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Original Article

Mucinous breast carcinoma with a lobular neoplasia component: A subset with aberrant expression of cell adhesion and polarity molecules and lack of neuroendocrine differentiation

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We investigated whether some mucinous carcinomas (MUCs) are associated with lobular neoplasia (LN) components, and if so, whether this subset has any distinct biological properties. MUC specimens from 41 patients were stratified into pure and mixed types. The LN components adjacent to MUC lesions were examined histopathologically. We also tested immunohistochemically for E-cadherin, β-catenin, and the neuroendocrine markers chromogranin A and synaptophysin; and compared results between MUCs with and without LN. Of 41 patients with MUC, LN was detected in 12 patients (29%); LN alone was the noninvasive component in 8 patients (20%). Decreased E-cadherin and β-catenin expression in the MUC component was detected in 2 (17%) and 7 (58%) cases, respectively, of MUC with LN, compared with 0% ($P = 0.080$) and 21% ($P = 0.018$) in MUCs without LN. Neuroendocrine factors were frequently detected in MUCs with LN (42%) and without LN (52%), but tended to be less frequent in MUCs with only LN components (25%) than in other MUCs (55%; $P = 0.133$). MUCs associated with LN components appear to be a biologically characteristic subset that frequently shows decreased cell–cell adhesion, cell polarity molecules and lack of neuroendocrine differentiation.

Key words: breast, E-cadherin, lobular neoplasia, mucinous carcinoma, neuroendocrine differentiation, β-catenin

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Mucinous carcinoma (MUC) comprises 1–6% of all breast cancers.[1–4] Up to 75% of MUCs are associated with the component of intraductal carcinoma, and it is generally believed that MUC of the breast originates from ductal carcinoma *in situ* (DCIS), usually of the common or endocrine (or solid-papillary) subtype.[5]

Neuroendocrine differentiation in MUC was reported in 1980 by Capella *et al.*, who used structural and cytological features to classify cases of MUC as type A (paucicellular), type B (hypercellular), and type AB (the intermediate form).[6] Type B MUC frequently shows neuroendocrine differentiation but type A MUC does not.[7] Neuroendocrine differentiation in MUC is immunohistochemically identified by the expression of chromogranin A and/or synaptophysin.[8] However, not many studies have been performed on the morphologic characteristics and differentiation of type A MUC.

Lobular neoplasia (LN) is composed of lobular carcinoma *in situ* (LCIS) and atypical lobular hyperplasia (ALH). It is thought that LN constitutes a risk factor for concurrent ipsilateral and/or contralateral breast cancers and a non-obligate precursor for the development of invasive carcinoma in either breast.[9] LN is found in 0.3–3.9% of otherwise benign breast biopsies.[10–15] Since we have experienced some cases of MUC accompanied by an extensive LN component, we speculated that a subset of MUC occurs in association with LN and may show unique biological properties. We were particularly interested in the percentage of MUC with an LN component and in its association with aberrant cell adhesion, cell polarity molecules, and neuroendocrine differentiation. In this study, we included cases of MUC with an LN component and characterized their biological characteristics by performing immunohistochemical analysis of the expression of E-cadherin,

β-catenin, and the neuroendocrine markers chromogranin A and synaptophysin and comparing the results with those for MUC without LN.

MATERIALS AND METHODS

The subjects comprised 41 consecutive patients who had MUC that had been surgically resected at National Cancer Center Hospital, Tokyo, between 2009 and 2011. Clinical and pathological T and N factors were registered according to the Cancer Staging Manual of the American Joint Committee on Cancer (AJCC), 7th edition. The cut-off value for ER and PR positivity was 10% positive cells, regardless of intensity. HER2 positivity was defined as an HER2 score of 3+ (>30% strong membrane immunoreaction-positive cells) or an HER2 gene/centromere 17 copy ratio of ≥2.0, as assessed by fluorescence *in situ* hybridization.

Mucinous carcinoma was also histopathologically stratified into pure and mixed types, with the former composed of >90% MUC in the invasive carcinoma component, and the latter being mixed with 50–90% MUC with 10–49% conventional invasive ductal carcinoma in the invasive carcinoma component.[5] The presence of the LN components LCIS or ALH adjacent to the MUC lesion was examined histopathologically. Simple lobular hyperplasia was not taken into account. The MUC component was also subclassified into type A (hypocellular) and type B (hypercellular) based on the cellularity and extracellular mucin status according to Capella's criteria.[6]

In the MUC and LN components, E-cadherin (1:100, mouse monoclonal, clone NCH-38, Dako, Glostrup, Denmark), β-catenin (1:500, mouse monoclonal, 14/β-catenin, BD Biosciences, San Jose, CA, USA), chromogranin A (1:100, rabbit polyclonal, A0430, Dako), and synaptophysin (ready-to-use, mouse monoclonal, 27G12, Nichirei, Tokyo) expression was examined by immunohistochemistry. Antigen retrieval was performed in TRS buffer (Dako) for E-cadherin, citrate buffer for β-catenin and chromogranin A, and TRS, pH 9.0 (Dako), for synaptophysin. Immunohistochemistry was performed with the Envision method and a Dako Autostainer.

This study was conducted under internal review board approval with written informed consent obtained from the patients.

Statistical analysis

The Mann–Whitney test was used to compare age between MUCs with and without the LN component, while the χ^2 test or Fisher's exact test was used to compare other variables. Differences were considered significant at *P*-values less than 0.05. SPSS statistical software (version 19, IBM SPSS Statistics, Chicago, IL, USA) was used for all statistical analyses.

RESULTS

Lobular neoplasia was detected in the background of MUC in twelve cases (29%), varying from 1 to 85 mm in diameter: eight with LN only and four with both LN and DCIS components. DCIS alone was detected in the background of MUC in 19 cases, but the noninvasive carcinoma component was not observed in the other 10 cases. Extensive intraductal spread (EIC[+]) was seen in 14 cases, with the DCIS component alone noted in nine, both DCIS and LN components in four, and LN component alone in one case. These LN and DCIS components were located in continuity with or around the main MUC lesion on the representative cut surface of the tumor. Therefore, we considered that there is a possibility that these LN and/or DCIS components might be precursors for the MUC component, although genetic studies are needed to draw a conclusion.

The clinicopathological characteristics of the 41 patients are listed in Table 1. The median age was 62.5 years (range, 35–81 years). There were no significant differences in clinicopathological characteristics between the 12 patients with MUC with the LN component and the 29 patients without, with regard to pure/mixed type, A/B type, and nuclear grade. In three cases of mixed type MUC with LN, the invasive component other than MUC did not contain invasive lobular carcinoma. The percentage of axillary lymph node metastases tended to be larger in the former group: three (25%) of 12 patients with MUC with the LN component showed histological lymph node metastases, whereas only three (10%) of 29 patients with MUC without the LN component showed histological lymph node metastases. All MUCs, both with LN and without LN, expressed ER/PgR and lacked HER2 overexpression, except in one case.

Figures 1 and 2 show photomicrographs of representative cases of MUC with the LN component.

Reduction and disappearance of membrane immunoreactivity in >50% of constituent tumor cells was judged abnormal for E-cadherin and β-catenin expression. Only two cases showed a decrease in E-cadherin expression; in the other 39 cases, E-cadherin was diffusely expressed and <5% of cells showed a decrease in expression. Likewise, 13 of 41 MUCs showed a significant decrease in β-catenin expression; in the other 28 cases, β-catenin was expressed diffusely and <5% of cells showed a decrease in expression. However, for both E-cadherin and β-catenin, it was unclear whether the decreased expression in <5% of cells was a focal decrease or uneven staining due to a technical artifact. Therefore, we set the cut-off value at 50% for both E-cadherin and β-catenin.

Table 1 Correlations of clinicopathological parameters of mucinous carcinoma with/without lobular neoplasia component

	Number of cases (%)			
		Non-invasive carcinoma component		
Parameter	Total	With LN (n = 12)	Without LN (n = 29)	P
Age				
Average (range)	62.5 (35–81)	62.6 (42–78)	62.5 (35–81)	0.774
<50	9	1 (9)	8 (28)	0.124
≥50	32	11 (91)	21 (72)	
Histological type				
Pure type	28	9 (75)	19 (66)	0.553
Mixed type	13	3 (25)	10 (34)	
Morphological type				
Type A	19	5 (42)	14 (48)	0.699
Type B	22	7 (58)	15 (52)	
cT-factor				
T1	26	8 (67)	18 (62)	0.505
T2	12	4 (33)	8 (28)	
T3	3	0 (0)	3 (10)	
pT-factor				
T1	28	9 (75)	19 (66)	0.729
T2	12	3 (25)	9 (31)	
T3	1	0 (0)	1 (3)	
LVI				
Negative	35	11 (91)	24 (83)	0.463
Positive	6	1 (9)	5 (17)	
Nuclear grade				
1	33	10 (83)	23 (79)	0.767
2	8	2 (17)	6 (21)	
3	0	0 (0)	0 (0)	
Hormone receptor				
Positive	41	12 (100)	29 (100)	
Negative	0	0 (0)	0 (0)	—
HER2				
Positive	1	1 (9)	0 (0)	0.116
Negative	40	11 (91)	29 (100)	
Lymph node metastasis				
Positive	6	3 (25)	3 (10)	0.088
Negative	31	6 (50)	25 (86)	
Unknown	4	3 (25)	1 (4)	

LVI, Lymphovascular invasion.

Immunohistochemical analysis showed that the membrane expression of E-cadherin in the MUC component decreased in two (17%) MUCs with an LN component but not in MUCs without LN (P = 0.080; Table 2). Likewise, a decrease in the membrane expression of β-catenin in the MUC component was detected in seven (58%) MUCs with the LN component and in six (21%) MUCs without LN (P = 0.018; Table 2).

A decrease in the membrane expression of E-cadherin and β-catenin in the MUC component was detected in two (25%) and seven (87%) of eight MUCs with the LN component only, while the decrease was detected in zero (0%) and six (18%) of 33 MUCs with both LN and DCIS components or without the LN component (P = 0.034 and 0.00051; Table 3). In Figures 3 and 4, two cases of MUC showing different expression patterns of E-cadherin and β-catenin are presented.

Chromogranin A and/or synaptophysin positivity in the MUC component was >50% in 14 cases, >20–50% in zero cases, >10–20% in six cases, >0–10% in seven cases, and negative in 14 cases. Because chromogranin A- or synaptophysin-positive cells were easily identified, the expression of either chromogranin A or synaptophysin in 10% or more of tumor cells was judged as the neuroendocrine immunophenotype. Positive results were obtained for neuroendocrine marker expression in 20 (49%) of 41 MUCs, and this expression was observed in 42% (5 of 12) of MUC with LNs and 52% (15 of 29) of MUCs without LNs (Table 2).

Among the eight MUCs with only the LN component, the neuroendocrine immunophenotype was detected in two (25%). This tended to be lower than the rate of the neuroendocrine immunophenotype in MUCs with both LN and DCIS components or without the LN component (18 of 33 [55%]),

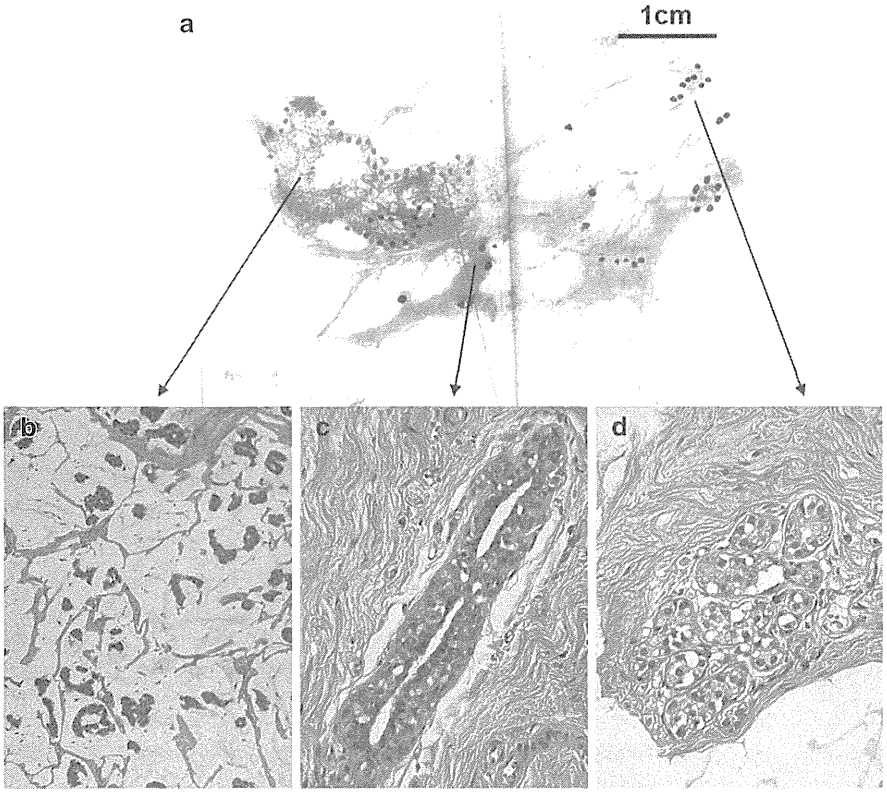

Figure 1 (a) A low magnified view of a case of mucinous carcinoma with lobular neoplasia (LN) component. The mucinous carcinoma component (**b**, ×100) is indicated in red dots, and the LN components (**c** and **d**, ×400) are indicated in blue dots. The extent of LN exceeds 3.0 cm. This case does not have the component of ductal carcinoma *in situ*.

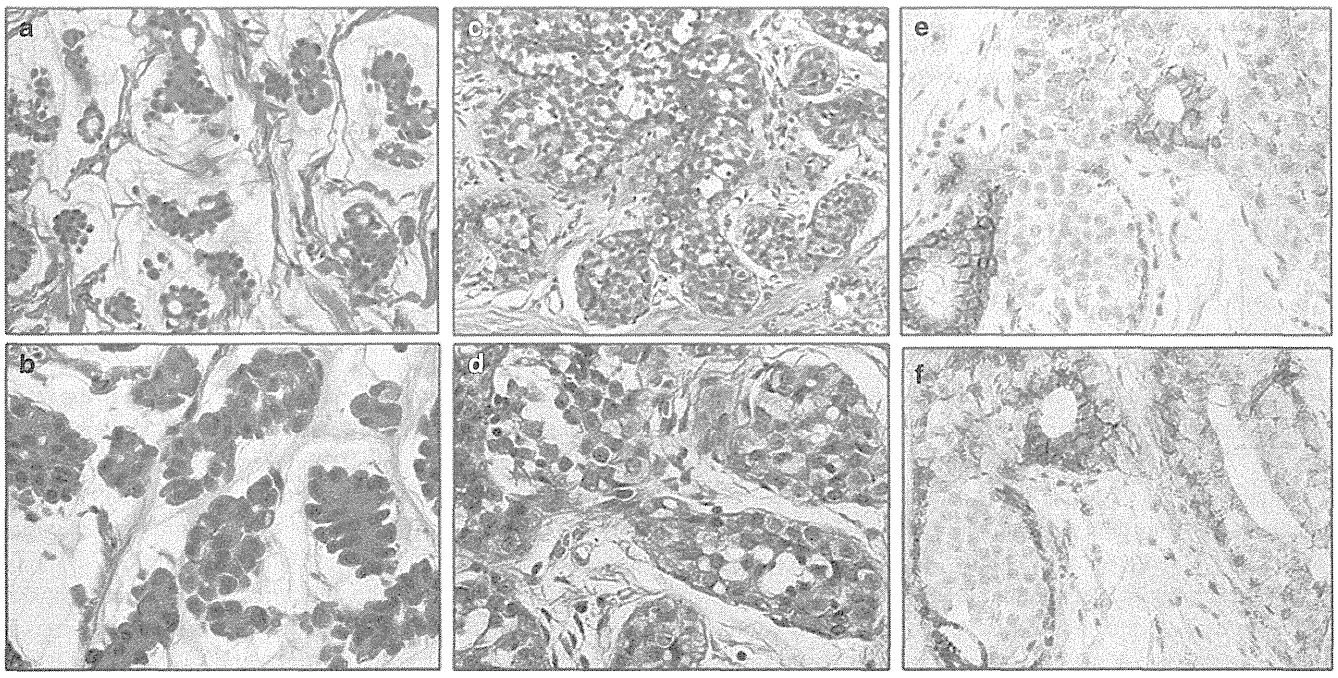

Figure 2 A case of mucinous carcinoma (MUC) with a lobular neoplasia (LN) component. (**a,b**) MUC component. (**c–f**) LN component. **a–d**, HE; **e**, E-cadherin; **f**, β-catenin. E-cadherin and β-catenin are not expressed in the LN component. **a** and **c**, ×200; **b**, **d–f**, ×400.

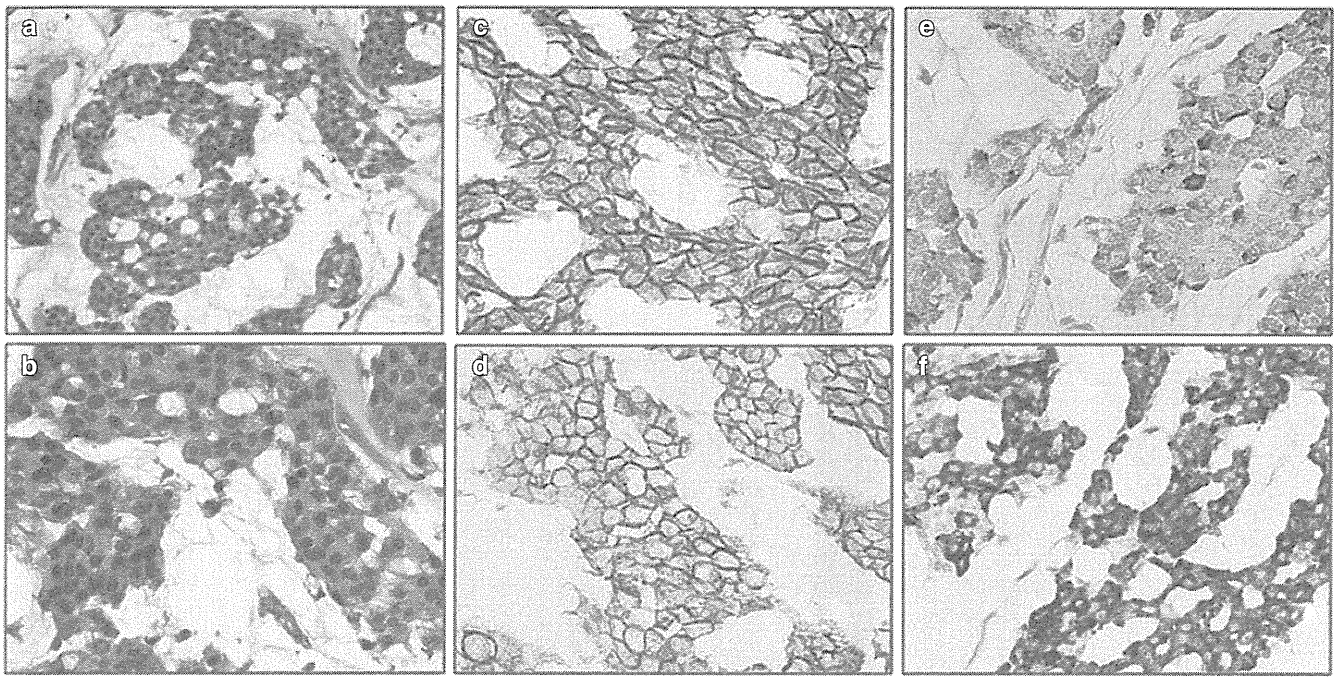

Figure 3 A case of mucinous carcinoma. (**a,b**) HE; (**c**) E-cadherin; (**d**) β-catenin; (**e**) Chromogranin A; (**f**) Synaptophysin; Expression of E-cadherin, β-catenin, chromogranin A, and synaptophysin were positive in each of these cases. **a**, ×200; **b** to **f**, ×400.

Table 2 Correlations of mucinous carcinoma with/without lobular neoplasia component with E-cadherin and β-catenin expression and neuroendocrine immunophenotype

	Number of cases (%)			
	Noninvasive carcinoma component			
Parameter in MUC component	Total	With LN (n = 12)	Without LN (n = 29)	P
E-cadherin				
Normal	39	10 (83)	29 (100)	0.08
Decreased	2	2 (17)	0 (0)	
β-catenin				
Normal	28	5 (42)	23 (79)	0.018
Decreased	13	7 (58)	6 (21)	
Neuroendocrine immunophenotype				
Present	20	5 (42)	15 (52)	0.558
Absent	21	7 (58)	14 (48)	

Table 3 Correlations of mucinous carcinoma with lobular neoplasia component only with E-cadherin and β-catenin expression and neuroendocrine immunophenotype

	Number of cases (%)			
	Noninvasive carcinoma component			
Parameter	Total	LN only (n = 8)	LN + DCIS, DCIS, or none (n = 33)	P
E-cadherin				
Normal	39	6(75)	33(100)	0.034
Decreased	2	2(25)	0 (0)	
β-catenin				
Normal	28	1(13)	27 (82)	0.00051
Decreased	13	7(87)	6 (18)	
Neuroendocrine immunophenotype				
Present	20	2(25)	18 (55)	0.133
Absent	21	6(75)	15 (45)	

but the difference was not significant (P = 0.133; Table 3). In Figures 3 and 4, two cases of MUC with different chromogranin A and synaptophysin expression patterns are presented.

For the 12 LN components in 12 cases, E-cadherin and β-catenin expression was always negative and the neuroendocrine immunophenotype was always absent (Fig. 1e,f).

DISCUSSION

Histological analysis of the noninvasive component may allow classification of MUC into biologically distinct subsets. In this study, we classified MUC according to the presence or absence of the LN component, and then compared the ratio of E-cadherin, β-catenin, chromogranin A, and synaptophysin expression in MUCs with and without the LN component. We observed a statistically significant difference in the rate of abnormal β-catenin expression in the MUC component for these two MUC groups. In addition, we observed a trend toward a higher rate of decreased E-cadherin expression in the MUC component in the MUC group with the LN component than in the MUC group without LN.

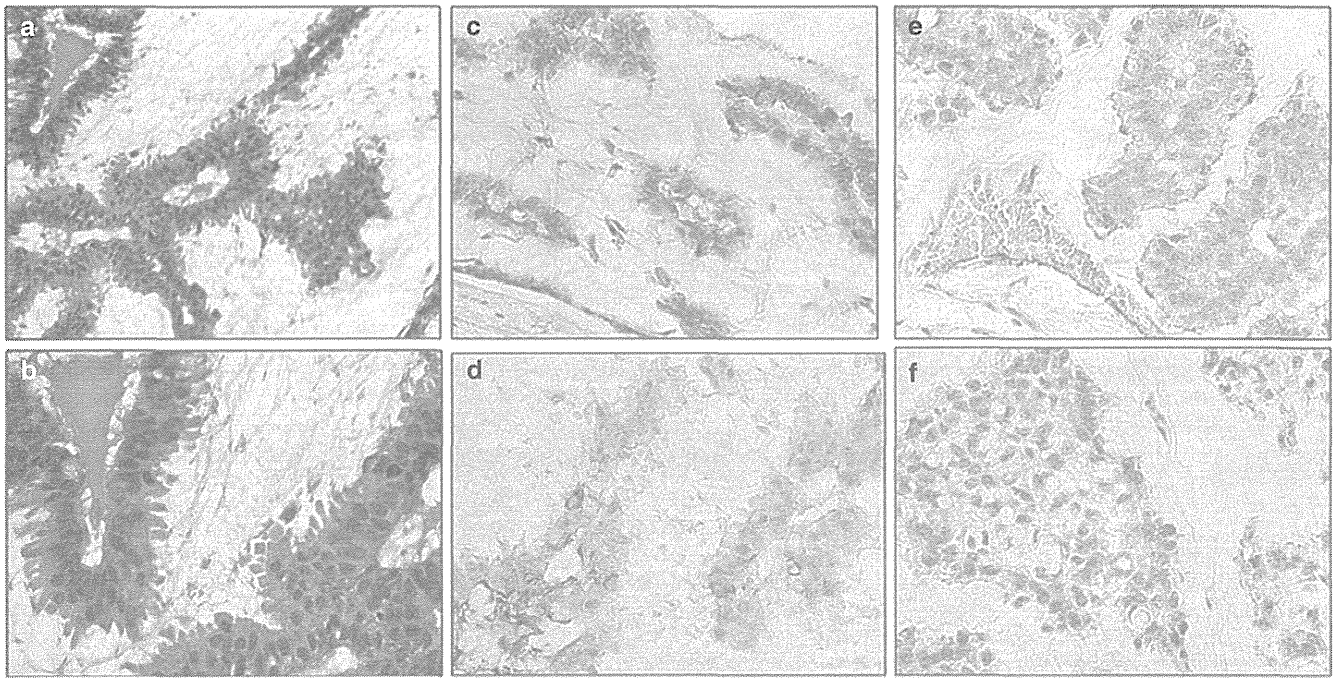

Figure 4 A case of mucinous carcinoma (MUC) with a lobular neoplasia component. (**a**,**b**) HE; (**c**) E-cadherin; (**d**) β-catenin; (**e**) Chromogranin A; (**f**) Synaptophysin; In the MUC component, negative results were obtained for the expression of E-cadherin, β-catenin, and the neuroendocrine markers. **a**, ×200; **b** to **f**, ×400.

E-cadherin, a transmembrane glycoprotein, is expressed on the cell surface in most epithelial tissues[16] and mediates the formation of the intercellular junctional complex and the establishment of cell polarization.[17] Its extracellular domain forms a molecular zipper mediating cell–cell adhesion, which transforms molecular adhesive interactions into strong intercellular bonds.[18] The cytoplasmic tail of E-cadherin is linked via catenins to the actin cytoskeleton.[19]

The ability of carcinomas to invade and metastasize largely depends on the degree of differentiation and tumor cell characteristics. Frixen *et al.* found that loss of E-cadherin expression promotes dedifferentiation and invasiveness of human carcinoma cells and suggested that E-cadherin acts as an invasion suppressor.[20] Aberrant β-catenin expression is also reported in these poorly differentiated carcinomas. On the other hand, a lack of E-cadherin and β-catenin expression occurs frequently in lobular breast carcinomas, including invasive lobular carcinoma and LN, regardless of the degree of differentiation.[21,22] These facts suggest that reduced E-cadherin and β-catenin expression is associated with the properties of lobular carcinoma cells, which have the potential to infiltrate the stroma as single cells.

The MUC subset with the LN component tended to show decreased E-cadherin and β-catenin expression. Abnormal cell adhesion and cell polarity in this MUC subset may produce a higher tendency to metastasize to lymph nodes or other organs. In fact, the rate of axillary lymph node metastasis in MUC with LN tended to be higher (25%) than in MUC without LN (10%). It is unclear whether MUC with LN produces worse clinical outcomes than MUC without LN.

DCIS of the endocrine type, or solid-papillary subtype, expresses chromogranin A and/or synaptophysin, and is a precursor for MUC. More than 70% of type B MUC cases show the expression of these neuroendocrine markers.[6] In this study, 48% (20 of 41) of MUCs were immunohistochemically positive for these markers. There was no significant difference in the expression of these neuroendocrine markers in the MUC groups with and without the LN component. However, positivity for neuroendocrine features in eight cases of MUC with LN and without the DCIS component was only 25%, whereas the rate in the other 33 cases of MUC was 55%. Although MUC with the LN component was found to be a minor subset, our data suggest it might be a distinct subset of MUC. Differentiation backgrounds may differ between MUC with the LN component only and MUC with the DCIS component, which usually occurs in association with neuroendocrine features. In contrast, MUC with an LN component only may occur without neuroendocrine differentiation. MUC with LN only might be included as a part of type A MUC.

The LN and DCIS components that were examined were located around the main MUC lesion. More than half of the cases were EIC(+), and these noninvasive carcinoma components were obviously direct precursors of the MUC component. It is unclear whether there is a progression from LN to MUC in all cases or if there is a common carcinogenic pathway for incidentally coexistent LN and MUC.

Alternative histogenetic significance of MUC with LN component could be considerable. There were cases of MUC with both LN and DCIS components and were cases of MUC with LN only with coexpression of E-cadherin, β-catenin, and neuroendocrine markers. The existence of these cases might also indicate a possibility that MUC with LN could be a subtype of invasive combined ductal and lobular carcinoma.

In conclusion, although it is unclear whether MUCs always occur from precursor LN, or if some of them were independent of coincident LN within a common field, MUC associated with LN showed characteristic biological properties, including decreased cell-cell adhesion and cell polarity. Although a significant correlation was not presented, there was a tendency of lower incidence of neuroendocrine differentiation in MUC with LN component only. It appeared that the accumulation of a larger number of cases might disclose a significant relationship between MUC with LN and lack of neuroendocrine differentiation.

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REFERENCES

1 Komaki K, Sakamoto G, Sugano H *et al.* Mucinous carcinoma of the breast in Japan. A prognostic analysis based on morphologic features. *Cancer* 1988; **61**: 989–96.
2 Andre S, Cunha F, Bernardo M *et al.* Mucinous carcinoma of the breast: A pathologic study of 82 cases. *J Surg Oncol* 1995; **58**: 162–7.
3 Diab SG, Clark GM, Osborne CK *et al.* Tumor characteristics and clinical outcome of tubular and mucinous breast carcinomas. *J Clin Oncol* 1999; **17**: 1442–8.
4 Hanagiri T, Ono K, Baba T *et al.* Clinicopathologic characteristics of mucinous carcinoma of the breast. *Int Surg* 2010; **95**: 126–9.
5 Kato N, Endo Y, Tamura G *et al.* Mucinous carcinoma of the breast: A multifaceted study with special reference to histogenesis and neuroendocrine differentiation. *Pathol Int* 1999; **49**: 947–55.
6 Capella C, Eusebi V, Mann B *et al.* Endocrine differentiation in mucoid carcinoma of the breast. *Histopathology* 1980; **4**: 613–30.
7 Scopsi L, Andreola S, Pilotti S *et al.* Mucinous carcinoma of the breast. A clinicopathologic, histochemical, and immunocytochemical study with special reference to neuroendocrine differentiation. *Am J Surg Pathol* 1994; **18**: 702–11.
8 Maluf HM, Koerner FC. Carcinomas of the breast with endocrine differentiation: A review. *Virchows Arch* 1994; **425**: 449–57.
9 Page DL, Dupont WD, Rogers LW *et al.* Atypical hyperplastic lesions of the female breast. A long-term follow-up study. *Cancer* 1985; **55**: 2698–708.
10 Piubello Q, Montemezzi S, D'Atri C. Breast calcifications with percutaneous vacuum-assisted biopsy diagnosis of malignancy or atypical hyerplasia: Correlations with surgical findings. *Pathologica* 2002; **94**: 299–305.
11 Shah-Khan MG, Geiger XJ, Reynolds C *et al.* Long-term follow-up of lobular neoplasia (atypical lobular hyperplasia/lobular carcinoma in situ) diagnosed on core needle biopsy. *Ann Surg Oncol* 2012; **19**: 3131–8.
12 Esserman LE, Lamea L, Tanev S *et al.* Should the extent of lobular neoplasia on core biopsy influence the decision for excision? *Breast J* 2007; **13**: 55–61.
13 Lavoue V, Graesslin O, Classe JM *et al.* Management of lobular neoplasia diagnosed by core needle biopsy: Study of 52 biopsies with follow-up surgical excision. *Breast* 2007; **16**: 533–9.
14 Meloni GB, Becchere MP, Soro D *et al.* Percutaneous vacuum-assisted core breast biopsy with upright stereotactic equipment. Indications, limitations and results. *Acta Radiol* 2002; **43**: 575–8.
15 Georgian-Smith D, Lawton TJ. Calcifications of lobular carcinoma in situ of the breast: Radiologic-pathologic correlation. *AJR Am J Roentgenol* 2001; **176**: 1255–9.
16 Takeichi M. Cadherins: A molecular family important in selective cell-cell adhesion. *Annu Rev Biochem* 1990; **59**: 237–52.
17 Gumbiner B, Stevenson B, Grimaldi A. The role of the cell adhesion molecule uvomorulin in the formation and maintenance of the epithelial junctional complex. *J Cell Biol* 1988; **107**: 1575–87.
18 Shapiro L, Fannon AM, Kwong PD *et al.* Structural basis of cell-cell adhesion by cadherins. *Nature* 1995; **374**: 327–37.
19 Cowin P. Unraveling the cytoplasmic interactions of the cadherin superfamily. *Proc Natl Acad Sci U S A* 1994; **91**: 10759–61.
20 Frixen UH, Behrens J, Sachs M *et al.* E-cadherin-mediated cell-cell adhesion prevents invasiveness of human carcinoma cells. *J Cell Biol* 1991; **113**: 173–85.
21 Kanai Y, Oda T, Tsuda H *et al.* Point mutation of the E-cadherin gene in invasive lobular carcinoma of the breast. *Jpn J Cancer Res* 1994; **85**: 1035–9.
22 Berx G, Cleton-Jansen AM, Strumane K *et al.* E-cadherin is inactivated in a majority of invasive human lobular breast cancers by truncation mutations throughout its extracellular domain. *Oncogene* 1996; **13**: 1919–25.

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CLINICAL TRIAL

Randomized phase II study of weekly paclitaxel with and without carboplatin followed by cyclophosphamide/epirubicin/5-fluorouracil as neoadjuvant chemotherapy for stage II/IIIA breast cancer without HER2 overexpression

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Abstract Addition of carboplatin to neoadjuvant chemotherapy in HER2-negative breast cancer may improve pathological complete response (pCR) rates. We evaluated the efficacy and safety of carboplatin and weekly paclitaxel (wPTX) followed by cyclophosphamide, epirubicin, and 5-fluorouracil (CEF) as neoadjuvant chemotherapy for HER2-negative breast cancer. Patients with stage II/IIIA HER2-negative breast cancer were randomly assigned to preoperatively receive CP-CEF (four 3-week cycles of carboplatin [area under the curve 5 mg/mL/min, day 1] and wPTX [80 mg/m^2, day 1, 8, 15] followed by four 3-week cycles of CEF [500/100/500 mg/m^2] or P-CEF (four cycles of wPTX followed by four cycles of CEF). The primary objective was pCR rate. Of 181 eligible patients, 89 were randomly assigned to the CP-CEF and 92 to the P-CEF. Two patients in each arm refused to receive neoadjuvant chemotherapy. Overall 88 patients in the CP-CEF and 91 patients in the P-CEF were assessable for efficacy and safety. The pCR rate in the CP-CEF was significantly higher than that in the P-CEF (31.8 vs. 17.6 %, one-sided $P = 0.01$). Among patients with triple-negative breast cancer, the pCR rate in the CP-CEF was significantly

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higher than that in the P-CEF [61.2 (23/37) vs. 26.3 % (10/38), $P = 0.003$]. Grade 3–4 neutropenia was observed in the CP-CEF more frequently than in the P-CEF (65.9 vs. 38.5 %). Adding carboplatin to neoadjuvant wPTX followed by CEF for HER2-negative breast cancer improved the pCR rate and exacerbated hematotoxicity.

Keywords Breast cancer · Carboplatin · HER2 negative · Neoadjuvant chemotherapy

Introduction

Neoadjuvant chemotherapy is a widely accepted treatment option for patients with operable breast cancer [1, 2]. Currently, anthracyclines and taxanes in sequence or in combination are recommended for patients with HER2-negative disease, and anthracyclines followed by combinations of taxanes and trastuzumab are recommended for patients with HER2-positive disease [3–5]. Pathological complete response (pCR), which is defined as disappearance of all invasive carcinomas in primary and axillary nodes and is associated with long-term survival, occurs in about 15–20 % of patients with HER2-negative disease treated with anthracyclines and taxanes [3, 4].

Several new chemotherapeutic regimens have been evaluated in patients with HER2-negative disease. Adding capecitabine or gemcitabine to epirubicin and cyclophosphamide followed by taxane therapy did not improve pCR rates in the neoadjuvant setting [6, 7]. Carboplatin, a platinum compound, has yielded response rates of 20–35 % in phase II studies of previously untreated patients with metastatic breast cancer (MBC) [8–10]. In patients with HER2-positive disease, combinations of carboplatin, taxanes, and trastuzumab are active in both the adjuvant and metastatic settings [11, 12]. In a phase III study of MBC patients who previously received anthracycline-based adjuvant chemotherapy, ~70 % of whom had HER2-negative disease, first-line therapy consisting of triweekly carboplatin and paclitaxel resulted in similar progression-free survival as gemcitabine plus docetaxel [13]. Weekly paclitaxel (wPTX) followed by cyclophosphamide, epirubicin, and 5-fluorouracil (CEF) is a commonly used neoadjuvant chemotherapy regimen for patients with HER2-negative breast cancer [14]. Recently, triple-negative breast cancers (TNBC) were classified into six subtypes depending on gene profiles, and basal-like 1–2 subtypes were suggested as highly sensitive to cisplatin in the vitro study [15]. The previous randomized phase II study suggested a potential benefit of platinum for metastatic TNBC [16].

We hypothesized that carboplatin would enhance the anti-tumor activity of wPTX and that this combination would improve pCR rates over the conventional regimens of wPTX followed by CEF. We conducted this randomized phase II trial to assess the efficacy and safety of adding carboplatin to wPTX followed by CEF in the neoadjuvant setting for patients with HER2-negative breast cancer.

Patients and methods

Patient eligibility

Eligible patients had previously untreated, unilateral, histologically confirmed, invasive, non-inflammatory, breast carcinoma. Histologic confirmation of invasive cancer was performed by core needle biopsy (CNB). HER2-negative disease was defined as a score of 0 or 1 + by immunohistochemistry (IHC) or HER2 gene copy: chromosome 17 ratio of <2.0 by fluorescence in situ hybridization (FISH). Patients with a tumor >2.0 cm at the largest dimension by ultrasonography, or ≤2.0 cm with axillary lymph node metastasis clinically diagnosed as positive, were eligible (clinical stage II and IIIA). Patients with axillary nodes enlarged by >1 cm at the largest dimension according to ultrasonography were considered to be clinically node positive. Patients with T4, N3, (supraclavicular lymph node), or distant metastatic disease (M1) were excluded from this study.

Other requirements included age 18–70 years, ECOG performance status 0–2, adequate bone marrow function (absolute granulocyte count ≥1,500/mm^3 and platelet count ≥100,000/mm^3), liver function (total bilirubin ≤1.5 mg/dL and liver transaminase [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)] ≤60 IU/L), and renal function (serum creatinine ≤1.5 mg/dL), and written informed consent. Patients with a history of ischemic cardiac disease were excluded. Patients with clinically negative axillary lymph nodes had the option of undergoing pretreatment sentinel lymph node biopsy (SLNB).

Study design and neoadjuvant chemotherapy

This was a randomized, multicenter (10 institutions), non-blinded phase II study. The study design is shown in Fig. 1. Enrolled patients were randomly assigned to receive either wPTX (P) followed by CEF (P-CEF arm) or combination carboplatin and wPTX (CP) followed by CEF (CP-CEF arm) by the minimization method, with balancing of the treatment arms according to disease status (stage II vs. IIIA), hormone receptor (HR) status, and institution. Paclitaxel was administered at 80 mg/m^2 IV over 1 h on days 1, 8, and 15 every 3 weeks for four cycles. Carboplatin and wPTX were administered at area under blood concentration

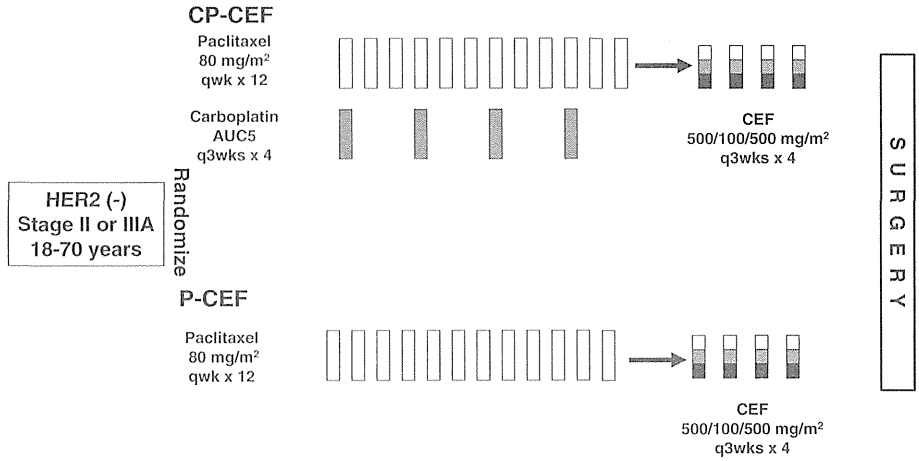

Fig. 1 Study design. *CPA* cyclophosphamide, *EPI* epirubicin, *5-FU* 5-fluorouracil, and *HER2* human epidermal growth factor receptor-2

time curve (AUC) 5 mg/mL/min IV over 1 h on day 1 and at 80 mg/m^2 IV over 1 h on days 1, 8, and 15, respectively, every 3 weeks for four cycles. CEF consisted of CEF (500/100/500 mg/m^2) IV on day 1 every 3 weeks for four cycles. Carboplatin was provided by Bristol-Myers Squibb K.K., Tokyo, Japan as an investigational drug.

If a patient developed grade $\geq$3 febrile neutropenia, thrombocytopenia <25,000/mm^3, or grade $\geq$3 non-hematologic toxicity while receiving CP or CEF, the doses of carboplatin and epirubicin were reduced by 20 and 25 %, respectively, in subsequent cycles. The doses of paclitaxel during CP and P were reduced by 25 % in subsequent cycles if a patient developed grade 3 neurotoxicity. Before administration of the following cycle of CP, P, or CEF, patients were required to have a granulocyte count $\geq$1,500/mm^3, platelet count $\geq$75,000/mm^3, and no non-hematologic toxicity of grade $\leq$2 (excluding alopecia). Before administration of CP on day 8 and 15, patients were required to have a granulocyte count $\geq$500/mm^3, platelet count $\geq$75,000/mm^3, and peripheral neuropathy of grade $\leq$2. If toxicity did not improve within 2 weeks on the P or CP regimen, chemotherapy was discontinued and initiation of CEF was recommended. If toxicity did not improve within 2 weeks on CEF, chemotherapy was discontinued and surgery was recommended.

Therapy after neoadjuvant chemotherapy

Patients who were considered candidates for breast-conserving therapy (BCT) were offered lumpectomy. Axillary lymph node dissection (AxLND) was mandatory, except in patients diagnosed as having no metastases by SLNB before neoadjuvant chemotherapy. Surgery was performed within 8 weeks after completion of preoperative chemotherapy. All patients who underwent BCT received whole-breast irradiation. After completion of neoadjuvant chemotherapy and surgery, patients with HR-positive disease received adjuvant endocrine therapy.

Study evaluation and criteria

The HER2 status of CNB specimens was determined by IHC and/or FISH performed at each institution before study enrollment, and was not subject to central review. HR status [estrogen receptor (ER) and progesterone receptor (PgR)] of CNB specimens was assessed by IHC, for which $\geq$10 % staining of cancer cell nuclei was diagnosed as positive. HR positivity was defined as ER-positive and/or PgR-positive disease. Histological grade was scored according to the modified Scarff–Bloom–Richardson classification [17]. After completion of neoadjuvant chemotherapy, resected specimens and CNB specimens were evaluated centrally by 3 breast pathologists. A pCR was defined as the absence of viable invasive tumor in both the breast and axillary nodes. Patients with residual ductal carcinoma in situ (DCIS) in the breast and no viable invasive tumor in the axillary nodes were also classified as having a pCR. Clinical response was evaluated by palpation and caliper after each cycle according to the Response Evaluation Criteria In Solid Tumors version 1.1. All adverse events were evaluated according to the Common Terminology Criteria for Adverse Events version 4.03.

Endpoints and statistical analysis

The primary endpoint was the pCR rate. Secondary endpoints included disease-free survival, clinical response rate, breast conservation rate, and safety. Efficacy and safety analysis were performed in the intent-to-treat (ITT) population, which consisted of subjects fulfilling the study inclusion criteria who had received at least one dose of study chemotherapy. The per-protocol population consisted of subjects who had completed chemotherapy and underwent surgery in this study without serious violations of the inclusion criteria.

Based on previous studies of neoadjuvant anthracyclines and taxanes, patients with HER2-positive disease account

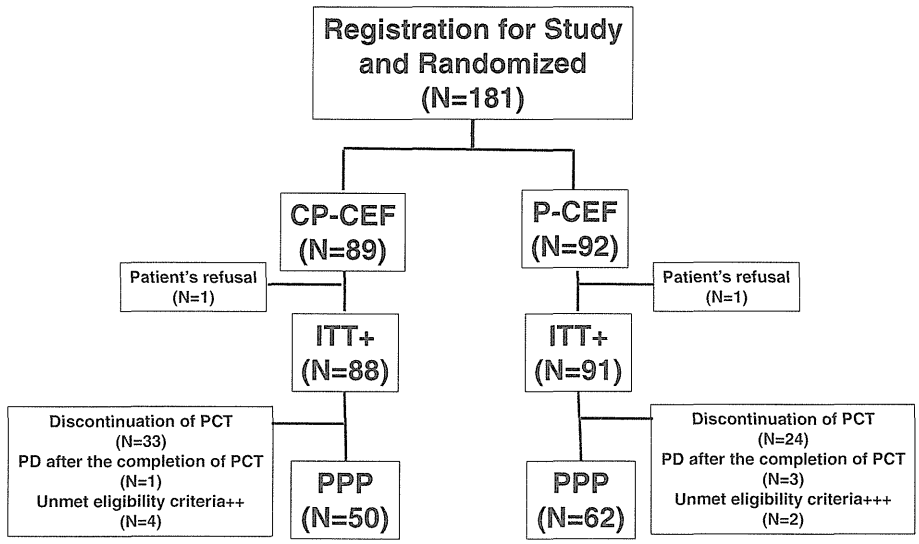

Fig. 2 CONSORT flow diagram. Disposition of study participants. + 6 of the 61 patients who discontinued neoadjuvant chemotherapy or showed disease progression after the completion of chemotherapy were diagnosed as ineligible by pathological central review (3 patients in the CP-CEF and 3 patients in the P-CEF arm), ++ patients who were diagnosed as ineligible by pathological central review, and +++ patients who were determined to have Stage IIIC disease after enrollment. *ITT* intent to treat, *PCT* preoperative chemotherapy by study protocol, and *PPP* per-protocol population

for 6–30 % of the treatment population, and pCR rates (defined in the same manner as the present study) ranged from 16 to 26 % [4, 6, 13]. The present study was designed for patients with HER2-negative disease, and P-CEF was expected to produce a pCR rate of 15 %. The study was originally planned to enroll 110 patients in each treatment arm in order to detect a 30 % increase in pCR in the CP-CEF arm with 90 % power using the Pearson's chi squared test and one-sided 10 % significance level. Due to an administrative reason (the termination of financial support due to the end of a government-sponsored clinical trial program), the revised sample size with 87 % power was a total of 180 patients. Study accrual was not stopped on the basis of an interim analysis. An exploratory logistic regression analysis was conducted to examine the influence of clinical stage (II, IIIA), clinical nodal status (positive, negative), histological grade (grade 1, 2, 3), HR status (positive, negative), and age (<50, ≥50 years) on pCR. The primary test of the pCR rate was reported as one-sided and other reported *P* values were two-sided tests. Analyses were conducted using JMP® software version 8.0.2 (SAS Institute Inc., Cary, NC, USA).

Results

Patient characteristics

Between March 2010 and September 2011, 181 patients entered into this study. Of these, 88 patients treated with CP-CEF and 91 treated with P-CEF were evaluable in the ITT population. Two patients in each arm refused to receive neoadjuvant chemotherapy. Furthermore 38 patients in the CP-CEF arm and 29 patients in the P-CEF arm were excluded from the per-protocol population (Fig. 2). According to central review, 9 patients were considered ineligible [HER2 score 2 + by IHC and FISH not done ($n = 6$), CNB specimen not evaluable for invasive component ($n = 1$), and CNB specimen not evaluable ($n = 2$)]. Two patients had proven stage IIIC disease after enrollment.

Characteristics of the ITT population are shown in Table 1. The median age was 47 years old. Distributions of tumor size, nuclear grade, and clinical axillary node status were similar; and more than 95 % of patients were diagnosed with invasive ductal carcinoma in the two arms. In the both arms, 42 % of patients had HR-negative (and thus triple-negative) tumors and 41 % had ER- and PgR-positive disease.

Treatment exposure

In the CP-CEF and P-CEF arms, 55 of 88 patients (62.5 %) and 67 of 91 patients (73.6 %), respectively, received all of the planned treatment cycles. In the CP-CEF arm, 64 patients (72.7 %) completed four cycles of CP; while in the P-CEF arm, 82 patients (90.1 %) completed four cycles of P (Table 2). In the CP-CEF arm, 33 patients did not complete chemotherapy due to adverse events ($n = 29$) or disease progression ($n = 4$). In the P-CEF arm, 24 patients

Table 1 Characteristics of eligible patients treated with neoadjuvant chemotherapy ($n = 179$)

	CP-CEF ($n = 88$)	P-CEF ($n = 91$)
Age (range; years)	47 (30–69)	47 (30–70)
<50	52 (59.1 %)	51 (66.0 %)
≥50	36 (40.9 %)	40 (44.0 %)
Menopausal status		
Premenopausal	60 (68.8 %)	54 (59.3 %)
Postmenopausal	28 (31.2 %)	37 (40.7 %)
Performance status 0	88 (100 %)	91 (100 %)
Clinical stage		
II	71 (80.7 %)	75 (82.4 %)
IIIA	17 (19.3 %)	14 (15.4 %)
IIIC	0 (0 %)	2 (2.2 %)[a]
Clinical tumor size (cm)		
≤2.0	2 (2.3 %)	4 (4.4 %)
2.1–5.0	64 (72.7 %)	63 (69.2 %)
≥5.1	22 (25.0 %)	24 (26.4 %)
Median, cm (range)	4.0 (1.0–11.0)	4.0 (1.5–8.0)
Clinical axillary nodal status		
Negative	32 (36.4 %)	30 (33.0 %)
Positive	56 (63.6 %)	61 (67.0 %)
Pathology		
Invasive ductal carcinoma	84 (95.5 %)	89 (97.8 %)
Invasive lobular carcinoma	3 (3.4 %)	0 (0 %)
Others	1 (1.1 %)	2 (2.2 %)
Histological grade		
1	16 (18.2 %)	13 (14.3 %)
2	29 (33.0 %)	35 (38.5 %)
3	43 (48.9 %)	43 (47.3 %)
Hormone receptor status		
ER−/PgR−	37 (42.0 %)	38 (41.8 %)
ER+/PgR+	36 (40.9 %)	37 (40.7 %)
ER+/PgR−	13 (14.8 %)	14 (15.4 %)
ER−/PgR+	2 (2.2 %)	2 (2.2 %)

CP-CEF carboplatin and weekly paclitaxel followed by cyclophosphamide/epirubicin/5-fluorouracil, *P-CEF* weekly paclitaxel followed by cyclophosphamide/epirubicin/5-fluorouracil, *ER* estrogen receptor, *PgR*, progesterone receptor

[a] These 2 patients were determined to have clinical stage IIIC disease after enrollment

did not complete chemotherapy due to adverse events ($n = 6$), refusal ($n = 6$), ineligibility ($n = 2$), or disease progression ($n = 10$).

Of 88 patients treated with CP, 65 (73.9 %) required delayed administration or at least one dose reduction of paclitaxel, 18 of whom required one dose reduction of carboplatin. Of 91 patients treated with P, 28 (30.8 %) required delayed administration or at least one dose reduction of paclitaxel. Sixteen patients in each treatment arm required at least one dose reduction of CEF.

Table 2 Treatment exposure, clinical response, breast surgery, and adjuvant therapy

	CP-CEF ($n = 88$)	P-CEF ($n = 91$)
Completion of each treatment cycle		
CP or P 1st cycle	87 (98.9 %)	89 (97.8 %)
CP or P 2nd cycle	81 (92.0 %)	88 (96.7 %)
CP or P 3rd cycle	73 (83.0 %)	85 (93.4 %)
CP or P 4th cycle	64 (72.7 %)	82 (90.1 %)
CEF 1st cycle	59 (67.0 %)	76 (83.5 %)
CEF 2nd cycle	58 (65.9 %)	75 (82.4 %)
CEF 3rd cycle	58 (65.9 %)	69 (75.8 %)
CEF 4th cycle	55 (62.5 %)	67 (73.6 %)
Clinical response rate		
CR	40 (45.5 %)	30 (33.0 %)
PR	34 (38.6 %)	34 (37.4 %)
SD	6 (6.8 %)	5 (5.5 %)
PD	5 (5.7 %)	13 (14.3 %)
NE	3 (3.4 %)	9 (9.9 %)
Breast surgery	88 (100 %)	89 (98.9 %)
Breast-conserving surgery	54 (61.4 %)	59 (64.8 %)
Axillary lymph nodes dissection	59 (67.0 %)	64 (70.3 %)
No. of nodes		
Negative	21	27
1–3 nodes	20	27
4–9 nodes	11	9
≥10 nodes	7	1
Adjuvant radiotherapy	50 (56.8 %)	56 (61.5 %)
Adjuvant endocrine therapy	37 (42.0 %)	33 (36.3 %)

CP carboplatin and weekly paclitaxel, *CEF* cyclophosphamide/epirubicin/5-fluorouracil, *P* weekly paclitaxel, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease

Efficacy

After chemotherapy, 88 patients in the CP-CEF arm and 89 patients in the P-CEF arm underwent breast surgery. Two patients in the P-CEF arm did not undergo surgery due to proven stage IIIC disease after enrollment ($n = 1$), and patient refusal to continue treatment due to adverse events experienced during CEF ($n = 1$). The breast conservation rates were 61.4 % in the CP-CEF arm and 64.8 % in the P-CEF arm. Fifty-nine patients (67.0 %) in the CP-CEF arm and fifty-nine patients (67.0 %) in the P-CEF arm underwent AxLND (Table 2).

The overall clinical response rate to CP-CEF was significantly higher than that to P-CEF (84.1 vs. 70.3 %, $P = 0.03$). Disease progression was observed in 4 patients who received CP-CEF (3 during CP and 1 during CEF) and 10 patients who received P-CEF (8 during P and 2 during CEF). After completion of neoadjuvant chemotherapy, 1

Table 3 Odds ratios for pCR rates according to subgroups

Subgroup	Non-pCR[a] No.	pCR No. (%)	Univariative analysis Odds ratio (95 % CI)	*P* value
Arm				
CP-CEF	60	28 (31.8)	2.19 (1.08–4.41)	0.04
P-CEF	75	16 (17.6)	1.00	
Age (years)				
<50	75	28 (27.2)	0.71 (0.35–1.44)	0.38
≥50	60	16 (21.1)	1.00	
Clinical T stage				
T1–2	95	39 (29.1)	1.00	0.02
T3	40	5 (11.1)	0.30 (0.11–0.83)	
Clinical N status				
Negative	43	19 (30.6)	1.00	0.20
Positive	92	25 (21.3)	0.61 (0.31–1.24)	
Histological grade				
1	26	3 (10.3)	1.00	0.06
2–3	109	41 (27.3)	3.26 (0.94–11.35)	
Hormone receptor status				
Negative	42	33 (44.0)	0.15 (0.07–0.33)	<0.01
Positive	93	11 (10.6)	1.00	
ER+/PgR+	68	5 (6.8)		
ER+/PgR−	21	6 (22.2)		
ER−/PgR+	2	2 (50)		

ER estrogen receptor, *pCR* pathological complete response, *PgR* progesterone receptor, *T* tumor size, *T1* (≤2.0 cm), *T2* (2.1–5.0 cm), and *T3* (≥5.1 cm)

[a] Including 3 patients in the P-CEF arm (1 patient with stage IIIC disease and 2 patients who did not undergo breast surgery)

patient in the CP-CEF arm and 3 patients in the P-CEF arm experienced disease progression. All 3 patients in the CP-CEF arm and 10 of 13 patients in the P-CEF arm who experienced disease progression had HR-negative disease.

The pCR rate in the CP-CEF arm was significantly higher than that in the P-CEF arm (31.8 vs. 17.6 %, one-sided $P = 0.01$). Among these pCR patients, 9 of 28 patients in the CP-CEF arm and 4 of 16 patients in the P-CEF arm had DCIS. In the per-protocol population, the difference in pCR rates between the two arms was not significant [28.0 % (14/50) in the CP-CEF arm vs. 24.2 % (15/62) in the P-CEF arm, one-sided $P = 0.179$]. By univariate analysis, treatment arm, clinical tumor size, and HR status were significantly associated with pCR (Table 3), and these were all shown to be independent factors by multivariate analysis. Among HR-negative patients, 23 of 37 patients (61.2 %) in the CP-CEF arm achieved a pCR; this rate was significantly higher than that in the P-CEF arm [26.3 % (10/38), $P = 0.003$, Fig. 3]. Among patients with HR-positive and histological grade 1 disease, 0 of 12 patients in the CP-CEF arm and 1 of 11 patients in the P-CEF arm experienced a pCR. In contrast, among patients with HR-positive and histological grade 2–3 disease, 5 of 39 patients (12.8 %) in the CP-CEF arm and 5 of 42 patients (11.9 %) in the P-CEF arm experienced a pCR. Other factors associated with significantly higher pCR rates in the CP-CEF arm included age (≥50 years), clinical tumor size (T1–2), and histological grade (grade 2–3). After a median follow-up of 12.0 months, 4 and three patients experienced disease recurrence in the CP-CEF and P-CEF arms, respectively.

Safety

Grade 3–4 hematologic toxicities were more common in patients treated with CP than in those treated with P (neutropenia 58.0 vs. 9.9 %, anemia 15.9 vs. 0 %, and thrombocytopenia 1.1 vs. 0 %, respectively, Table 4). Non-hematologic toxicities were similar between the two treatment arms. In the CP-CEF arm, 26 patients discontinued CP due to adverse events, which were predominantly hematologic toxicities [prolonged neutropenia ($n = 19$), febrile neutropenia ($n = 1$), thrombocytopenia ($n = 2$), peripheral sensory neuropathy ($n = 2$), infection ($n = 1$), and elevation of liver transaminase ($n = 1$)], and 3 patients discontinued CEF due to adverse events. Five and six patients in the P-CEF arm discontinued P and CEF, respectively, due to adverse events. One patient in the CP-CEF arm developed acute monocytic leukemia 1.5 years after completion of neoadjuvant chemotherapy.

Discussion

The addition of carboplatin to wPTX followed by CEF significantly improved the pCR rates in the ITT population in the present study. No difference in pCR rates was observed in the per-protocol population, although this could be due to the high rate of discontinuation of neoadjuvant chemotherapy in the CP-CEF arm (37.5 %) and the small sample size.

A meta-analysis of 12 randomized neoadjuvant trials for breast cancer (12,993 patients total) suggested that pCR rates differed by tumor subtype [18]. In patients with HER2-negative and HR-positive disease, the pCR rates of patients with grade 1–2 and 3 were 7 and 16 %, respectively. The pCR rate of patients with TNBC was 34 %. Furthermore, the association between pCR and event-free survival in patients with HR-positive and grade 3 disease or TNBC was significant. In the present study, the difference in pCR rates between the two arms was not significant in patients with HR-positive disease. However, in patients with TNBC, the pCR rate in the CP-CEF arm was

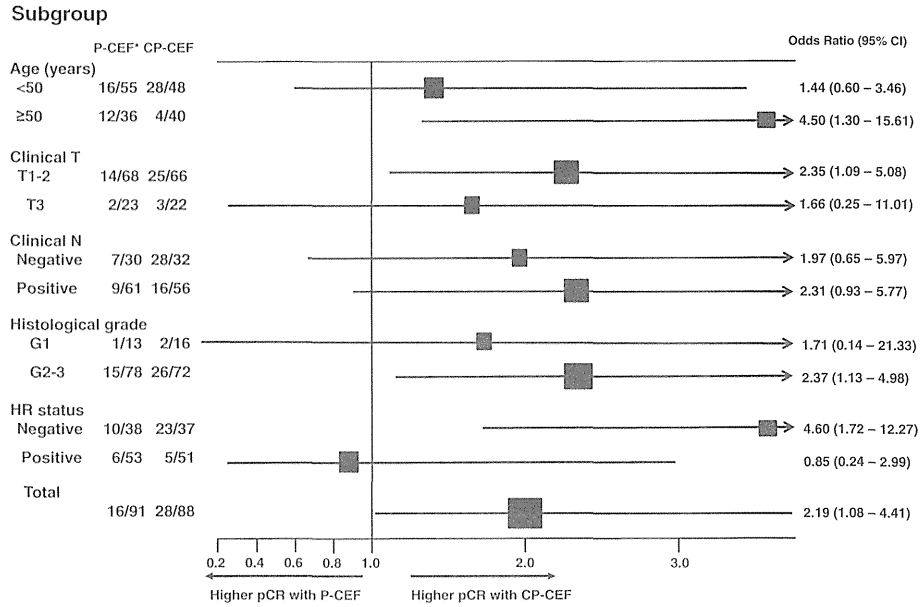

Fig. 3 Odds ratios for pCR rates between the two treatment arms by subgroup. *pCR* pathological complete response, *T* tumor size, *T1* (≤2.0 cm), *T2* (2.1–5.0 cm), and *T3* (≥5.1 cm). *Asterisk* including 3 patients in the P-CEF arm (1 patient with stage IIIC disease and 2 patients who did not undergo breast surgery)

Table 4 Grade 3–4 adverse events (NCI-CTCAE version 4.03)

Treatment arm	CP-CEF				P-CEF			
Adverse events	All		CP phase		All		P phase	
	G3 %	G4 %	G3 %	G4 %	G3 %	G4 %	G3 %	G4 %
Anemia	18.2	1.1	14.8	1.1	1.1	0	0	0
Neutropenia	46.6	19.3	52.3	5.7	17.6	20.9	8.8	1.1
Thrombocytopenia	1.1	0	1.1	0	0	0	0	0
Febrile neutropenia	20.5	0	2.3	0	15.4	0	0	0
Abdominal pain	1.1	0	1.1	0	0	0	0	0
Oral mucositis	1.1	0	0	0	1.1	0	0	0
Nausea	3.4	0	2.3	0	2.2	0	0	0
Vomiting	2.3	0	1.1	0	0	0	0	0
Fatigue	2.3	0	2.3	0	1.1	0	0	0
Infection	4.4	0	2.2	0	1.1	0	0	0
Elevation of ALT	2.3	0	2.3	0	2.2	0	1.1	0
Elevation of AST	1.1	0	1.1	0	1.1	0	1.1	0
Elevation of GGT	1.1	0	1.1	0	0	0	0	0
Anorexia	2.3	0	2.3	0	0	0	0	0
Dehydration	1.1	0	1.1	0	0	0	0	0
Hypertriglyceridemia	1.1	0	1.1	0	0	0	0	0
Hypokalemia	1.1	0	0	0	0	0	0	0
Arthritis	1.1	0	1.1	0	0	0	0	0
Peripheral motor neuropathy	1.1	0	1.1	0	0	0	0	0
Peripheral sensory neuropathy	1.1	0	1.1	0	1.1	0	1.1	0
Syncope	1.1	0	1.1	0	0	0	0	0

ALT alanine aminotransferase, *AST* aspartate aminotransferase, *CP-CEF* carboplatin and weekly paclitaxel followed by cyclophosphamide/epirubicin/5-fluorouracil, *P-CEF* weekly paclitaxel followed by cyclophosphamide/epirubicin/5-fluorouracil, *GGT* gamma-glutamyl transpeptidase, *CP* carboplatin and weekly paclitaxel, and *P* weekly paclitaxel

significantly higher than that in the P-CEF arm (Fig. 3). In the randomized studies of the addition of carboplatin to anthracycline and taxane for TNBC in neoadjuvant settings, one study showed no improvement of the pCR rate by addition of carboplatin (GEICAM/2006-03: $n = 93$, 29.8 vs. 3.48 %, $P = 0.606$) and the other two studies suggested any improvement of the pCR rates (GeparSixto: $n = 315$, 58.7 vs. 37.9 %, $P < 0.05$; CALGB40603: $n = 233$, 60 vs. 46 %, $P < 0.0018$) [19–21]. The present results combined with those of previous studies suggested

an advantage associated with the addition of platinum compounds to anthracyclines and taxanes as neoadjuvant therapy for TNBC.

The dosage and schedule of carboplatin and wPTX in the experimental arm of our study were chosen on the basis of the results of a previous study in advanced ovarian cancer, in which improved survival was observed in patients who received wPTX compared with the conventional triweekly schedule. In that study, 312 patients were treated with carboplatin (AUC of 6 on day 1) plus wPTX (80 mg/m^2 on day 1, 8, and 15) every 3 weeks, and carboplatin doses were reduced for hematologic toxicities in 48 % of patients. Therefore, the AUC of carboplatin in the present study was reduced to 5 [22]. In the present study, hematologic toxicities were more common in the CP-CEF arm, and they resulted in delayed administration or at least one dose reduction of paclitaxel (73.9 %) and dose reduction of carboplatin (20.5 %). In the CALGB 40603 trial, 4 cycles of triweekly administration of carboplatin (AUC6) with wPTX increased grade 3/4 neutropenia and thrombocytopenia [21]. In the 18 weekly administrations of liposomal doxorubicin, paclitaxel, and carboplatin (AUC2) of the GeparSixto study, all treatments were completed by 52.2 % and discontinuations due to adverse events occurred in 37.7 % [20]. The optimum dosage and schedule of carboplatin and wPTX have not yet been established. The frequency of neutropenia in patients who received paclitaxel and carboplatin, which were given every week, was lower than that reported in the present study. A weekly carboplatin and paclitaxel may be an alternative regimen with mild hematologic toxicities. A randomized trial of sequential taxane and anthracycline neoadjuvant regimens showed no significant difference in pCR rates between the two sequences, although the regimen of a taxane followed by an anthracycline was associated with milder hematologic toxicity [23]. In the present study, due to concerns about hematologic toxicities associated with the combination of carboplatin and wPTX, a sequence of a taxane followed by an anthracycline was chosen.

The present study has a number of limitations, and was stopped early before full accrual keeping with 87 % power and one-sided 10 % significance level. In the present study, the definition of HR negativity was <10 % staining of cancer cell nuclei by IHC. Out of concerns about false negative or positive, The ER- and PgR-negativities are recommended <1 % staining of cancer nuclei irrespective of staining intensity with the objectives of clinical trial eligibility for TNBC [24]. In the vitro study, basal-like subtypes of TNBC depending on gene profiles were suggested a highly sensitive to cisplatin, and pragmatic selection method of basal-like subtypes is an issue in the future [15]. The primary endpoint was a pCR rate rather than indicative of long-term outcome. A meta-analysis of neoadjuvant breast cancer trials showed that the magnitude of improvement in pCR did not predict long-term outcomes. However, in patients with TNBC, improvement of pCR was significantly associated with improvement of event-free and overall survival [18]. Therefore, the improvement of pCR associated with the addition of carboplatin in patients with TNBC in the present study may contribute to improved long-term outcomes.

In conclusion, the addition of carboplatin to wPTX followed by CEF for HER2-negative breast cancer improved the pCR rate but resulted in more hematologic toxicity.

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References

1. Mauri D, Pavlidis N, Ioannidis HPA (2005) Neoadjuvant versus adjuvant systemic treatment in breast cancer. J Natl Cancer Inst 97:188–194
2. Kaufmann M, von Minckwitz G, Mamounas EP et al (2012) Recommendations from an international consensus conference on the current status and future of neoadjuvant systemic therapy in primary breast cancer. Ann Surg Oncol 19:1508–1516
3. Bear HD, Anderson S, Brown A et al (2003) The effect on tumor response of adding sequential preoperative docetaxel to preoperative doxorubicin and cyclophosphamide: preliminary results

from National Surgical Adjuvant Breast and Bowel Project Protocol B-27. J Clin Oncol 21:4165–4174
4. von Minckwitz G, Kummel S, Vogel P et al (2008) Intensified neoadjuvant chemotherapy in early-responding breast cancer: phase III randomized Gepar Trio Study. J Natl Cancer Inst 100:552–562
5. Gianni L, Eiermann W, Semiglazov V et al (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 randomized controlled superiority trial with a parallel HER-negative cohort. Lancet 375:377–384
6. von Minckwitz G, Rezai M, Loibl S et al (2010) Capecitabine in addition to anthracycline- and taxane-based neoadjuvant treatment in patients with primary breast cancer: phase III Gepar Quattro study. J Clin Oncol 28:2015–2023
7. Earl HM, Vallier AL, Hiller L et al (2014) Effects of the addition of gemcitabine, and paclitaxel-first sequencing, in neoadjuvant sequential epirubicin, cyclophosphamide, and paclitaxel for woman with high-risk early breast cancer (Neo-tAnGo): an open-label, 2 × 2 factorial randomized phase 3 trial. Lancet Oncol 15:201–212
8. Kolaric K, Vukas D (1991) Carboplatin activity in untreated metastatic breast cancer patients—results of a phase II study. Cancer Chemother Pharmacol 27:409–412
9. Martin M, Diaz-Rubio E, Casado A et al (1992) Carboplatin: an active drug in metastatic breast cancer. J Clin Oncol 10:433–437
10. O'Brien ME, Talbot DC, Smith IE (1993) Carboplatin in the treatment of advanced breast cancer: a phase II study using a pharmacokinetically guided dose schedule. J Clin Oncol 11: 2112–2117
11. Slamon D, Eiermann W, Robert N et al (2011) Adjuvant trastuzumab in HER2-positive breast cancer. N Engl J Med 365: 1273–1283
12. Robert N, Leyland-Jones B, Asmar L et al (2006) Randomized phase III study of trastuzumab, paclitaxel, and carboplatin compared with trastuzumab and paclitaxel in women with HER-2 overexpressing metastatic breast cancer. J Clin Oncol 24: 2786–2792
13. Fountzilas G, Dafni U, Dimopoulos MA et al (2009) A randomized phase III study comparing three anthracycline-free taxane-based regimens, as first-line chemotherapy, in metastatic breast cancer: a Hellenic Cooperative Oncology Group study. Breast Cancer Res Treat 115:87–99
14. Kelly CM, Green MC, Broglio K et al (2012) Phase III trial evaluating weekly paclitaxel versus docetaxel in combination with capecitabine in operable breast cancer. J Clin Oncol 30:930–935
15. Lehmann BD, Bauer JA, Chen X et al (2011) Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest 121: 2750–2767
16. Fan Y, Xu BH, Yuan P et al (2013) Docetaxel-cisplatin might be superior to docetaxel–capecitabine in the first-line treatment of metastatic triple-negative breast cancer. Ann Oncol 24:1219–1225
17. Elston CW, Ellis IO (1991) Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology 19:403–410
18. Cortazar P, Zhang L, Untch M et al (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
19. Alba E, Chacon JI, Llush A et al (2012) A randomized phase II trial of platinum salts in basal-like breast cancer patients in the neoadjuvant seeting. Results from the GEICAM/2006-03, multicenter study. Breast Cancer Res Treat 136:487–493
20. Sikow WM, Berry DA, Perou CM et al (2013) Impact of the addition of carboplatin and/or bevacizumab to neoadjuvant weekly paclitaxel followed by dose-dense AC on pathologic complete response rates in triple-negative breast cancer: CALGB/Alliance 40603. In: 36th annual San Antonio Breast Cancer Symposium abstract S5-01
21. von Minckwitz G, Schneeweiss A, Salat C et al (2013) A randomized phase II trial investigating the addition of carboplatin to neoadjuvant therapy for triple-negative and HER2-positive early breast cancer (GeparSixto). J Clin Oncol 31:860–867
22. Katsumata N, Yasuda M, Takahashi F et al (2009) Dose-dense paclitaxel once a week in combination with carboplatin every 3 weeks for advanced ovarian cancer: a phase 3, open-label, randomised controlled trial. Lancet 374:1331–1338
23. Thiery-Vuillemin A, Llombart-Cussac A, Chaiqneau L et al (2011) Sequential taxane and anthracycline-containing neoadjuvant regimens: the sequential order impact. Breast 20:46–49
24. Eiermann W, Bergh J, Cardoso F et al (2012) Triple negative breast cancer: proposals for a pragmatic definition and implications for patient management and trial design. Breast 21:20–26

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Original article

The effect of molecular subtype and body mass index on neo-adjuvant chemotherapy in breast cancer patients

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ABSTRACT

The aim of the present study was to analyze the effect of subtype and body mass index (BMI) on neo-adjuvant chemotherapy (NAC) and postoperative prognosis. Two-hundred and forty nine patients who underwent surgery after NAC were included. A multivariate analysis and survival analysis were used to clarify the relationship between BMI, subtype, and NAC. In the logistic regression model, the pCR rate had a significant relationship with the subtype and tumor stage. In the non-pCR group, more overweight patients had significantly a worse disease-free survival (DFS) compared to normal range patients (Log lank test, $p < 0.05$). In the Cox proportional hazards model, subtype and tumor stage were significantly associated with decreased DFS. In conclusion, patients with the ER (+), HER (−) type and a high BMI had a high risk for recurrence when they achieved non-pCR after NAC.

Introduction

Neo-adjuvant chemotherapy (NAC) plays an important role in breast cancer therapy. It is well known that the recurrence rate after NAC is significantly reduced in patients who achieved a pathologic complete response (pCR) [1,2]. On the other hand, the optimal approach to treat patients with a non-pCR is still uncertain. Moreover, detecting high risk groups among these patients and preventing cancer recurrence is also an important concern.

From the results of major clinical trials on NAC which held in Germany (GeparDuo, GeparTrio, GeparQuattro, AGO1, PREPARE, TECHNO), Minckwitz et al. reported that disease-free survival (DFS) was significantly prolonged in patients with a pCR whose subtype was luminal B, human epidermal growth factor 2 (HER2) negative, non-luminal with HER2 positive, and triple negative (TN) compared to other subtypes. On the contrary, there were no associations between pCR and DFS in the group whose subtype was luminal A and luminal B-HER2 positive. [3] Considering these results, it may be difficult to predict the prognosis after NAC in patients with luminal A, as well as luminal B, HER2 positive subtypes by using the results of the chemotherapeutic effect of NAC. Further analysis is needed on these groups to detect other risk factors for recurrence.

In addition to the subtype, several risk factors for recurrence were reported in non-pCR groups, such as tumor size, number of metastatic lymph nodes, and body mass index (BMI) [3,4]. It is easy to understand why large tumors and many metastatic lymph nodes are associated with recurrence; however, the relationship between BMI and the NAC effect is still unclear. Many studies have shown that obese breast cancer patients showed resistance to breast cancer therapy and that this led to worse overall survival (OS) and disease-free survival (DFS) compared to normal weight patients [4–9]. The meta-analysis report which included 43 studies between 1963 and 2005 also showed poorer survival among obese compared with non-obese women with breast cancer, which was similar for overall (HR = 1.33; 95% confidence interval (CI): 1.21, 1.47) and breast cancer-specific (HR = 1.33; 95% CI: 1.19, 1.50) survival [10]. Recent research in molecular biology confirmed that obesity promoted cancer progression by elevating the serum estrogen level and insulin-like growth factor, which stimulated cancer progression through a growth regulatory pathway [11,12]. Evidently, obesity has a strong influence on the progression of breast cancer based on conventional reports. To the best of our knowledge, there is no report which analyzes the effect of the subtype and BMI on the chemotherapeutic effect, as well as prognosis after NAC. It is highly probable that clarifying the relationship

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between the subtype and BMI would help to detect high risk groups for recurrence after NAC. Moreover, an appropriate intervention for such high risk groups may improve the prognosis of non-pCR patients. From that point of view, we set out to analyze the effect of subtype and BMI on NAC.

Patients & methods

Patient selection

From April 2000 to November 2010, two-hundred and sixty-nine patients with breast cancer who underwent surgery after NAC at our institution were included. The patients who were diagnosed with stage IV due to the presence of distant metastasis were excluded.

BMI

Patients were categorized into Underweight (BMI < 18.5 kg/m^2), Normal range ($18.5 \leq$ BMI < 25 kg/m^2), Overweight ($25 \leq$ BMI < 30 kg/m^2), and Obese (BMI ≥ 30 kg/m^2) as defined by WHO BMI classification. Since the obese population is smaller in Asians than in Europeans, the BMI cut-off value for Asians corresponding to the risk recently proposed by WHO experts (Asian Adjusted BMI Classification, AABC) was also used [13]. Based on AABC, the patients were classified into underweight (BMI < 18.5), increasing but acceptable risk ($18.5 \leq$ BMI < 23), increased risk ($23 \leq$ BMI < 27.5), and high-risk (BMI ≥ 27.5) groups. BMI was calculated before the time of drug initiation.

Application for NAC

Inclusion criteria for NAC were as follows, 0–1 in performance status, 2 cm or more in diameter, and pathologically confirmed lymph node metastasis in the axillar area by fine needle aspiration biopsy. All patients underwent a core needle biopsy of the main tumor, and information regarding pathological type, hormone receptor, HER2, and pathological grade were obtained before NAC. Furthermore, computed tomography (CT) ranging from the neck to the abdomen, as well as bone scintigraphy with ^{99m}Tc, were performed in order to evaluate metastasis in regional lymph nodes and internal organs.

NAC regimen

Two hundred and forty-five patients received Anthracycline-based therapy, and 221 patients received Taxanes followed by Anthracycline. Fifteen HER2 positive patients received Trastuzumab combined with Taxanes. In statistical analysis, whether the patient received Taxanes or not was included in factor based analysis on a report that Taxanes increased the chemotherapeutic effect on NAC [13].

Drug dosage

The drug dosage was determined according to the body surface area (BSA) regardless of the patient's BMI [14]. BSA was calculated based on the Dubois & Dubois formula using the following equation: BSA (m^2) $= 0.007184 \times$ actual weight (kg)$^{0.425} \times$ actual height (cm)$^{0.725}$. Adverse events and side effects were carefully assessed based on the national cancer institute-common terminology criteria for adverse events version 3.0 (NCI-CTCAE v.3.0), and the dosage and duration of administration were controlled according to their severity [15]. The dose intensity in NAC was evaluated using the Relative Dose Intensity (RDI). RDI was calculated using the following equation: RDI = (dose intensity/planned dose intensity) $\times$ 100, Dose intensity (mg/m^2/week) = total dose (mg/m^2)/duration of administration (weeks).

Operative method

Breast-conserving surgery was performed when the tumor downsized to 30 mm or less and was considered to be safely resectable with an adequate margin. All patients received axillar lymph node dissection regardless of the NAC effect in the axillar area.

Histopathological evaluation

The histopathological evaluation was performed by six pathologists at the same institution. The therapeutic effect of preoperative chemotherapy was evaluated using the histologic breast regression score (RS) reported by Sinn et al. RS was classified based on the following criteria: RS 4: indicating no viable tumor cell residuals in the breast. RS 3: indicating only noninvasive residuals in the breast, RS 2: indicating only focal (<5 mm) invasive residuals in the breast, RS 1: indicating minimal signs of tumor regression, RS 0: indicating no signs of regression [3].

The definition of pCR for the present study was no residual invasive carcinoma in the breast, regardless of the axillary lymph nodes. Residual ductal carcinoma in situ was regarded as pCR. Immunohistochemistry (IHC) was performed to evaluate hormone receptor based on the American society of clinical oncology and the college of American pathologists (ASCO/CAP) guidelines [17]. If the HER2 study was equivocal, a 2.2 or higher HER2/CEP17 ratio calculated by fluorescence in situ hybridization (FISH) was considered as positive [18].

Adjuvant therapy

An aromatase inhibitor was administered to postmenopausal patients whose estrogen receptor (ER) or progesterone receptor (PgR) was positive more than 1% by IHC. Premenopausal patients with amenorrhea as a result of chemotherapy started adjuvant therapy with Tamoxifen. Radiation therapy (RT) was performed for patients who underwent breast-conserving therapy. If the number of metastatic lymph nodes was four or more, RT with 50 Gy was also performed to the chest wall, as well as the regional lymph node area. Furthermore, a total 10 Gy of boost radiation therapy to the regional lymph node area was performed for patients who were suspected of four or more metastatic lymph nodes on CT evaluation before NAC, even if that finding disappeared as a result of the NAC effect.

Statistical methods

Prior to the application of multivariate analysis, univariate analysis was performed for a preliminary exploration of marked associations. In univariate analysis, each statistical test was performed according to the type of variables and distribution of populations. To clarify the effect of independent clinicopathological factors related to NAC, a logistic regression model was applied. OS was calculated from the date of the first visit to our facility to the date of death or last follow-up. DFS was calculated from the date of the first visit to our facility to the time disease metastasis or recurrence was recorded. The Kaplan–Meier method was used for survival analysis, and each survival curve was constructed according to BMI, subtype, or both. Each curve was statistically compared by log rank test. The hazard ratio (HR) and 95% CI were measured using the Cox proportional hazards model, which was applied to

evaluate survival analysis. All analysis data was reported by using Dr. SPSSII® for Windows (SPSS Japan Inc. Tokyo, Japan). $P < 0.05$ was considered as statistically significant. All reported P-values were two-sided.

Results

Patient characteristics

Thirty-seven patients (15%) achieved pCR, and the results of the chemotherapeutic effects were as follows, grade 0 (1 case, 1%), grade 1 (69 cases, 28%), grade 2 (124 cases, 49%), grade 3 (45 cases, 18%), and grade 4 (9 cases, 4%). The WHO BMI classification demonstrated underweightness (17 cases, 7%), a normal range (163 cases, 65%), overweightness (57 cases, 23%), and obesity (11 cases, 4%) (Table 1). When applying ABCC, patients were categorized into underweight (17 cases, 7%), increasing but acceptable risk (118 cases, 47%), increased risk (80 cases, 32%) and high-risk (33 cases, 13%) groups. In the present study, the number of overweight/obese patients tended to be smaller compared to Western studies. The subtype was ER (+)/HER2 (−) in 131 patients (53%), ER (+)/HER2 (+) in 26 (10%), HER2 in 31 (12%), and triple-negative in 60 (24%),

Table 1
Patient demographics. BMI: body mass index, WHO: world health organization, AABC: Asian adjusted body mass index classification, ER: estrogen receptor, PgR: pregesterone receptor, HER2: human epidermal growth factor receptor 2, LN: lymph node, RS: histologic breast regression score.

Characteristics		(N = 248)	
		No.of patients	%
Age (year ± SD)		50.3 ± 9.7	
pCR	Yes	37	15
	No	211	85
BMI		23.2 ± 3.7	
BMI (WHO)	<18.5 (Underweight)	17	7
	18.5 ≤ BMI < 25 (Normal range)	163	66
	25 ≤ BMI < 30 (Overweight)	57	23
	≥30 (Obesity)	11	4
BMI (AABC)	<18.5 (Underweight)	17	7
	18.5 ≤ BMI < 23 (Increasing but acceptable risk)	118	48
	23 ≤ BMI < 27.5 (Increased risk)	80	32
	≥27.5 (High risk)	33	13
Subtype	ER (+), HER2 (−)	131	53
	ER (+), HER2 (+)	26	10
	HER2 type	31	13
	Triple negative	60	24
Tumor stage	1	24	10
	2	139	56
	3	31	13
	4	54	22
Ax LN metastasis	Positive	136	55
	Negative	112	45
Menopausal status	Pre	125	50
	Post	115	46
	Removed	4	2
	Unknown	4	2
Histological type	Ductal	120	48
	Lobular	2	1
	Other	126	51
Operation method	Mastectomy	141	57
	Lumpectomy	106	43
	Other	1	0
Chemotherapy	Taxane	221	89
	No taxane	27	11
Chemotherapeutic effect (RS)	0	1	0
	1	69	28
	2	124	50
	3	45	18
	4	9	4

Table 2
Patients and tumor characteristics by pCR. BMI: body mass index, WHO: world health organization, AABC: Asian adjusted body mass index classification, ER: estrogen receptor, PgR: pregesterone receptor, HER2: human epidermal growth factor receptor 2, LN: lymph node, RS: histologic breast regression score.

Characteristics		pCR (N = 37)		Non-pCR (N = 211)		p*
		No.of patients	%	No.of patients	%	
Age (year ± SD)		50.8 ± 9.8		50.2 ± 9.7		0.73[a]
BMI		22.0 ± 3.1		23.4 ± 3.8		<0.05[a]
BMI (WHO)	<18.5 (Underweight)	5	14	12	6	0.19
	18.5 ≤ BMI < 25 (Normal range)	24	65	139	66	
	25 ≤ BMI < 30 (Overweight)	8	22	49	23	
	≥30 (Obesity)	0	0	11	5	
BMI (AABC)	<18.5 (Underweight)	5	14	12	6	0.24
	18.5 ≤ BMI < 23 (Increasing but acceptable risk)	19	51	99	47	
	23 ≤ BMI < 27.5 (Increased risk)	10	27	70	33	
	≥27.5 (High risk)	3	8	30	14	
Subtype	ER (+), HER2 (−)	9	24	122	58	<0.05
	ER (+), HER2 (+)	2	5	24	11	
	HER2 type	7	19	24	11	
	Triple negative	19	51	41	19	
Tumor stage	1	9	24	15	7	<0.05
	2	19	51	121	57	
	3	4	11	26	12	
	4	5	14	49	23	
Ax LN metastasis	Positive	35	5	134	64	<0.05
	Negative	0	0	77	36	
Menopausal status	Pre	17	46	108	51	0.84
	Post	18	48	97	46	
	Removed	1	3	3	1	
	Unknown	1	3	3	1	
Histological type	Ductal	21	57	99	47	0.48
	Lobular	0	0	2	1	
	Other	16	43	110	52	
Operation method	Mastectomy	12	32	129	61	<0.05
	Lumpectomy	25	68	81	38	
	Other	0	0	1	0	
Chemotherapy	Taxane	35	95	186	88	0.39
	No taxane	2	5	25	12	
Chemotherapeutic Effect (RS)	0	0	0	1	0	
	1	0	0	69	33	
	2	0	0	124	59	
	3	0	0	8	4	
	4	37	100	9	4	

*P values are from Fisher's Exact test unless otherwise indicated.
[a] Student's T test.

showing that the triple-negative type was the second most frequent following the ER (+)/HER2 (−) type.

Table 2 shows patients and tumor characteristics by pCR. BMI was significantly correlated with pCR when analyzed as a continuous variable, but not a categorical one (WHO BMI classification or ABCC) (22.0 ± 3.1 in pCR group, 23.3 ± 3.8 in non-pCR group; $P < 0.05$). Tumor stage, Ax lymph node metastasis, and operation method were also associated with pCR. More non-pCR patients received mastectomy due to having larger tumors (Table 2). In addition, significantly more patients were positive for axillary lymph node metastasis in the non-pCR than pCR group.

Drug dosage

The relationships of the RDI and hematological adverse events with the BMI are shown in Table 3. The RDI was within the range of 97–98%, showing that the dose intensity was maintained at a high level regardless of the BMI. No significant difference was noted in

Table 3
Relative dose intensity and Hematological adverse events based on two BMI classification. Hematological adverse events were assessed referring NCI-CTCAE v3.0.

		RDI (%, average ± SD)	P	Hematological adverse events (Grade 3/4)	P
BMI			0.78[a]		0.36[b]
(WHO)	<18.5 (Underweight, N = 17)	98.2 ± 4.4		4/18	
	18.5 ≤ BMI < 25 (Normal range, N = 163)	98.6 ± 3.5		21/163	
	25 ≤ BMI < 30 (Overweight, N = 57)	98.3 ± 6.9		11/57	
	≥30 (Obesity, N = 11)	99.2 ± 1.6		3/11	
BMI			0.29[a]		0.76[b]
(AABC)	<18.5 (Underweight, N = 17)	98.2 ± 4.4		4/18	
	18.5 ≤ BMI < 23 (Increasing but acceptable risk, N = 118)	98.7 ± 3.9		16/118	
	23 ≤ BMI < 27.5 (Increased risk, N = 80)	99.3 ± 1.9		13/80	
	≥27.5 (High risk, N = 33)	97.6 ± 8.8		6/33	

*P values are from.
[a] One-way analysis of variance.
[b] χ2 test.

the RDI using either the WHO BMI classification or ABCC. Grade 3 or more severe hematological adverse events, such as neutropenia, thrombocytopenia, anemia, infections, febrile neutropenia, and bleeding, were noted in 39 patients in total. No significant difference was noted in hematological adverse events using either the WHO BMI classification or ABCC (Table 3).

Predictors of pCR

In the univariate model, when BMI was categorized into four groups applying the WHO BMI classification or AABC, BMI showed no significant correlation with either pCR ($p = 0.23$) or the chemotherapeutic effect ($p = 0.56$) (Data applying AABC not shown in table) (Table 4). Underweight patients tended to be younger (average age = 46.0) and premenopausal (72%) compared to the other groups.

In the multivariate model, the pCR rate had a significant relationship with subtype and tumor stage. As for subtype, the HER2 type (odds ratio [OR] 4.46; 95% CI, 1.42 to 14.0; $P < 0.05$) and triple negative type (OR 6.29; 95% CI, 2.52 to 15.71; $P < 0.05$) had a more significant association with pCR compared with ER (+), HER (−) Type (Table 5). Regarding BMI, the associations of the WHO classification-based overweight (OR 0.34; 95% CI, 0.08 to 1.46; $P = 0.10$) and obese (OR 0.001; 95% CI, 0.00 to 3.75E + 11; $P = 0.67$) and AABC-based increased risk (OR 0.26; 95% CI, 0.06 to 1.06; $P = 0.06$) and high risk (OR 0.21; 95% CI, 0.04 to 1.14; $P = 0.07$) were not significant, showing that BMI was not extracted as a predictor of pCR using either classification.

Effect of pCR, BMI and subtype on breast cancer outcomes

After a median of 1292 days of follow-up, a total of 62 patients had a breast cancer recurrence. Fig. 1 presents DFS and OS by pCR and BMI. DFS significantly extended in patients who achieved pCR, but OS was not influenced (Fig. 1A and B). Using the WHO classification, DFS was significantly shorter in the overweight and obese

Table 4
Results of Univariate Analysis.Patients were divided into four categories based on their BMI.

Characteristics		BMI <18.5 (N = 17)		18.5 ≤ BMI (N = 163)		25 ≤ BMI < (N = 57)		BMI ≥ 30 (N = 11)		P
		No.of patients	%	No.of patients	%	No.of patients	%	No.of patients	%	
Age (year ± SD)		46.0 ± 7.9		50.5 ± 10.0		50.9 ± 9.1		51.4 ± 9.1		0.27[a]
pCR	Yes	5	29	24	15	8	14	0	0	0.23
	No	12	71	139	85	49	86	11	100	
Subtype	ER (+), HER2	8	47	8	5	33	58	5	45	<0.05
	ER (+), HER2	3	18	16	10	5	9	2	18	
	HER2 type	3	18	18	11	10	18	0	0	
	Triple negative	3	18	44	27	9	16	4	36	
Tumor stage	1	0	0	16	10	8	14	0	0	0.15
	2	15	88	89	55	30	53	5	45	
	3	2	12	20	12	6	11	2	18	
	4	0	0	37	23	13	23	4	36	
Ax LN metastasis (Before NAC)	Positive	6	35	90	55	33	58	7	64	0.49
	Negative	11	65	73	45	24	42	4	36	
Menopausal status	Pre	12	71	81	50	25	44	7	64	0.78
	Post	5	29	76	47	30	53	4	36	
	Removed	0	0	3	2	1	2	0	0	
	Unknown	0	0	3	2	1	2	0	0	
Histological type	Ductal	11	65	80	49	24	42	5	45	0.82
	Lobular	0	0	1	1	1	2	0	0	
	Other	6	35	82	50	32	56	6	55	
Operation method	Mastectomy	9	53	98	60	27	47	8	73	
	Lumpectomy	8	47	64	39	30	53	3	27	
	Other	0	0	1	1	0	0	0	0	
Chemotherapy	Taxane	16	94	143	88	53	93	9	82	0.63
	No taxane	1	6	20	12	4	7	2	18	
Chemotherapeutic effect (RS)	0	0	0	1	1	0	0	0	0	0.56
	1	0	0	47	29	18	32	4	36	
	2	10	59	81	50	28	49	5	45	
	3	6	35	28	17	10	18	1	9	
	4	1	6	6	4	1	2	1	9	

*P values are from Fisher's Exact test unless otherwise indicated.
[a] One-way analysis of variance.