. Buzdar AU, Valero V, Ibrahim NK, Francis D, Broglio KR, Theriault RL, Pusztai L, Green MC, Singletary SE, Hunt KK, Sahin AA, Esteva F, Symmans WF, Ewer MS, Buchholz TA, Hortobagyi GN (2007) Neoadjuvant therapy with paclitaxel followed by 5-fluorouracil, epirubicin, and cyclophosphamide chemotherapy and concurrent trastuzumab in human epidermal growth factor receptor 2-positive operable breast cancer: an update of the initial randomized study population and data of additional patients treated with the same regimen. Clin Cancer Res 13(1):228–233. doi:10.1158/1078-0432.CCR-06-1345

12. Kim MM, Allen P, Gonzalez-Angulo AM, Woodward WA, Meric-Bernstam F, Buzdar AU, Hunt KK, Kuerer HM, Litton JK, Hortobagyi GN, Buchholz TA, Mittendorf EA (2013) Pathologic complete response to neoadjuvant chemotherapy with trastuzumab predicts for improved survival in women with HER2-overexpressing breast cancer. Ann Oncol 24(8):1999–2004. doi:10.1093/annonc/mdt131

13. Untch M, Loibl S, Bischoff J, Eidtmann H, Kaufmann M, Blohmer JU, Hilfrich J, Strumberg D, Fasching PA, Kreienberg R, Tesch H, Hanusch C, Gerber B, Rezai M, Jackisch C, Huober J, Kuhn T, Nekljudova V, von Minckwitz G (2012) Lapatinib versus trastuzumab in combination with neoadjuvant anthracycline-taxane-based chemotherapy (GeparQuinto, GBG 44): a randomised phase 3 trial. Lancet Oncol 13(2):135–144. doi:10. 1016/s1470-2045(11)70397-7

14. Baselga J, Bradbury I, Eidtmann H, Di Cosimo S, de Azambuja E, Aura C, Gomez H, Dinh P, Fauria K, Van Dooren V, Aktan G, Goldhirsch A, Chang TW, Horvath Z, Coccia-Portugal M, Domont J, Tseng LM, Kunz G, Sohn JH, Semiglazov V, Lerzo G, Palacova M, Probachai V, Pusztai L, Untch M, Gelber RD, Piccart-Gebhart M (2012) Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): a randomised, open-label, multicentre, phase 3 trial. Lancet 379(9816):633–640. doi:10.1016/s0140-6736(11)61847-3

15. Gianni L, Pienkowski T, Im YH, Roman L, Tseng LM, Liu MC, Lluch A, Staroslawska E, de la Haba-Rodriguez J, Im SA, Pedrini JL, Poirier B, Morandi P, Semiglazov V, Srimuninnimit V, Bianchi G, Szado T, Ratnayake J, Ross G, Valagussa P (2012) Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial. Lancet Oncol 13(1):25–32. doi:10.1016/ S1470-2045(11)70336-9

16. Cortazar P, Zhang L, Untch M, Mehta K, Costantino JP, Wolmark N, Bonnefoi H, Cameron D, Gianni L, Valagussa P, Swain SM, Prowell T, Loibl S, Wickerham DL, Bogaerts J, Baselga J, Perou C, Blumenthal G, Blohmer J, Mamounas EP, Bergh J, Semiglazov V, Justice R, Eidtmann H, Paik S, Piccart M, Sridhara R, Fasching PA, Slaets L, Tang S, Gerber B, Geyer CE, Pazdur R, Ditsch N, Rastogi P, Eiermann W, von Minckwitz G (2014) Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. Lancet. doi:10.1016/s0140-6736(13)62422-8

17. Partridge AH, Gelber S, Piccart-Gebhart MJ, Focant F, Scullion M, Holmes E, Winer EP, Gelber RD (2013) Effect of age on breast cancer outcomes in women with human epidermal growth factor receptor 2-positive breast cancer: results from a herceptin adjuvant trial. J Clin Oncol 31(21):2692–2698. doi:10.1200/JCO. 2012.44.1956

18. Ahn SH, Son BH, Kim SW, Kim SI, Jeong J, Ko SS, Han W, Korean Breast Cancer Society (2007) Poor outcome of hormone receptor-positive breast cancer at very young age is due to tamoxifen resistance: nationwide survival data in Korea–a report from the Korean Breast Cancer Society. J Clin Oncol 25(17):2360–2368. doi:10.1200/JCO.2006.10.3754

19. Colleoni M, Rotmensz N, Peruzzotti G, Maisonneuve P, Orlando L, Ghisini R, Viale G, Pruneri G, Veronesi P, Luini A, Intra M, Cardillo A, Torrisi R, Rocca A, Goldhirsch A (2006) Role of endocrine responsiveness and adjuvant therapy in very young women (below 35 years) with operable breast cancer and node negative disease. Ann Oncol 17(10):1497–1503. doi:10.1093/ annonc/mdl145

20. Parker JS, Mullins M, Cheang MC, Leung S, Voduc D, Vickery T, Davies S, Fauron C, He X, Hu Z, Quackenbush JF, Stijleman IJ, Palazzo J, Marron JS, Nobel AB, Mardis E, Nielsen TO, Ellis MJ, Perou CM, Bernard PS (2009) Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol 27(8):1160–1167. doi:10.1200/JCO.2008.18.1370

21. Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, Thorsen T, Quist

H, Matese JC, Brown PO, Botstein D, Lonning PE, Borresen-Dale AL (2001) Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA 98(19):10869–10874. doi:10.1073/pnas.191367098

22. Perou CM (2011) Molecular stratification of triple-negative breast cancers. Oncologist 16(Suppl 1):61–70. doi:10.1634/theoncologist.2011-S1-61

23. Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thurlimann B, Senn HJ, Panel M (2013) Personalizing the treatment of women with early breast cancer: highlights of the St.

Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. Ann Oncol 24(9):2206–2223. doi:10.1093/annonc/mdt303

24. Metzger-Filho O, Sun Z, Viale G, Price KN, Crivellari D, Snyder RD, Gelber RD, Castiglione-Gertsch M, Coates AS, Goldhirsch A, Cardoso F (2013) Patterns of Recurrence and outcome according to breast cancer subtypes in lymph node-negative disease: results from international breast cancer study group trials VIII and IX. J Clin Oncol 31(25):3083–3090. doi:10.1200/JCO.2012.46.1574

Evaluating the 21-gene assay Recurrence Score® as a predictor of clinical response to 24 weeks of neoadjuvant exemestane in estrogen receptor-positive breast cancer

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Abstract

Background The aim of this study was to investigate the association between the results of the Recurrence Score (RS) assay and the clinical response to neoadjuvant endocrine therapy in postmenopausal women with breast cancer.

Methods Core biopsy samples at baseline and post-treatment surgical samples were obtained from 80 and 77 of 116 patients, respectively, enrolled in the multicenter prospective study of neoadjuvant exemestane therapy (JFMC34-0601). The 21-gene assay was performed after appropriate manual microdissection. The estrogen receptor

(ER), progesterone receptor, HER2 and Ki-67 were assayed by immunohistochemistry at a central laboratory. Clinical response was assessed based on the RECIST (Response Evaluation Criteria In Solid Tumors) guideline.

Results Sixty-four core biopsy samples and 52 resection samples met the RS quality requirements. The clinical response rate in those patients with a low RS result (low RS group; 19/32, 59.4 %) was significantly higher than that in those patients with a high RS result (high RS group; 3/15, 20.0 %) ($P = 0.015$) and similar to that in patients with an intermediate RS result (intermediate RS group; 10/17, 58.8 %). The rates of breast-conserving surgery (BCS) were 90.6 % (29/32) in the low RS group, 76.5 % (13/17) in the intermediate RS group and 46.7 % (7/15) in the high RS group. The odds ratio for BCS adjusted for continuous

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baseline Ki-67 was 0.114 [95 % confidence interval (CI) 0.014–0.721; $P = 0.028$] between the high and low RS groups. RS values in pre-treatment samples were highly correlated with those in post-treatment samples (Spearman correlation coefficient 0.745, 95 % CI 0.592–0.846).

Conclusion Our results demonstrate the predictive value of the RS for clinical response to neoadjuvant exemestane therapy in postmenopausal women with ER-positive breast cancer.

Keywords Recurrence Score · Neoadjuvant endocrine therapy · Ki-67 · Clinical response · Breast-conserving surgery rate

Introduction

There are several potential advantages to neoadjuvant therapy of breast cancer in terms of improving outcomes in women with operable and inoperable early-stage disease [1, 2]. Both neoadjuvant chemotherapy and endocrine therapy have been shown to enable less extensive resection and improve rates of breast-conserving surgery (BCS) [3–6]. The ACOSOG Z1031 trial, which compared three aromatase inhibitors (AIs) in neoadjuvant settings, showed that 51 % (81/159) of the patients who were designated candidates for mastectomy experienced downstaging to BCS [7]. Neoadjuvant endocrine therapy is now an acceptable option for postmenopausal patients with endocrine-responsive disease [8].

Despite the use of standard biomarkers, the considerable heterogeneity of response to therapy still represents a challenge to clinicians in terms of choosing the most suitable neoadjuvant therapy. As such, tools to improve the identification of those patients who will respond to therapy would represent a major clinical advance. Although the Ki-67 labeling index (LI) shows some consistency in predicting response to chemotherapy, its ability to predict response to neoadjuvant endocrine therapy is controversial [9, 10].

We previously reported results from a neoadjuvant exemestane study in postmenopausal women [11]. In that study, the target response rate was 51 % (59/116), and 40 (77 %) of 59 patients who would have required mastectomy were converted to BCS. Neither baseline Ki-67 LI nor changes in Ki-67 LI were associated with clinical response in the study.

The Onco*type* DX® assay (Genomic Health, Redwood City, CA) has been shown to be able assess recurrence risk in women with hormone receptor-positive (HR+), lymph node-negative or -positive, early stage breast cancer who are treated with adjuvant endocrine therapy [12–15]. It has also been shown to predict the likelihood of benefit from

adjuvant chemotherapy [12, 16]. Accordingly, the assay is included in clinical guidelines for use in patients with HR+ lymph node-negative disease; however, its applicability to HR+ postmenopausal women with lymph node positive disease is considered controversial, pending results of the RxPONDER trial [8, 17–19]. Additionally, studies in the neoadjuvant setting have shown that the test can be used to predict the response to chemotherapy [20, 21]. More recently, a study suggested that the Recurrence Score (RS) value may predict responses to neoadjuvant endocrine therapy with either tamoxifen or anastrozole [22]. The Onco*type* DX assay may improve the clinician's ability to discriminate between clinically similar tumors based on the tumor's underlying biology. Consequently, the aim of this study was to investigate the clinical usefulness of the RS assay results in the prediction of response to neoadjuvant endocrine therapy.

Methods

Study design

This was a prospectively designed study using archived tumor tissues from the previously conducted JFMC34-0601 study. The primary objective was to assess the association between the results of the RS assay at baseline and clinical response, by comparing the response rates between patients with a low RS result (<18; low RS group) and those with a high RS result (≥31; high RS group). Secondary objectives included assessment of the associations of continuous baseline RS, quantitative estrogen receptor (ER) by reverse transcriptase (RT)-PCR and Ki-67 with clinical response and with BCS, as well as associations of changes from baseline to post-treatment values of these markers with clinical response. The study protocol was approved by the Ethics Committee of each participating institution. Informed consent was obtained from all patients. The study was performed in accordance with the Helsinki Declaration.

Patient cohort and tumor samples

Eligibility criteria for the parent JFMC34-0601 study included age 55–75 years, ER+ and stage II or IIIa invasive breast cancer (T2-3, N0-2, M0). Patients were confirmed positive for ER or progesterone receptor (PgR) by immunohistochemistry (≥10 % nuclear staining). The study treatment was 25 mg/day exemestane for 16 weeks, with a possible 8-week extension based on the assessment of clinical response. Patients with progressive disease (PD) were withdrawn from the study. At week 24, patients underwent surgery, except those with PD, who had the option of selecting another treatment approach.

Clinical outcomes measures

Clinical response

Clinical response was assessed by comparing the longest diameter of the target lesions with the baseline measurement, based on the Response Evaluation Criteria in Solid Tumors (RECIST) guideline version 1.0, by caliper measurement of palpable lesions and ultrasound as previously described [11]. Briefly, complete response (CR) was defined by the disappearance of all target lesions; partial response (PR) by at least a 30 % decrease in the sum of diameters of the target lesions; PD by at least a 20 % increase in the sum of diameters of the target lesions; stable disease (SD) by neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD.

Biomarker assessments

The Onco*type* DX® 21-gene assay was performed on core biopsy and resection samples by Genomic Health [14].

Immunohistochemistry assays of Ki-67, ER and PgR were performed at one central location and the results assessed by three independent pathologists as described previously [11]. In brief, immunohistochemistry staining was performed using a Histofine kit (Nichirei, Tokyo, Japan). Ki-67 was stained using the following antibody dilution: 1:100 (Dako, Glostrup, Denmark), and the Ki-67 LI was obtained by counting 500–1,000 tumor cells at the sites of hot spots. Ki-67 groups were defined post hoc as <10, 10–30 and >30 %, respectively. ER and PgR immunoreactivity were scored according to Allred's procedure.

Expression of HER2 was determined by the HercepTest (Dako, Glostrup, Denmark). Positive HER2 status was defined as either 3+ or 2+ with confirmed c-erbB2 gene amplification by the fluorescence in situ hybridization (FISH) test.

Statistical analyses

Analyses of baseline markers included all patients with an evaluable RT-PCR result from core biopsies. Analyses of changes from baseline to post-treatment markers included the subset of patients with results from both core biopsies and surgical resections. Changes in continuous markers were defined as "post-treatment value–pre-treatment value". In the primary analysis, the rates of clinical response were compared between the high and low baseline RS groups using Fisher's exact test. Logistic regression models were fit to both clinical response and surgery type. Odds ratio (OR) estimates are presented with Wald *p* values and 95 % confidence intervals (CIs). All *P* values are two-sided. In exploratory analyses, the Spearman rank correlation coefficient (and associated 95 % CI) was calculated for the baseline continuous RS and either the post-treatment RS or baseline continuous Ki-67 as determined by immunohistochemistry. A paired *t* test was applied to compare the baseline and post-treatment RS values. A two-sample *t* test was used to compare the percentage reduction in tumor size between the high and low RS groups. Fisher's exact test was used to compare the conversion rate from mastectomy to BCS among risk groups.

Results

A total of 116 patients were enrolled in JFMC34-0601 between March 2006 and December 2007, of whom 102 completed 24 weeks of neoadjuvant exemestane treatment [11]. Core biopsy and resection samples were obtained for 80 (69 %) and 77 (66 %) patients, respectively. Of the 157 samples sent for Onco*type* DX testing, two were deemed ineligible based on the blinded Genomic Health pathology review, insufficient RNA (<375 ng) was extracted from 18 samples (15 core biopsy and 3 resection samples), and standard quality metrics were not met for eight samples (all resections). This left 64 core biopsy samples, of which 52 had matching resection samples with evaluable RT-PCR results.

Baseline characteristics and clinical outcomes for the 64 patients are shown in Table 1. Forty-nine (76.6 %) patients had BCS, and 32 patients (50 %) had been candidates for BCS before the treatment. Four patients refused surgery after exemestane therapy and are treated as not BCS patients.

In the primary analysis, the clinical response rate in the low RS group (19/32, 59.4 %) was significantly higher than that in the high RS group (3/15, 20.0 %) (*P* = 0.015) (Table 2). The clinical response rate in the intermediate risk group (10/17, 58.8 %) was similar to that in the low risk group. Logistic regression revealed that the OR for clinical response between the intermediate and low RS groups was 0.977 (95 % CI 0.296–3.233, *P* = 0.970) and that the OR between the high and low RS groups was 0.171 (95 % CI 0.040–0.728, *P* = 0.017). In an exploratory analysis, the percentage reduction in tumor size determined by ultrasound was compared between the low and high RS groups. Patients in the low RS group showed an average reduction in tumor size of 31.8 % while those in the high RS group showed an average reduction of 12.5 %; this difference was significant between the groups (*P* = 0.045). The average reduction (27.6 %) in patients in the intermediate risk group was similar to that in the low risk group.

When treated as a continuous variable, the baseline RS Score was significantly associated with clinical response in a logistic regression analysis (*P* = 0.042; Table 3). There

Table 1 Baseline patient characteristics and clinical outcomes (n = 64)

Feature	n (%)
Age (years)	
55–64	34 (53.1)
65–74	25 (39.1)
75–77	5 (7.8)
Tumor stage at baseline	
T2	62 (96.9)
T3	2 (3.1)
Stage	
IIA	47 (73.4)
IIB	15 (23.4)
IIIA	2 (3.1)
ER by IHC (Allred score)	
4	1 (1.6)
5	3 (4.7)
6	5 (7.8)
7	14 (21.9)
8	41 (64.1)
ER status by RT-PCR	
ER− ($\leq 6.5 C_T$)	1 (1.5)
ER+ ($> 6.5 C_T$)	63 (98.4)
PgR by IHC (Allred score)	
0	4 (6.25)
4	7 (10.94)
5	4 (6.25)
6	8 (12.5)
8	12 (18.75)
NE	10 (15.63)
PgR status by RT-PCR	
PgR− ($\leq 5.5 \; C_T$)	14 (21.9)
PgR+ ($> 5.5 \; C_T$)	50 (78.1)
HER2 by IHC/FISH	
Negative	50 (78.1)
Positive	2 (3.1)
Unknown	12 (18.8)
RS risk group	
Low (<18)	32 (50.0)
Intermediate (18–30)	17 (26.6)
High (≥ 31)	15 (23.4)
Ki-67 by IHC (%)	
<10	28 (43.8)
10–30	23 (35.9)
>30	13 (20.3)
Clinical response	
Complete response (CR)	0
Partial response (PR)	32 (50.0)
Stable disease (SD)	24 (37.5)
Progressive disease (PD)	5 (7.8)
NE	3 (4.7)

Table 1 continued

Feature	n (%)
Surgery type	
Breast-conserving	49 (76.6)
Mastectomy	11 (17.2)
No surgery	4 (6.3)

ER estrogen receptor, *IHC* immunohistochemistry, *RT* reverse transcriptase, *PgR* progesterone receptor, *NE* not evaluable, *FISH* fluorescence in situ hybridization, C_T cycling threshold score, *RS* recurrence Score

was a trend between continuous baseline ER as determined by RT-PCR and clinical response (P = 0.076). Continuous baseline Ki-67 by IHC was not associated with clinical response (P = 0.273).

The associations between changes from baseline to post-treatment values of continuous markers and clinical response were examined in logistic regression analyses. Changes in the RS, ER as determined by RT-PCR, and Ki-67 as determined by IHC were not associated with clinical response (P = 0.240, 0.343 and 0.629, respectively).

Analysis of the RS categories and BCS is shown in Table 2. The OR for BCS between the intermediate and low RS groups was 0.336 (95 % CI 0.066–1.722, P = 0.19) and that between the high and low RS groups was 0.091 (95 % CI 0.019–0.432, P = 0.003). The logistic regression analyses of continuous baseline RS, ER by RT-PCR and Ki-67 by IHC with BCS are shown in Table 3. The continuous baseline RS was significantly associated with BCS in both the unadjusted (p = 0.001) and covariate-adjusted (for tumor size and PgR) (P = 0.004) analyses. The continuous baseline ER by RT-PCR was also significantly associated with BCS in both the unadjusted (P = 0.001) and covariate-adjusted (P = 0.023) analyses. Continuous baseline Ki-67 by IHC was significantly associated with BCS in the unadjusted analysis (P = 0.024) but lost its significance when adjusted for tumor size and PgR (P = 0.060). When both the continuous RS values and continuous Ki-67 were included in the logistic regression model for BCS, the RS retained its statistical significance (P = 0.012) whereas Ki-67 did not (P = 0.868). The conversion rate from mastectomy planned at baseline to BCS performed after the treatment was 88 % (15/17) in the low RS group, 70 % (7/10) in the intermediate RS group and 20 % (1/5) in the high RS group. The rate was significantly different among groups (P = 0.010).

The associations between RS and Ki-67, and their respective and joint associations with BCS were examined in exploratory analyses. Figure 1a shows a scatterplot of baseline Ki-67 as determined by IHC versus the baseline RS results. The Spearman correlation coefficient was 0.672 (95 % CI 0.506–0.785). All patients with PD had a high RS