(RR) of 10–30% in recurrent ovarian cancer, depending on the anticancer activity of the drug(s) used, cross-resistance with previously administered drugs, and the response of the primary tumor to platinum compounds. In general, primary platinum sensitivity is defined as a documented response to the initial platinum-based therapy for at least 6 months after the end of treatment. RR of 30% to >50% have been obtained in patients with longer treatment-free intervals or with platinum-sensitive primary tumors (primary platinum sensitivity) as compared with only <20% in patients with shorter treatment-free intervals (platinum resistance of the primary tumor) or refractory ovarian cancer (no remission in response to first-line therapy) (Blackledge et al. 1989; Markman et al. 1991; Thigpen et al. 1994). Most previous studies have focused on the overall tumor response and time to treatment failure for specific drugs rather than attempting to evaluate the overall response to second-, third-, or fourth-line chemotherapy. The aim of this retrospective study was to investigate the relations of clinicopathological factors to important clinical endpoints such as the RR, time to progression (TTP), and overall survival (OS) in response to third-line chemotherapy in women with recurrent ovarian, fallopian tube, and primary peritoneal cancer who received platinum/taxane regimens as first-line therapy.

## Patients and methods

### Patients

We retrospectively reviewed the medical records of patients with ovarian, fallopian tube, and primary peritoneal cancer treated at the National Cancer Center Hospital between 1999 and 2005. All the patients had received platinum/taxane regimens as first-line therapy. Treatment decisions were usually made by the attending clinician. Patients in whom the tumor was considered possibly platinum-sensitive usually received a platinum agent, a taxane, or both. In general, combination chemotherapy was not administered as salvage treatment for recurrent disease, and most patients with recurrent disease received a single chemotherapeutic agent. Drug-free interval (DFI) was measured from the date of the last dose of chemotherapy until disease progression. Primary DFI was measured from the date of last dose of first-line chemotherapy until disease progression, and secondary DFI was measured from the date of the last dose of second-line chemotherapy until disease progression. Patients participated in clinical trials if they were eligible. The imaging criteria for treatment response were based on two-dimensional measurements of the lesions. Serum CA125 levels were not used as a primary measure of the response, but were referred to in the evaluation of response. Complete response was defined as no evidence of disease on physical examination or imaging studies, with normalization of the serum CA125 level. Partial response was defined as a >50% reduction in tumor size. Stable disease was defined as a 25–50% decrease or increase, or as no change in tumor size. Patients with an increase in the serum CA125 level were not evaluated to have had a partial response or stable disease. Progressive disease was defined as a >25% increase in tumor size. The Response Evaluation Criteria in Solid Tumors (RECIST) criteria were not used because most patients received treatment before this system was adopted by our hospital.

### Statistical analysis

The main outcome measures for drug efficacy were RR, TTP, and OS. TTP was defined as the interval from the first day of third-line chemotherapy to the day of documented disease progression. For patients who were alive at the end of the study, the TTP data were right-censored to the time of the last evaluation or the time of the last contact at which the patient was progression-free. OS was defined as the interval from the first day of third-line chemotherapy to the day of death. For patients who were alive at the end of the study, the OS data were right-censored to the time of the last evaluation or contact. Data were analyzed by parametric and nonparametric statistics using SAS, version 9.1.3 (SAS Institute, Cary, NC, USA). Descriptive statistics were used for demographic data; such data are presented as mean with standard deviations or as medians with ranges. Survival was estimated using the Kaplan–Meier method, and differences between survival curves were evaluated with the log-rank test. A multivariate logistic regression analysis was performed to determine predictive factors of the response to chemotherapy. A Cox regression analysis was performed to determine factors influencing TTP and OS.

## Results

A total of 172 patients received first-line platinum/taxane regimens during the study period, of whom 111 had disease progression after first-line chemotherapy. Eighty-one of these 111 patients received second-line chemotherapy, among whom 73 had disease progression. Fifty-four of these 73 patients received third-line chemotherapy (Fig. 1). Mean age at the time of diagnosis of the primary cancer was 54 years (26–75 years), and mean age at the start of second- and third-line chemotherapy was 55 (28–76 years) and 55 (31–77 years) years, respectively. There were 46 cases (85.1%) of ovarian carcinoma, 7 (13.1%) of primary peritoneal carcinoma, and 1 (1.8%) of fallopian tube carcinoma. The patients' characteristics are shown in Table 1.

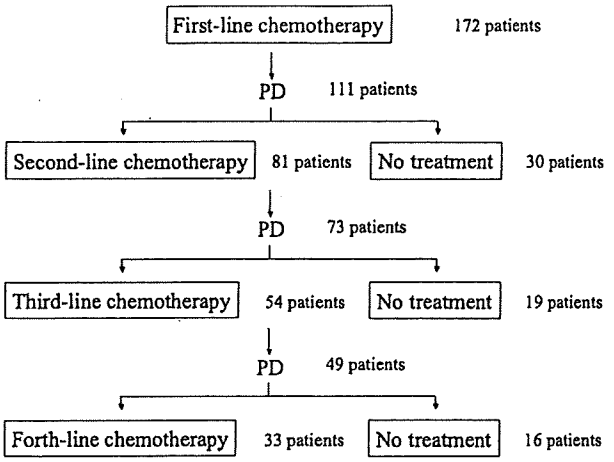


**Fig. 1** Schema of treatment

At the time of initial recurrence, 37 patients (69%) had primary platinum sensitivity, and 17 (31%) had primary platinum resistance. All patients treated with second-line drugs had either stable or progressive disease and received third-line treatment within 1–2 months after the last cycle of second-line treatment. The most commonly used regimen was weekly paclitaxel/carboplatin for second-line treatment and carboplatin for third-line treatment. The numbers of patients and chemotherapeutic drugs used in each setting are listed in Table 2. The median number of cycles of third-line treatment was 6 (range 1–18 cycles). The median TTP was 4.4 months (range 0–19.5 months), and the median OS was 10.4 months (range 1.5–44.3 months) (Fig. 2). The RR to third-line chemotherapy was 40.7% (95% CI; 27.6–53.8%). Five patients (9.2%) had complete responses and 17 (31.5%) had partial responses. Disease remained stable in 18 patients (33.3%) and progressed in 12 (22.2%). Two patients discontinued treatment because of hypersensitivity reactions to carboplatin. Overall, 49 patients had disease progression and 33 subsequently received fourth-line chemotherapy. The RR to fourth-line chemotherapy was 36.3% (95% CI, 19.9–52.7%); 2 patients had complete responses, 7 had partial responses, 9 had stable disease, and 12 had progressive disease. At the time of data analysis, 38 of the 54 patients (70.3%) had died. We studied the relations between the response to third-line drug therapy and clinical factors such as age, performance status (PS), histopathological type of cancer, number of target lesions, primary and secondary DFI, response to second-line chemotherapy, and the use of platinum/taxane regimens. The RR to third-line treatment was found to be significantly better in patients with a good PS (0 or 1) and a primary DFI of >6 months ($P = 0.04$ and $P = 0.009$, respectively, Table 3). Patients with a good PS and a primary DFI of >6 months also had a longer TTP and better OS ($P = 0.006$, $P = 0.005$ and $P = 0.01$, $P = 0.004$, respectively, Table 4). Median OS was slightly but not significantly better in the patients who responded to third-line chemotherapy (15.1 months; range 2.4–33.7 months) than in those who did not (9.4 months; range 1.5–44.3 months) ($P = 0.054$, Fig. 3). Median OS was significantly longer in the patients who received fourth-line chemotherapy (8.2 months; range 2.1–25.2 months) than in those who did not receive chemotherapy (2.4 months; range 0.2–16.2 months) ($P < 0.0001$, Fig. 4).

**Table 1** Patient characteristics ($n = 54$)

| | |
|---|---|
| Age (year) | Median (range) |
| At primary diagnosis | 54 (26–76) |
| At second-line chemotherapy | 55 (28–77) |
| At third-line chemotherapy | 55 (31–78) |
| Performance status | |
| 0 | 6 |
| 1 | 22 |
| 2 | 24 |
| 3 | 2 |
| Stage | |
| I | 4 |
| II | 5 |
| III | 30 |
| IV | 15 |
| Organ | |
| Ovarian carcinoma | 46 |
| Primary peritoneal carcinoma | 7 |
| Fallopian tube carcinoma | 1 |
| Pathology | |
| Serous adenocarcinoma | 40 |
| Endometrioid adenocarcinoma | 2 |
| Mucinous adenocarcinoma | 1 |
| Clear cell carcinoma | 5 |
| Undifferentiated carcinoma | 6 |
| No. of target lesions | |
| 1 | 38 |
| 2 | 10 |
| 3 | 6 |
| Drug free-interval (month) | |
| Primary | 8.2 (0.9–39.3) |
| Secondary | 8.3 (0.1–21.5) |
| 3rd-line regimens | |
| Platinum/taxane-containing regimens | 36 |
| Other regimens | 18 |

## Discussion

In women with ovarian cancer, treatment goals after failure to respond to first-line therapy are (1) the control or prevention

**Table 2** First-, second- and third- line chemotherapeutic regimens used

| First-line | | Second-line | | Third-line | |
|---|---|---|---|---|---|
| Paclitaxel/carboplatin | 35 | Weekly paclitaxel/carboplatin | 28 | Carboplatin | 14 |
| Docetaxel/carboplatin | 12 | Docetaxel/carboplatin | 12 | Weekly paclitaxel/carboplatin | 10 |
| Paclitaxel/cisplatin | 7 | Irinotecan | 5 | Irinotecan | 8 |
| | | Topotecan | 2 | Irinotecan/etoposide | 4 |
| | | Carboplatin | 2 | Docetaxel | 4 |
| | | Liposomal doxorubicin | 1 | Liposomal doxorubicin | 4 |
| | | Paclitaxel/carboplatin | 1 | Docetaxel/carboplatin | 3 |
| | | Irinotecan/carboplatin | 1 | Cisplatin | 2 |
| | | Irinotecan/etoposide | 1 | Paclitaxel/carboplatin | 1 |
| | | Docetaxel | 1 | Paclitaxel/cisplatin | 1 |
| | | | | Irinotecan/mitomycin | 1 |
| | | | | Paclitaxel | 1 |
| | | | | Etoposide | 1 |

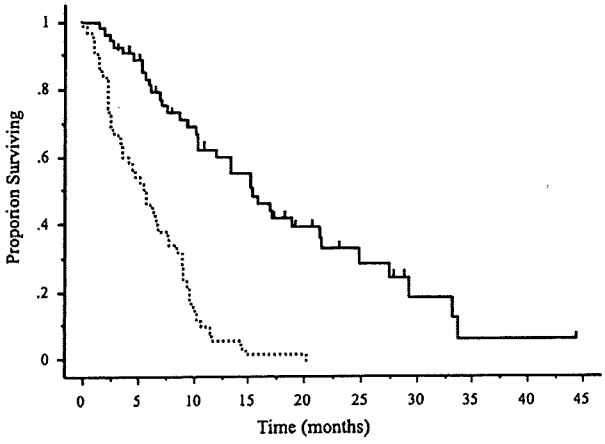


**Fig. 2** Kaplan–Meier analysis of time to progression (*solid line*) and overall survival (*dotted line*) following third-line chemotherapy. *Vertical bars* indicate censored cases

of disease-related symptoms, (2) the maintenance of a good quality of life, and (3) the prolongation of progression-free survival. The aims of salvage treatment have long been a matter of debate. The possibility of achieving an OS benefit in these patients is very limited. RRs are generally similar to or poorer than those with previous treatments are. Moreover, the increased risk of toxicity in patients with a history of previous treatment(s) and of negatively affecting performance status makes some physicians reluctant to continue drug treatment.

Patients who have good performance status without clinically significant comorbidity may wish to continue treatment (Doyle et al. 2001). Donovan et al. (2002) evaluated the treatment preferences of women with recurrent ovarian cancer and reported that most patients (86%) initially prefer subsequent therapy, with 25% never considering the withdrawal of chemotherapy, even when the expected median survival was <1 week. Physicians must therefore take into account patients' wishes along with other clinical data when planning treatment.

Most studies of salvage therapy have focused on the response to a particular single- or combined-drug regimen.

**Table 3** Multivariate analysis of response rates to third-line chemotherapy

| Clinical factors | No. of patients | Response rate (95% CI) | *P* value |
|---|---|---|---|
| Age | | | |
| <60 | 35 | 52.6% (30.1–75.0%) | 0.50 |
| ≥60 | 19 | 44.0% (23.7–56.2%) | |
| PS | | | |
| 0.1 | 28 | 57.1% (38.8–75.4%) | 0.04 |
| 2.3 | 26 | 30.7% (13.0–48.5%) | |
| Pathology | | | |
| Mucinous/clear cell | 6 | 33.3% (4.3–71.0%) | 0.22 |
| Non-mucinous/clear cell | 48 | 45.8% (31.7–59.9%) | |
| No. of target lesions | | | |
| 1 | 40 | 45.0% (29.5–60.4%) | 0.37 |
| 2.3 | 14 | 42.8% (16.9–68.7%) | |
| Primary DFI | | | |
| <6 months | 17 | 17.6% (0.4–35.7%) | 0.009 |
| ≥6 months | 37 | 51.3% (35.2–67.4%) | |
| Secondary DFI | | | |
| <6 months | 33 | 42.4% (25.5–59.2%) | 0.70 |
| ≥6 months | 21 | 47.6% (26.2–68.9%) | |
| Response to second-line therapy | | | |
| Responders | 35 | 45.7% (29.2–62.2%) | 0.09 |
| Non-responders | 19 | 31.5% (10.6–52.4%) | |
| PT regimens | | | |
| PT regimens | 36 | 38.8% (22.9–54.8%) | 0.75 |
| Non-PT regimens | 18 | 44.4% (21.4–67.4%) | |

*PT* Platinum/taxane

**Table 4** Multivariate analysis of TTP and OS following third-line chemotherapy

| Clinical factors | TTP *P* value | OS *P* value |
|---|---|---|
| Age (<60 vs. ≥60) | 0.59 | 0.76 |
| PS (0.1 vs. 2.3) | 0.006 | 0.005 |
| Pathology (mucinous/clear cell vs. non-mucinous/clear cell) | 0.34 | 0.29 |
| No. of target lesions (1 vs. 2.3) | 0.85 | 0.79 |
| Primary DFI (<6 months vs. ≥6 months) | 0.01 | 0.004 |
| Secondary DFI (<6 months vs. ≥ 6 months) | 0.61 | 0.34 |
| Response to second-line therapy (responder vs. non-responder) | 0.43 | 0.17 |
| PT regimens (PT regimens vs. non-PT regimens) | 0.84 | 0.36 |

*DFI* Drug free-interval, *PT* Platinum/taxane

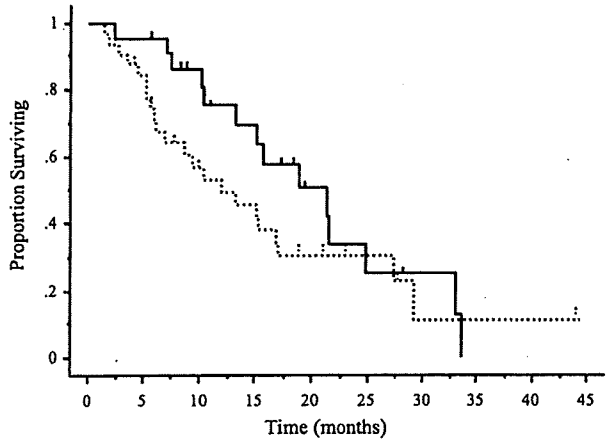


**Fig. 3** Kaplan–Meier analysis of overall survival (*bottom*) following third-line chemotherapy. The difference between third-line responders (*solid line*) and third-line non-responders (*dotted line*) was not statistically significant ($P = 0.054$). *Vertical bars* indicate censored cases

Without well-designed controlled studies, however, it is difficult to determine outcomes that would be obtained if a drug were used earlier or later in the course of salvage treatment.

In our study, the RR to third-line chemotherapy was 40.7% (95% CI; 27.6–53.8%). To date, only a few authors have distinguished between second- and third-line treatments when evaluating drug response and survival rates. In a study by Villa et al. (1999), 49 patients with recurrent ovarian cancer received third-line drugs after complete or partial responses to second-line chemotherapy. The overall RR was 48%, and median survival was 6 months. The 1-year survival rate differed significantly between patients who responded and those who did not respond to second-line treatment (82 vs. 39%, $P < 0.05$).

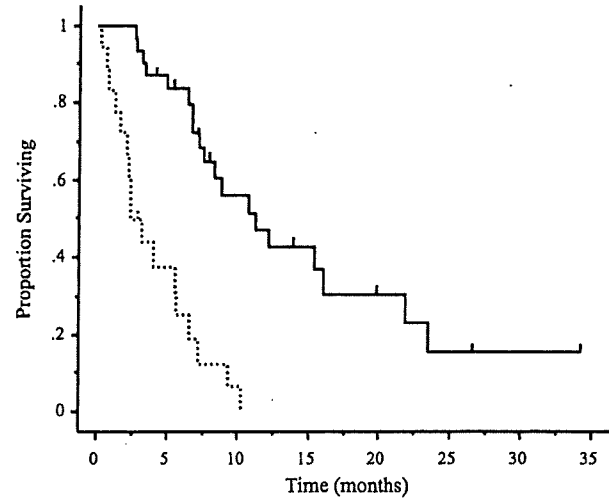


**Fig. 4** Kaplan–Meier analysis of overall survival following fourth-line chemotherapy. The chemotherapy group (*solid line*) had significantly better survival ($P < 0.0001$) than the non-chemotherapy group (*dotted line*). *Vertical bars* indicate censored cases

We obtained good RRs to third-line treatment in patients with a good PS and those with a primary DFI of >6 months. The response to third-line chemotherapy is influenced by the response to second-line chemotherapy. However, third-line chemotherapy has only a modest RR with a marginal prolongation of progression-free interval, but no obvious effect on survival in patients with ovarian cancer (Tangjitgamol et al. 2004).

In our study, there was no significant difference in the survival rate between patients responded to third-line chemotherapy and those who did not. In terms of cost-effectiveness, best supportive care is the only cost-effective strategy, followed perhaps by second-line monotherapy, given currently available chemotherapeutic options (Rocconi et al. 2006).

Patients with recurrent disease are often retreated with the same primary drug(s), most often platinum agents, but might also receive other drugs (Markman et al. 1991; Thigpen et al. 1993; Bookman 2003; Fung et al. 2002). One of the most important considerations in selecting second-line therapy is platinum sensitivity status, as defined by the response of the primary disease to a platinum drug and the progression-free interval after the completion of treatment (Blackledge et al. 1989; Thigpen et al. 1993; Thigpen et al. 1994). Some researchers have argued that there is no definite treatment-free interval, which can reliably distinguish platinum sensitivity from platinum resistance (Markman 1998; Markman et al. 1998). Nonetheless, it is generally accepted that the longer the treatment-free interval, the better is the expected response to retreatment (Markman et al. 1991; Thigpen et al. 1994). One study reported that women with recurrent ovarian cancer who had a treatment-free

interval of between 5 and 12 months showed a RR of only 27% to second-line platinum-based therapy, as compared with 59% in those with a treatment-free interval of longer than 24 months (Markman et al. 1991). However, the duration of secondary response to platinum therapy is less well documented; in particular, the relation between the duration of secondary response and that of the initial response is poorly understood.

Our results showed that PS and primary DFI were useful predictors of the response to third-line chemotherapy. Eisenhauer et al. (1997) conducted a multivariate analysis to determine predictors of clinical response to subsequent chemotherapy in 704 patients with platinum-pretreated ovarian cancer. Their initial univariate analysis revealed that response was significantly associated with many factors, including the drug used, time since diagnosis, tumor size, histology, and the presence or absence of liver metastasis. In contrast, their multivariate analysis showed that only serous histologic type, number of disease sites $\leq 2$, and maximum size of the largest lesion <5 cm were associated with a favorable response. They concluded that drug activity might not be the only determinant of response, and that tumor characteristics are also important factors.

In our study, two patients had hypersensitivity reactions to carboplatin. Patients who receive multiple courses of carboplatin have increased rates of hypersensitivity reactions (Zanotti et al. 2001). The incidence of such reactions is 27% in patients receiving 7 or more cycles of carboplatin, with more moderate to severe symptoms developing in more than 50% of these patients (Markman et al. 1999).

The time to treatment failure (4.4 months) and the OS (10.4 months) in our study were consistent with those reported by other studies assessing individual drugs (Villa et al. 1999; Heintz et al. 2001; Tangjitgamol et al. 2004; Rocconi et al. 2006). The survival of patients given fourth-line and subsequent treatment was significantly longer than that of patients who received no further therapy after third-line treatment (8.3 months vs. 2.4 months, respectively; $P < 0.0001$). Administration of fourth-line chemotherapy to patients who might tolerate such treatment may also improve OS; however, the analysis of OS in this setting has its limitations and is prone to potential bias. One study reported that giving additional lines of chemotherapy may not improve OS and that the inclusion of paclitaxel in treatment regimens may have a significant effect on survival (Findley et al. 2005).

In conclusion, our study suggested that PS and primary DFI may be useful predictors of the response to third-line chemotherapy in women with recurrent ovarian, fallopian tube, and primary peritoneal cancer. Our findings will hopefully help physicians make treatment recommendations and inform patients about expected benefits and risks, outcomes, and survival rates in this setting. Finally, the decision whether to use third-line chemotherapy should be based on a comprehensive assessment of patients' wishes, drug efficacy and toxicity, and treatment expertise of the clinician.

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ORIGINAL ARTICLE

# Clinicopathological significance of cervical adenocarcinoma associated with lobular endocervical glandular hyperplasia

Shin Nishio[a,b,*], Hitoshi Tsuda[a,c], Naoki Fujiyoshi[b], Shun-Ichiro Ota[b], Kimio Ushijima[b], Yuko Sasajima[c], Takahiro Kasamatsu[d], Toshiharu Kamura[b], Osamu Matsubara[a]

[a]*Department of Basic Pathology, National Defense Medical College, 3-2 Namiki, Tokorozawa, Saitama 359-8513, Japan*
[b]*Department of Obstetrics and Gynecology, Kurume University School of Medicine, 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan*
[c]*Pathology Section, Clinical Laboratory Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan*
[d]*Department of Gynecologic Oncology, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan*

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## Abstract

Lobular endocervical glandular hyperplasia (LEGH) is usually assumed to be a benign tumor-like lesion of the glands of the uterine cervix. However, LEGH has been associated with obvious cervical adenocarcinoma. The clinicopathological significance of coexistence of LEGH with adenocarcinoma remains unclear. We microscopically examined the presence or absence of LEGH components in 95 stage Ib cervical adenocarcinomas. Gastric mucin was detected with the use of clone HIK1083. Associations of the coexistence of LEGH components with clinicopathological variables were analyzed. LEGH components were present in 16 cases (16.8%). Gastric mucin was positive in all 16 LEGH components, as compared with only 6 of the 95 adenocarcinoma components. Of the 16 adenocarcinomas with LEGH components, 15 were well-differentiated mucinous adenocarcinomas, and one was poorly differentiated adenocarcinoma. The mortality rate of tumor recurrence was 25% (4 of 16) in patients whose tumors had LEGH components, and 21.5% (17 of 79) in those whose tumors had no LEGH components. There was no significant difference in survival. Early cervical adenocarcinoma was relatively frequently associated with LEGH components. LEGH may be one of the factors related to the development of cervical adenocarcinoma, but adenocarcinoma with LEGH components does not necessarily develop into a highly aggressive "adenoma malignum."


*Keywords:* LEGH; Cervical adenocarcinoma; Prognostic factor

## Introduction

Adenocarcinoma is detected in approximately 10% of all uterine cervix cancers. Among these lesions, minimal deviation adenocarcinoma (MDA), initially described by Gusserow in 1870, is characterized by a watery vaginal discharge clinically, an extremely

*Corresponding author at: Department of Obstetrics and Gynecology, Kurume University School of Medicine, 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan. Tel.: +81 942317573; fax: +81 942350238.
*E-mail address:* shinshin@med.kurume-u.ac.jp (S. Nishio).

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well-differentiated adenocarcinoma histologically, and high-grade malignancy biologically [1–3,15]. Indeed, MDA is usually associated with metastasis and dissemination at the time of diagnosis and poorly responds to treatment, resulting in poor outcomes.

In 1999, Nucci et al. [13] proposed a new disease entity called lobular endocervical glandular hyperplasia (LEGH) to describe a benign disease closely akin to MDA. Of the 13 reported cases, 4 had watery vaginal discharge, a characteristic symptom of MDA. Their report clearly distinguished LEGH from MDA: all affected glands appeared to be benign in LEGH, whereas MDA consistently had some regions that were clearly cancerous within the affected area. Subsequently, it was questioned whether LEGH could be accurately differentiated from MDA. However, recent studies have provided compelling evidence that MDA can be clearly distinguished from LEGH, and that LEGH and MDA are distinct disease entities [5,9,16,17].

The natural history of LEGH is still poorly understood. Some investigators have suggested that LEGH is a precancerous lesion, based on the occasional coexistence of LEGH and obvious adenocarcinoma [8,10]. However, it is not known how often adenocarcinoma develops from LEGH, and the biological characteristics of such cases need to be clarified. The present study was designed to clarify the frequency and the clinicopathological significance of LEGH components in early (stage Ib) cervical adenocarcinoma.

## Patients and methods

### Patients

The study group comprised 95 patients with stage Ib cervical adenocarcinoma (Ib1: 65 cases, Ib2: 30 cases) according to the diagnostic criteria proposed by the International Federation of Gynaecology and Obstetrics (FIGO). All cases were diagnosed and treated surgically at the Kurume University Hospital and National Cancer Center Hospital between 1989 and 2004. Postoperative radiotherapy was administered to patients who had lymph node metastasis, lymphovascular invasion, a tumor-invasion depth of more than two thirds (>2/3 invasion) of the cervical stroma, or poorly differentiated tumors. The resected tissue specimens were processed into formalin-fixed, paraffin-embedded sections for pathological examination, and sections containing a representative part of the tumor were studied.

Table 1 summarizes the characteristics of the adenocarcinomas studied. The histopathological factors suggesting high-grade malignancy were poor differentiation in 9 cases, a longitudinal tumor size of >4 cm in 30 cases, >2/3 invasion of the cervical stroma in 44 cases, lymphovascular invasion in 48 cases, and lymph node metastasis in 20 cases. The histological subtypes of adenocarcinoma were endocervical-type mucinous in 60 cases, endometrioid in 20 cases, intestinal-type mucinous in 10 cases, serous in 3 cases, and clear cell in 2 cases.

**Table 1.** Characteristics of 95 patients with stage Ib cervical adenocarcinoma.

| | |
|---|---|
| Age (years) median (range) | 47 (28–74) |
| <35 | 15 |
| 35–50 | 44 |
| ⩾50 | 36 |
| FIGO stage | |
| Ib1 | 65 |
| Ib2 | 30 |
| Tumor diameter (mm) median (range) | 25 (4–118) |
| Depth of stromal invasion (mm) median (range) | 10 (1.5–25) |
| Differentiation | |
| Well | 82 |
| Moderate | 4 |
| Poorly | 9 |
| Histopathology | |
| Endocervical-type mucinous | 60 |
| Intestinal-type mucinous | 10 |
| Endometrioid type | 20 |
| Serous | 3 |
| Clear cell | 2 |

### Histopathological evaluation

The presence or absence of LEGH was judged by two histopathologists (SN and HT). Cases that met the following criteria on examination of sections stained with hematoxylin and eosin (HE) were classified as adenocarcinoma with LEGH components: (1) the tumor is composed of a distinct area of LEGH and one area of obvious adenocarcinoma, e.g., endocervical-type mucinous, intestinal-type mucinous, endometrioid, serous, or clear cell adenocarcinoma. (2) The LEGH component shows the following characteristics: glands are arranged in certain directions and grow towards the musculature in a compressive manner while retaining the lobular structure; growth of the cervical glands is associated with scant evidence of nuclear atypia; glands assume a circular or oval form, with a regular margin; and clear demarcation from the surrounding musculature and no evidence of stromal invasion [9,13]. (3) The LEGH component shows the following characteristics: cells

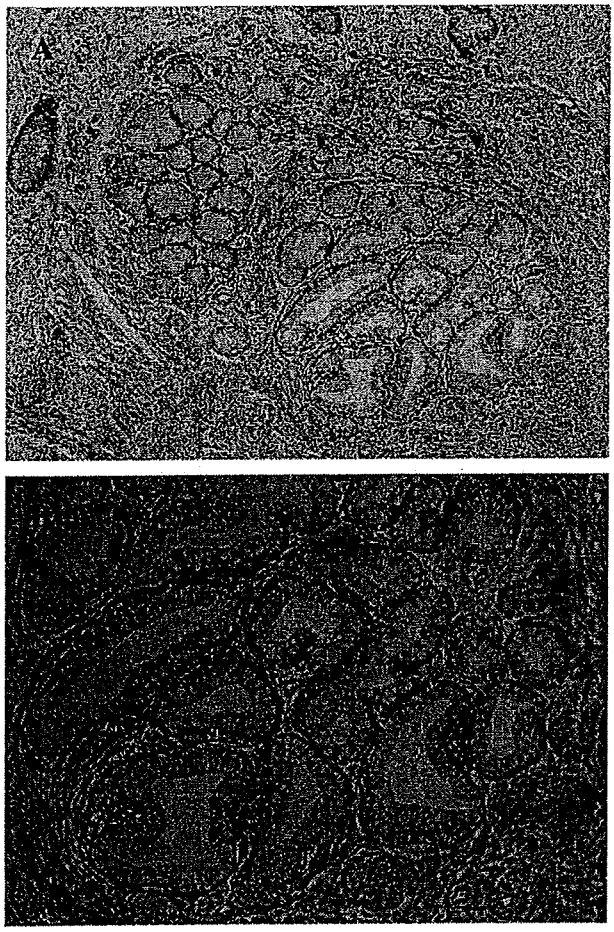


Fig. 1. Histological findings of the lobular endocervical glandular hyperplasia (LEGH) component in obvious cervical adenocarcinoma. (A) Lobular proliferation of small-to-medium-sized rounded glands surrounding larger glands. (B) Hyperplastic glandular lesions are arranged in a lobular fashion, without desmoplastic stomal reactions. These features are identical to those of pure LEGH. HE stain. Original magnification: (A) × 100; and (B) × 200.

with small nuclei (circular or oval) relatively uniform in size; nuclear chromatin not dense; minimal or no nucleoli; basally located nuclei, with no stratification; and no visible evidence of nuclear division or apoptosis [9,13] (Fig. 1).

## Immunohistochemistry

Tissue blocks were cut into 4-μm-thick sections, mounted on silane-coated glass slides, and studied immunohistochemically using the following primary monoclonal antibodies and dilutions: clone HIK1083, recognizing gastric mucin (1:200, Kanto Kagaku, Tokyo, Japan) [6,7,18], and anti-$p16^{INK4a}$ (clone sc-56330, 1:500, Santa Cruz, CA) [4,19]. The tissue sections were deparaffinized, subjected to antigen retrieval by autoclaving in sodium citrate buffer (pH 6.0) for 15 min at 121 °C for clone HIK1083 and anti-$p16^{INK4a}$, and allowed to cool at room temperature. Endogenous peroxidase was blocked with 5% hydrogen peroxide. Non-specific staining was blocked with 2% normal swine serum (Dako, Grostrup, Denmark). The slides were incubated with primary antibodies overnight at 4 °C and then allowed to react with a dextran polymer reagent mixed with secondary antibodies and peroxidase (Envision Plus; Dako) for 1 h at room temperature. Specific antigen–antibody reactions were visualized with 0.2% diaminobenzidine tetrahydrochloride (Muto Chemical, Tokyo, Japan) and hydrogen peroxide. Counterstaining was performed using Mayer's hematoxylin. For HIK1083, cases showing any degree of cytoplasmic immunoreactivity were judged as positive (Fig. 2).

On the basis of the literature [4,19], $p16^{INK4a}$ was regarded as nuclear immunostaining and was classified

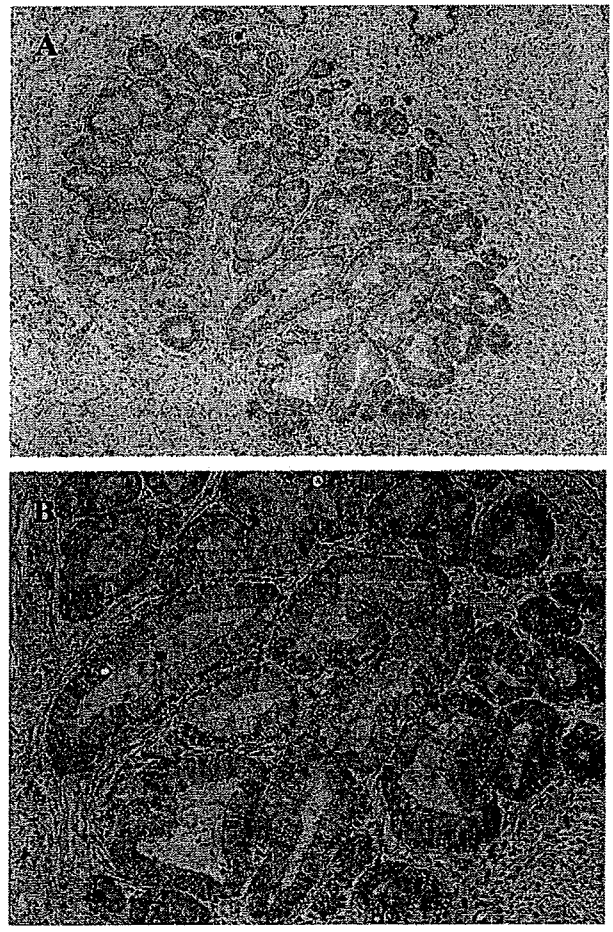


Fig. 2. Gastric mucin expression in lobular endocervical glandular hyperplasia (LEGH) components, detected on immunostaining with HIK1083. (A, B) Gastric mucin is diffusely positive in the cytoplasm of the LEGH component. Immunoperoxidase stain. Original magnification: (A) × 100; (B) × 200.

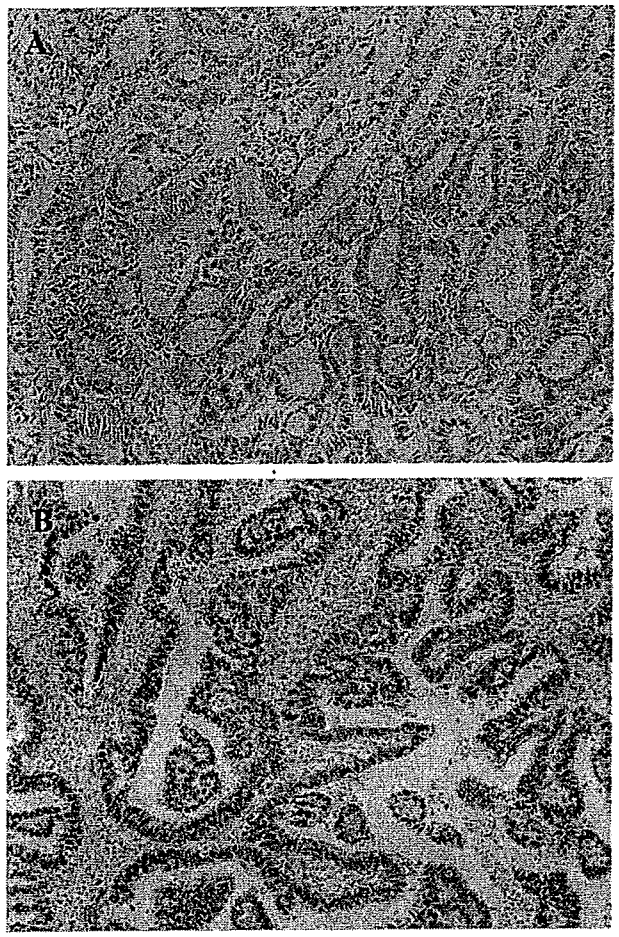


**Fig. 3.** $p16^{INK4a}$ expression in obvious cervical adenocarcinoma. (A) More than 10% of carcinoma cells show weak nuclear immunoreactivity, scored as 1+. (B) More than 10% of carcinoma cells show strong nuclear immunoreactivity, scored as 3+. Immunoperoxidase stain. Original magnification: (A) × 100; (B) × 200.

as +1 if less than 1% of the cells showed positive staining, as +2 if 1–10% of the cells showed positive staining, and as +3 if more than 10% of the cells showed positive staining. Scores of 0, 1+, or 2+ were defined as negative staining, whereas a score of 3+ was defined as positive staining.

As positive controls, we used gastric mucosal tissue for HIK1083 and a case of squamous cell carcinoma for $p16^{INK4a}$. As negative controls, the primary antibodies were omitted from the respective reactions (Fig. 3).

## Statistical analysis

Correlations between the presence of LEGH components and clinicopathological characteristics were analyzed using the chi-square test or Fisher's exact test. Cumulative survival curves were drawn by the Kaplan–Meier method, and differences between curves were tested by the log-rank test. Prognostic significance was computed by univariate and multivariate analyses with a Cox proportional-hazards model. Independent effects of the following variables were assessed by multivariate analysis: presence/absence of LEGH, $p16^{INK4a}$, tumor differentiation, tumor size, invasion depth of the cervical stroma, lymphovascular invasion, and lymph node metastasis.

## Results

The patients' characteristics are shown in Table 1. The median follow-up time was 66.3 months. At the time of analysis, tumor recurrence had been diagnosed in 21 patients, and 19 had died. Microscopic examination of HE-stained specimens revealed the presence of LEGH in 16 cases (16.8%). LEGH was localized to defined area(s) and did not intermingle with obvious adenocarcinoma components (Fig. 4). The mean maximal diameter of the LEGH component was 27 mm, ranging from 8 to 42 mm (standard deviation 8.01). The ratios of the area of the LEGH component in the 16 tumors ranged from 1% to 80%, with an average of 18.6% and a standard deviation of 24.92. The $p16^{INK4a}$ scores were 0, 1+, 2+, and 3+ in 13, 12, 10, and 63 cases, respectively. When classified according to the criteria used in this study, 63 cases (66.3%) with components of adenocarcinoma were positive for $p16^{INK4a}$. Staining for $p16^{INK4a}$ was positive in 5 (31.2%) of the 16 cases with LEGH components and in 58 (73.4%) of the 79 cases without LEGH components. Of the 16 cases with LEGH components, the cancer component was well-differentiated adenocarcinoma in 15 and poorly differentiated adenocarcinoma in one. The presence of LEGH components correlated with

**Fig. 4.** Histological findings of cervical adenocarcinoma. Apparent cellular atypia (left upper side) is seen in carcinoma cells, and atypical small glands (right lower side) are part of LEGH. Original magnification: × 20.

**Table 2.** Clinicopathological characteristics of 95 stage Ib cervical adenocarcinomas associated with or without lobular endocervical glandular hyperplasia (LEGH) components.

| Variable | No. of patients (%) | | | *P* value |
|---|---|---|---|---|
| | Total | LEGH component | | |
| | | Present | Absent | |
| Patient age (years old) | | | | |
| <50 | 40 | 8 | 32 | 0.15 |
| ⩾50 | 55 | 8 | 47 | |
| $p16^{INK4a}$ | | | | |
| Positive | 63 | 5 | 58 | 0.026 |
| Negative | 32 | 11 | 21 | |
| Maximal tumor diameter (cm) | | | | |
| <4.0 | 65 | 10 | 55 | 0.56 |
| ⩾4.0 | 30 | 6 | 24 | |
| Cervical stromal invasion | | | | |
| <2/3 in depth | 51 | 9 | 42 | 0.99 |
| ⩾2/3 in depth | 44 | 7 | 37 | |
| Lymphovascular space invasion | | | | |
| Negative | 47 | 7 | 30 | 0.78 |
| Positive | 48 | 9 | 39 | |
| Lymph node metastasis | | | | |
| Negative | 75 | 13 | 62 | 0.99 |
| Positive | 20 | 3 | 17 | |
| Differentiation of adenocarcinoma component | | | | |
| Well/moderate | 86 | 15 | 71 | 0.99 |
| Poorly | 9 | 1 | 8 | |
| Histological type of adenocarcinoma component | | | | |
| Endocervical/intestinal-type | 79 | 16 | 63 | 0.048 |
| Other histological type | 16 | 0 | 16 | |

$p16^{INK4a}$ ($P = 0.026$) and histological type ($P = 0.048$), respectively (Table 2).

In all 16 cases with LEGH components, immunoreactivity with clone HIK1083 was positive in the LEGH component. In 7 cases, HIK1083 immunoreactivity was also positive in the adenocarcinoma component. In 72 cases without LEGH components, immunoreactivity to HIK1083 was negative in the adenocarcinoma component.

In the study group as a whole, median disease-free survival was 61 months, and median overall survival was 62.1 months. Of the 16 patients who had cervical adenocarcinoma with LEGH, 4 died of tumor recurrence, and the remaining 12 were alive without recurrence. Of the 79 patients who had cervical adenocarcinoma without LEGH, 17 died. The survival curves did not differ significantly according to the presence or absence of LEGH.

Univariate analyses of prognostic factors potentially related to OS revealed that the following factors were associated with poorer clinical outcome: tumor size of >4 cm ($P = 0.0002$), >2/3 invasion of the cervical stroma ($P = 0.0052$), lymphovascular invasion ($P = 0.0018$), lymph node metastasis ($P < 0.0001$), and poor differentiation ($P = 0.039$). In a multivariate analysis, including those five factors as well as the presence of LEGH and $p16^{INK4a}$, lymph node metastasis ($P = 0.0018$, hazard ratio: 8.45, 95% confidence interval: 2.21–32.3) and poor differentiation ($P = 0.0049$, hazard ratio: 5.74, 95% confidence interval: 1.33–24.7) were associated with poor outcomes (Table 3). The presence/absence of LEGH was not an independent prognostic indicator.

## Discussion

Several cases of adenocarcinoma in association with LEGH have been reported [8,10]. Kondo et al. [8] analyzed 4 cases of endocervical adenocarcinoma coexisting with LEGH. In our study, a LEGH component was detected in a relative percentage (16.8%) of stage Ib cervical adenocarcinomas. The LEGH component was contiguous with the adenocarcinoma component and comprised part of the tumor. However, the LEGH and adenocarcinoma components were sharply demarcated.

The coexistence of LEGH and adenocarcinoma in a tumor may arise through two mechanisms: one possibility is that adenocarcinoma develops from LEGH in a multistep manner. The relatively frequent coexistence of LEGH components and stage Ib cervical adenocarcinoma components in the same tumor supports the mechanism of LEGH giving rise to cervical adenocarcinoma. However, many cases of LEGH grow into large tumors without malignant components. The relative risk of LEGH as a precancerous lesion thus remains unclear.

The other mechanism is that the adenocarcinoma component arises in the vicinity of LEGH, where common environmental factors promote the development of both LEGH and adenocarcinoma. However, such environmental factors have yet to be identified. In cervical cancer, human papillomavirus (HPV) infection induces $p16^{INK4a}$ expression [12]. LEGH has been shown to be associated with $p16^{INK4a}$ expression, but

**Table 3.** Impact of variables on overall survival of patients with stage Ib cervical adenocarcinoma, computed by univariate and multivariate analyses.

| Factor | Univariate | Multivariate | | |
|---|---|---|---|---|
| | *P* value | Hazard ratio | 95%CI | *P* value |
| LEGH component (absent vs. present) | 0.96 | 1.95 | 0.47–8.08 | 0.35 |
| $p16^{INK4a}$ (positive vs. negative) | 0.14 | 0.47 | 0.14–1.52 | 0.20 |
| Tumor diameter (≥4 cm vs. <4 cm) | 0.0002 | 1.91 | 0.55–6.57 | 0.30 |
| Cervical stromal invasion (≥2/3 vs. <2/3) | 0.0052 | 1.82 | 0.40–8.32 | 0.43 |
| Lymphovascular invasion (positive vs. negative) | 0.0018 | 1.62 | 0.27–9.69 | 0.59 |
| Lymph node metastasis (positive vs. negative) | <0.0001 | 8.45 | 2.21–32.3 | 0.0018 |
| Tumor differentiation (poorly vs. well/moderate) | 0.049 | 5.74 | 1.33–24.7 | 0.0189 |

LEGH, lobular endocervical glandular hyperplasia; CI, confidence interval.

not with HPV [4,19]. In our series, $p16^{INK4a}$ expression in the adenocarcinoma component correlated with the presence of an LEGH component.

Before LEGH became an established clinical entity, adenocarcinoma with LEGH components might have been included in MDA, because the LEGH component was believed to constitute malignant glands. In our previous studies, both true MDA and adenocarcinoma with LEGH components were included in MDA [5,17]. True MDA, extremely well-differentiated mucinous adenocarcinoma, is composed mainly of well-formed glands resembling LEGH. Foci of obvious adenocarcinoma are sparsely distributed among the LEGH-like glands and the tumor infiltrates into the cervical stroma [5].

The 16 cases of adenocarcinoma with LEGH components in the present study were stage Ib cases, and tumor extension was limited to the uterine cervix. In contrast, MDA is usually a widespread lesion, with a mean maximal tumor diameter of 62 mm (range 37–110 mm) in 6 cases and extension to the uterine corpus and vagina [14]. After surgery, the clinical outcomes of patients with MDA were poor, whereas the clinical outcomes of adenocarcinoma with LEGH components were slightly better than those of adenocarcinoma without LEGH components. These findings suggest that LEGH-associated cervical adenocarcinoma could differ from MDA with respect to the biological aggressiveness of tumor cells.

In our study, the area of LEGH components associated with adenocarcinoma showed an immunoreactivity pattern to HIK1083 that was identical to that of pure LEGH [6,7,8]. Therefore, the mucin profile in the LEGH components of our 16 cases of adenocarcinoma might be consistent with that of pure LEGH.

Mikami et al. attempted to differentiate LEGH and MDA on the basis of immunohistochemical properties of stromal cells [11]. Their findings suggested that MDA is characterized by positive immunoreactivity of stromal cells to alpha-smooth muscle actin and by weak or no response to estrogen receptor. Perhaps LEGH can be distinguished from MDA on the basis of these properties [11].

In conclusion, our study showed that early cervical adenocarcinomas were relatively frequently associated with LEGH. Cervical adenocarcinomas with LEGH components were almost always well-differentiated tumors and had no significantly better clinical outcomes than cervical adenocarcinomas without LEGH. Our findings suggested that LEGH may serve as a basis for the development of cervical adenocarcinoma, but obvious adenocarcinomas with LEGH components appear to differ from MDA because the former is not destined to develop into a highly aggressive "adenoma malignum."

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SURGERY TODAY


*Original Articles*

# Clinical and Pathological Features of Intracystic Papillary Carcinoma of the Breast

TOMONORI AKAGI[1], TAKAYUKI KINOSHITA[1], TADAHIKO SHIEN[1], TAKASHI HOJO[1], SADAKO AKASHI-TANAKA[1], and YUSUKE MURATA[2]

[1]Division of Breast Surgery and [2]Pathological Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan

**Abstract**

***Purpose.*** To evaluate the clinicopathological features of intracystic papillary carcinoma (ICPC), which have not been established given its rarity and lack of standard diagnostic criteria.

***Methods.*** We reviewed the clinicopathological findings and treatment outcomes of 14 patients with ICPC diagnosed between 2002 and 2006.

***Results.*** Intracystic papillary carcinoma was diagnosed by fine-needle aspiration biopsy in three patients and by core-needle biopsy in six patients. A preoperative diagnosis was not made in five patients. Three patients underwent magnetic resonance imaging preoperatively, which helped to differentiate benign tumors and maintain free surgical margins. The final pathological diagnosis was invasive carcinoma in 2 (14.2%) of the 14 patients. The patients were followed up for 1–72 months, during which time only one died, of a cancer-unrelated cause.

***Conclusion.*** Our results show that ICPC is more difficult to diagnose than common breast cancer preoperatively. Excisional biopsy was necessary when fine-needle aspiration and core-needle biopsy could not provide a diagnosis. Magnetic resonance imaging is helpful to differentiate a benign tumor from invasive disease.

**Key words** Intracystic papillary carcinoma · Magnetic resonance imaging

## Introduction

Intracystic papillary carcinoma (ICPC) of the breast is a rare malignant tumor, accounting for fewer than 2% of breast cancers.[1] According to the Japanese Society for Breast Cancer, ICPC includes ductal carcinoma in situ (DCIS). Several reports have described invasive ICPC with synchronous liver metastases.[2–6] Intracystic papillary carcinoma is more difficult to diagnose than common breast cancer. Because of the lack of standard criteria for diagnosis and treatment, the clinicopathological features and treatments of this type of breast cancer have not been defined. We reviewed the clinical and pathological features of 14 patients who underwent surgery for ICPC between 2002 and 2006.

## Patients and Methods

Between 2000 and 2006, 2700 cases of primary breast cancer were diagnosed at the National Cancer Center Hospital, 14 of which were diagnosed as ICPC based on clinicopathological analysis. We reviewed the clinical features, pathological findings, and treatments of these 14 patients. Immunohistochemical evaluation was performed according to the DAKO criteria, with the ABC staining method. Immunohistochemical examinations for ER and PgR were defined as positive when there was positive nuclear reactivity. Positivity was scored as follows: 0, 0% positive cells; 1+, less than 10% positive cells; 2+, 10%–50% positive cells; and 3+, more than 50% positive cells. Immunohistochemical examinations for p53 were also defined as positive when there was positive nuclear reactivity. Positivity was scored as follows: 0, 0% of positive cells; +/–, less than 10%; 1+, 10 to 50%; and 2+, more than 50%. HER2 was defined as positive depending on the cytoplasmic membrane reactivity. The grading system for HER2 was also scored from 0 to 3+ by the immunohistological method reported by Bilous et al.[7]

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**Table 1.** Clinicopathological features of the 14 patients with intracystic papillary carcinoma

| No. | Age (years)/sex | Duration from detecting the tumor to operation (months) | Location | US cystic size (mm) | US solid size (mm) | US shape of solid component | MMG shape of mass | MMG calcification | MRI | FNA | CNB | Preoperative diagnosis |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 84/F | 2 | A | 22 | 5 | Irregular | Irregular | None | — | — | − | Not given |
| 2 | 83/F | 2 | D | 11 | 6 | Regular | Regular | None | — | Class 5 | − | DC |
| 3 | 75/F | 3 | A | 22 | 7 | Irregular | Irregular | A | — | Class 3 | + | Not given |
| 4 | 60/F | 4 | B | 36 | 10 | Regular | Regular | None | — | Class 2 | + | Not given |
| 5 | 43/F | 3 | A | 15 | 3 | Regular | Regular | None | — | — | + | Not given |
| 6 | 36/F | 9 | C | 34 | 17 | Irregular | No mass | None | — | — | + | ICPC |
| 7 | 57/F | 4 | E | 10 | 4 | Regular | Regular | None | — | Class 5 | − | DC |
| 8 | 70/M | 6 | E | 50 | 15 | Irregular | Irregular | None | — | — | + | ICPC |
| 9 | 75/F | 2 | A | 28 | 20 | Regular | Regular | A | — | Class 5 | − | DC |
| 10 | 48/F | 3 | A | 23 | 5 | Regular | Regular | P | — | Class 2 | + | Not given |
| 11 | 74/F | 8 | A | 14 | 14 | Regular | — | — | — | — | + | ICPC |
| 12 | 82/F | 24 | C | 200 | 30 | Regular | — | — | BCP | Class 2 | + | ICPC |
| 13 | 81/F | 2 | A | 170 | 52 | Irregular | Irregular | None | BCP | Class 2 | + | ICPC |
| 14 | 71/F | 2 | E | 60 | 21 | Irregular | Regular | None | BCP | — | + | ICPC |

US, ultrasonography; MMG, mammography; MRI, magnetic resonance imaging; FNA, fine-needle aspiration; CNB, core-needle biopsy; A, amorphous; P, pleomorphic; BCP, breast cancer type in MRI; DC, ductal carcinoma

## Results

The clinical data are summarized in Table 1. The patients consisted of one man and 13 women and their ages ranged from 36 to 82 years (median 72.5 years). The initial manifestation was a breast lump in all patients, 13 of whom noticed the breast lump, whereas it was detected by breast cancer screening in 1 patient. The time from tumor detection to treatment ranged from 2 to 24 months (median, 5.2 months). The size of the cystic component ranged from 1 to 20 cm (mean, 4 cm), and the size of the solid component ranged from 3 to 52 mm (median, 12 mm). The tumor was located in areas A, B, C, D, and E in seven, one, two, one, and three patients, respectively.

Ultrasonography showed a multicystic lesion in one patient, and a unicystic lesion in 13 patients. All patients had solid components with intracystic growth. The cystic component ranged from 11 to 220 mm (median, 22.5 mm), and the solid component ranged from 3 to 52 mm (median, 12 mm). The solid components were variable, regular, or irregular in shape.

Twelve patients underwent mammography, which showed a smooth mass in seven, an irregular mass in four, and no mass in one. Four patients had amorphous or pleomorphic calcifications. Magnetic resonance imaging (MRI) showed a breast cancer pattern in all three patients who underwent this examination. It also showed invasion of the cystic wall in one patient.

Fine-needle aspiration was done in 8 of the 14 patients and the tumor was designated as class 5 in 3 (37%) patients, class 3 in 1, and class 2 in 4. Core-needle biopsy was done of five of the tumors designated as class 3 or class 2. Five other patients underwent core-needle biopsy without fine-needle aspiration. A diagnosis of ICPC was made in six (60%) of these ten patients. A diagnosis was not able to be made by core-needle biopsy in four patients, who required excisional biopsy for a definite diagnosis. One patient did not undergo fine-needle aspiration or core-needle biopsy preoperatively.

The pathological features are summarized in Table 2. Thirteen patients underwent mastectomy or partial mastectomy; with axillary lymph node dissection in five, without axillary lymph node dissection in four, and with sentinel lymph node dissection in four. The intracystic fluid was either serous or bloody. Pathological findings revealed invasive ICPC in 2 (14.2%) patients and DCIS was detected around the ICPC in 3 (21.4%) patients. Axillary lymph node metastasis was found in one patient. Estrogen receptor, progesterone receptor, HER2, and p53 were positive in 14 (100%), 13 (92.8%), 3 (21.4%) and 2 (14.2%) patients, respectively.

Thirteen patients were treated with tamoxifen postoperatively, and three of the eight who underwent

**Table 2.** Pathological findings of intracystic papillary carcinoma (ICPC)

| No. | Proposed operation | Invasion of cystic wall | DCIS around ICPC | Lymph node metastasis | ER PgR HER2 | p53 | Histologic grade | Nuclear grade |
|---|---|---|---|---|---|---|---|---|
| 1 | Bp | – | – | No dissection | ER2 PgR2 HER2 1 | – | 1 | 1 |
| 2 | Bp | – | – | No dissection | ER2 PgR0 HER2 0 | – | 1 | 1 |
| 3 | Bt+sampling | – | – | 0/2 | ER2 PgR2 HER2 0 | + | 2 | 2 |
| 4 | Bq | – | – | No dissection | ER2 PgR1 HER2 0 | – | 2 | 2 |
| 5 | Bp+Ax | – | – | 0/11 | ER2 PgR2 HER2 0 | – | 2 | 2 |
| 6 | Bp+Ax | – | + | 0/22 | ER2 PgR2 HER2 0 | – | 1 | 1 |
| 7 | Bt+Ax | – | – | 0/20 | ER2 PgR2 HER2 1 | – | 2 | 2 |
| 8 | Bp | – | – | No dissection | ER2 PgR2 HER2 2 | 2+ | 2 | 2 |
| 9 | Bt+Ax | – | – | 0/18 | ER1 PgR1 HER2 0 | – | 2 | 3 |
| 10 | Bq+SLN | – | – | 0/4 | ER1 PgR2 HER2 0 | – | 1 | 1 |
| 11 | Bp | + | – | No dissection | ER2 PgR2 HER2 0 | – | 1 | 1 |
| 12 | Bt+SLN | – | – | 1/5 | ER3 PgR3 HER2 0 | – | 1 | 1 |
| 13 | Bt+SLN | – | + | 0/5 | ER3 PgR3 HER2 0 | – | 1 | 1 |
| 14 | Bt+SLN | + | + | 0/3 | ER3 PgR2 HER2 0 | – | 1 | 1 |

DCIS, ductal carcinoma in situ

partial mastectomy were also treated with radiation therapy. All 14 patients were followed up for 1–72 months. At the time of writing, 13 patients were alive without evidence of recurrence and one had died of a cause unrelated to cancer.

## Discussion

Intracystic papillary carcinoma is a rare type of breast cancer characterized by papillary growth within a macroscopic cyst. It accounts for fewer than 2% of all breast cancers.[1] Generally, ICPC shows no invasive growth outside of the cyst and is treated similarly to DCIS. However, there are reports of invasive ICPC with synchronous liver metastases.[2–6] Yet, because of its rarity and the lack of diagnostic criteria, the clinicopathological features and treatments of ICPC have not been established.

The average age of onset is higher than that for the more common types of breast cancer, at about 65 years old (range, 34–92 years).[8–10] The average age of onset in this series was 36–82 years old (median, 72.5). Some studies have reported a longer period from tumor detection to treatment for ICPC than for common breast cancer.[8,9] In our study, it ranged from 1 to 24 months (median, 5 months), which suggests that ICPC grows more slowly than common breast cancer, and that it has a lower pathological grade and a tendency not to form ulcerations.

Intracystic papillomas are difficult to diagnose. Previous studies reported that the average age of onset was 40.7–47 years, and that 81% of intracystic papillomas in patients older than 60 years old were carcinoma.[8–10] Intracystic papillary carcinoma tumors tend to be larger than intracystic papillomas, but this does not necessarily help differentiate malignancy from benign growth.[11]

Ultrasonography was thought to be a useful modality to differentiate malignant from benign tumors, but as seen in this series, the shapes of the solid components can be variable, regular, or irregular in malignant and benign tumors. Thus, several studies have found that ultrasonography is not useful for identifying benign tumors.[11,12] Although ultrasonography can differentiate malignancy from benign tumors relatively easily when there is invasion, ICPC without invasion is difficult to diagnose with ultrasonography.

Magnetic resonance imaging is one of the most useful diagnostic techniques for common breast cancer, as it shows the patterns of the time–intensity curves of the lesion, allowing us to differentiate cancerous from benign tumors. Naoshige et al. reported that dynamic MRI imaging is very useful in the differential diagnosis of ICPC.[6] Kusuma et al. also reported that the MRI findings correlated with the pathological findings.[13] Only three of our patients underwent MRI, which showed malignant patterns in the time–intensity curve in all three. Moreover, in one patient it showed invasive growth outside of the cyst, corresponding to the pathological findings (Fig. 1). This finding demonstrates the strong potential of MRI to differentiate benign tumors from invasive disease.

Fine-needle aspiration or core-needle biopsy is important if the preoperative image indicates a potential malignancy. Fine-needle aspiration should be done initially, followed by core-needle biopsy, unless the fine-needle biopsy reveals class 5. In this series excisional biopsy was necessary when fine-needle aspiration or core-needle biopsy could not provide a diagnosis. It is more difficult to diagnose ICPC than common breast

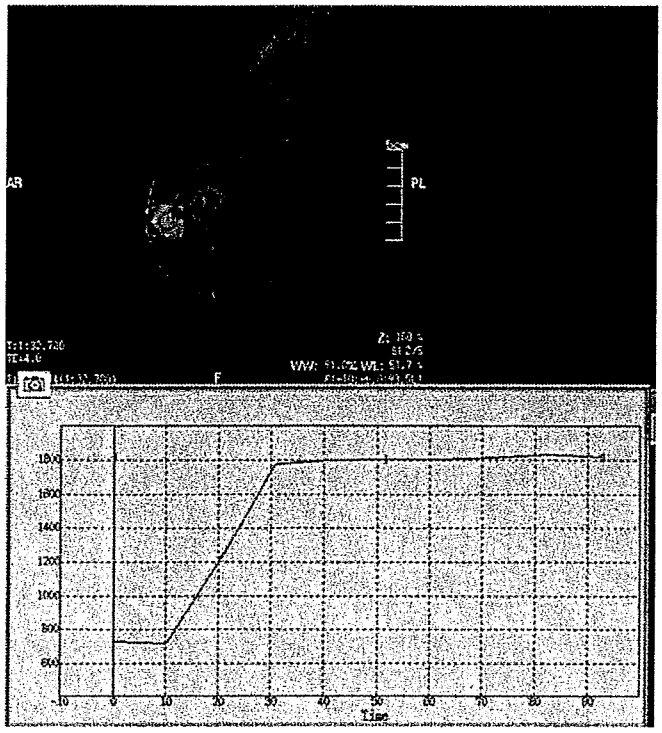

**Fig. 1.** Dynamic magnetic resonance imaging showed malignant patterns in the time–intensity curve of the lesion

cancer because nuclear atypicality of ICPC is not prominent. Therefore, a correct diagnosis is dependent on an adequate preoperative biopsy specimen.

The treatments for ICPC and DCIS are generally the same, although cases of invasive ICPC with synchronous liver metastases have been reported.[2–6] According to Yamashita et al., invasive ICPC is no longer rare and intraductal spread beyond 2 cm from the cystic wall is possible.[10] In this series, two patients had invasive ICPC and another patient had axillary lymph node metastases despite no evidence of invasion in any pathologic section. It is likely that this patient had invasive disease that was missed on the available pathologic sections. Thus, it is important to obtain negative pathological surgical margins. Intracystic papillary carcinoma has the potential to be invasive, which can be evaluated by MRI. Standard neoadjuvant and adjuvant treatments have not been established and surgical resection remains the first line of treatment. The frequency of lymph node metastasis of ICPC has been reported as 0%–36%, which is lower than that of common breast cancer.[9,14] Four patients in this series were treated with sentinel biopsy, which we have been performing in our department since 2004. It is now reasonable not to perform axillary lymph node dissection, as sentinel biopsy is an accepted indicator of ICPC.

All of the tumors in this series were positive for estrogen and progesterone receptors, and the patients were given tamoxifen as adjuvant therapy. Eight patients who underwent breast-conserving treatment received radiation. Although no definitive conclusions about adjuvant treatments have been made, ICPC should g enerally be treated like DCIS.

Based on our experience and review of the literature, we conclude that it is critical to evaluate the malignant potential of ICPC and to decide on the most appropriate adjuvant treatment for each individual patient.

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CLINICAL TRIAL

# Comparison among different classification systems regarding the pathological response of preoperative chemotherapy in relation to the long-term outcome

**Tadahiko Shien · Chikako Shimizu · Kunihiko Seki · Taro Shibata · Takashi Hojo · Masashi Ando · Tsutomu Kohno · Noriyuki Katsumata · Sadako Akashi-Tanaka · Takayuki Kinoshita · Yasuhiro Fujiwara**

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**Abstract** Neoadjuvant chemotherapy (NAC) is increasingly used for operable disease. However there are several pathological response classification systems and the correlation between the pathological response to NAC according to each system and the patient outcome is still under debate. From 1998 to 2006, 370 primary breast cancer patients underwent curative surgical treatment after NAC containing both anthracycline and taxane at the National Cancer Center Hospital. We retrospectively evaluated the clinical and pathological response using the cTMN, Fisher's, Chevailler's, and the Japanese Breast Cancer Society classification systems (JBCS) respectively, and analyzed the correlation between each pathological response and disease free survival (DFS). Ninety-five (26%) patients had tumor recurrence. The five-year DFS according to Fisher's system was pCR, 80% and pINV, 63%. The five-year DFS according to Chevallier's system was Grade 1, 83%, Grade 2, 85%, Grade 3, 62%, and Grade 4, 65%. The five-year DFS according to the JBSC system was Grade 3, 77%, Grade 2, 68%, Grade 1a, 68%, Grade 1b, 58%, and Grade 0, 52%. None of the pathological response systems reached a statistically significant difference. In the classification by the post-treatment number of metastatic axillary lymph nodes, the 5-year DFS was $n = 0$, 86%; $n = 1$–3, 64%; $n = 4$–9, 44%; and $n > 10$ positive: 25% ($P < .0001$). In pathologically node negative patients, there were no significant differences in the DFS among all the classification systems. All three classifications analyzed were considered inadequate as the prognostic marker of the long-term outcome after NAC and further studies are warranted to optimize the prediction.

**Keywords** Breast cancer · Neoadjuvant · Chemotherapy · Response · Predictor

T. Shien · T. Hojo · S. Akashi-Tanaka · T. Kinoshita
Department of Breast Surgery, National Cancer Center Hospital, Tokyo, Japan

T. Shien · C. Shimizu (✉) · M. Ando · T. Kohno · N. Katsumata · Y. Fujiwara
Breast and Medical Oncology Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan
e-mail: cshimizu@ncc.go.jp

K. Seki
Department of Pathology, National Cancer Center Hospital, Tokyo, Japan

T. Shibata
Statistics and Cancer Control Division, National Cancer Center, Tokyo, Japan

## Introduction

Breast cancer has recently become the most common malignancy among Japanese women. Approximately 40,000 women are annually affected and breast cancer mortality has been increasing. National efforts to establish an early detection system by screening mammography has begun, but many of the primary cases still present with a palpable mass in the breast.

Neoadjuvant chemotherapy (NAC) has been accepted as one of the standards of care not only for locally advanced breast cancer but also for primary operable breast cancer. The disease free survival (DFS) and overall survival (OS) of patients treated with NAC is at least equivalent to those treated with post-operative adjuvant chemotherapy and the chance of breast conservation increases in patients with larger tumors [1, 2]. Although the benefit of the addition of taxane to anthracycline in the preoperative setting in terms of long-term outcome remains controversial, regimens that

combine both anthracycline and taxane, either sequentially or concomitantly, are widely used.

Prognostic factors after primary chemotherapy include the clinical and pathological response to primary chemotherapy, the cTNM stage, and axillary lymph-node status after chemotherapy. "Pathological complete response (pCR)" correlates with an improved DFS and OS and has often been used as the surrogate primary endpoint for NAC. However, the classification systems for pathological response vary among studies and the system that best reflects the long-term outcome remains unidentified. Thus in this study, we applied various pathological response systems in the published literature to the same patient cohort treated with NAC including anthracycline and taxane to compare their usefulness in the prediction of the long-term outcome after NAC.

## Patients and methods

### Patients and treatments

All breast cancer patients treated with NAC containing both anthracycline and taxane between May 1998 and October 2006 at the National Cancer Center Hospital were extracted from the surgical database to be included in this retrospective study. NAC was indicated in patients with clinical stage II or III primary breast cancer with tumors larger than 3 cm. Core needle biopsy was performed before NAC to obtain a pathological diagnosis. The NAC regimens included (1) four cycles of doxorubicin (DOX, 50 mg/m$^2$) and docetaxel (DOC, 60 mg/m$^2$) (AT) followed by additional adjuvant treatment with two cycles of AT or four cycles of iv CMF (cyclophosphamide, methotrexate and 5FU), (2) four cycles of fluorouracil (500 mg/m$^2$)/epirubicin (100 mg/m$^2$)/cyclophosphamide (500 mg/m$^2$) plus 12 weekly cycles of paclitaxel (80 mg/m$^2$) (FECT), (3) four cycles of doxorubicin (60 mg/m$^2$)/cyclophosphamide (600 mg/m$^2$) plus 12 weekly cycles of paclitaxel (80 mg/m$^2$) (ACT). After November 2002, in patients with HER2-overexpression tumors, trastsuzumab (initially 4 mg/kg and 2 mg/kg weekly) was administered with paclitaxel for 12 weeks in the ACT and FECT treated populations (ACTH and FECTH, respectively). Five years of endocrine therapy was scheduled when either the pretreatment biopsy specimen or surgical specimen post-chemotherapy were positive for the estrogen or progesterone receptor.

### Evaluation of pathological factors

Pretreatment diagnosis was established by pathologists from a core needle biopsy specimen. Surgical specimens were sectioned at about 7–10 mm and the pathological response was evaluated by pathologists. The expression levels of ER (1D5, Dako Cytomation), PgR (1A6, Novocastra) and HER2 (HercepTest®, Dako Cytomation) were examined with immunohistological staining. ER and PgR were classed as positive when more than 10% of cancer cell nuclei were stained, regardless of the intensity of the staining. HER2 was scored as follows: (0): negative for cells, (1+): slightly positive in more than 10% of cancer cells, (2+): moderately positive in more than 10% of cancer cells, (3+): markedly positive in more than 10% of cancer cells. Additional fluorescent in situ hybridization (FISH) for HER2 amplification (Pathvision, Vysis) was performed and when IHC (3+) or FISH-positive (HER2/CEP17 signal ratio $\geq$ 2.0) were defined as HER2-positive.

The response criteria used in this study included Fisher's system [1], Chevalier's system [3] and the histological response criteria of the Japanese Breast Cancer Society (JBCS) [4, 5]. The key definitions of each response classification system are described in Table 1. To summarize, Fisher's system evaluated only the histological evidence of invasive disease in the primary tumor, Chevallier's system incorporated nodal status and the JBSC system measured morphological changes in of the tumor cells and the proportion of histological changes in the primary tumor. The histological effect in both the primary tumor and axillary lymph node should be separately evaluated in the JBCS system, but the standard of how to combine the effect is not mentioned. Therefore we used the pathological response in only the primary lesion in the present study.

In addition, we evaluated pretreatment clinical staging, the clinical response to preoperative chemotherapy and postoperative pathological lymph node status. The clinical response to preoperative chemotherapy was decided from the two greatest perpendicular diameters (before each chemotherapy treatment and before surgery) of tumors in the breast and axillary lymph nodes. No clinical evidence of palpable tumor in the breast and axillary lymph nodes was defined as a clinical complete response (cCR). A reduction in the total tumor size of 30% or greater was graded as clinical partial response (cPR). An increase in total tumor size of more than 20% or appearance of new suspicious ipsilateral axillary adenopathy was considered progressive disease (cPD). Tumors that did not meet the criteria for objective response and progression were considered as stable disease (cSD).

### Statistical analysis

Disease free survival (DFS) was calculated from the date that NC was initiated to the date of the first relapse including loco-regional recurrence or the last visit without

**Table 1** Pathological response classification systems

| Classification system | Key definitions |
|---|---|
| Fisher et al. [1] | Classification based on microscopic evidence |
| | pCR = no histological evidence of invasive tumor cells (specimens with only noninvasive cells included) |
| | pINV = histological evidence of invasive disease of any extent |
| Chevallier et al. [3] | Classification using both microscopic and macroscopic evidence |
| | Grade 1 (pCR) = disappearance of all tumor on either macroscopic or microscopic assessment |
| | Grade 2 = presence of in situ carcinoma in the breast, no invasive tumor, no tumor in the ALNs |
| | Grade 3 = presence of invasive carcinoma with stromal alteration |
| | Grade 4 = no/few modifications of the tumor appearance |
| JBCS | Classification using both microscopic and macroscopic evidence in primary tumor |
| | Grade 0 = no therapeutic effect |
| | Grade 1 = <66% therapeutic effect, but >33% effect evident |
| | Grade 2 = subjectively >66% therapeutic effect, but <near total therapeutic effect |
| | Grade 3 = disappearance of all tumor on either macroscopic or microscopic assessment |

pCR, pathological complete response; ALN, Axillary lymph node; pINV, pathological invasive disease; JBCS, Japanese breast cancer society

relapse. Kaplan–Meier plots and the log-rank test were used to assess the difference in survival. All comparisons were two-tailed. Cox-proportional hazards models were fitted for OS and DFS and included variables identified a priori as being associated with survival and the ALN status. Other variables not identified a priori were entered into the model one at a time and assessed for statistical significance. All pair-wise interactions were tested. The fit of the model and the proportional hazards assumption were assessed visually with residual plots. The statistical significance level ($P$) was taken as a measure of the strength of evidence against the null hypothesis, and $P < .05$ was considered statistically significant.

## Results

Three hundred and seventy patients with operable breast cancer were included in this study. Table 2 lists the patient and tumor characteristics. The median age was 50 years (26–71) and 192 (52%) patients were over the age of 50. Clinical staging at diagnosis was IIA in 104 (28%), IIB in 114 (31%), IIIA in 75 (20%) and IIIB in 77 (21%). ER and PgR positive patients were respectively 148 (40%) and 152 (41%). 183 (49%) patients were treated with AT, 73 (20%) with ACT and 90 (24%) with FECT. Trastuzumab as administered to four patients among the ACT-treated patients (ACTH) and 20 among the FECT-treated patients. Ten percent of patients with HER2-positive breast cancer received trastuzumab in this study.

Ninety-six patients (26%) had tumor recurrence with a median follow-up of 45 months (range 4–104). Nine patients had only loco-regional recurrence without distant metastasis. Only 42 patients died within this period.

The clinical and pathological response results are shown in Table 3. The overall clinical response rate to NAC was 88% (cCR + cPR) and the cCR rate was 28%. According to Fisher's classification, pCR and pINV was 65 (18%) and 305 (82%). According to Chevallier's classification, 30 (8%) patients achieved a Grade 1 (disappearance of all

**Table 2** Patient and tumor characteristics

| Parameter | No. of patients (%) |
|---|---|
| Total | 370 |
| Age | |
| Age <50 | 179 (48) |
| Age >51 | 191 (52) |
| Pretreatment pathology | |
| Invasive ductal carcinoma | 347 (94) |
| Invasive lobular carcinoma | 13 (4) |
| Mucinous carcinoma | 7 (2) |
| Others | 3 (1) |
| Hormone receptors | |
| ER positive | 148 (40) |
| PgR positive | 152 (41) |
| HER2 | |
| Positive (>2+) | 132 (36) |
| Neoadjuvant chemotherapy | |
| AT | 183 (49) |
| ACT | 73 (20) |
| ACTH | 4 (1) |
| FECT | 90 (24) |
| FECTH | 20 (5) |
| Surgery | |
| Partial mastectomy | 136 (37) |
| Total mastectomy | 234 (63) |