Acknowledgments

This study was supported by the National Cancer Center Research and Development Fund (23-A-17) and the Grant-in-Aid for Cancer Research (No. 18-06, No. 10103749) from the Ministry of Health, Labour, and Welfare of Japan.

We thank the following participating institutions and investigators: National Cancer Center Hospital: Noriyuki Katsumata, Maki Tanioka; Saitama Cancer Center: Nao Kino, Yoko Hasumi; National Hospital Organization Shikoku Cancer Center: Masamichi Hiura, Takashi Matsumoto; Kagoshima City Hospital: Masayuki Hatae, Yoshitaka Onishi, Takayo Kawabata; The Cancer Institute Hospital of the Japanese Foundation of Cancer Research: Ken Takizawa, Nobuhiro Takeshima, Kiyoshi Fujiwara; The University of Tokyo: Shunsuke Nakagawa, Takahide Arimoto; Kyushu University: Hiroaki Kobayashi, Kaoru Okugawa; Tohoku University: Nobuo Yaegashi, Tadao Takano; Hyogo Cancer Center: Ryuichiro Nishimura, Satoshi Yamaguchi, Yasunori Hashiguchi; Kurume University School of Medicine: Kimio Ushijima; Osaka City General Hospital: Naoki Kawamura, Sadako Nishimura; National Hospital Organization Kure Medical Center-Chugoku Cancer Center: Tomoya Mizunoe, Kazuhiro Takehara; Niigata Cancer Center: Shoji Kodama; Kyoto University Graduate School of Medicine: Masaki Mandai; Sapporo Medical University: Tsuyoshi Saito, Masahiro Iwasaki; Tottori University: Junzo Kigawa, Jun Naniwa; Osaka City University Graduate School of Medicine: Osamu Ishiko, Yoshinari Matsumoto; National Defense Medical College: Masashi Takano; Saga University: Tsuyoshi Iwasaka, Yoshifumi Nakao; Juntendo University School of Medicine: Satoru Takeda, Daiki Ogishima, Yasuhisa Terao; Osaka Prefectural Hospital Organization Osaka Medical Center for Cancer and Cardiovascular Diseases: Shoji Kamiura, Yukinobu Ohta; The Jikei University School of Medicine: Kazunori Ochiai, Hiroshi Tanabe; Hokkaido University Graduate School of Medicine: Noriaki Sakuragi, Hidemichi Watari; Shimane University School of Medicine: Koji Miyazaki, Kentaro Nakayama; Sakai Hospital, Kinki University School of Medicine: Kaichiro Yamamoto, Kinki University School of Medicine: Hiroshi Hoshiai, Yoh Watanabe; Kitasato University: Shinpei Tsunoda, Miwa Kawaguchi.

We thank Marguerite Elgin of Edanz (http://www.edanzediting.com) and Linda Saza for editing the manuscript.

References

[1] Amant F, Moerman P, Neven P, Timmerman D, Van Limbergen E, Vergote I. Endometrial cancer. Lancet 2005;366(9484):491-505.
[2] Rose PG. Endometrial carcinoma. N Engl J Med 1996;335(9):640-9.
[3] Creasman WT, Odicino F, Maisonneuve P, Quinn MA, Beller U, Benedet JL, et al. Carcinoma of the corpus uteri. FIGO 26th annual report on the results of treatment in gynecological cancer. Int J Gynaecol Obstet 2006;95(Suppl. 1):S105-43.
[4] Ayhan A, Taskiran C, Celik C, Yuce K, Kucukali T. The influence of cytoreductive surgery on survival and morbidity in stage IVB endometrial cancer. Int J Gynecol Cancer 2002;12(5):448-53.
[5] Numazaki R, Miyagi E, Konnai K, Ikeda M, Yamamoto A, Onose R, et al. Analysis of stage IVB endometrial carcinoma patients with distant metastasis: a review of prognoses in 55 patients. Int J Clin Oncol 2009;14(4):344-50.
[6] Ueda Y, Enomoto T, Miyatake T, Egawa-Tanaka T, Ugaki H, Yoshino K, et al. Endometrial carcinoma with extra-abdominal metastasis: improved prognosis following cytoreductive surgery. Ann Surg Oncol 2010;17(4):1111-7.
[7] Bristow RE, Zerbe MJ, Rosenshein NB, Grumbine FC, Montz FJ. Stage IVB endometrial carcinoma: the role of cytoreductive surgery and determinants of survival. Gynecol Oncol 2000;78(2):85-91.
[8] Goff BA, Goodman A, Muntz HG, Fuller Jr AF, Nikrui N, Rice LW. Surgical stage IV endometrial carcinoma: a study of 47 cases. Gynecol Oncol 1994;52(2):237-40.
[9] Chi DS, Welshinger M, Venkatraman ES, Barakat RR. The role of surgical cytoreduction in stage IV endometrial carcinoma. Gynecol Oncol 1997;67(1):56-60.
[10] Shih KK, Yun E, Gardner GJ, Barakat RR, Chi DS, Leitao Jr MM. Surgical cytoreduction in stage IV endometrioid endometrial carcinoma. Gynecol Oncol 2011;122(3):608-11.
[11] Bristow RE, Duska LR, Montz FJ. The role of cytoreductive surgery in the management of stage IV uterine papillary serous carcinoma. Gynecol Oncol 2001;81(1):92-9.
[12] Thomas MB, Mariani A, Cliby WA, Keeney GL, Podratz KC, Dowdy SC. Role of cytoreduction in stage III and IV uterine papillary serous carcinoma. Gynecol Oncol 2007;107(2):190-3.
[13] Barlin JN, Puri I, Bristow RE. Cytoreductive surgery for advanced or recurrent endometrial cancer: a meta-analysis. Gynecol Oncol 2010;118(1):14-8.
[14] Aalders JG, Abeler V, Kolstad P. Stage IV endometrial carcinoma: a clinical and histopathological study of 83 patients. Gynecol Oncol 1984;17(1):75-84.
[15] du Bois A, Reuss A, Pujade-Lauraine E, Harter P, Ray-Coquard I, Pfisterer J. Role of surgical outcome as prognostic factor in advanced epithelial ovarian cancer: a combined exploratory analysis of 3 prospectively randomized phase 3 multicenter trials: by the Arbeitsgemeinschaft Gynaekologische Onkologie Studiengruppe Ovarialkarzinom (AGO-OVAR) and the Groupe d'Investigateurs Nationaux Pour les Etudes des Cancers de l'Ovaire (GINECO). Cancer 2009;115(6):1234-44.
[16] Makar AP, Baekelandt M, Trope CG, Kristensen GB. The prognostic significance of residual disease, FIGO substage, tumor histology, and grade in patients with FIGO stage III ovarian cancer. Gynecol Oncol 1995;56(2):175-80.
[17] Greer BE, Hamberger AD. Treatment of intraperitoneal metastatic adenocarcinoma of the endometrium by the whole-abdomen moving-strip technique and pelvic boost irradiation. Gynecol Oncol 1983;16(3):365-73.
[18] Lambrou NC, Gomez-Marin O, Mirhashemi R, Beach H, Salom E, Almeida-Parra Z, et al. Optimal surgical cytoreduction in patients with stage III and stage IV endometrial carcinoma: a study of morbidity and survival. Gynecol Oncol 2004;93(3):653-8.
[19] Memarzadeh S, Holschneider CH, Bristow RE, Jones NL, Fu YS, Karlan BY, et al. FIGO stage III and IV uterine papillary serous carcinoma: impact of residual disease on survival. Int J Gynecol Cancer 2002;12(5):454-8.
[20] Randall ME, Filiaci VL, Muss H, Spirtos NM, Mannel RS, Fowler J, et al. Randomized phase III trial of whole-abdominal irradiation versus doxorubicin and cisplatin chemotherapy in advanced endometrial carcinoma: a Gynecologic Oncology Group Study. J Clin Oncol 2006;24(1):36-44.
[21] Fleming GF, Brunetto VL, Cella D, Look KY, Reid GC, Munkarah AR, et al. Phase III trial of doxorubicin plus cisplatin with or without paclitaxel plus filgrastim in advanced endometrial carcinoma: a Gynecologic Oncology Group Study. J Clin Oncol 2004;22(11):2159-66.
[22] Secord AA, Havrilesky LJ, Bae-Jump V, Chin J, Calingaert B, Bland A, et al. The role of multi-modality adjuvant chemotherapy and radiation in women with advanced stage endometrial cancer. Gynecol Oncol 2007;107(2):285-91.
[23] Homesley HD, Filiaci V, Gibbons SK, Long HJ, Cella D, Spirtos NM, et al. A randomized phase III trial in advanced endometrial carcinoma of surgery and volume directed radiation followed by cisplatin and doxorubicin with or without paclitaxel: a Gynecologic Oncology Group study. Gynecol Oncol 2009;112(3):543-52.
[24] Hoskins PJ, Swenerton KD, Pike JA, Wong F, Lim P, Acquino-Parsons C, et al. Paclitaxel and carboplatin, alone or with irradiation, in advanced or recurrent endometrial cancer: a phase II study. J Clin Oncol 2001;19(20):4048-53.

THE JOURNAL OF
Obstetrics and Gynaecology Research

doi:10.1111/j.1447-0756.2012.01862.x J. Obstet. Gynaecol. Res. Vol. 38, No. 10: 1260–1265, October 2012

Combination of squamous cell carcinoma-antigen, carcinoembryonic antigen, and carbohydrate antigen 19-9 predicts positive pelvic lymph nodes and parametrial involvement in early stage squamous cell carcinoma of the uterine cervix

Shun-ichi Ikeda[1], Kenichi Yoshimura[3], Takashi Onda[1], Takahiro Kasamatsu[1], Tomoyasu Kato[1], Mitsuya Ishikawa[1], Yuko Sasajima[2] and Hitoshi Tsuda[2]

Divisions of [1]Gynecology and [2]Diagnostic Pathology, National Cancer Center Hospital, Tokyo, and [3]Translational Research Center, Kyoto University Hospital, Kyoto, Japan

Abstract

Aim: We examined the correlations between the pretreatment values of four tumor markers (squamous cell carcinoma [SCC]-antigen, carcinoembryonic antigen [CEA], carbohydrate antigen [CA]19-9, and CA125) and postsurgical high-risk factors (parametrial involvement and positive pelvic lymph nodes) in women with SCC of the uterine cervix who had International Federation of Gynecology and Obstetrics clinical stage IB and IIA disease and underwent radical hysterectomy.

Material and Methods: In this retrospective study, we reviewed 291 patients between April 1989 and December 2008. The first 200 subjects, studied between 1989 and 2001, served as the training set, and another 91 subjects, studied between 2002 and 2008, comprised the test set. To evaluate the correlations between pretreatment tumor markers and postsurgical high-risk factors, the χ^2-test and logistic regression analysis were used for univariate and multivariate analysis, respectively.

Results: Multivariate analysis with receiver–operator curves showed that the combination of SCC-antigen, CEA, and CA19-9 strongly predicted postsurgical high-risk factors. Analysis of the training set showed that 66.7% (95% confidence interval, 52.6–84.8%) of patients who tested positive for at least two of these three tumor markers had postsurgical high-risk factors. Similar results were obtained with the test set.

Conclusions: Preoperative levels of SCC-antigen, CEA, and CA19-9 are useful for predicting the status of postsurgical high-risk factors in women with SCC of the uterine cervix who undergo radical hysterectomy.

Key words: prognostic factor, receiver–operator curve, squamous cell carcinoma, tumor marker, uterine cervical cancer.

Introduction

Various pathological prognostic factors have been proposed in women with early stage uterine cervical cancer who undergo radical hysterectomy. Parametrial involvement and positive pelvic lymph nodes are considered to indicate a particularly high postsurgical risk.[1–3] The ability to more accurately predict postsurgical high-risk factors before therapy would facilitate prognosis in individual patients. Several circulating

Received: August 19 2011.
Accepted: December 26 2011.
Reprint request to: Dr Shun-ichi Ikeda, Division of Gynecology, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, 104-0045 Tokyo, Japan. Email: shuikeda@ncc.go.jp

tumor markers have been proposed as predictors of outcomes.[4–6] The usefulness of these markers remains uncertain; however, squamous cell carcinoma (SCC)-ag levels are widely used to monitor recurrence in women with SCC of the uterine cervix.[7] We studied the correlation between the pretreatment status of four tumor markers and the postsurgical status of high-risk factors in women with uterine cervical SCC who underwent radical hysterectomy.

Methods

Patients

From April 1989 through December 2008, 301 patients with International Federation of Gynecology and Obstetrics (FIGO) stage IB or IIA SCC of the uterine cervix (245 with FIGO stage IB disease and 46 with stage IIA disease) underwent radical hysterectomy with pelvic lymphadenectomy (Class III of the Piver–Rutledge classification) at the Division of Gynecology, National Cancer Center Hospital, Tokyo, Japan. Systemic lymphadenectomy, including inguinal, external iliac, internal iliac obturator and common iliac lymph nodes, was performed and all lymph nodes were submitted for pathological examination. Ten (3.0%) of these patients were excluded from analysis because three or fewer tumor markers were measured, and the remaining 291 patients were retrospectively evaluated. These patients were divided into a training set (200 patients; 162 with FIGO stage IB disease and 38 with stage IIA disease), studied from April 1989 through December 2001, and a test set (91 patients; 83 with FIGO stage IB disease and eight with stage IIA disease), studied from January 2002 through December 2008. In accordance with the treatment policy of our hospital, patients with histologically proven positive pelvic lymph nodes or parametrial involvement additionally received postoperative adjuvant radiotherapy. All tumors were staged according to the FIGO clinical staging system (1994) for cervical cancer, based on the results of physical examination, chest radiography, drip infusion pyelography, and cystoscopy. To examine the influence of tumor markers on postsurgical high-risk factors, serum levels of SCC-antigen (SCC-ag), carcinoembryonic antigen (CEA), carbohydrate antigen (CA)19-9, and CA125 were measured in the 200 patients in the training set.

Postsurgical risk was classified as high in patients who had parametrial involvement, positive pelvic lymph nodes, or both, and low in patients who had none of these prognostic factors.

Laboratory analysis

All serum samples were routinely analyzed before operation by the central laboratory in our hospital. In patients who underwent conization, peripheral blood samples were also obtained before the procedure. CEA, CA125, CA19-9, and SCC-ag were measured by enzyme immunoassay. The cut-off levels recommended by the manufacturers of the test kits were used, that is, 5 ng/mL for CEA, 35 U/mL for CA125, 37 U/mL for CA19-9, and 1.5 ng/mL for SCC-ag.

Pathological evaluation

Tissue sections stained with hematoxylin–eosin were examined to establish the histopathological diagnosis. Parametrial invasion was defined as tumor in parametrial tissues, including direct invasion and lymphovascular spread. Lymph node metastasis in the parametrium was included in positive pelvic lymph nodes in the present study. Lymphovascular involvement was defined as the presence of tumor cells in the luminal space lined by flattened endothelial cells. The depth of cervical stromal invasion was classified as ≤two-thirds or >two-thirds.

Statistical analysis

To assess the correlation between tumor markers and postsurgical risk, the χ^2-test and logistic regression analysis were used for univariate and multivariate analyses, respectively. Significant factors on univariate analysis were included in multivariate analysis. To evaluate the predictive value of tumor markers, the areas under receiver–operator curves (AUC) were estimated using a logistic regression model, in which tumor markers were analyzed as variables according to the cut-off levels recommended by the manufacturers. The combination of tumor markers with highest AUC was retested in the test set.

In the training set, 5-year survival rates and 95% confidence intervals (CI) were calculated by the Kaplan–Meier method. Deaths from any other causes and loss to follow-up were treated as censored data for survival analysis. The log–rank test was used to detect the differences in survival curves among different subgroups.

All statistical analyses were performed using SAS, version 9.1.3. All *P*-values were two-sided, and *P*-values of less than 0.05 were considered to indicate statistical significance.

Table 1 Characteristics of patients in the training set and test set

Factor		Training set Number of patients	%	Test set Number of patients	%
Total		200	100	91	100
FIGO stage					
IB		162	81	83	91
IIA		38	19	8	9
Age					
<46		102	51	48	53
≥46		98	49	43	47
Pathological stage					
pT	1b	132	66	62	68
	2a	46	23	14	15
	2b	22	11	15	17
Tumor diameter					
≤4 cm		151	75.5	79	86.8
>4 cm		49	24.5	12	13.2
Cervical stromal invasion					
<2/3		87	43.5	45	49.5
≥2/3		113	56.5	46	50.5
Lymphovascular space involvement					
Absent		64	32	33	36.3
Present		136	68	58	63.7
Postsurgical high-risk factors		53	26.5	25	27.5
Only parametrial invasion		5	2.5	7	7.7
Only positive pelvic lymph nodes		31	15.5	10	11
Parametrial invasion and positive pelvic lymph nodes		17	8.5	8	8.8

FIGO, International Federation of Gynecology and Obstetrics.

Results

Table 1 shows the patients' characteristics in the training set and the test set. In the training set, 53 (26.5%) patients had postsurgical high-risk factors: five (2.5%) had only parametrial invasion, 31 (15.5%) had only positive pelvic lymph nodes, and 17 (8.5%) had parametrial invasion plus positive pelvic lymph nodes. In the test set, 25 (27.5%) patients had postsurgical high-risk factors: seven (7.7%) had only parametrial invasion, 10 (11.0%) had only positive pelvic lymph nodes, and eight (8.8%) had parametrial invasion plus positive pelvic lymph nodes.

Univariate and multivariate analyses of the training set showed that the four tumor markers (SCC-ag, CEA, CA19-9, and CA125) significantly correlated with postsurgical high-risk factors (Tables 2, 3). Four tumor markers (SCC, CEA, CA19-9, and CA125) were analyzed on multivariate analysis in Table 3.

Table 4 shows the AUC values of preoperative SCC-ag, CEA, CA19-9, and CA125 for predicting postsurgical high-risk factors in the training set. The combination of SCC-ag, CEA, and CA19-9 was related to postsurgical high-risk factors as strongly as was the combination of SCC-ag, CEA, CA19-9, and CA125 (Table 4). The estimated logistic regression model on ROC analysis was as follows: log odds = $-5.15 + 0.80$*I(SCC-ag ≥ 1.5 ng/mL) + 1.27*I(CEA ≥ 5.0 ng/mL) + 1.24*I(CA19-9 ≥ 37 U/mL), which gives the predicted probability of postsurgical high risk, where I(A ≥ a) = 1 if A ≥ a and I(A ≥ a) = 0 if A < a. We used c-statistics to estimate the AUC of the ROC.

Postsurgical high-risk factors were present in 22 (66.7%) of 33 patients with at least two positive tumor markers, as compared with 19 (26.4%) of 72 patients with only one positive tumor marker and 13 (13.7%) of 95 patients in whom all three tumor markers were negative (Table 5). The value of these three markers for the prediction of postsurgical high-risk factors was validated in 92 patients with stage IB and IIA disease who underwent radical hysterectomy from January 2002 through December 2008 at the National Cancer Center Hospital, Tokyo, Japan. In the test set, postsurgical high-risk factors were present in 11 (68.8%) of 16 patients with at least two positive tumor markers, as compared with 11 (37.9%) of 29 patients with one

Table 2 Preoperative tumor markers according to high-risk-factor status in the training set

	No. of patients	High-risk factors – *n* (%)		High-risk factors + *n* (%)		*P*-value*
SCC (ng/mL)						<0.001
≤1.5	109	91	(83.5)	18	(16.5)	
>1.5	91	55	(60.4)	36	(39.6)	
CEA (ng/mL)						<0.001
≤5.0	168	133	(79.2)	35	(20.8)	
>5.0	32	13	(40.6)	19	(59.4)	
CA19-9 (U/mL)						<0.001
≤37	175	136	(77.7)	39	(22.3)	
>37	25	10	(40.0)	15	(60.0)	
CA125 (U/mL)						0.02
≤35	182	137	(75.3)	45	(24.7)	
>35	18	9	(50.0)	9	(50.0)	

*Pearson's χ^2-test. CA, carbohydrate antigen; CEA, carcinoembryonic antigen; SCC, squamous cell carcinoma.

Table 3 Multivariate logistic regression analysis of preoperative predictors of high-risk factors in the training set

Factor	Category	Relative hazard ratio	95%CI	*P*-value
SCC	≤1.5 vs >1.5	2.258	1.101–4.631	0.03
CEA	≤5.0 vs >5.0	3.512	1.465–8.417	0.005
CA19-9	≤37 vs >37	3.437	1.315–8.987	0.01
CA125	≤35 vs >35	3.077	1.053–8.997	0.04

CA, carbohydrate antigen; CEA, carcinoembryonic antigen; CI, confidence interval; SCC, squamous cell carcinoma.

Table 4 AUC of different combinations of tumor markers for predicting postsurgical high-risk factors in the training set

	AUC
Univariate	
SCC	0.65
CEA	0.63
CA19-9	0.61
CA125	0.55
Multivariate	
CEA, CA19-9	0.69
SCC, CA19-9	0.68
SCC, CEA	0.70
CEA, CA19-9, CA125	0.70
SCC, CA19-9, CA125	0.69
SCC, CEA, CA125	0.70
SCC, CEA, CA19-9	0.73
SCC, CEA, CA19-9, CA125	0.73

AUC, area under the receiver–operator curve; CA, carbohydrate antigen; CEA, carcinoembryonic antigen; SCC, squamous cell carcinoma.

positive tumor marker and three (6.4%) of 47 patients in whom all three tumor markers were negative (Table 6). The results obtained with the test set were similar to those obtained with the training set.

In the training set, the tumor markers SCC-ag, CEA, and CA19-9 were found to be significantly related to the 5-year survival rate, that is, patients in whom two or three markers were positive had poorer survival (93.6% in patients in whom all three markers were negative, 91.7% in those with one positive marker, and 60.6% in those with two or three positive markers, $P < 0.001$, log–rank test).

Discussion

Parametrial involvement and positive pelvic lymph nodes have been established to be important prognostic factors in early stage uterine cervical cancer.[1–3] However, these prognostic factors cannot be accurately evaluated preoperatively, even on exhaustive studies with computed tomography, magnetic resonance imaging, and positron emission tomography. In particular, microscopic parametrial metastases, positive pelvic lymph nodes, or both cannot be detected on currently available imaging techniques.

Several studies have shown by multivariate analysis that elevated preoperative SCC-ag levels are associated with lymph node metastasis among high-risk factors. Lin *et al.* found on multivariate analysis that only lymph node metastasis was significantly related to SCC-Ag levels exceeding 8 ng/mL.[8] Takeshima *et al.* also reported that SCC-Ag levels greater than 4 ng/mL were associated with nodal metastasis.[9] Only one study

Table 5 Positive postoperative high-risk factors according to the combination of SCC, CEA, and CA19-9 in the training set

SCC (ng/mL)	CEA (ng/mL)	CA19-9 (U/mL)		*n*			Positive HRF			%	(95%CI)
—	—	—		95			13			13.7	(6.8–20.6)
>1.5	—	—	58				14				
—	>5.0	—	7	72			3	19		26.4	(16.2–36.6)
—	—	>37	7				2				
—	>5.0	>37	0								
>1.5	>5.0	—	15	23			9	15			
>1.5	—	>37	8		33		6		22	66.7	(52.6–84.8)
>1.5	>5.0	>37		10			7				

—, under cut-off value; CA, carbohydrate antigen; CEA, carcinoembryonic antigen; CI, confidence interval; HRF, high-risk factors; SCC, squamous cell carcinoma.

Table 6 Validation of positive postoperative high-risk factors according to the combination of SCC, CEA, and CA19-9 in the test set

SCC (ng/mL)	CEA (ng/mL)	CA19-9 (U/mL)		*n*			Positive HRF			%	(95%CI)
—	—	—		47			3			6.4	(0.0–13.4)
>1.5	—	—	26				10	11			
—	>5.0	—	2	29			1			37.9	(20.3–50.6)
—	—	>37	1				0				
—	>5.0	>37	0				0				
>1.5	>5.0	—	8	15			7	10			
>1.5	—	>37	7		16		3		11	68.8	(46.0–91.5)
>1.5	>5.0	>37		1				1			

—, under cut-off value; CA, carbohydrate antigen; CEA, carcinoembryonic antigen; CI, confidence interval; HRF, high-risk factors; SCC, squamous cell carcinoma.

performed by Takeda *et al.* examined the usefulness of pretreatment levels of multiple tumor markers for predicting the status of high-risk factors. A combination of SCC-ag and CA125 levels was shown to be related to lymph node status.[10] However, these studies did not assess parametrial involvement.

Reesink-Peters *et al.* investigated the correlation between preoperative SCC-ag levels and postsurgical high-risk factors in early stage SCC of the uterine cervix. They proposed that an SCC-ag level of >1.9 ng/mL could be used to identify patients who required adjuvant radiotherapy because postsurgical high-risk factors were present in 73 (66.4%) of 110 patients with stage IB and IIA disease who had elevated SCC-ag levels;[11] however, their study excluded from analysis 184 patients who underwent previous loop excision or conization. The exclusion of these patients might have resulted in the high proportion of patients with postsurgical high-risk factors.

The present study was designed to identify prognostic variables related to postsurgical high-risk factors in early stage SCC of the uterine cervix. We performed multivariate analysis of ROC curves to assess the value of various combinations of four tumor markers for the prediction of postsurgical high-risk factors. Our results showed that positivity for at least two of three markers (i.e., elevated preoperative levels of SCC-ag, CEA, and CA19-9 in serum) is strongly related to high-risk factors in women with SCC of the uterine cervix.

One important question is why the combination of tumor markers correlates with high-risk factors. Unfortunately, the answer to this question is beyond the scope of the present investigation. The biologic functions of tumor markers are largely unknown and must be elucidated to answer this question in the future.

Using a combination of SCC-antigen, CEA, and CA19-9 as determined by the training set, we confirmed that 11 (68.8%; 95%CI, 46.0%–91.5%) patients with at least two positive tumor markers had postoperative high-risk factors in the test set. A combination of at least two positive tumor markers had a sensitivity of 40% and a specificity of 92.5% in the training set. This combination of tumor markers cannot be used for screening because of its low sensitivity and high specificity, but confirms our results. Physicians want to know the probability of high-risk factors in a patient

with positive test results. One way of expressing this probability is the positive predictive value (PPV). PPV is influenced by the prevalence of disease in the population being tested. Few studies have assessed the incidences of pelvic lymph node metastasis, parametrial involvement, or both in patients undergoing radical hysterectomy for early stage uterine cervical cancer. The incidence of pelvic lymph node metastasis, however, is estimated to range between 20% and 30%.[12,13] Because our combination of tumor markers had a sensitivity of 40% and a specificity of 92.5%, the PPV was estimated to be 57% given a 20% prevalence of high-risk factors and 70.6% given a 30% prevalence. On the basis of the literature,[12,13] the proportion of patients with stage IB–II disease who have high-risk factors is estimated to be 20–30%. Historically in Japan, radical hysterectomy was aggressively performed to treat stage IB-II cervical cancer. Internationally, surgery and radiotherapy have been demonstrated to have similar effectiveness in stage IB–IIA cervical cancer. If a method was available to identify patients with stage IB–II disease who have high-risk factors with a probability of 60–70% before treatment, such a method might be used to decide whether patients should receive surgery or radiotherapy.

Varying the cut-off value of the tumor markers, we analyzed sensitivity and specificity as continuous levels using ROC, and the AUC for the combination of the three and four tumor markers were 0.8. On multivariate analysis of the ROC in the present study, it was difficult to determine the cut-off level for each tumor marker. Therefore, we used the cut-off levels recommended by the manufacturers of the test kits. These cut-off levels could identify patients with high-risk factors, and reproducibility was confirmed in the test set.

Our study also showed that survival was poorer in patients in whom two or three markers were positive. This finding is attributed to the close correlation between two or three positive markers and the presence of high-risk factors.

Our results suggest that the combination of three tumor markers (SCC-antigen, CEA, and CA19-9) might be useful for identifying subgroups of patients with early stage cervical cancer associated with different risk levels before treatment.

In conclusion, the combination of the preoperative serum levels of SCC-antigen, CEA, and CA19-9 is useful for predicting the status of postsurgical high-risk factors in women with SCC of the uterine cervix who undergo radical hysterectomy. Our study is limited by its retrospective nature; however, our findings will hopefully contribute to more accurate prediction of clinical outcomes before treatment.

Disclosure

The authors make no disclosures.

References

1. Peters WA 3rd, Liu PY, Barrett RJ *et al.* Concurrent chemotherapy and pelvic radiation therapy compared with pelvic radiation therapy alone as adjuvant therapy after radical surgery in high-risk early stage cancer of the cervix. *J Clin Oncol* 2000; **18**: 1606–1613.
2. Inoue T, Okumura M. Prognostic significance of parametrial extension in patients with cervical carcinoma stage IB, IIA, and IIB. A study of 628 cases treated by radical hysterectomy and lymphadenectomy with or without postoperative irradiation. *Cancer* 1984; **54**: 1714–1719.
3. Fuller AF Jr, Elliott N, Kosloff C, Hoskins WJ, Lewis JL Jr. Determinants of increased risk for recurrence in patients undergoing radical hysterectomy for stage IB and IIA carcinoma of the cervix. *Gynecol Oncol* 1989; **33**: 34–39.
4. Borras G, Molina R, Xercavins J, Ballesta A, Iglesias J. Tumor antigens CA 19.9, CA 125, and CEA in carcinoma of the uterine cervix. *Gynecol Oncol* 1995; **57**: 205–211.
5. Bae SN, Namkoong SE, Jung JK *et al.* Prognostic significance of pretreatment squamous cell carcinoma antigen and carcinoembryonic antigen in squamous cell carcinoma of the uterine cervix. *Gynecol Oncol* 1997; **64**: 418–424.
6. Schwartz PE, Chambers SK, Chambers JT, Gutmann J, Katopodis N, Foemmel R. Circulating tumor markers in the monitoring of gynecologic malignancies. *Cancer* 1987; **60**: 353–361.
7. Gadducci A, Tana R, Fanucchi A, Genazzani AR. Biochemical prognostic factors and risk of relapses in patients with cervical cancer. *Gynecol Oncol* 2007; **107** (1 Suppl 1): 23–26.
8. Lin H, ChangChien CC, Huang EY, Tseng CW, Eng HL, Huang CC. The role of pretreatment squamous cell carcinoma antigen in predicting nodal metastasis in early stage cervical cancer. *Acta Obstet Gynecol Scand* 2000; **79**: 140–144.
9. Takeshima N, Hirai Y, Katase K, Yano K, Yamauchi K, Hasumi K. The value of squamous cell carcinoma antigen as a predictor of nodal metastasis in cervical cancer. *Gynecol Oncol* 1998; **68**: 263–266.
10. Takeda M, Sakuragi N, Okamoto K *et al.* Preoperative serum SCC, CA125, and CA19-9 levels and lymph node status in squamous cell carcinoma of the uterine cervix. *Acta Obstet Gynecol Scand* 2002; **81**: 451–457.
11. Reesink-Peters N, van der Velden J, Ten Hoor KA *et al.* Preoperative serum squamous cell carcinoma antigen levels in clinical decision making for patients with early-stage cervical cancer. *J Clin Oncol* 2005; **23**: 1455–1462.
12. Sakuragi N. Up-to-date management of lymph node metastasis and the role of tailored lymphadenectomy in cervical cancer. *Int J Clin Oncol* 2007; **12**: 165–175.
13. Gien LT, Covens A. Lymph node assessment in cervical cancer: Prognostic and therapeutic implications. *J Surg Oncol* 2009; **99**: 242–247.

Journal of Radiation Research, 2012, 53, 588–593
doi: 10.1093/jrr/rrs010 Advance Access Publication 15 June 2012

Regular Paper

Impact of concurrent chemotherapy on definitive radiotherapy for women with FIGO IIIb cervical cancer

Yuuki KURODA[1,*], Naoya MURAKAMI[1], Madoka MOROTA[1], Shuhei SEKII[1], Kana TAKAHASHI[1], Koji INABA[1], Hiroshi MAYAHARA[1], Yoshinori ITO[1], Ryo-ichi YOSHIMURA[1], Minako SUMI[1], Yoshikazu KAGAMI[1], Noriyuki KATSUMATA[2], Takahiro KASAMATSU[3] and Jun ITAMI[1]

[1]Department of Radiation Oncology, National Cancer Center Hospital, Tokyo, Japan
[2]Department of Medical Oncology, National Cancer Center Hospital, Tokyo, Japan
[3]Department of Gynecologic Oncology, National Cancer Center Hospital, Tokyo, Japan
*Corresponding author. Department of Radiation Oncology, National Cancer Center Hospital, 5-1-1 Tsukiji Chuo-ku, Tokyo, Japan, 104-0045; Tel: 81-(3)3542-2511; Fax: 81-(3)3545-3567; Email: yuuki.kuroda.96@gmail.com

(Received 18 October 2011; revised 20 January 2012; accepted 7 February 2012)

The purpose of this retrospective study is to investigate the impact of concurrent chemotherapy on definitive radiotherapy for the International Federation of Gynecology and Obstetrics (FIGO) IIIb cervical cancer. Between 2000 and 2009, 131 women with FIGO IIIb cervical cancer were treated by definitive radiotherapy (i.e. whole pelvic external beam radiotherapy for 40–60 Gy in 20–30 fractions with or without center shielding and concomitant high-dose rate intracavitary brachytherapy with 192-iridium remote after loading system for 6 Gy to point A of the Manchester method). The concurrent chemotherapy regimen was cisplatin (40 mg/m^2/week). After a median follow-up period of 44.0 months (range 4.2–114.9 months) and 62.1 months for live patients, the five-year overall survival (OS), loco-regional control (LRC) and distant metastasis-free survival (DMFS) rates were 52.4, 80.1 and 59.9%, respectively. Univariate and multivariate analyses revealed that lack of concurrent chemotherapy was the most significant factor leading to poor prognosis for OS (HR = 2.53; 95% CI 1.44–4.47; $P = 0.001$) and DMFS (HR = 2.53; 95% CI 1.39–4.61; $P = 0.002$), but not for LRC (HR = 1.57; 95% CI 0.64–3.88; $P = 0.322$). The cumulative incidence rates of late rectal complications after definitive radiotherapy were not significantly different with or without concurrent chemotherapy (any grade at five years 23.9 vs 21.7%; $P = 0.669$). In conclusion, concurrent chemotherapy is valuable in definitive radiotherapy for Japanese women with FIGO IIIb cervical cancer.

Keywords: cervical cancer; IIIb; chemotherapy; radiotherapy; HDR

INTRODUCTION

External beam radiotherapy (EBRT) combined with intracavitary brachytherapy (ICBT) is the standard treatment for women with cervical cancer [1–3]. A combination of EBRT plus high-dose rate (HDR) ICBT for Japanese women with cervical cancer has provided acceptable outcomes and late complication rates despite the lower dose prescription in Japan than in the US [4–9]. In 2000s concurrent chemoradiotherapy (CCRT) became standard after the National Cancer Institute (NCI) announcement recommending concurrent chemotherapy in 1999 [10], however, the benefits of concurrent chemotherapy on definitive radiotherapy might not be applicable to concomitant EBRT plus HDR-ICBT and are not clear yet in Japan and other Asian countries [9]. We therefore performed a retrospective analysis in a mono-institutional group with newly diagnosed International Federation of Gynecology and Obstetrics (FIGO) IIIb cervical cancer treated by definitive radiotherapy, the purpose of this study being to investigate the impact of concurrent chemotherapy on definitive radiotherapy for Japanese women.

MATERIALS AND METHODS

Patients

We reviewed our database looking for women with newly diagnosed FIGO IIIb uterine cervical cancers with a

maximum diameter over 4 cm treated with definitive radiotherapy at the National Cancer Center Hospital between 2000 and 2009. Patients who received palliative EBRT alone, postoperative radiotherapy, interstitial brachytherapy or an experimental regimen of concurrent chemotherapy were excluded. A total of 131 women treated with EBRT plus HDR-ICBT were admitted to this retrospective analysis. All patients underwent pelvic examination, cystoscope, urography, computed tomography (CT), magnetic resonance imaging (MRI), ultrasound (US) and blood tests. Maximum tumor diameters were measured based on the MRI findings and/or US. FIGO staging was allocated for tumor boards of gynecological, medical and radiation oncologists. The pathological diagnosis was carried out with a central pathology review at our pathological division.

Treatment

Treatment selection was determined by the gynecological cancer board, our treatment policy for FIGO IIIb cervical cancer is CCRT to aim for loco-regional control (LRC) even if distant metastasis is not ruled out. Neoadjuvant chemotherapy was prohibited. The concurrent chemotherapy regimen was cisplatin (40 mg/m^2/week). Supportive treatments such as blood transfusions were encouraged during radiotherapy.

Radiotherapy

The radiotherapy field selected was the whole pelvis but exceptions were as follows: para-aortic node (PAN) area irradiation was acceptable in cases with suspicions of PAN metastasis, bilateral inguinal node area irradiation was acceptable in cases with vaginal involvement of more than two-thirds of total vaginal length. Radiotherapy doses of 40–60 Gy in 20–30 fractions were carried out with a 4-field box or the anterior–posterior technique. Center shield radiotherapy (CS) was performed for a shorter overall treatment time (OTT) reducing organ at risk (OAR) exposure depending on tumor shrinkage. CS was carried out 3–4 days/week, and HDR-ICBT 1–2 days/week, but both therapies were not carried out on the same day. All patients underwent EBRT with 10-, 15- and 20-MV X-rays from linear accelerators (Clinac IX, Varian, Palo Alto, CA, USA). Two-dimensional conventional radiotherapy (2DCRT) was employed between 2000 and 2005, and three-dimensional conformal radiotherapy (3DCRT) was used between 2005 and 2010. All patients underwent HDR-ICBT with 192-iridium remote after loading system (RALS, Microselectron). The point A dose prescription for 6 Gy using the Manchester method was performed with the ICBT planning system (Plato®, Nucletron). Image-guided optimization was not applicable even in the case of CT-based ICBT planning. A tandem-cylinder was used only in cases with vaginal involvement of more than one-third of total vaginal length or of an extraordinarily narrow vagina.

Follow-up

All patients were evaluated weekly for toxicity during radiotherapy through physical examinations and blood tests. CT and/or MRI scans and cytology were performed 1–3 months after radiotherapy for initial response, physical examination and blood tests were performed regularly every 1–6 months. Disease progression was defined by the response evaluation criteria in solid tumours (RECIST) version 1.1, new clinical symptoms or observable pelvic deficits.

Statistical analysis

Patient and treatment characteristics were compared using the Mann-Whitney U test and Pearson's chi-square test. OS was estimated from the beginning of radiotherapy to the date of death considered as an event, and censored at the time of last follow-up. LRC rate was estimated from the beginning of radiotherapy to the date of LRC failure including both central and lateral pelvic relapse considered as an event, and censored at the time of death or last follow-up. DMFS rate was estimated from the beginning of radiotherapy to the date of distant metastasis considered as an event, and censored at the time of death or last follow-up. The cumulative incidence rate of late rectal complication was estimated from the beginning of radiotherapy to the date of any grade rectal hemorrhage according to common terminology criteria for adverse events (CTCAE) version 4.0. [11] OS, LRC and DMFS, and the cumulative incidence rates of late rectal complication were calculated using the Kaplan–Meier method [12].

As a measure of radiotherapeutic intensity to point A, we used the equivalent dose in 2-Gy fractions (EQD_2) calculated from total irradiated dose (D) and each dose (d) with α/β for 10 Gy and potential doubling time (Tpot) defined as five days' subtraction from EQD_2 with correction for tumor proliferation associated with OTT (EQD_2T) as shown in the following formula:

$$EQD_2 = D\left(\frac{d+\alpha/\beta}{2+\alpha/\beta}\right)$$

$$\mathrm{EQD_2}T = \mathrm{EQD_2} - \frac{\log_{e^2}\mathrm{T}-\mathrm{T}}{\alpha}\frac{}{T_{pot}}\Big/\left(1+\frac{2}{\alpha/\beta}\right)$$

T_K is the kick-off time of accelerated repopulation and was defined as 21 days, and 0.3 for α [13]. These parameters are not well estimated for cervical cancer so we used those for head and neck squamous cell carcinoma (SCC) and extrapolated them. The survival curves were compared using the log-rank test and Cox's proportional hazards model. In order to carry out univariate and/or multivariate

analysis comparing OS, LRC and DMFS rates, patients were categorized as follows: age (<60 vs ≥60), tumor bulk (<55 vs ≥55 mm), OTT (<6 vs ≥6 weeks), hemoglobin (Hb) before (<11.9 vs ≥11.9 mg/dl) and concurrent chemotherapy. We added univariate and multivariate analysis to assess the impact of concurrent chemotherapy on OS, LRC and DMFS after stratified analysis for age and tumor bulk. All statistical analyses were performed using PASW statistics (Version 18.0, SPSS Japan Inc., an IBM company, Chicago, IL, USA). A P value of <0.05 was considered significant.

RESULTS

Patient and treatment characteristics are shown in Table 1. There were differences in age and Hb level after treatment between the radiotherapy alone and CCRT groups. After a median follow-up period of 44.0 months (range 4.2–114.9 months) collectively and 62.1 months for live patients, five-year OS, LRC and DMFS rates were 52.4, 80.1 and 59.9%, respectively. Univariate and multivariate analyses revealed that default of concurrent chemotherapy was the most significant factor leading to poor prognosis for OS (HR = 2.53; 95% CI 1.44–4.47; $P = 0.001$) and DMFS (HR = 2.53; 95% CI 1.39–4.61; $P = 0.002$), but not for LRC (HR = 1.57; 95% CI 0.64–3.88; $P = 0.322$). (Table 2). The cumulative incidence rates of late rectal complications after definitive radiotherapy were not significantly different with or without concurrent chemotherapy (any grade at five years 23.9 vs 21.7%; $P = 0.669$) (Fig. 1). After stratifying 131 patients for age and tumor bulk, subgroup analysis with or without concurrent chemotherapy revealed that non-elderly women (HR = 2.78; 95% CI 1.25–6.18; $P = 0.012$) with even bulky length (HR = 2.53; 95% CI 1.26–5.07; $P = 0.009$) clearly benefit from concurrent chemotherapy (Table 3).

DISCUSSION

Various predictors such as treatment duration and anemia had been reported in the last decade before CCRT [14–18]. Concomitant EBRT with HDR-ICBT, which requires shorter treatment duration, was originally the mainstream treatment for women with cervical cancer in Japan [5]. Treatment durations of gross tumor irradiation had a median of 42 days, and were mostly 6 weeks, which is much shorter than the 8 weeks recommended by the American brachytherapy society (ABS) [14]. Concurrent chemotherapy has the potential hazard of treatment interruption associated with acute toxicities, however OTT was not significantly different between radiotherapy alone and CCRT (42 (30–69) vs 42 (36–62) days; $P = 0.217$). In this situation, OTT is no longer a prognostic factor [17]. Similarly, a low Hb value before radiotherapy has no impact on survival, and is no longer a prognostic factor if anemia has been actively corrected using blood transfusion during radiotherapy [18].

Randomized trials have shown survival benefits of CCRT for cervical cancer [19–23]. Incorporating concurrent chemotherapy contributed to improvement in both LRC and DMFS [19–23]. This impact is less in stages III–IV than in stages I–II [20–23]. Our study also supported this impact on OS and DMFS even in cases of FIGO IIIb, but not on LRC (Table 2). The cumulative incidence rates of late rectal complications after definitive radiotherapy were not significantly different with or without chemotherapy (any grade at five years 23.9 vs 21.7%; $P = 0.669$) and reached a plateau (Fig. 1), though limited by the short follow-up period for late radiation-induced complications of other organs such as bladder or small intestine [7].

There were important limitations on this retrospective analysis: the advantage of concurrent chemotherapy might merely indicate that the reasons for not undergoing concurrent chemotherapy were associated with poor prognosis. Forty-two women with FIGO IIIb cervical cancer did not undergo concurrent chemotherapy in our study because of advanced age (77 (72–85) years) for 17 patients (40.4%), and the other half (53 (36–70)) had the following reasons for not undergoing concurrent chemotherapy:PAN irradiation for eight patients (19.0%), renal failure for three patients (7.2%), lack of patient's consent for five patients (11.9%), chronic hepatitis for two patients (4.8%), active pyometra, uncontrolled anemia, synchronous double cancer, hypertrophic cardiomyopathy, low white blood cell counts and sequential chemotherapy for one patient each (2.4%). These reasons not to perform concurrent chemotherapy seem to be clinically ordinary and acceptable, but could indicate a potential selection bias that modified the impact of concurrent chemotherapy. Our study revealed that concurrent chemotherapy is the most significant predictor of definitive radiotherapy, thus we conclude that concurrent chemotherapy combined with definitive radiotherapy for FIGO IIIb cervical cancer is advantageous for survival improvement.

Development of the optimal chemotherapy regimen and schedule to increase chemotherapeutic intensity as a cytotoxic agent but not a radiosensitizer seems to be warranted because our results indicated concurrent chemotherapy has impacts on DMFS but not on LRC. It is not reasonable for Japanese women with cervical cancer to undergo increased intensity of dose-dense concurrent chemotherapy due to a lack of relevant feasibility [24]. There is no evidence that platinum-doublet is superior to platinum-alone as concurrent chemotherapy for cervical cancer [22–23]. Therefore, devising the best form of concurrent chemotherapy is considered to be a limitation. The efficacy of adjuvant chemotherapy after definitive CCRT is unclear but worth testing as it is a feasible method [25].

Table 1. Patient and treatment characteristics for RT alone and CCRT

		RT alone ($n=42$)	CCRT ($n=89$)	P
Age	Median (range)	66 (36–85)	55 (29–73)	0.000
Tumor bulk	mm	55 (45–87)	55 (40–95)	0.302
Pathology	SCC	37 (88.1%)	82 (92.1%)	0.454
	non-SCC	5 (11.9%)	7 (7.9%)	
Hb before RT	mg/dl	11.9 (6.4–14.2)	11.9 (7.1–14.5)	0.653
Hb after RT	mg/dl	11.3 (7.6–14.4)	10.3 (6.9–12.3)	0.002
OTT	days	42 (30–69)	42 (36–62)	0.217
EQD_2	Gy	56.4 (44.0–74.0)	54.0 (52.2–74.0)	0.128
EQD_2T	Gy	50.0 (40.9–66.2)	48.2 (39.2–61.2)	0.177
wCDDP courses	1	0	5 (5.6%)	0.000
	2	0	6 (6.8%)	
	3	0	12 (13.5%)	
	4	0	23 (25.8%)	
	5	0	30 (33.7%)	
	6	0	13 (14.6%)	
Reason for RT alone	Advanced age	17 (40.4%)	0	0.000
	PAN irradiation	8 (19.0%)	0	
	No consent	5 (11.9%)	0	
	Renal function	3 (7.2%)	0	
	Hepatitis	2 (4.8%)	0	
	Others	7 (16.7%)	0	
Follow-up	months	30.7 (4.2–100.3)	48.8 (7.3–114.9)	0.001

RT = radiotherapy, CCRT = concurrent chemoradiotherapy, FIGO = International Federation of Gynecology and Obstetrics, SCC = squamous cell carcinoma, Hb = hemoglobin, OTT = overall treatment time, EQD_2 = the equivalent dose in 2-Gy fractions, $EQD_2T = EQD_2$ with correction for tumor proliferation associated with OTT, wCDDP = weekly cisplatin, ns = not significant.

Table 2. Univariate and multivariate analyses on OS, LRC and DMFS

		OS				LRC			DMFS		
Variants		n	**Five years**	**uni**	**multi**	**Five years**	**uni**	**multi**	**Five years**	**uni**	**multi**
Age	<60	72	51.4	0.631	0.121	73.3	0.129	0.076	56.0	0.173	0.033
	≥60	59	53.7			89.2			64.8		
Tumor bulk	<55 mm	54	59.8	0.358	0.486	79.5	0.768	0.856	74.4	0.010	0.027
	≥55 mm	77	47.6			80.6			50.2		
OTT	<6 weeks	75	53.1	0.789	0.639	78.5	0.532	0.258	63.5	0.626	0.918
	≥6 weeks	56	50.8			82.6			56.0		
Hb before RT	<11.9 mg/dl	62	53.1	0.627	0.934	74.5	0.380	0.599	59.3	0.527	0.988
	≥11.9 mg/dl	69	52.2			84.8			60.6		
Concurrent chemotherapy	Yes	89	60.4	0.002	0.001	82.6	0.583	0.322	66.6	0.005	0.002
	No	42	33.5			68.3			44.7		

OS = overall survival, LRC = loco-regional control, DFMS = distant metastasis free survival, uni = univariate analysis, multi = multivariate analysis, OTT = overall treatment time, Hb = hemoglobin, ns = not significant.

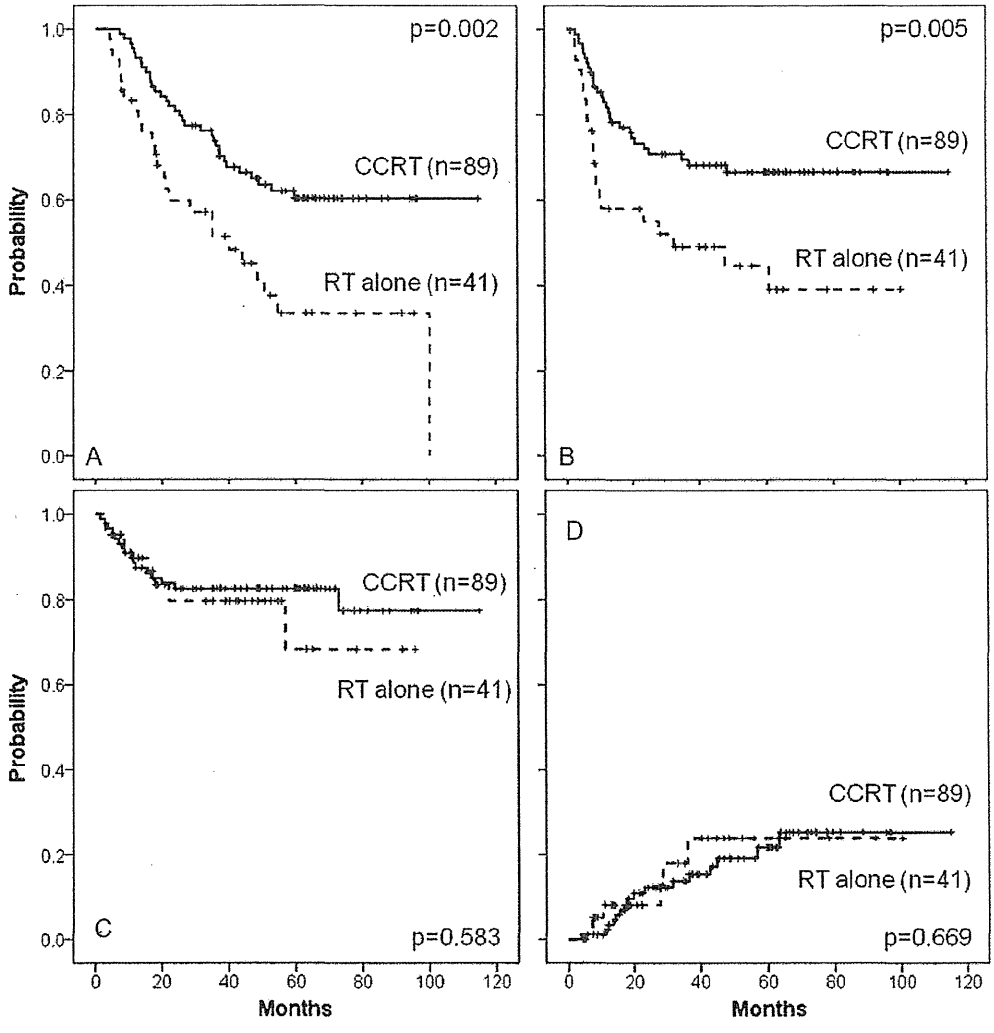

Fig. 1. OS (**A**), DMFS (**B**), LRC (**C**) and the cumulative incidence rates of late rectal complication (**D**) of women with FIGO IIIb cervical cancer after definitive radiotherapy with or without concurrent chemotherapy. Solid line for CCRT, dashed line for RT alone. OS = overall survival, DMFS = distant metastasis free survival, LRC = loco-regional control, CCRT = concurrent chemoradiotherapy, RT = radiotherapy.

Table 3. Impact of concurrent chemotherapy on OS, LRC and DMFS in the stratified analysis

		OS			LRC			DMFS		
		Log-rank	Cox's		Log-rank	Cox's		Log-rank	Cox's	
Variates		*P*	HR (95%CI)	*P*	*P*	HR (95%CI)	*P*	*P*	HR (95%CI)	*P*
Age	<60	0.005	2.78 (1.25–6.18)	0.012	0.145	2.31 (0.76–6.96)	0.136	0.001	2.83 (1.32–6.05)	0.007
	≥60	0.023	2.55 (1.10–5.89)	0.028	0.942	1.05 (0.23–4.85)	0.942	0.079	2.29 (0.88–5.94)	0.087
Tumor bulk	<55 mm	0.118	2.36 (0.85–6.52)	0.096	0.108	5.87 (1.27–27.0)	0.023	0.043	3.46 (1.01–11.9)	0.049
	≥55 mm	0.018	2.53 (1.26–5.07)	0.009	0.587	0.75 (0.22–2.49)	0.645	0.085	2.23 (1.12–4.44)	0.021

OS = overall survival, DMFS = distant metastasis free survival, ns = not significant.

In conclusion, though limited to a mono-institutional retrospective analysis, this study revealed that concurrent chemotherapy is valuable in definitive radiotherapy for Japanese women with FIGO IIIb cervical cancer. A randomized controlled trial is needed to establish the optimal chemotherapy combined with definitive radiotherapy for women with advanced cervical cancer.

ACKNOWLEDGEMENTS

We declare no conflict of interest. This work was partly supported by a Grant-in-Aid from the Tohoku Cancer EBM project of Yamagata University Faculity of Medicine, Cancer Research Development Fund, National Cancer Center.

REFERENCES

1. Thoms WW, Jr, Eifel PJ, Smith TL *et al.* Bulky endocervical carcinoma: a 23-year experience. *Int J Radiat Oncol Biol Phys* 1992;**23**:491–9.
2. Perez CA, Grigsby PW, Nene SM *et al.* Effect of tumor size on the prognosis of carcinoma of the uterine cervix treated with irradiation alone. *Cancer* 1992;**69**:2796–806.
3. Landoni F, Maneo A, Colombo A *et al.* Randomised study of radical surgery versus radiotherapy for stage Ib-IIa cervical cancer *Lancet* 1997;**350**:535–40.
4. Eifel PJ, Moughan J, Erickson B *et al.* Patterns of radiotherapy practice for patients with carcinoma of the uterine cervix: a patterns of care study. *Int J Radiat Oncol Biol Phys* 2004;**60**:1144–53.
5. Toita T, Kodaira T, Shinoda A *et al.* Patterns of radiotherapy practice for patients with cervical cancer (1999–2001): patterns of care study in Japan. *Int J Radiat Oncol Biol Phys 2008*;**70**:788–94.
6. Toita T, Kato S, Niibe Y *et al.* Prospective multi-institutional study of definitive radiotherapy with high-dose-rate intracavitary brachytherapy in patients with nonbulky (<4-cm) stage i and ii uterine cervical cancer (JAROG0401/JROSG04-2). *Int J Radiat Oncol Biol Phys* 2012;**82**:49–56.
7. Nakano T, Kato S, Ohno T *et al.* Long-term results of high-dose rate intracavitary brachytherapy for squamous cell carcinoma of the uterine cervix. *Cancer* 2005;**103**:92–101.
8. Toita T, Moromizato H, Ogawa K *et al.* Concurrent chemoradiotherapy using high-dose-rate intracavitary brachytherapy for uterine cervical cancer. *Gynecol Oncol* 2005;**96**:665–70.
9. Nagase S, Inoue Y, Umesaki N *et al.* Evidence-based guidelines for treatment of cervical cancer in Japan: Japan Society of Gynecologic Oncology (JSGO) 2007 edition. *Int J Clin Oncol* 2010;**15**:117–24.
10. NCI Issues Clinical Announcement on Cervical Cancer: Chemotherapy Plus Radiation Improves Survival. http://www.nih.gov/news/pr/feb99/nci-22.htm (11 April 2012, date last accessed).
11. National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.03, 2010. http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf (10 April 2012, date last accessed).
12. Kaplan EL, Meier P Nonparametric estimation from incomplete observations *J Am StatAssoc* 1958;**53**:457–81.
13. Hall EJ, Giaccia AJ. *Radiobiology for the Radiologist: Time, Dose, and Fractionation in Radiotherapy, 6th edn.* Philadelphia: Lippincott Williams & Wilkins, 2006, 378–97.
14. Nag S, Erickson B, Thomadsen B *et al.* The American Brachytherapy Society recommendations for high-dose-rate brachytherapy for carcinoma of the cervix. *Int J Radiat Oncol Biol Phys* 2000;**48**:201–11.
15. Lanciano RM, Martz K, Coia LR *et al.* Tumor and treatment factors improving outcome in stage III-B cervix cancer. *Int J Radiat Oncol Biol Phys* 1991;**20**:95–100.
16. Toita T, Kakinohana Y, Ogawa K *et al.* Combination external beam radiotherapy and high-dose-rate intracavitary brachytherapy for uterine cervical cancer: analysis of dose and fractionation schedule. *Int J Radiat Oncol Biol Phys* 2003;**56**:1344–53.
17. Chatani M, Matayoshi Y, Masaki N *et al.* High-dose rate intracavitary irradiation for carcinoma of the uterine cervix. The adverse effect of treatment prolongation. *Strahlenther Onkol* 1997;**173**:379–84.
18. Grogan M, Thomas GM, Melamed I *et al.* The importance of hemoglobin levels during radiotherapy for carcinoma of the cervix. *Cancer* 1999;**86**:1528–36.
19. Rose PG, Bundy BN, Watkins EB *et al.* Concurrent cisplatin-based radiotherapy and chemotherapy for locally advanced cervical cancer. *N Engl J Med* 1999;**340**:1144–53.
20. Morris M, Eifel PJ, Lu J *et al.* Pelvic radiation with concurrent chemotherapy compared with pelvic and para-aortic radiation for high-risk cervical cancer. *N Engl J Med* 1999;**340**:1137–43.
21. Eifel PJ, Winter K, Morris M *et al.* Pelvic irradiation with concurrent chemotherapy versus pelvic and para-aortic irradiation for high-risk cervical cancer: an update of radiation therapy oncology group trial (RTOG) 90-01. *J Clin Oncol* 2004;**22**:872–80.
22. Green JA, Kirwan JM, Tierney JF *et al.* Survival and recurrence after concomitant chemotherapy and radiotherapy for cancer of the uterine cervix: a systematic review and meta-analysis. *Lancet* 2001;**358**:781–6.
23. Chemoradiotherapy for Cervical Cancer Meta-Analysis Collaboration. Reducing uncertainties about the effects of chemoradiotherapy for cervical cancer: a systematic review and meta-analysis of individual patient data from 18 randomized trials. *J Clin Oncol* 2008;**26**:5802–12.
24. Watanabe Y, Nakai H, Shimaoka M *et al.* Feasibility of concurrent cisplatin use during primary and adjuvant chemoradiation therapy: a phase I study in Japanese patients with cancer of the uterine cervix. *Int J Clin Oncol* 2006;**11**:309–13.
25. Dueñas-González A, Zarbá JJ, Patel F *et al.* Phase III, open-label, randomized study comparing concurrent gemcitabine plus cisplatin and radiation followed by adjuvant gemcitabine and cisplatin versus concurrent cisplatin and radiation in patients with stage IIB to IVA carcinoma of the cervix. *J Clin Oncol* 2011;**29**:1678–85.

Cancer Science
The offical journal of the Japanese Cancer Association JCA

Clinicopathological and prognostic impact of human epidermal growth factor receptor type 2 (HER2) and hormone receptor expression in uterine papillary serous carcinoma

Shinichi Togami,[1,5] Yuko Sasajima,[2,3] Takateru Oi,[1] Mitsuya Ishikawa,[1] Takashi Onda,[4] Shun-Ichi Ikeda,[1] Tomoyasu Kato,[1] Hitoshi Tsuda[2] and Takahiro Kasamatsu[1]

[1]Division of Gynecology, National Cancer Center Hospital, Tokyo; [2]Pathology Section, Clinical Laboratory Division, National Cancer Center Hospital, Tokyo; [3]Department of Pathology, Teikyo University School of Medicine, Tokyo; [4]Department of Gynecology, Kitasato University School of Medicine, Kitasato, Kanagawa, Japan

(Received December 2, 2011/Revised February 2, 2012/Accepted February 2, 2012/Accepted manuscript online February 13, 2012/Article first published online March 27, 2012)

Uterine papillary serous carcinoma (UPSC) is a rare and aggressive variant of endometrial carcinoma. Little is known about the pathological and biological features of this tumor. Human epidermal growth factor receptor 2 (HER2) and hormone receptor (HR) expression have an important role in tumor behavior and clinical outcome, but their relevance in UPSC is not clear. In the present study, the immunohistochemical expression of HER2 and HR was assessed in 27 patients with Stage I disease, 13 with Stage II disease, 25 with Stage III disease, and 6 with Stage IV disease. Correlations between HER2 and HR expression and the clinicopathological parameters of UPSC were evaluated using Cox's univariate and multivariate analyses. For all patients, the 5-year recurrence-free survival (RFS) and overall survival (OS) rates were 51% and 66%, respectively; in patients with Stage I, II, III and IV disease, the RFS and OS were 67%/81%, 59%/77%, 43%/54% and 0%/0%, respectively. Of all 71 patients, 14% (10/71) were positive for HER2 and 52% (37/71) were positive for HR. Overexpression of HER2 was correlated with lower OS ($P = 0.01$), whereas HR overexpression was correlated with higher OS ($P = 0.008$). In multivariate models, HER2, HR, and histologic subtype were identified as independent prognostic indicators for RFS ($P = 0.022$, $P = 0.018$, and $P = 0.01$, respectively), but HR was the only independent factor associated with OS ($P = 0.044$). Thus, HER2 and HR are prognostic variables in UPSC, with HR an independent prognostic factor for OS. (*Cancer Sci* 2012; 103: 926–932)

Endometrial carcinoma is the most common gynecological malignancy in the US and its occurrence in Japan has increased recently.[1,2] Endometrial carcinomas are conventionally divided into two subgroups, Types I and II. Type I endometrial carcinomas is associated with a hyperestrogenic state and tends to be well differentiated, whereas Type II endometrial carcinomas is not associated with a hyperestrogenic status and is high grade.[3] Uterine papillary serous carcinoma (UPSC), initially described by Hendrickson *et al.*,[4] is one of the Type II endometrial carcinomas. It comprises <10% of all endometrial carcinomas, but accounts for over 50% of all recurrences and deaths caused by this disease.[5–7] Despite aggressive treatment, locoregional and distant failures are common, even in patients treated by surgery for early stage disease.[5,8–10] One study reported a 5-year overall survival (OS) rate for 129 patients with UPSC of 45.9%, with recurrence even occurring in four of 21 (19%) patients with Stage IA.[7] Nonetheless, there is little information regarding the molecular basis for the aggressive biological behavior of UPSC.

Human epidermal growth factor receptor type 2 (HER2) is a well-established tumor biomarker that is overexpressed in a wide variety of carcinomas, including breast, ovary, prostate, and lung.[11,12] Overexpression of HER2 is a significant factor in aggressive tumor behavior and poor clinical outcome.[13]

Estrogen and progesterone are important hormones secreted by the ovary that act through specific receptors, namely the estrogen (ER) and progesterone (PR) receptors. The expression of ER and PR is important in endometrial cancers, especially in low-grade endometrioid adenocarcinoma,[14–17] but the relevance of ER and PR expression in UPSC is not known.

Thus, the purpose of the present study was to investigate the clinicopathological significance and prognostic value of HER2, ER, and PR status in a large cohort of UPSC patients.

Materials and Methods

Patients and tissue samples. The present study was performed in 71 patients with UPSC (mean age 63.6 years; range 47–81 years). Patients with serous adenocarcinoma of the uterine cervix were excluded from the study. No patient was lost to follow-up. All subjects initially underwent surgery in the Gynecology Division of the National Cancer Center Hospital (Tokyo, Japan) between 1997 and 2008. All patients underwent hysterectomy, bilateral salpingo-oophorectomy, and pelvic lymph node evaluation. Staging was according to the 1988 International Federation of Gynecology and Obstetrics (FIGO, http://www.figo.org/files/figo-corp/docs/staging_booklet.pdf, accessed 01 Jul 2011) surgical staging system. Following primary surgical treatment, asymptomatic patients underwent pelvic examination, Pap smear, ultrasound, and serial determination of tumor markers (carbohydrate antigen [CA] 125, CA19-9 and carcinoembryonic antigen) every 2–6 months. Symptomatic patients underwent the appropriate examinations, as indicated, including chest radiography, computed tomography (CT), and magnetic resonance imaging (MRI).

For the present study, all surgically removed pathology samples were reviewed by two gynecological pathologists. Postoperative classification was according to the 2002 revision of the International Union Against Cancer (UICC) TNM classification of malignant tumors.[18] Based on World Health Organization classification,[19] at least 10% of the tumor area had to have serous papillary features for inclusion in the present study. Cases were identified as pure UPSC if the serous component

[5]To whom correspondence should be addressed.
E-mail: togami@m3.kufm.kagoshima-u.ac.jp

doi: 10.1111/j.1349-7006.2012.02240.x

occupied all of the tumor area. Otherwise, cases were identified as mixed UPSC. Of the 71 patients included in the present study, 50 were determined to have pure-type UPSC, whereas 21 had mixed-type UPSC. The clinicopathological factors examined included patient age, histologic subtype of UPSC (pure or mixed), FIGO stage, myometrial invasion, lymph node status, and lymph-vascular space involvement (LVSI).

Immunohistochemistry. After being formalin fixed and embedded in paraffin, all tumor tissue specimens were cut into 4-μm serial sections for immunohistochemical staining, in addition to the usual H&E staining. The present study was performed with the approval of the Internal Review Board (Division of Gynecology, National Cancer Center Hospital, Tokyo, Japan) on Ethical Issues. The antibodies and test kits used for immunohistochemistry were as follows: the Hercep Test (Dako, Glostrup, Denmark), anti-ER mouse monoclonal antibody (Clone 1D5; Dako), and anti-PR mouse monoclonal antibody (Clone PgR636; Dako). An autoimmunostainer (Autostainer Link 48; Dako) was used for immunohistochemical staining of HER2/neu, ER, and PR.

Scoring of the results. The results of the immunohistochemical staining were evaluated as the percentage of positively stained neoplastic cells. In mixed-type tumors, only the serous component was evaluated. Immunoreactivity for HER2 was scored semiquantitatively as follows: 0, no immunostaining or membrane staining in <10% of cells; 1+, weak or barely perceptible staining in >10% of cells, with the cells stained in only part of the membrane; 2+, weak or moderate staining in the whole membrane in >10% of tumor cells; and 3+, strong staining in the whole membrane in >10% of tumor cells. Scores of 2+ and 3+ were defined as HER2 positive (Fig. 1a, b). Hormone receptor (HR) expression was scored by assigning proportion and intensity scores according to procedures described by Allred *et al.*[20] Briefly, the proportion score represented the estimated proportion of tumor nuclei staining positive: 0, no immunostaining; 1, <1% immunostaining; 2, 1–10% immunostaining; 3, 11–33% immunostaining; 4, 34–66% immunostaining; and 5, 67–100% immunostaining. The intensity score represented the average intensity of positive nuclei: 0, no immunostaining; 1, weak immunostaining; 2, moderate immunostaining; and 3, strong immunostaining. The proportion and intensity scores were then summed to obtain a total score, which could range from 0 to 8. In the present study, samples were defined as HR positive when either total scores for either ER or PR were >4 (Fig. 1c,d). Immunohistochemical evaluations were performed independently by two observers (ST and YS), with the median value used in analyses.

Statistical analysis. Inter-group comparisons were made using the Chi-squared test. Recurrence-free survival (RFS) and overall survival (OS) were calculated using the Kaplan–Meier method and differences were analyzed by log-rank test. Independent prognostic significance was computed using Cox's proportional hazards general linear model for RFS and OS. The relative risk for relapse or death was estimated by hazard ratios (HR) with a 95% confidence interval (CI). JMP software (SAS Institute, Cary, NC, USA) was used for statistical analyses. $P < 0.05$ was considered significant. These analyses were performed for all 71 cases of UPSC and for the 50 cases of pure-type UPSC.

Results

Patients' clinicopathological information is presented in Table 1. Based on the FIGO classification system, 27, 13, 25 and 6 patients had Stage I, II, III, and IV disease, respectively. Of all 71 patients, 10 (14%) were HER2 positive and 37 (52%) were HR positive. Seven of 10 (70%) HER2-postivite cases had FIGO Stage III or IV disease, compared with 24 of 61 (39%) HER2-negative cases ($P = 0.04$). Furthermore, the recurrence rate was significantly higher in the HER2-positive group than in the HER2-negative group ($P = 0.0024$). In contrast, the incidence of lymph node metastasis, LVSI, and recurrence was significantly lower in HR-positive patients ($P = 0.005$, $P = 0.012$, and $P = 0.024$, respectively) than in HR-negative patients. There were no significant differences between the HER2-positive and HER2-negative groups with regard to histologic type, myometrial invasion, lymph node status, and LVSI. Similarly, there were no significant differences between the HR-positive and HR-negative groups regarding histologic type, stage, and myometrial invasion.

The median follow-up time for all patients was 49.7 months (range 4–125 months). During this time, recurrence occurred in 34 patients (48%). Initial recurrence sites, either single or multiple, were as follows: lung ($n = 12$ patients), abdominal wall or cavity ($n = 9$), para-aortic lymph nodes ($n = 8$), cervical or supraclavicular lymph nodes ($n = 6$), vagina ($n = 3$),

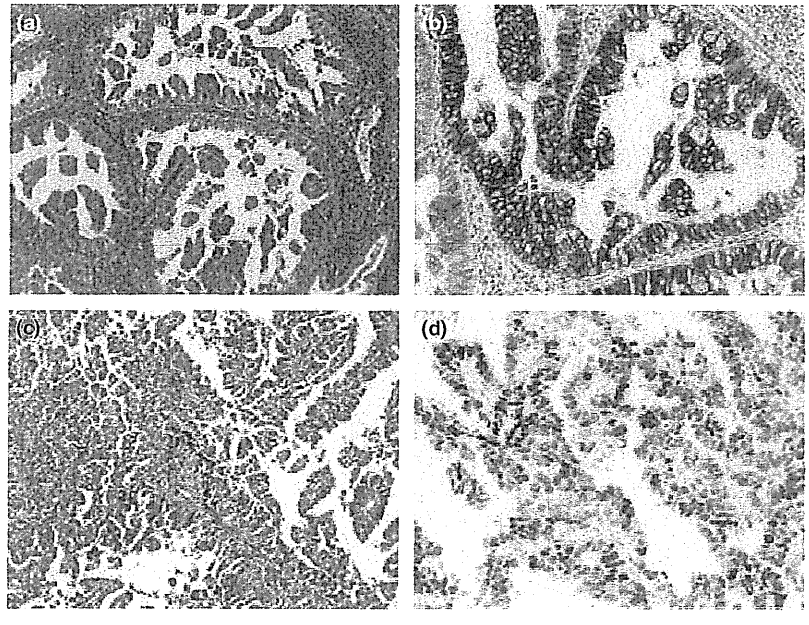

Fig. 1. Histopathological presentation of uterine papillary serous carcinoma. (a,c) Hematoxylin and eosin staining, original magnification ×200. (b) Immunohistochemical staining showing overexpression of human epidermal growth factor receptor type 2 (HER2; score 3+). Original magnification ×200. (d) Immunohistochemical staining showing expression of the estrogen receptor (Allred score 5 + 3 = 8). Original magnification ×200.

Table 1. Correlation between clinicopathological parameters in uterine papillary serous carcinoma and human epidermal growth factor receptor type 2 and hormone receptor status

Total no. patients		HER2			Hormone receptor*		
		Positive (n = 10)	Negative (n = 61)	P-value	Positive (n = 37)	Negative (n = 34)	P-value
Histologic subtype							
Pure	50	8 (80)	42 (69)	0.46	26 (70)	24 (71)	0.98
Mixed	21	2 (20)	19 (31)		11 (30)	10 (29)	
FIGO stage							
I	27	1 (10)	26 (42)	0.04	14 (38)	13 (38)	0.27
II	13	2 (20)	11 (18)		7 (19)	6 (18)	
III	25	7 (70)	18 (30)		15 (40)	10 (29)	
IV	6	0 (0)	6 (10)		1 (3)	5 (15)	
Myometrial invasion (%)							
0	10	0 (0)	10 (16)	0.10	7 (19)	3 (9)	0.12
<50	28	3 (30)	25 (41)		17 (46)	11 (32)	
>50	33	7 (70)	26 (43)		13 (35)	20 (59)	
Lymph node metastasis							
Positive	13	2 (20)	11 (18)	0.35	2 (6)	11 (32)	0.005
Negative	30	6 (60)	24 (39)		16 (43)	14 (41)	
Unknown	28	2 (20)	26 (43)		19 (51)	9 (27)	
Lymph–vascular space involvement							
Positive	35	7 (70)	28 (46)	0.15	13 (35)	22 (65)	0.012
Negative	36	3 (30)	33 (54)		24 (65)	12 (35)	
Recurrence							
Yes	34	9 (90)	25 (41)	0.0024	13 (35)	21 (62)	0.024
No	37	1 (10)	36 (59)		24 (65)	13 (38)	

Data show the number of patients in each group, with percentages given in parentheses. *Hormone receptor expression was classified as positive when the patient was positive for either estrogen or progesterone receptors. HER2, human epidermal growth factor receptor type 2; FIGO, International Federation of Gynecology and Obstetrics.

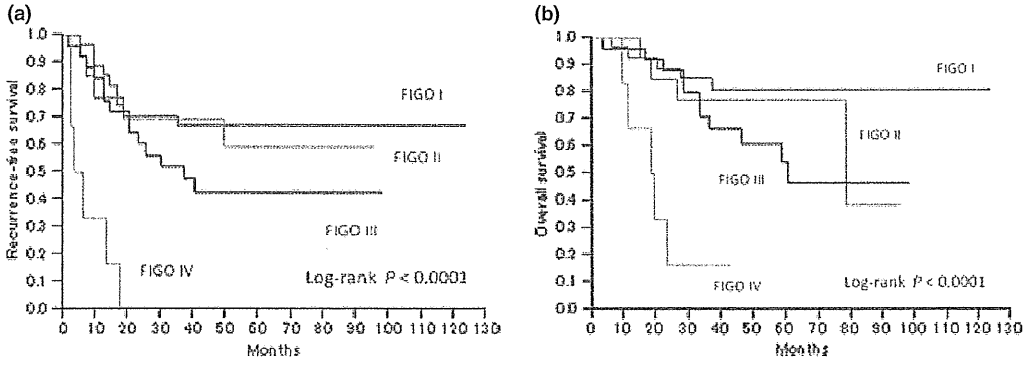

Fig. 2. Kaplan–Meier survival curves for patients with uterine papillary serous carcinoma stratified according to International Federation of Gynecology and Obstetrics (FIGO) stages. Significant differences were observed between the groups for both (a) recurrence-free survival and (b) overall survival (P < 0.0001, log-rank test).

liver (n = 3), bone (n = 3), pelvic cavity (n = 2) and brain (n = 1). Figure 2 shows Kaplan–Meier curves for RFS and OS stratified according to FIGO stage. The RFS rates after 3 and 5 years were 56% and 51%, respectively, with 5-year RFS significantly better for patients with early stage disease (Stage I, 67%; Stage II, 59%) than those with advanced stages of the disease (Stage III, 43%; Stage IV, 0%; P < 0.0001). The OS rates after 3 and 5 years were 73% and 66%, respectively; after 5 years, OS was again significantly higher in patients with early stage disease (Stage I, 81%; Stage II, 77%) than in patients at advanced stages of the disease (Stage III, 54%; Stage IV, 0%; P < 0.0001).

Figure 3 shows Kaplan–Meier curves for RFS and OS in the HER2-positive and -negative groups. Patients with HER2-positive tumors had a significantly lower RFS and OS than patients with HER2-negative tumors (P = 0.0008 and P = 0.01, respectively). In contrast, patients with HR-positive tumors had significantly higher RFS and OS than patients with HR-negative tumors (P = 0.01 and P = 0.008, respectively; Fig. 4).

Patients were stratified into four groups according to both HER2 and HR status. The clinical outcome was best for patients in the HER2-negative HR-positive group, followed by the HER2-negative HR-negative group, the HER2-positive HR-positive group, and finally the HER2-positive HR-negative group, with corresponding 5-year RFS/OS rates in these four groups of 68%/86%, 45%/58%, 43%/51%, and 0%/20%, respectively. The OS of HER2-positive HR-negative patients and HER2-negative HR-positive patients differed significantly (P = 0.0004). Similarly, OS for HER2-negative HR-negative patients and HER2-positive HR-negative patients differed significantly (P = 0.02). However, there were no significant

doi: 10.1111/j.1349-7006.2012.02240.x

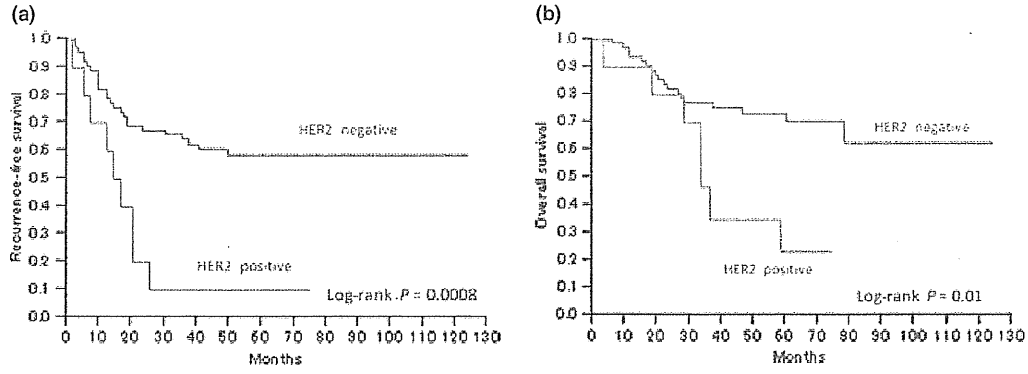

Fig. 3. Kaplan–Meier survival curves for patients with uterine papillary serous carcinoma stratified according to human epidermal growth factor receptor type 2 (HER2) status. Significant differences were observed between the groups for both (a) recurrence-free survival ($P < 0.0008$) and (b) overall survival ($P = 0.01$).

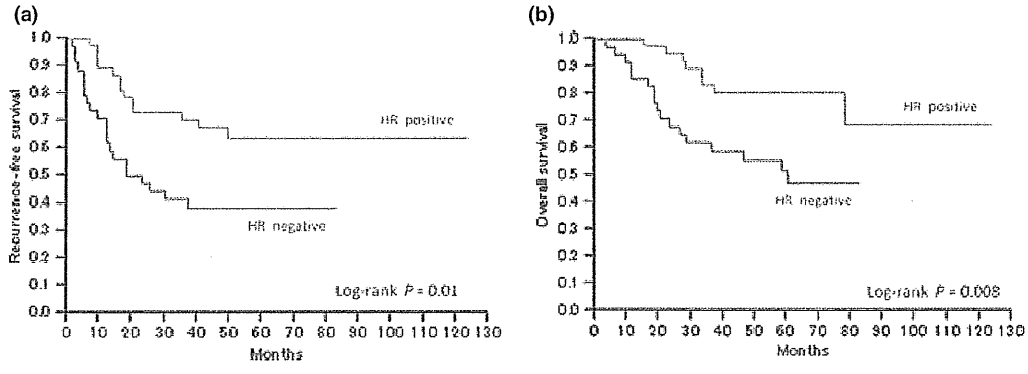

Fig. 4. Kaplan–Meier survival curves for patients with uterine papillary serous carcinoma stratified according to hormone receptor (HR) status. Note, HR expression was classified as positive when the patient was positive for either estrogen or progesterone receptors. Significant differences were observed between the groups for both (a) recurrence-free survival ($P = 0.01$) and (b) overall survival ($P = 0.008$).

differences in OS between the HER2-negative HR-negative group and any of the three other groups. The results of univariate analysis of the effects of various clinicopathological factors on RFS are given in Table 2. All factors examined, except lymph node status, were significantly associated with a worse patient outcome. We also performed multivariate analysis using Cox's proportional hazards model including HER2, HR, histologic type, stage, myometrial invasion, and LVSI. Multivariate analysis identified HER2, HR, and histologic type as independent prognostic factors for RFS ($P = 0.022$, $P = 0.018$, and $P = 0.01$, respectively). The results of univariate analysis of the effects of various clinicopathological factors on OS are given in Table 3. All factors examined, except lymph node status, were significantly associated with a worse patient outcome. We also performed multivariate analysis including HER2, HR, histologic type, stage, myometrial invasion, and LVSI. This analysis revealed that HR was the only independent factor associated with OS ($P = 0.044$).

Separate univariate and multivariate analyses using the Cox's proportional hazards model were also performed for the 50 cases of pure-type UPSC (data not shown). In these patients, HER2, HR, and LVSI were identified as independent prognostic factors for RFS ($P = 0.006$, $P = 0.018$, and $P = 0.04$, respectively). Similarly, HR tended towards being an independent prognostic factor for OS ($P = 0.06$).

Discussion

Uterine papillary serous carcinoma is an aggressive variant of endometrial carcinoma but, because it is so rare, little is known about the prognostic markers for this disease. In the present study, we found that HER2 and HR were prognostic markers of UPSC.

In the present study, the 5-year OS rate after initial surgery for UPSC was 66%; furthermore, the 5-year OS was significantly better for patients in the early stages of the disease (Stages I and II) than in the advanced stages (Stages III and IV). Other studies have reported OS rates of 37–57%, with better rates for patients in the early stages of the disease.[7,21,22] Based on the FIGO annual report,[23] 76.5% of endometrial cancer patients are alive at 5 years. We confirmed the poor prognosis of UPSC compared with endometrioid adenocarcinoma of the uterine corpus. Assessing patterns of recurrence revealed that lung and abdominal failure occurred frequently. Wang *et al.*[24] reported that more than half of the patients who developed recurrence had abdominal failure. Furthermore, UPSC has a propensity to recur at distant sites, with metastases including lung, lymph nodes, liver, bone, and brain. In contrast, vaginal recurrence is less frequent than in endometrioid adenocarcinomas. An interesting aspect of UPSC is that even patients with no myometrial invasion have extrauterine disease.[7,8,10,25,26] In the present series, 40% (4/10) of patients without myometrial invasion had extrauterine disease. Therefore, it is very important to perform accurate surgical staging for all patients with UPSC, regardless of the extent of myometrial invasion.

Overexpression of HER2 is frequently associated with more aggressive disease in cancer patients[27] and a worse prognosis in breast cancer.[28–30] However, there is little information regarding the relationship between HER2 expression and prognosis in patients with UPSC. In the present study, the

Table 2. Cox model estimates of the prognostic significance of each parameter for recurrence-free survival

Variables	Univariate			Multivariate		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
HER2						
Positive	3.43	1.50–7.23	0.005	2.94	1.18–6.90	0.022
Negative	1.00			1.00		
Hormone receptor*						
Positive	0.41	0.20–0.82	0.011	0.41	0.18–0.86	0.018
Negative	1.00			1.00		
Histologic subtype						
Pure	1.00		0.013			0.01
Mixed	0.58	0.34–0.90		0.54	0.30–0.87	
FIGO stage						
I or II	1.00		0.013	1.00		0.19
III or IV	1.54	1.09–2.19		1.28	0.88–1.88	
Myometrial invasion (%)						
≤50	1.00		0.0001	1.00		0.97
>50	2.00	1.40–2.97		1.02	0.49–1.98	
Lymph node metastasis						
Positive	1.86	1.07–3.03	0.085			
Negative	1.00					
Unknown	0.78	0.48–1.24				
Lymph–vascular space involvement						
Positive	1.87	1.31–2.77	0.0005	1.51	0.80–3.06	0.21
Negative	1.00			1.00		

*Hormone receptor expression was classified as positive when the patient was positive for either estrogen or progesterone receptors. HR, hazards ratio; CI, confidence interval; HER2, human epidermal growth factor receptor type 2; FIGO, International Federation of Gynecology and Obstetrics.

Table 3. Cox model estimates of the prognostic significance of each parameter for overall survival

Variables	Univariate			Multivariate		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
HER2						
Positive	3.00	1.15–7.01	0.026	2.06	0.77–5.04	0.144
Negative	1.00			1.00		
Hormone receptor*						
Positive	0.34	0.14–0.76	0.0084	0.41	0.16–0.98	0.044
Negative	1.00			1.00		
Histologic subtype						
Pure	1.00		0.039	1.00		0.087
Mixed	0.60	0.32–0.98		0.63	0.33–1.07	
FIGO stage						
I or II	1.00		0.011	1.00		0.246
III or IV	1.68	1.13–2.59		1.29	0.84–2.05	
Myometrial invasion (%)						
≤50	1.00		0.0001	1.00		0.385
>50	2.65	1.68–4.60		1.45	0.64–3.38	
Lymph node metastasis						
Positive	2.08	1.14–3.59	0.062			
Negative	1.00					
Unknown	0.63	0.34–1.10				
Lymph–vascular space involvement						
Positive	2.38	1.51–4.13	0.0001	1.47	0.68–3.47	0.344
Negative	1.00			1.00		

*Hormone receptor expression was classified as positive when the patient was positive for either estrogen or progesterone receptors. HR, hazards ratio; CI, confidence interval; HER2, human epidermal growth factor receptor type 2; FIGO, International Federation of Gynecology and Obstetrics.

frequency of HER2 overexpression in UPSC was found to be 14% (10/71). Halperin *et al.*[31] reported that 45% (10/22) of UPSC samples were positive for HER2. Santin *et al.*[32] noted HER2 expression in 16 of 26 (62%) UPSC samples. In the present study, we observed a low rate of HER2 overexpression compared with the rates reported in these earlier, smaller studies. Other larger scale studies have also reported a lower rate of HER2 overexpression, similar to the findings in the present study. For example, Slomovitz *et al.*[33] and Konecny *et al.*[34] have reported HER2 overexpression in only 18% (12/68) and 17% (18/105) of tumors.

With regard to clinicopathological parameters, patients in the HER2-positive group were at more advanced stages of the disease and experienced a higher rate of recurrence than patients in the HER2-negative group. Furthermore, the HER2-positive group had lower RFS and OS than the HER2-negative group. Few other studies have evaluated the prognostic value of HER2 in UPSC. Slomovitz *et al.*[33] reported that HER2 overexpression was associated with a lower OS ($P = 0.008$). They also reported that, in multivariate analysis, HER2 overexpression, lymph node status, and stage were each correlated with OS ($P < 0.05$).[33]

In our multivariate analysis, HER2 overexpression was an independent prognostic factor for RFS (HR 2.94; 95% CI 1.18–6.90; $P = 0.022$), but not for OS. The latter negative result may have been due to the small number of UPSC patients in the follow-up, and the small number with HER2 overexpression (10 cases).

Both ER and PR are present in normal endometrial tissue as well as in endometrial carcinoma. It is known that HR-positive breast cancers and Type I endometrial carcinomas, both associated with a hyperestrogenic state, have a better clinical outcome than HR-negative breast cancers and Type II endometrial cancers, which are not associated with a hyperestrogenic state. In endometrial cancers, the absence of HRs is considered an indicator of aggressive tumor growth and poor patient prognosis.[16,35–37] Therefore, we propose that Type II endometrial carcinoma with HR expression is associated with a good prognosis. To the best of our knowledge, few studies have evaluated the prognostic value of ER and PR in UPSC. The present study is the largest correlating ER and PR expression with the clinical outcome of UPSC patients. The frequency of HR expression in our UPSC cohort was 52% (37/71). Reported frequencies of ER and PR expression in previous studies from 23.8% to 50% for ER and from 19% to 45.5% for PR.[38–40] Therefore, UPSC frequently express ER and PR despite being unrelated to estrogenic stimulation.

In terms of clinicopathological parameters, the HR-positive group did not have pelvic lymph node metastasis and had negative LVSI and lower recurrence rates than the HR-negative group. Moreover, HR expression was significantly associated with higher RFS and OS. Fukuda *et al.*[15] reported that ER/PR-positive cases had significantly higher disease-free survival rates in endometrioid adenocarcinoma of the uterine corpus. However, there appears to be no information in the literature regarding the significance of HRs as prognostic parameters in UPSC. In the present study, multivariate analysis revealed that HR expression was an independent prognostic indicator for RFS (HR 0.41; 95% CI 0.18–0.86; $P = 0.018$) and OS (HR 0.41; 95% CI 0.16–0.98; $P = 0.044$). Furthermore, HR expression was the only independent favorable prognostic factor for OS: Other factors, such as FIGO stage, histologic subtype, myometrial invasion, lymph node status, and LVSI, lost their prognostic significance. No other studies have investigated HR expression as a prognostic factor for RFS and OS in patients with UPSC. In the present study, HRs were expressed less frequently in UPSC than reported for endometrioid carcinoma,[38,40] but still seem clinically useful for prognosis.

doi: 10.1111/j.1349-7006.2012.02240.x

In the present study, there were 21 cases (30%) of mixed-type UPSC. Mixed serous and endometrioid carcinomas containing at least 25% of the serous component have been shown to behave in the same way as pure serous carcinomas.[41] Using univariate and multivariate analyses, we also showed that HER2 and HR were important prognostic factors for pure-type UPSC. We assume that the results of the present study hold true for both pure- and mixed-type UPSC.

The breast cancer group that lacks ER and PR expression and has HER2 protein overexpression is classified as the "triple-negative" subtype, with the tumors characterized as aggressive and more likely to recur and metastasize than those of other subtypes.[42] Kothari *et al.*[3] reported that the triple-negative phenotype in endometrial cancer was also associated with poor prognostic factors. However, in the present study, the prognosis of patients with triple-negative UPSC was intermediate between those with HR-positive HER2-negative UPSC and HR-negative HER2-positive UPSC.

In conclusion, UPSC is an aggressive variant of endometrial carcinoma and the optimal therapy for this condition has not yet been developed. The significance of HER2 and HR for prognosis in UPSC was not sufficiently clear, but the present study has demonstrated that clinicopathological factors and molecular biological prognostic factors, such as HER2 and HR, are related to RFS and/or OS in patients with UPSC. Although UPSC is a rare tumor, it is mandatory to establish novel therapies, including chemotherapy, endocrine therapy and molecular-targeted drug therapy, based on the findings of the status of these molecular biological markers.

Disclosure Statement

None of the authors have any financial relationships relevant to this publication.

References

1 Jemal A, Siegel R, Ward E *et al.* Cancer statistics, 2006. *CA Cancer J Clin* 2006; **56**: 106–30.
2 Nakao Y, Yokoyama M, Hara K *et al.* MR imaging in endometrial carcinoma as a diagnostic tool for the absence of myometrial invasion. *Gynecol Oncol* 2006; **102**: 343–7.
3 Kothari R, Morrison C, Richardson D *et al.* The prognostic significance of the triple negative phenotype in endometrial cancer. *Gynecol Oncol* 2010; **118**: 172–5.
4 Hendrickson M, Ross J, Eifel PJ, Cox RS, Martinez A, Kempson R. Adenocarcinoma of the endometrium: analysis of 256 cases with carcinoma limited to the uterine corpus. Pathology review and analysis of prognostic variables. *Gynecol Oncol* 1982; **13**: 373–92.
5 Hendrickson M, Ross J, Eifel P, Martinez A, Kempson R. Uterine papillary serous carcinoma: a highly malignant form of endometrial adenocarcinoma. *Am J Surg Pathol* 1982; **6**: 93–108.
6 Nicklin JL, Copeland LJ. Endometrial papillary serous carcinoma: patterns of spread and treatment. *Clin Obstet Gynecol* 1996; **39**: 686–95.
7 Slomovitz BM, Burke TW, Eifel PJ *et al.* Uterine papillary serous carcinoma (UPSC): a single institution review of 129 cases. *Gynecol Oncol* 2003; **91**: 463–9.
8 Bristow RE, Asrari F, Trimble EL, Montz FJ. Extended surgical staging for uterine papillary serous carcinoma: survival outcome of locoregional (Stage I–III) disease. *Gynecol Oncol* 2001; **81**: 279–86.
9 Carcangiu ML, Chambers JT. Uterine papillary serous carcinoma: a study on 108 cases with emphasis on the prognostic significance of associated endometrioid carcinoma, absence of invasion, and concomitant ovarian carcinoma. *Gynecol Oncol* 1992; **47**: 298–305.
10 Grice J, Ek M, Greer B *et al.* Uterine papillary serous carcinoma: evaluation of long-term survival in surgically staged patients. *Gynecol Oncol* 1998; **69**: 69–73.
11 Meric-Bernstam F, Hung MC. Advances in targeting human epidermal growth factor receptor-2 signaling for cancer therapy. *Clin Cancer Res* 2006; **12**: 6326–30.
12 Rabindran SK. Antitumor activity of HER-2 inhibitors. *Cancer Lett* 2005; **227**: 9–23.
13 Friedlander E, Barok M, Szollosi J, Vereb G. ErbB-directed immunotherapy: antibodies in current practice and promising new agents. *Immunol Lett* 2008; **116**: 126–40.
14 Creasman WT. Prognostic significance of hormone receptors in endometrial cancer. *Cancer* 1993; **71**: 1467–70.
15 Fukuda K, Mori M, Uchiyama M, Iwai K, Iwasaka T, Sugimori H. Prognostic significance of progesterone receptor immunohistochemistry in endometrial carcinoma. *Gynecol Oncol* 1998; **69**: 220–5.
16 Nyholm HC, Christensen IJ, Nielsen AL. Progesterone receptor levels independently predict survival in endometrial adenocarcinoma. *Gynecol Oncol* 1995; **59**: 347–51.
17 Pertschuk LP, Masood S, Simone J *et al.* Estrogen receptor immunocytochemistry in endometrial carcinoma: a prognostic marker for survival. *Gynecol Oncol* 1996; **63**: 28–33.
18 Sobin LH, Wittekind C, eds. *TNM Classification of Malignant Tumours*, 6th edn. Hoboken, NJ: John Wiley & Sons, 2002.
19 Silverberg S, Mutter. GL, Kurman RJ, Kubik-Huch RA, Nogales F, Tavassoli FA. Tumours of the uterine corpus. In: Tavassoli FA, Devilee P, eds. *World Health Organization Classification of Tumours. Tumours of the Breast and Female Genital Organs*. Lyon: IARC Press, 2003; 224–7.
20 Allred DC, Harvey JM, Berardo M, Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. *Mod Pathol* 1998; **11**: 155–68.
21 Goldberg H, Miller RC, Abdah-Bortnyak R *et al.* Outcome after combined modality treatment for uterine papillary serous carcinoma: a study by the Rare Cancer Network (RCN). *Gynecol Oncol* 2008; **108**: 298–305.
22 Low JS, Wong EH, Tan HS *et al.* Adjuvant sequential chemotherapy and radiotherapy in uterine papillary serous carcinoma. *Gynecol Oncol* 2005; **97**: 171–7.
23 FIGO (International Federation of Gynecology and Obstetrics) annual report on the results of treatment in gynecological cancer. *Int J Gynaecol Obstet* 2003; **83**(Suppl. 1): ix–xxii, 1–229.
24 Wang W, Do V, Hogg R *et al.* Uterine papillary serous carcinoma: patterns of failure and survival. *Aust N Z J Obstet Gynaecol* 2009; **49**: 419–25.
25 Bristow RE, Duska LR, Montz FJ. The role of cytoreductive surgery in the management of Stage IV uterine papillary serous carcinoma. *Gynecol Oncol* 2001; **81**: 92–9.
26 Goff BA, Kato D, Schmidt RA *et al.* Uterine papillary serous carcinoma: patterns of metastatic spread. *Gynecol Oncol* 1994; **54**: 264–8.
27 Odicino FE, Bignotti E, Rossi E *et al.* HER-2/neu overexpression and amplification in uterine serous papillary carcinoma: comparative analysis of immunohistochemistry, real-time reverse transcription-polymerase chain reaction, and fluorescence *in situ* hybridization. *Int J Gynecol Cancer* 2008; **18**: 14–21.
28 Konecny G, Pauletti G, Pegram M *et al.* Quantitative association between HER-2/neu and steroid hormone receptors in hormone receptor-positive primary breast cancer. *J Natl Cancer Inst* 2003; **95**: 142–53.
29 Menard S, Valagussa P, Pilotti S *et al.* Response to cyclophosphamide, methotrexate, and fluorouracil in lymph node-positive breast cancer according to HER2 overexpression and other tumor biologic variables. *J Clin Oncol* 2001; **19**: 329–35.
30 Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 1987; **235**: 177–82.
31 Halperin R, Zehavi S, Habler L, Hadas E, Bukovsky I, Schneider D. Comparative immunohistochemical study of endometrioid and serous papillary carcinoma of endometrium. *Eur J Gynaecol Oncol* 2001; **22**: 122–6.
32 Santin AD, Bellone S, Van Stedum S *et al.* Determination of HER2/neu status in uterine serous papillary carcinoma: comparative analysis of immunohistochemistry and fluorescence *in situ* hybridization. *Gynecol Oncol* 2005; **98**: 24–30.
33 Slomovitz BM, Broaddus RR, Burke TW *et al.* Her-2/neu overexpression and amplification in uterine papillary serous carcinoma. *J Clin Oncol* 2004; **22**: 3126–32.
34 Konecny GE, Santos L, Winterhoff B *et al.* HER2 gene amplification and EGFR expression in a large cohort of surgically staged patients with nonendometrioid (Type II) endometrial cancer. *Br J Cancer* 2009; **100**: 89–95.
35 Ferrandina G, Ranelletti FO, Gallotta V *et al.* Expression of cyclooxygenase-2 (COX-2), receptors for estrogen (ER), and progesterone (PR), p53, ki67, and neu protein in endometrial cancer. *Gynecol Oncol* 2005; **98**: 383–9.
36 Jazaeri AA, Nunes KJ, Dalton MS, Xu M, Shupnik MA, Rice LW. Well-differentiated endometrial adenocarcinomas and poorly differentiated mixed

mullerian tumors have altered ER and PR isoform expression. *Oncogene* 2001; **20**: 6965–9.
37 Kadar N, Malfetano JH, Homesley HD. Steroid receptor concentrations in endometrial carcinoma: effect on survival in surgically staged patients. *Gynecol Oncol* 1993; **50**: 281–6.
38 Alkushi A, Irving J, Hsu F *et al.* Immunoprofile of cervical and endometrial adenocarcinomas using a tissue microarray. *Virchows Arch* 2003; **442**: 271–7.
39 Alkushi A, Kobel M, Kalloger SE, Gilks CB. High-grade endometrial carcinoma: serous and Grade 3 endometrioid carcinomas have different immunophenotypes and outcomes. *Int J Gynecol Pathol* 2010; **29**: 343–50.
40 Kounelis S, Kapranos N, Kouri E, Coppola D, Papadaki H, Jones MW. Immunohistochemical profile of endometrial adenocarcinoma: a study of 61 cases and review of the literature. *Mod Pathol* 2000; **13**: 379–88.
41 Sherman ME, Bitterman P, Rosenshein NB, Delgado G, Kurman RJ. Uterine serous carcinoma. A morphologically diverse neoplasm with unifying clinicopathologic features. *Am J Surg Pathol* 1992; **16**: 600–10.
42 Badve S, Dabbs DJ, Schnitt SJ *et al.* Basal-like and triple-negative breast cancers: a critical review with an emphasis on the implications for pathologists and oncologists. *Mod Pathol* 2011; **24**: 157–67.

doi: 10.1111/j.1349-7006.2012.02240.x