the difference was not statistically significant, grade 3 or worse gastrointestinal toxicities were more frequent in the CPT-P arm. Grade 2 or worse peripheral sensory and motor neuropathy occurred in 26.0% and 18.0%, respectively, of the patients in the TC arm in FAS, and in 6.3% and 10.4%, respectively, of the patients in the CPT-P arm in FAS. Grade 2 or worse peripheral sensory neuropathy occurred more frequently in the TC arm (odds ratio, 0.19; 95% CI, 0.05–0.72; $P = 0.0015$).

Efficacy

Clinical response was assessed in the 13 patients in the PPS with clinically measurable disease. Clinical response results are listed in Table 4. There were 2 CR, 2 NC, and 4 PD among the 8 assembled patients in the CPT-P group, and overall response rate was 25% (95% CI, 3.2%–65.1%) in the CPT-P group. There were 1 CR, 1 PR, and 3 PD among the 5 assembled patients in the TC group, and overall response rate was 40% (95% CI, 5.3%–85.3%). No significant difference was observed in overall response rate between the 2 treatment groups.

Survival results are shown in Figure 1. The PFS was compared for all patients in PPS and FAS. No significant difference was observed between the 2 treatment groups (PPS, $P = 0.9089$ by the log-rank test, Fig. 1A; FAS, $P = 0.9035$ by the log-rank test, data not shown), and the relative risk of disease progression in the TC group as compared with that in the CPT-P group was 1.034 (95% CI, 0.583–1.835) in PPS and 0.964 (95% CI, 0.544–1.710) in FAS. Because there were more patients in the CPT-P arm (11 patients in PPS and FAS) than in the TC arm (2 patients in PPS; 4 patients in FAS) with larger residual disease greater than or equal to 2 cm, we performed a subset analysis by removing those patients and then compared the PFS with patients without residual disease or with residual disease less than 2 cm. The PFS tended to be longer in the CPT-P group, although the difference was not statistically significant (PPS, $P = 0.2702$ by the log-rank test, Fig. 1B; FAS, $P = 0.3176$ by the log-rank test, data not shown), and the relative risk of disease progression in the TC group as compared with that in the CPT-P group was 1.465 (95% CI, 0.757–2.836) in PPS and 1.414 (95% CI, 0.730–2.739) in FAS.

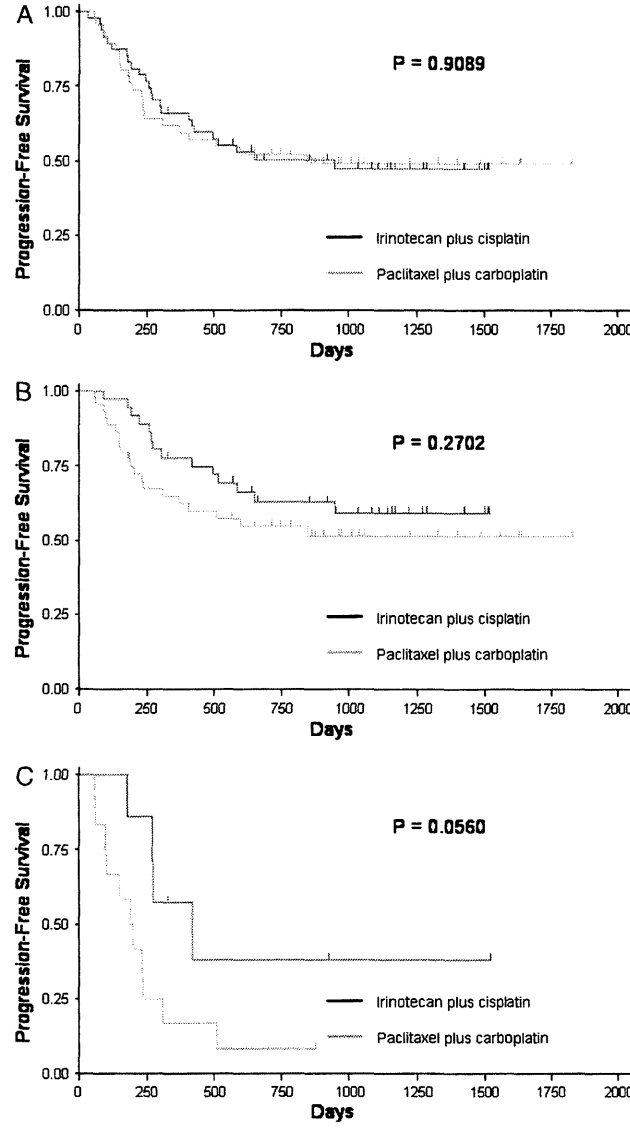

FIGURE 1. Progression-free survival by treatment group. A, Progression-free survival in all patients in PPS by treatment group. B, Progression-free survival in patients without residual disease or with residual disease of less than 2 cm in PPS by treatment group. C, Progression-free survival in patients with residual disease of less than 2 cm in PPS by treatment group.

TABLE 4. Objective tumor response

	CPT-P (n = 8)		TC (n = 5)	
	No. Patients	%	No. Patients	%
CR	2	25	1	20
PR	0	0	1	20
Overall response:				
CR + PR	2	25	2	40
95% CI		3.2–65.1		5.3–85.3
NC	2	25	0	0
PD	4	50	3	60

Furthermore, we compared PFS in patients with residual disease less than 2 cm. There was a strong tendency that PFS was longer in the CPT-P group, although the difference was not statistically significant (PPS and FAS, $P = 0.056$ by the log-rank test, Fig. 1C), and the relative risk of disease progression in the TC group was significantly higher than that in the CPT-P group (2.945; 95% CI, 1.052–8.246) in PPS and FAS.

We also compared PFS in patients with no residual disease. No significant difference was observed between the

2 treatment groups (PPS, $P = 0.8479$ by the log-rank test, data not shown; FAS, $P = 0.7774$ by the log-rank test, data not shown).

A comparison of OS in all patients in PPS and FAS revealed no significant difference between the 2 treatment groups (PPS, $P = 0.2834$ by the log-rank test, data not shown; FAS, $P = 0.2217$ by the log-rank test, data not shown).

DISCUSSION

The CCC has been suggested to lack sensitivity compared with conventional platinum-based chemotherapy.[1,4,5] Paclitaxel plus carboplatin is generally considered to be the gold standard regimen for epithelial ovarian carcinomas. However, the survival benefit of TC compared with conventional platinum-based regimens in CCC patients is controversial.[12,13] On the other hand, it was reported that CPT-P therapy was effective for primary advanced and recurrent or resistant CCC.[2,3,15–17] We conducted a randomized phase II study, JGOG3014, to compare the efficacy and toxicity of CPT-P against TC in patients with CCC.

Although the toxicities of CPT-P and TC were well tolerated, the toxicity profile of each treatment differed. Paclitaxel plus carboplatin produced more thrombocytopenia and peripheral sensory neuropathy. Although the difference was not statistically significant, CPT-P produced more gastrointestinal toxicities. The toxicity results for the TC and CPT-P regimens were similar to those obtained in several phase II and phase III studies for advanced ovarian cancer.[8,18,19]

One retrospective study reported that PFS in patients with optimally resected stages II to IV CCC treated with CPT-P therapy was significantly better than that with paclitaxel plus platinum; that no significant difference was observed in PFS in patients with stage I CCC and patients with suboptimally resected CCC between the 2 treatment groups; and that multiple regression survival analysis revealed that CPT-P regimen and residual tumor diameter were both independent prognostic factors in stages II to IV CCC.[17] The authors suggested that CPT-P had a potential therapeutic benefit for advanced CCC, especially in cases with optimal debulking surgery.[17] Another retrospective study also reported that the estimated 2- and 5-year PFS rates for 35 patients with stage Ic (ascites/malignant washing) to IV CCC treated with CPT-P were 55% and 55%, respectively, and those for 82 patients treated with TC were 48% and 40%, respectively ($P = 0.31$).[20] The authors suggested that CPT-P showed a potential therapeutic effect of at least no less than that of TC therapy.[20] In our study, PFS showed no significant difference between the 2 treatment groups. Nevertheless, in a subset analysis, PFS for patients without residual disease or with residual disease less than 2 cm tended to be longer in the CPT-P group, although the difference was not statistically significant ($P = 0.2702$). This is probably caused by the small sample size. Moreover, in a small subset analysis for patients with residual disease less than 2 cm, the relative risk of disease progression in the TC group was significantly higher than that in the CPT-P group (2.945; 95% CI, 1.052–8.246). These results suggest that CPT-P has a potential therapeutic benefit greater than that of TC therapy for CCC. A phase III randomized trial is required to elucidate the efficacy of CPT-P combination chemotherapy in CCC.

Taken together with those from earlier reports, our data suggest that CPT-P is a candidate first-line chemotherapy regimen for CCC. However, TC is still generally considered to be the standard first-line chemotherapy for ovarian cancer. At present, the JGOG and the Gynecologic Cancer Intergroup is performing an international cooperative randomized phase III trial of TC therapy versus CPT-P therapy as a first-line chemotherapy for CCC (GCIG/JGOG 3017 ovarian trial), and the results are eagerly awaited.

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Distinguishing primary from secondary mucinous ovarian tumors: an algorithm using the novel marker DPEP1

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Distinguishing primary mucinous ovarian cancers from ovarian metastases of digestive organ cancers is often challenging. Dipeptidase 1 was selected as the candidate novel marker of colorectal cancer based on an analysis of a gene expression microarray. Immunohistochemical analysis indicated that 13/16 ovarian metastases of colorectal cancers, but only 1/58 primary mucinous ovarian cancers, were dipeptidase 1-positive (threshold; ≧25% expression, $P<0.0001$). Next, five immunohistochemical markers (dipeptidase 1, estrogen receptor-α, cytokeratin 7, cytokeratin 20, and caudal type homeobox 2) were analyzed in combination. In a hierarchical clustering analysis, the mutually exclusive expression of cytokeratin 7 and dipeptidase 1 specifically identified the ovarian metastases of colorectal cancers ($P<0.0001$). In a decision tree analysis, cytokeratin 7, caudal type homeobox 2, and dipeptidase 1 classified primary mucinous ovarian cancers and ovarian metastases of digestive organ cancers with 90% accuracy. Finally, the five immunohistochemical markers were combined with six preoperative factors (patient's age, tumor size, laterality, serum CEA, CA19-9, and CA125) and combinations were analyzed. Of the 11 factors, 4 (dipeptidase 1, cytokeratin 7, caudal type homeobox 2, and tumor size) were used to generate a decision tree to classify primary mucinous ovarian cancers and metastases of digestive organ cancers with 93% accuracy. In conclusion, we identified a novel immunohistochemical marker, dipeptidase 1, to distinguish primary mucinous ovarian cancers from ovarian metastasis of colorectal cancers. The algorithm using immunohistochemical and clinical factors to distinguish metastases of digestive organ cancers from primary mucinous ovarian cancers will be useful to establish a protocol for the diagnosis of ovarian metastasis.
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Keywords: differential diagnosis; dipeptidase 1(DPEP1); metastasis; mucinous adenocarcinoma; ovary

The ovary is a common site of metastasis,[1] and in many cases, there is a known history of primary tumors outside the ovary. However, ovarian masses are sometimes found in patients with no known history of malignancy and the primary tumor is not diagnosed until some time later. The morphology of a metastatic tumor in the ovary can mimic a primary ovarian tumor by a so-called maturation phenomenon.[2] A majority of the tumors exhibit cystic pattern when they metastasize to the ovary, whereas primary tumor in the original site is not cystic at all. Furthermore, many metastatic mucinous adenocarcinomas, especially from digestive organ cancers, contain a mixture of mucinous epithelium with a range of atypia, resembling primary mucinous carcinomas or borderline tumors. Many metastatic mucinous carcinomas of the ovary have been and continue to be misdiagnosed as primary ovarian mucinous adenocarcinomas.[3]

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In problematic cases, the diagnosis has been made by integrating clinical, radiological, serological, and pathological features.[4] Recently, immunohistochemistry, especially cytokeratin 7 (CK7) and cytokeratin 20 (CK20) staining, has been widely used to distinguish primary from secondary ovarian tumors.[5,6] Although immunohistochemistry has been useful in diagnosis of some cases, current immunohistochemical markers are not sufficient to diagnose all secondary ovarian tumors.[7] Therefore, further investigation of immunohistochemical markers is required.

In this study, we utilized genome-wide gene expression analysis to identify a novel molecular marker, dipeptidase 1 (DPEP1). We also developed an algorithm to distinguish primary mucinous ovarian tumors from ovarian metastases of digestive organ cancers. This study will be useful to establish a protocol to improve the diagnosis of ovarian metastasis in problematic cases.

Table 1 Number of cases used for the analyses

	Number of cases	
Primary ovarian tumors	86	
Mucinous adenoma	28	
Mucinous borderline tumor	28	
Intestinal type	15	
Endocervical type	13	
Mucinous adenocarcinoma (stage I)	24	
Mucinous adenocarcinoma (stage III)	6	
Secondary ovarian tumors	36	
Colorectal cancer	16	
Appendix cancer	2	Digestive organ cancer other than colorectal cancer
Small intestine cancer	1	
Pancreatic cancer	2	
Bile duct cancer	3	
Gastric cancer	8	
Breast cancer	4	

Materials and methods

Tissue Samples

We collected formalin-fixed and paraffin-embedded tissue sections of primary ovarian mucinous tumors and secondary ovarian carcinomas operated between 1985 and 2008 at Kyoto University Hospital, Shinshu University Hospital, and Otsu Red Cross Hospital (104, 10, and 18 samples, respectively). We received written consent from each patient and approval by the ethics committee of each institute. In this study, only the cases with clinically evident primary sites were used. Tumors were classified as metastatic when they exhibited the same morphologic features as primary site. A total of 122 tumor samples, 86 from primary mucinous ovarian tumors and 36 from metastatic ovarian tumors (Table 1), were used for the following analyses.

Microarray Data Set

Microarray data (GSE2109) was obtained from the Gene Expression Omnibus (GEO) website (http://www.ncbi.nlm.nih.gov/geo/). This data set contains 247 epithelial ovarian cancers and 394 colorectal adenocarcinomas arrayed on the Affymetrix U133 plus 2 GeneChip.

Immunohistochemistry

Immunohistochemical stains were performed using the streptavidin–biotin–peroxidase method. Samples were deparaffinized and antigen retrieval was performed as indicated below. For CK20, estrogen receptor-α (ER), and caudal type homeobox 2 (CDX2) staining, the samples were boiled in 10 mM citrate buffer, pH 6.0, for 20 min (CK20 and ER) or 10 min (CDX2). For CK7 staining, the samples were incubated for 20 min at 37 °C with pepsin (Dako, Glostrup, Denmark). For DPEP1 staining, the samples were heated at 98 °C for 15 min in Dako solution. To block endogenous peroxidase activity, 100% methanol containing 0.3% H_2O_2 was added for 15 min. Nonspecific binding of IgG was blocked using normal rabbit (for CK20, CK7, ER, and CDX2) or goat serum (for DPEP1) (Nichirei, Tokyo, Japan). The sections were incubated overnight at 4 °C with the following antibodies: CK20; clone Ks20.8 (Novocastra, New Castle, UK; ready-to-use), CK7; clone OV-TL 12/30 (DAKO; ready-to-use), ER; clone 6F11 (DS Pharma Biomedical, Japan, 1:40), CDX2; clone CDX2-88 (Biogenex, San Ramon, CA, USA; ready-to-use), and DPEP1; HPA012783 (Sigma-Aldrich, St Louis, MO, USA; 1:50). Next, sections were incubated with biotinylated anti-mouse (for CK20, CK7, ER, and CDX2) or anti-rabbit (DPEP1) secondary antibodies (Nichirei). Subsequently, sections were incubated with streptavidin–peroxidase complex solution for 30 min. Signals were generated by incubation with 3,3′-diaminobenzidine. Finally, the sections were counterstained with hematoxylin.

Hierarchical Clustering

Hierarchical clustering was performed using Cluster 3.0 software (http://rana.lbl.gov/EisenSoftware.htm). The green–black–red heatmap was drawn by Java TreeView 1.1.2 (http://jtreeview.sourceforge.net/). The blue–yellow–red heatmap was drawn by R 2.8.1 (http://www.R-project.org.).

Decision Tree Analyses

The WEKA data mining software was downloaded from http://www.cs.waikato.ac.nz/~ml/index.html. For the decision tree analyses, the WEKA J48 tree classifier was used.

Statistical Analysis

All the statistical analyses were performed using GraphPad Prism 4.0b (GraphPad Software, La Jolla, CA, USA). ROC curves were generated using Microsoft Excel 2007 (Microsoft, Tokyo, Japan).

Results

Investigation of a Novel Immunohistochemical Marker to Distinguish Primary from Secondary Ovarian Cancer

We analyzed microarray data set GSE2109, which contains 247 epithelial ovarian cancers and 394 colorectal cancers. The fold difference of average expression between the ovarian and the colorectal cancers were determined for each of the U133 plus 2 gene probes ($n=54\,613$). A threshold of tenfold difference identified 40 probes (28 genes) upregulated in ovarian cancers and 114 probes (85 genes) upregulated in colorectal cancers (Supplementary Table 1). Several of these genes encode known immunohistochemical markers used to distinguish primary from secondary ovarian cancers, such as *CK7*, *ER*, *CK20*, and *CDX2*[8–10] (Figure 2). *FOLR1*,[11] *MUC16*,[12] *PAX8*,[13] *WT1*,[14] *CEACAM-1, 5, 6, 7*,[12] *MEP1A*,[15] and *MUC2*[16] were also identified (Supplementary Table 1). Therefore, this gene list was expected to include novel marker genes that could be used to discriminate primary from secondary ovarian cancers. *DPEP1*, which is reported to be expressed in colorectal cancer,[17,18] was among the genes identified (Figure 2).

We next determined if DPEP1 protein expression could be used to distinguish primary mucinous ovarian carcinomas (including borderline tumors) from ovarian metastases of colorectal cancers. DPEP1 expression was localized to the brush border (Figure 1b) and was observed in varying percentages of tumor cells in different samples. We quantified the percentage of positive cells in each sample. The majority of metastatic colorectal cancer samples contained >25% DPEP-1-positive cells (13/16), whereas nearly all primary mucinous ovarian carcinomas samples contained <25% DPEP-1-positive cells (1/58; $P<0.0001$, Fisher's exact test; Figure 1c). These results indicate that DPEP1 may be useful as an immunohistochemical marker to distinguish primary from secondary mucinous ovarian carcinoma.

Combined Analysis Using Multiple Immunohistochemical Markers

Expression levels of CK7, ER, CK20, and CDX2 were analyzed by immunohistochemistry. ER and CDX2 were found in the nucleus, whereas CK7 and CK20 were found in the cytoplasm (Figure 2a and b). Most samples had heterogeneous protein expression. In each sample, we quantified the percentage of tumor cells positive for each protein. In preliminary analyses, the best threshold of the percentage of expression of positive cells to distinguish primary from secondary ovarian cancers varied from protein to protein (data not shown). Therefore, we utilized the actual percent expression rather than determining arbitrary thresholds in the subsequent analyses. We performed a hierarchical clustering analysis to examine the associations between the five molecules. Notably, ovarian metastases of colorectal cancers were distinguished from other tumors by the mutually exclusive expression of CK7 and DPEP1 ($P<0.0001$, Fisher's exact test; Figure 2c). Ovarian metastases of non-colorectal digestive organ cancers exhibited modest expression of CK7, CK20, and CK20. Ovarian metastases of breast cancer were distinguished from other metastases by abundant ER and CK7 expression and lack of CK20, CDX2, and DPEP1 expression. DPEP1 was negatively correlated with CK7 ($r=-0.66$, $P<0.0001$, Pearson's correlation) and positively correlated with CK20 ($r=0.26$, $P=0.0037$) and CDX2 ($r=0.49$, $P<0.0001$).

Next, we generated dot plot graphs for the percent expression of each protein in each tumor type (Figure 2d). The percent positivity was different among groups (ER, $P=0.0017$; others, $P<0.0001$, Kruskal–Wallis test). The ovarian metastases of colorectal cancers showed the least prevalent expression of CK7 and the most abundant expression of CK20, CDX2, and DPEP1. The ovarian metastases of non-colorectal digestive organ cancers had expression similar to but less striking than the metastases of colorectal cancers. A comparison of primary mucinous ovarian carcinomas (including borderline tumors) and metastases of digestive organ cancers (colorectal plus non colorectal) indicated that CK7 expression was more abundant in primary mucinous ovarian carcinomas, whereas CK20, CDX2, and DPEP1 expression was more abundant in metastases of digestive organ cancers (Figure 3b; CK7, CDX2, and DPEP1, $P<0.0001$; CK20, $P=0.0003$, Mann–Whitney U test). ER expression tended to be more abundant in primary mucinous ovarian carcinomas than the metastases of digestive organ cancers, but the difference was not statistically significant ($P=0.11$).

Given the distinct immunohistochemical patterns in ovarian metastases of colorectal cancers and the modest but the similar pattern in metastases of non-colorectal digestive organ cancers, a combination of the immunohistochemical markers was expected to distinguish primary mucinous ovarian carcinomas (including borderline tumors) from metastases of digestive organ cancer. The Weka J48 software determines a decision tree that best discriminates between different groups using the minimum number of factors. Among the five molecules, three (CDX2, CK7, and DPEP1) were selected to draw a decision tree (Figure 2e) with the net accuracy of 90% (81/90). In all, 97% (56/58) of the primary mucinous ovarian carcinomas and 100% (16/16) of the ovarian metastases of colorectal cancers were

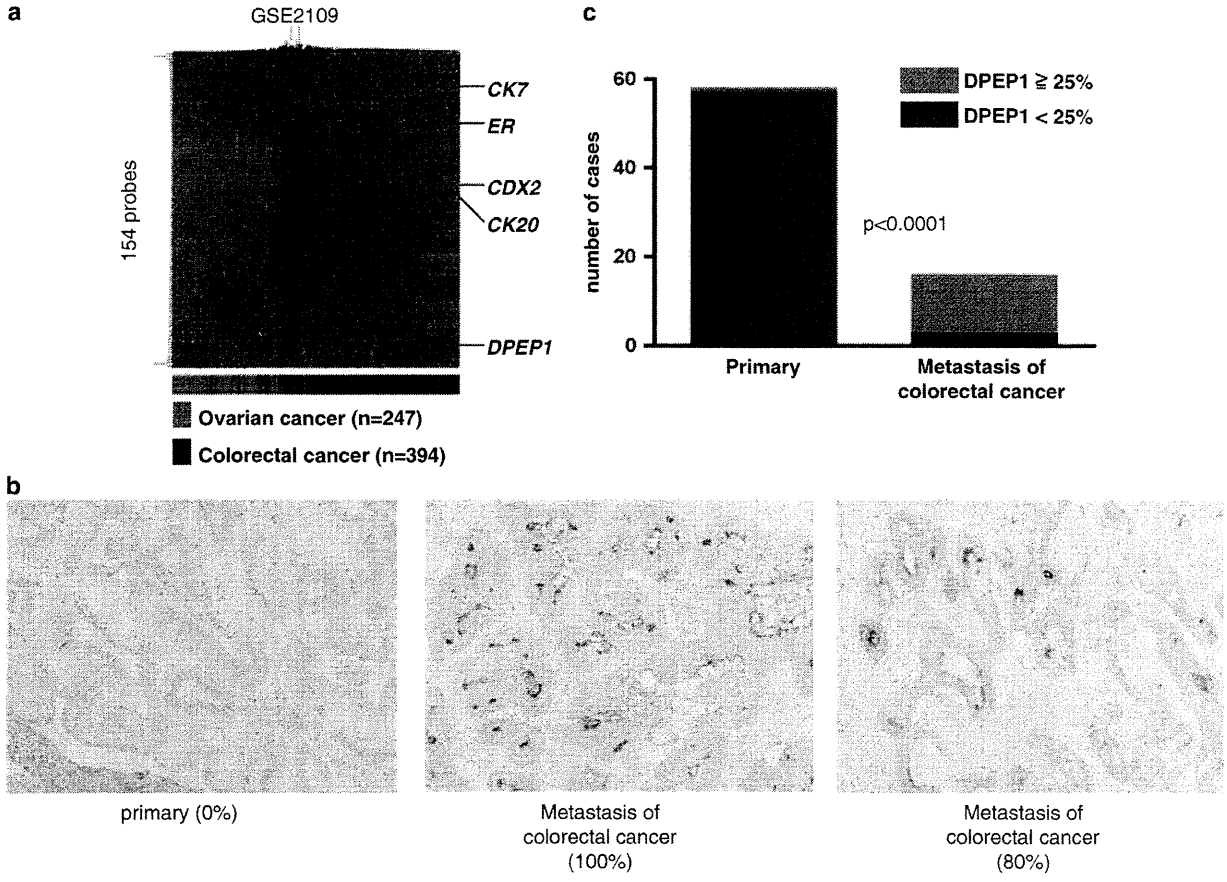

Figure 1 Identification of a novel immunohistochemical marker DPEP1 to distinguish primary mucinous ovarian carcinomas from ovarian metastases of colorectal cancers. (**a**) Identification of genes differentially expressed in ovarian cancer and colorectal cancer. A total of 154 probes were selected using a cutoff of tenfold difference in expression. Gene expressions are $\log_2$ transformed, centered, and normalized, and then the genes and tumor samples are ordered by an average-linkage hierarchical clustering. (**b**) Representative images of DPEP1 immunostaining in ovarian metastases of colorectal cancers and primary mucinous ovarian carcinomas. The percentage of positive tumor cells in the sample is indicated. (**c**) Number of samples with ≧25% or <25% DPEP1-positive tumor cells in primary mucinous ovarian carcinomas and ovarian metastases of colorectal cancers cases is shown.

correctly discriminated. Among the ovarian metastases of non-colorectal digestive organ cancers, the accuracy was 56% (9/16).

Combined Analysis Using Immunohistochemical Markers and Preoperative Clinical Information

The preoperative clinical information was added to improve the accuracy with which primary mucinous ovarian carcinomas (including borderline tumors) were distinguished from ovarian metastases of digestive organ cancers. The patient's age at the date of surgery was significantly different ($P=0.032$, Kruskal–Wallis test) between the groups (Figure 3a). The patient's age of the metastatic digestive organ cancers was significantly higher than that of primary mucinous ovarian carcinomas (median age: 57 *vs* 46.5 years old, $P=0.0027$, Mann–Whitney U test). The longest diameters of the tumors were significantly different between the groups ($P=0.0003$). Tumor sizes of ovarian metastases of digestive organ cancers were significantly smaller than that of primary mucinous ovarian carcinomas (Figure 3a; median size: 10 *vs* 15 cm, $P<0.0001$). The percentage of bilateral tumors was significantly higher in ovarian metastases of digestive organ cancers than in primary mucinous ovarian carcinomas (Figure 3a; 35 *vs* 14%, $P=0.020$). Seidman *et al*[3] previously reported that laterality and tumor size correctly classified 90% of the primary and secondary ovarian cancers. In our data set, Seidman's rule identified primary mucinous ovarian carcinomas and ovarian metastases of digestive organ cancers with the accuracy of 71% (62/87).

We next examined the preoperative serum concentration of CEA, CA19-9, and CA125. Each was different among the groups (Figure 3b; CEA, $P<0.0001$; CA19-9, $P=0.0010$; and CA125, $P=0.0002$, Kruskal–Wallis test). Comparison of ovarian metastases of digestive organ cancers and

primary mucinous ovarian carcinomas (including borderline tumors) indicated that the former showed a higher serum CEA values than the latter (median 6.5 *vs* 1.3 ng/ml, $P<0.0001$). CA19-9 and CA125 values were not significantly different between samples.

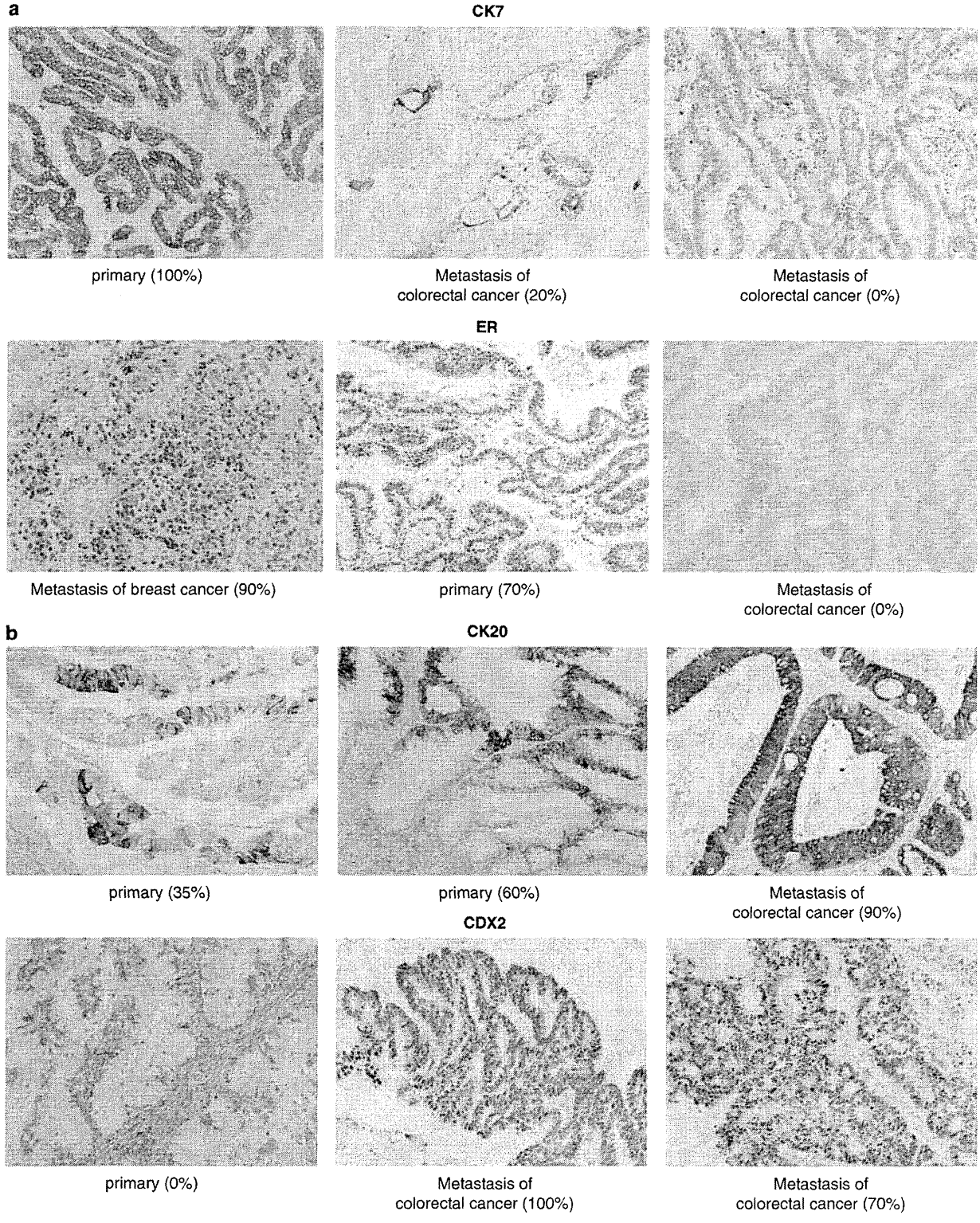

Figure 2 For caption see next page.

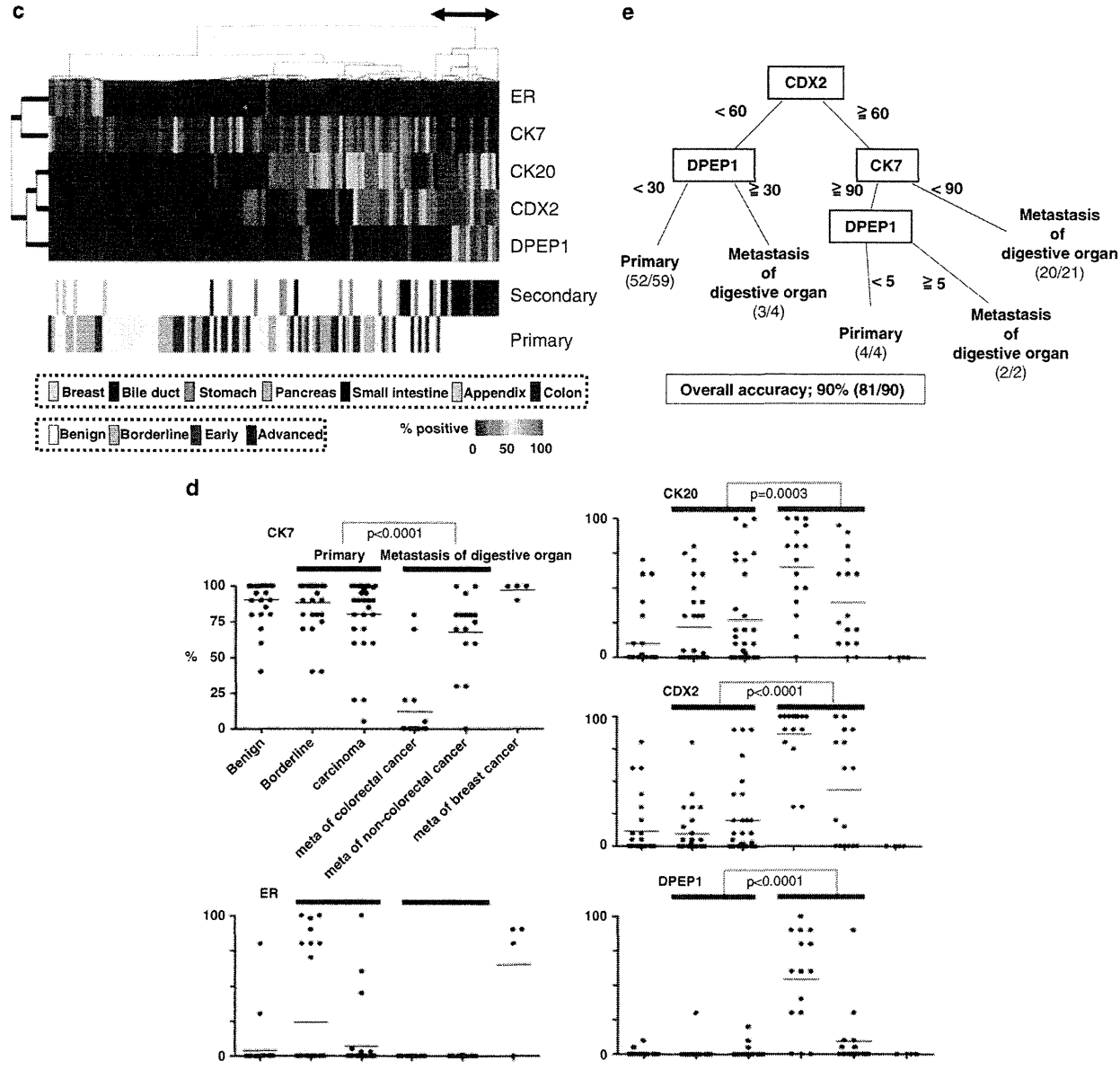

Figure 2 Analyses using multiple immunohistochemical markers. (**a**) Representative images of CK7, ER, CK20, and CDX2 immunostaining. (**b**) An average-linkage hierarchical clustering analysis by the percentage immunohistochemical positivity of the five molecules in the individual samples. The percentage positivity is shown by the blue–yellow–red heatmap. The double-headed arrow indicates a tumor cluster determined by scarce CK7 and abundant DPEP1, enriching ovarian metastases of colorectal cancers ($P<0.0001$, Fisher's exact test). Secondary; secondary ovarian tumors. The primary sites are indicated by the color bar. Primary; primary mucinous ovarian tumors. Benign tumor, borderline tumor, early-stage (stage I) carcinoma, and advanced-stage (stage III) carcinoma are indicated by the color bar. (**c**) The percentage positivity of the five molecules in the individual samples is shown on dot graphs. Ovarian metastasis of adenocarcinomas originating in bile duct, stomach, pancreas, small intestine, and appendix are designated as 'ovarian metastases of non-colorectal digestive organ cancers'. (**d**) Decision tree that best distinguishes primary mucinous ovarian carcinomas from the metastases of digestive organ cancers. Among the five molecules, CDX2, CK7. and DPEP1 were selected to draw the decision tree. 'Primary mucinous ovarian carcinomas (52/59)' indicates that 52 samples were primary mucinous ovarian carcinomas among the 59 samples classified. The numbers on the connecting lines indicate percent positivity. (**e**) Decision tree using immunohistochemical data for five proteins, among which three factors (CDX2, CK7 and DPEP) were selected to draw the decision trees.

Finally, we employed all factors analyzed in this study (immunohistochemical expression of five protein markers, three clinical features (Figure 3a), and three serum biomarkers (Figure 3b)) to draw a decision tree. Among the 11 factors, 4 (CDX2, CK7, DPEP1, and tumor size) were used to draw a tree that best discriminates between primary mucinous ovarian carcinomas and ovarian metastases of digestive organ cancers with the net accuracy of 93% (84/90; Figure 3c).

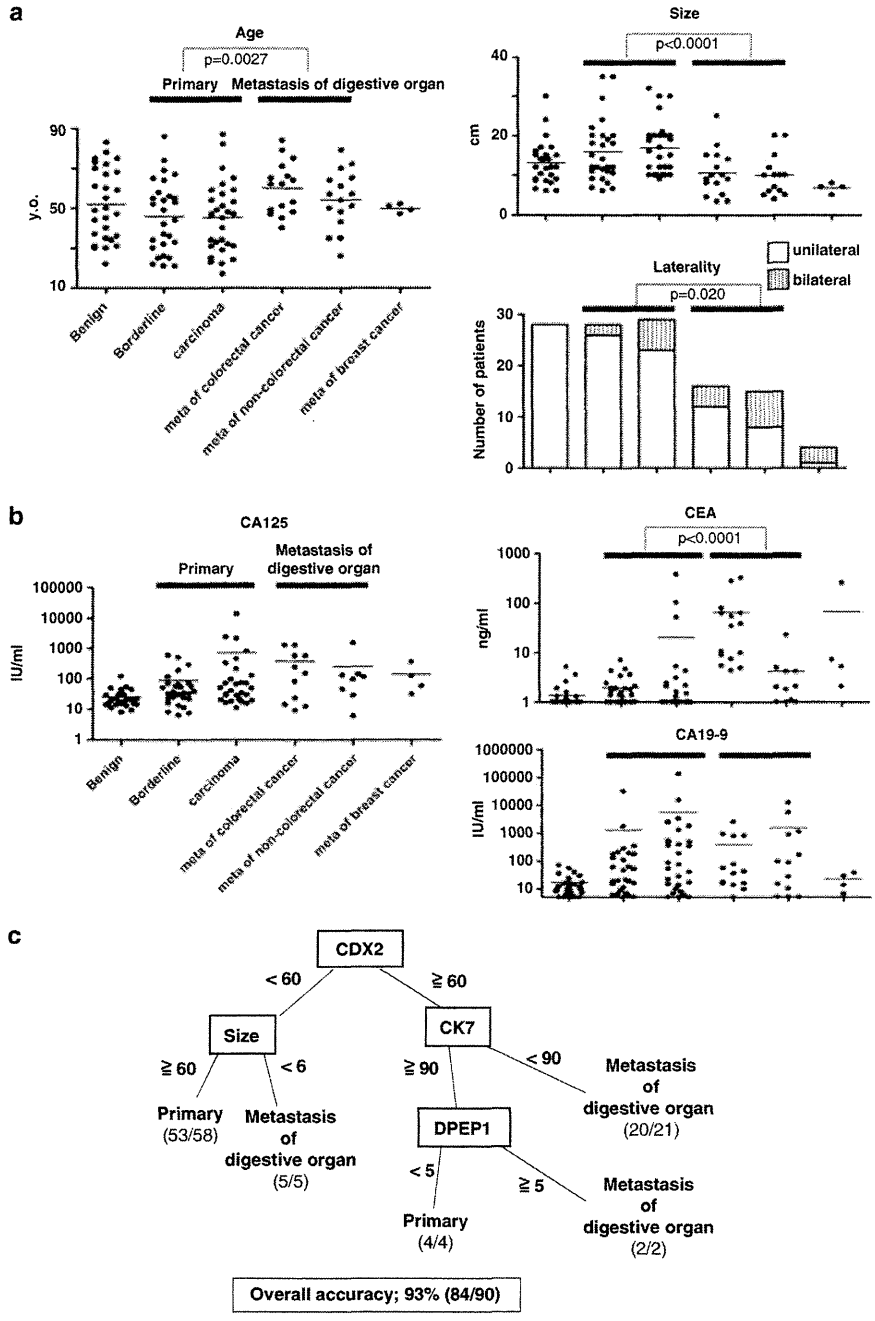

Figure 3 Analysis using preoperative clinical information and immunohistochemical markers. (**a**) Patient's age at the operation, tumor size, and tumor laterality are represented on dot plots. (**b**) Preoperative serum concentrations of CA125, CEA, and CA19-9 are represented on dot plots. (**c**) Decision tree using immunohistochemical data for five proteins and six preoperative factors. Among the 11 factors, 4 (CDX2, CK7, DPEP1, and tumor size) were selected to draw the decision tree.

Discussion

The distinction of primary mucinous ovarian cancers from ovarian metastasis of digestive organ cancers is often difficult. Features common among primary ovarian mucinous neoplasms include unilaterality, size >150 mm, smooth capsule, and lack of extraovarian spread. In addition, primary ovarian mucinous carcinomas are often accompanied by borderline mucinous tumors. Confluent glandular or expansile invasion is observed in many cases and destructive stromal invasion may also be detected. On the other hand, features common among metastatic mucinous adenocarcinomas of the ovary include bilaterality, small size, surface involvement, nodular involvement, and destructive

stromal invasion.[3,4,19,20] Unfortunately, in many cases, the diagnosis is not straightforward because many morphological features are shared by primary and secondary ovarian tumors.[7] The aim of this research was to develop a protocol to more accurately distinguish primary mucinous ovarian cancer from ovarian metastasis of digestive organ cancer, including colorectal and non-colorectal cancer.

We first sought a novel molecular marker of metastatic ovarian cancer using microarray analysis. A list of genes differentially expressed in epithelial ovarian cancers and colorectal cancers, one of the most common metastatic tumors to involve the ovary,[7] contained several genes encoding molecular markers known to distinguish primary from secondary ovarian neoplasms (Figure 1a, Supplementary Table 1). Thus, we expected that the list of differentially expressed genes might include novel immunohistochemical markers. Among them, we selected and investigated DPEP1 as a candidate marker because specific antibody was commercially available and its expression pattern in colorectal cancer is known.[17,18] DPEP1 is a zinc-dependent metallopeptidase that hydrolyzes a variety of dipeptides and is involved in glutathione metabolism.[21] As expected, positive DPEP1 expression was strongly associated with ovarian metastases of colorectal cancer (Figure 1c). Particularly, its high specificity (98%, 57/58, Figure 1c) to ovarian metastases of colorectal cancer suggests its clinical usefulness as the immunohistochemical marker.

Immunohistochemical expression of CK7, CK20, ER, and CDX2 has been used to distinguish primary from secondary ovarian cancers.[8,9,22] To evaluate the accuracy that the positivity of each marker predicts primary mucinous ovarian cancer *vs* ovarian metastasis of digestive organ cancer, we calculated the cutoff value by ROC curve. Each cutoff value of CK7, CK20, CDX2, and DPEP1 classified our cases in the accuracy of 76, 64, 83, and 81%, respectively (Supplementary Figure 1). As CK7 and CK20 stainings are most commonly used for the identification of primary and metastatic tumors, we also calculated the accuracy by the combination of CK7/CK20 staining, which classified primary mucinous ovarian carcinomas and ovarian metastases of digestive organ cancers with only 82% accuracy in our cases (Supplementary Figure 2). In order to compare DPEP1 with the commonly used markers and to develop an algorithm using multiple markers, we conducted a combined analysis. Hierarchical clustering has commonly been used to analyze DNA microarray data. More recently, studies of protein expression, including ours, have taken advantage of this method.[23,24] The hierarchical clustering detected the mutually exclusive pattern of CK7 and DPEP1 (Figure 2c). Therefore, a combination of CK7 and DPEP1 staining is expected to distinguish ovarian metastases of colorectal cancers from primary mucinous ovarian carcinomas very accurately. Contrary to the case of colorectal cancers, it is known that CK7, CK20, and CDX2 are not useful as markers to distinguish primary mucinous ovarian carcinomas from ovarian metastases of non-colorectal digestive organ cancers.[8,22] However, few reports have tested their utility in combination. Therefore, we conducted an analysis by combining multiple immunohistochemical markers. The Weka J48 software, which determines a decision tree that best discriminates between different groups using the minimum number of factors, selected CDX2, CK7, and DPEP1 to draw a decision tree (Figure 2e) with the net accuracy of 90% (81/90). Using this method, 97% (56/58) of the primary mucinous ovarian carcinomas and 100% (16/16) of the ovarian metastases of colorectal cancers were correctly discriminated. Among the ovarian metastases of non-colorectal digestive organ cancers, the accuracy was 56% (9/16).The fact that DPEP1 expression was included in the decision tree (Figure 2e) indicates that DPEP1 expression provides unique information that was useful to improve prediction.

Recently, clinical information has been re-evaluated to distinguish primary mucinous ovarian carcinoma from ovarian metastasis of digestive organ cancers. Seidman *et al*[3] classified primary and secondary ovarian neoplasms with 90% accuracy based on tumor laterality and size. Although smaller, bilateral tumors were significantly associated with ovarian metastasis of digestive organ cancers in our data set (Figure 3a), the algorithm of Seidman *et al*[3] classified primary and secondary tumors with an accuracy of only 71% in our cases. The modified size criteria of Yemelyanova *et al*[25] classified our cases with only 71% accuracy. In addition to tumor size and laterality, increased age (Figure 3a) and preoperative serum CEA (Figure 3b) were significantly associated with ovarian metastasis of digestive organ cancers, consistent with a previous report.[26] To develop a decision tree that more accurately distinguishes primary mucinous ovarian carcinomas from ovarian metastasis of digestive organ cancers, we next attempted to combine all the preoperative clinical information and immunohistochemical data. Of the six preoperative factors (Figure 3a and b), only 'tumor size' contributed to the decision tree (Figure 3c), indicating that immunohistochemical markers are necessary in problematic cases. Notably, DPEP1 was included in the decision tree, again verifying the importance of DPEP1 as the immunohistochemical marker. After all criteria were considered, CDX2, CK7, DPEP1, and tumor size were used to draw a tree that best discriminates primary mucinous ovarian carcinomas from ovarian metastasis of digestive organ cancers with a net accuracy of 93% (84/90; Figure 3c). We also assessed the accuracy of the Weka J48 tree model by a cross-validation. The accuracy was 83% when all the 11 factors (5 immunohistochemical and 6 clinical factors) were used. When the five immunohistochemical markers were used, the accuracy was 81%. When just CK7

and CK20 were used, the accuracy was only 70%. Then, when Seidman's factors (tumor size and laterality) were used, the accuracy was only 67%. Therefore, the cross-validation analysis of the tree model again indicated the accuracy of the prediction by using multiple factors.

In conclusion, using gene expression microarray analysis, we found a novel immunohistochemical marker, DPEP1, which specifically detects metastatic colorectal cancer samples. We also developed an algorithm to distinguish primary mucinous ovarian carcinomas from ovarian metastasis of digestive organ cancers using clinical information and immunohistochemical markers, including DPEP1. This study will be useful to improve the problematic diagnosis of mucinous ovarian cancers and ovarian metastasis from digestive organ cancers. Unfortunately, the accuracy with which primary mucinous ovarian carcinomas were distinguished from ovarian metastasis of digestive organ cancers was not 100%, even when all the factors were used. Especially, distinction of primary mucinous ovarian carcinomas from ovarian metastases of non-colorectal digestive organ cancers is clinically important. For this purpose, it is necessary to identify specific immunohistochemical markers distinguishing them. Gene expression microarray analysis, like that we used to identify DPEP1, should be useful to identify such markers.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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Postoperative Pulmonary Embolism Including Asymptomatic Cases in Gynecologic Oncology

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Introduction: So far, there has been no report addressing the actual rate of asymptomatic pulmonary embolism (PE). The present study was conducted to clarify the incidence and the characteristics of postoperative PE including asymptomatic cases in gynecologic oncology.

Methods: A total of 2107 gynecologic surgery cases that were performed from January 1996 to December 2006 at the National Kyushu Cancer Center were included. Pulmonary embolism was diagnosed using a lung scan, multi–detector row computed tomography, or pulmonary angiography. The clinical factors, including prophylaxes, were analyzed by univariate and multivariate analyses.

Results: PE was diagnosed in 45 patients (2.14%). Six (13.3%) of the 45 patients had respiratory symptoms or signs, and 16 patients (35.6%) had no symptoms or signs except for a SpO_2 level decrease. PE was diagnosed within 4 days after the surgery in 42 patients (93.3%). There were 1 massive, 2 recurrent, and no fatal PEs. A multivariate analysis demonstrated the incidence of PE to be associated with age (odds ratio, 1.957; 95% confidence interval, 1.497–2.559), operation time (1.664; 1.180–2.346), body mass index (2.457; 1.735–3.479), surgical position (2.253; 1.468–3.458), and the use of a perioperative intermittent pneumatic compression device (0.389; 0.229–0.659).

Conclusions: A substantial number of postoperative PEs were occult, and identification of high-risk patients and routine SpO_2 level monitoring would reduce the diagnostic delay of PE after gynecologic surgery. Increasing age, longer operation time, and obesity were risks. The use of a perioperative intermittent pneumatic compression device in multimodal conditions might thus prevent PE.

Key Words: Pulmonary embolism, Asymptomatic cases, Gynecologic surgery, Risk factors, Prophylaxis

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Most hospitalized patients have 1 or more risk factors for venous thromboembolism (VTE) such as surgery, immobility, malignancy, cancer therapy, previous VTE, increasing age, oral contraception or hormone replacement therapy, and obesity.[1–7] These risk factors are generally cumulative,[1,8] and many of these are present in patients undergoing gynecologic surgery. Although high-risk groups for VTE, predominately deep-vein thrombosis (DVT), can be identified, it is difficult to predict which individual patients in a given risk group will develop a clinically important thromboembolic event.[1] Recently, Laporte et al conducted a multivariate analysis of symptomatic VTE and reported the risk of fatal pulmonary embolism (PE) to symptomatic PE, especially massive PE, immobilization for neurological disease, age older than 75 years, and cancer in the study.[9] However, the

risk of fatal PE remains unclear in conditions including non-VTE cases and asymptomatic VTE cases. Roderick et al[10] referred to asymptomatic PE in their study, but there has been no report addressing the actual rate of asymptomatic cases and no investigation of postoperative PE including asymptomatic cases in gynecologic oncology.

Postoperative patients have been managed according to the guidelines for the prevention of DVT and PE since 2001 in this gynecology service. The guidelines are similar to those outlined in the American College of Obstetricians and Gynecologists practice bulletin,[11] which were modified from the study of the Seventh American College of Chest Physicians Conference on Antithrombotic and Thrombolytic Therapy.[1] In addition, pulse oximetry has been used to manage patients undergoing gynecologic surgery since the mid-1990s, and this can detect hypoxic events without exception. This has led to the early detection of postoperative PE. It is important to examine the factors associated with postoperative PE to predict the high-risk patients and do everything possible to reduce the potential occurrence of a massive or fatal PE. This study investigated the incidence and the characteristics of postoperative PE, including a considerable number of early-detected asymptomatic cases, and evaluated the clinical factors and the effectiveness of prophylactic measures using univariate and multivariate analyses.

PATIENTS AND METHODS

The surgical database was reviewed to identify the patients who had a surgery performed by the Gynecology Service of the National Kyushu Cancer Center from January 1996 to December 2006. A total of 2132 patients who underwent a laparotomy, a laparoscopic surgery, a vaginal surgery with a hysterectomy, or a vulvar cancer surgery were identified in this database. The patients who underwent conization and dilatation and curettage were not included, and there were no PE cases in this population. Twenty-five patients with nongynecologic operations, including a hysterectomy in association with colon cancer and uterine involvement, and patients younger than 15 years who underwent gynecologic operations were excluded from the 2132 patients, and there were no PE cases among them. A total of 2107 patients were examined in this study.

A schematic illustration of the diagnostic protocol for PE is presented in Figure 1. The SpO_2 levels of all the patients were routinely monitored by pulse oximetry during and after surgery until the patients achieved complete ambulation.

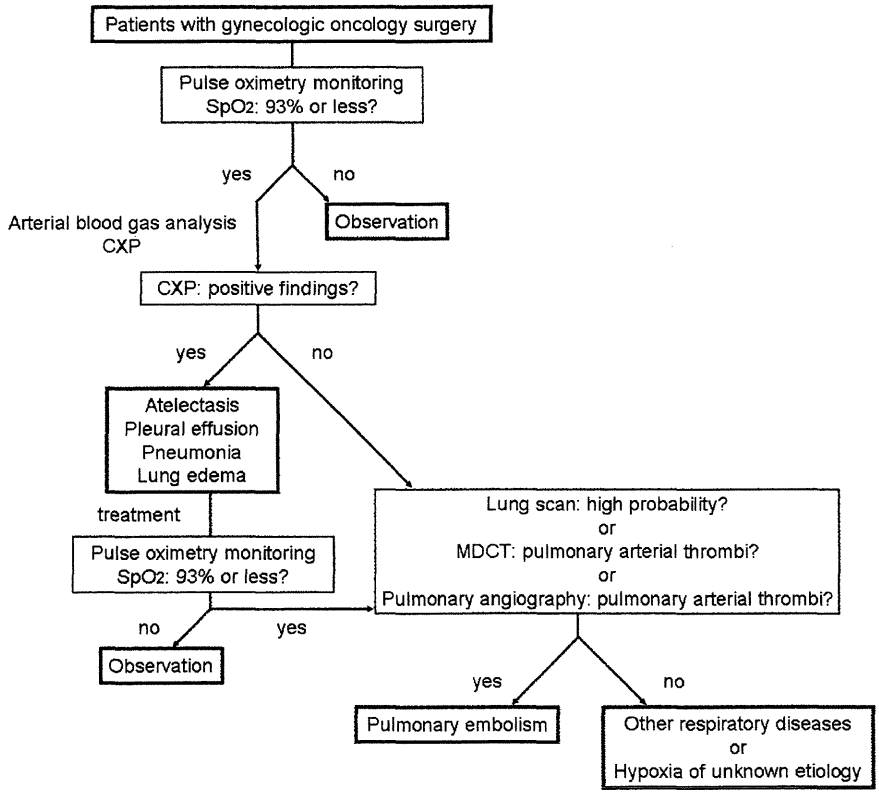

FIGURE 1. Schematic illustration of the diagnostic protocol for PE. When hypoxia with an SpO_2 level of 93% or lower under room air conditions was detected, further examinations were carried out. The diagnostic methods for PE were arterial blood gas analysis, CXP, lung perfusion scan, MDCT, and pulmonary angiography.

TABLE 1. Type of surgery

Type of Surgery	No. Cases
Laparotomy	1949
Vaginal surgery with hysterectomy	126
Laparoscopic surgery	26
Vulvar cancer surgery	6
Total	2107

Thereafter, pulse oximetry was performed once a day throughout the hospitalization. There was no unexplained dyspnea case without hypoxia during the study period. PE was confirmed by chest x-ray photography (CXP) and lung perfusion scan based on the criteria of the Prospective Investigation of Pulmonary Embolism Diagnosis.[12] Only high-probability cases on the Prospective Investigation of Pulmonary Embolism Diagnosis criteria were diagnosed to be PE. The cases where lung arterial thrombi were detected by multi–detector row computed tomography (MDCT) or pulmonary angiography were also diagnosed as PE.[13,14] All imaging studies were interpreted by the attending radiologists.

When patients with risks for DVT were recognized, the dimerized plasmin fragment (D-dimer) level was measured preoperatively. Either ultrasound tomography or venography on lower limb veins was performed in the patients with a higher D-dimer level range or with lower limb swelling. Upon detection of a DVT in the lower limb veins or pelvic veins, either an inferior vena cava (IVC) filter was inserted for PE prophylaxis or the operation was prolonged to treat the thrombi.

The mechanical prophylactic procedures involved the use of (1) graduated compression stockings (GCSs) that have been used since November 2000, (2) venous foot pumps (VFPs) that have been used since January 2001, (3) intermittent pneumatic compression (IPC) devices during the operation (intraoperative IPC) that have been used since June 2001, and (4) IPC devices through the operation to the postsurgical state (perioperative IPC) that have been used since June 2003. The GCSs have been used solely or combined with other prophylactic methods in most surgical cases. The perioperative IPC device was removed after the patient first walked, and most of the patients began to walk on the day after surgery.

Dalteparin has been used with these mechanical methods in most of the obese patients or the patients with malignant diseases since November 2000. The dalteparin dosage was 2500 units per day for 3 days after surgery, and in some cases, it was used from 12 hours before surgery. The dosage was increased to 5000 units per day when the body mass index (BMI) was 25 kg/m^2 or more. Dalteparin was used in those cases for up to 3 days after surgery with prophylactic use because of the cost. Dalteparin was not used in some of the cancer patients with relatively short operation time.

The preoperative complications or concomitant diseases were evaluated using a surgical database. The evaluated clinical factors were hypertension, diabetes mellitus (DM) including impaired glucose tolerance (IGT), DVT, pregnancy, varicose veins of the lower limbs, asthma, heart diseases, history of lung surgery, liver diseases, rheumatoid arthritis, and renal diseases.

Fisher exact test and the *t* test were used for the univariate analyses, and the stepwise logistic regression was used for the multivariate analysis. The analyses were performed using the SAS statistical software program version 9.0.

RESULTS

The surgical procedures performed on the 2107 patients are listed in Table 1. The laparoscopic patients were discharged within 4 days after the operations. Pulmonary embolism was diagnosed in the 45 patients (2.14%), and there were no PEs among the patients who underwent a vaginal hysterectomy, a vulvar cancer surgery, or a laparoscopic surgery.

Forty of the 45 PE cases were diagnosed by CXP and a lung perfusion scan; 3, by pulmonary angiography; and 2, by chest MDCT. The background characteristics of the PE and the non-PE patients are listed in Table 2.

There were 91 patients with suspicious cases who underwent a lung scan, a pulmonary angiography, or an MDCT. Among the cases with CXP and a lung perfusion scan, 40 (49.4%) of 81 cases were diagnosed as PE, and among the cases with MDCT, 2 (28.6%) of 7 cases were diagnosed as PE. The remaining 3 cases with hypoxia and symptoms were diagnosed as PE by pulmonary angiography. The causes of

TABLE 2. Background characteristics of the study population

Characteristics	With PE (n = 45), Median (Range)	Without PE (n = 2062), Median (Range)
Age, yr	61 (32–89)	50 (16–89)
Height, cm	153 (137.6–166.4)	155 (134–178)
Weight, kg	58 (40–89)	53 (31–110)
BMI, kg/m^2	25.2 (18.6–33.9)	21.9 (13.8–41.1)
Operation time, min	239 (50–500)	174 (25–680)
Blood loss, g	420 (10–2500)	210 (2–6640)
Malignant disease	34/45 (75.6%)	1234/2062 (59.8%)

TABLE 3. Causes of hypoxia in the patients without PE

Causes of Hypoxia	No. Patients (n = 46)
Atelectasis	20
Pleural effusion	4
Atelectasis and pleural effusion	4
Insufficient breathing due to sputum	3
Respiratory infection	2
Atelectasis and lung edema	2
Hypoxia of unknown etiology	11

hypoxia in the 46 cases without PE are listed in Table 3. Atelectasis was the main cause of hypoxia. All the hypoxic patients without PE recovered in 10 days. Some of these patients required treatment such as antibiotics for infection, postural drainage for atelectasis, and thoracocentesis for pleural effusion.

The mean (SD) SpO_2 level of the PE cases at diagnosis was 87.8% (6.2%) and the mean (SD) PO_2 level was 57.4 mm Hg (8.3 mm Hg). The symptoms and signs are listed in Table 4. Six (13.3%) of the 45 patients had respiratory findings, including 4 cases with dyspnea, 1 case with dyspnea and chest pain, and 1 case with hemoptysis. Some patients also had plural symptoms and signs such as pallor, perspiration, and fatigue. Sixteen patients (35.6%) had no symptoms and signs.

The interval between the operation and the diagnosis of PE is illustrated in Figure 2, and the interval between the appearance of symptoms or the detection of hypoxia and the diagnosis of PE is presented in Figure 3. Most of the PE cases were diagnosed soon after the detection of symptoms and signs or hypoxia.

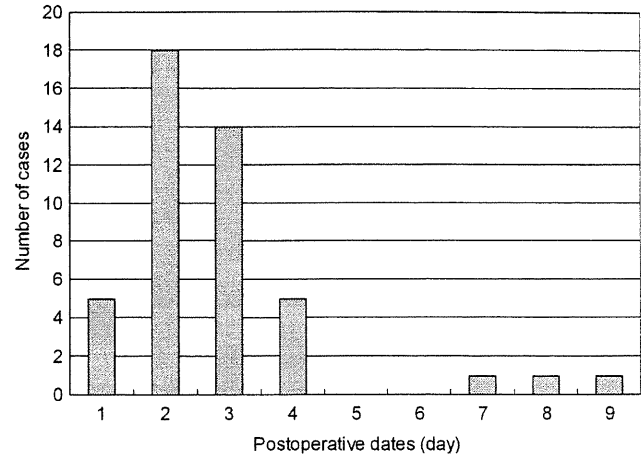

FIGURE 2. The interval between the operation and the diagnosis of PE is presented. Five of the 45 PE cases were diagnosed the day after surgery, and 42 (93.3%) of the 45 PE cases were diagnosed within the fourth day after surgery. All cases were diagnosed within 9 days after surgery.

The duration of SpO_2 measurement, which is equal to the length of hospitalization, was 10 to 246 days (median, 42 days) among the PE patients and 2 to 373 days (median, 24 days) among the patients without PE. In the study period in Japan, most of the cancer patients were not discharged from the hospital until at least 2 weeks after surgery.

Only 1 case was categorized as a massive PE,[15] with a systolic pressure lower than 90 mm Hg. This case was diagnosed as PE on the fourth postoperative day, and the patient was treated with anticoagulant medication. An IVC filter was inserted against the PE relapse. An abnormal electrocardiogram (ECG) finding, an inverted T wave, was also found in this case. Abnormal ECG findings were seen in 3

TABLE 4. Symptoms and signs of PE cases

Symptoms and Signs	Cases (n = 45)
None	16 (35.6%)
Symptoms and signs (+)	29 (64.4%)
Respiratory symptoms and signs	6*
Dyspnea	5
Hemoptysis	1
Chest pain	1
Others	23*
Pallor	8
Perspiration	7
Fatigue	7
Discomfort, not otherwise specified	6
Dizziness	6
Chill	2

*Some cases have multiple symptoms and signs.

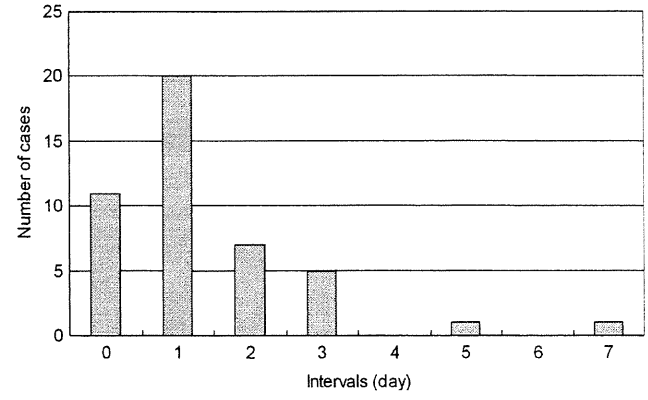

FIGURE 3. The interval between the appearance of symptoms or the detection of hypoxia and the diagnosis of PE is presented. Of the 45 patients with PE, 31 (68.9%) had conditions diagnosed as PE on either the day that symptoms appeared or hypoxia was detected or the next day. The longest interval was 7 days.

of the 45 cases. Other abnormal ECG findings included lower T waves.

One case was preoperatively diagnosed with DVT, and an IVC filter was inserted for PE prophylaxis. Postoperatively, there were 2 cases diagnosed as DVT among the PE population in which thrombi were detected in the pelvic vein and/or the lower limb vein. Among the patients without PE, there was 1 with symptomatic DVT. In that case, the lower limb was swollen without hypoxia and a lung scan was performed, but PE was not detected.

There were no patient deaths due to PE in this series. Among the PE cases, IVC filters were required for 4 patients. One of the 4 patients required an IVC filter because of increased perfusion defects on the lung scan, even after the anticoagulant regimen was started (the massive PE case). Two of them exhibited DVT and were thought to be high-risk for recurrent PE. The fourth patient received a temporary IVC filter because DVT was suspected, but the filter was soon removed when there was no venous thrombus detected on the lower limb venography.

The median follow-up period among the PE patients was 1571 days (range, 15–4446 days) and that of the patients without PE was 1212.5 days (range, 2–5132 days). There were 2 patients with recurrent PE cases that occurred 5 and 6 months after the first episodes. Both patients had been treated with warfarin potassium as secondary prophylaxis. However, one of them was treated for a relatively short period (approximately 2 months), and the other was treated with an ineffective dose of warfarin potassium. Both cases occurred during the adjuvant chemotherapy period, and they were successfully treated.

The incidence of PE was highest among ovarian cancer patients (3.1%), but the PE incidences were not significantly different among the reported gynecologic diseases (Table 5).

Of the 45 PE patients, 11 had benign diseases and 34 had malignant tumors. The PE incidence in the malignant diseases was significantly higher than that of benign diseases ($P = 0.0332$). Operations that took 3 hours or longer were performed on 31 of the 45 PE patients. The PE incidence for the operations of 3 hours or longer was significantly higher than that of operations shorter than 3 hours ($P = 0.0055$; Table 5).

Regarding the surgical position, PE occurred among 2.0% (36/1799) of the patients who underwent surgery under the gynecologic supine position, which meant supine astride; 5.1% (9/176) of the patients under the general surgical supine position, which was supine straightening of

TABLE 5. Univariate analyses of the patient characteristics

Characteristics	Total (n = 2107), n	Without PE (n = 2062), n (%)	With PE (n = 45), n (%)	*P*
Diseases				0.2593
Endometrial cancer	430	418 (97.2)	12 (2.8)	
Cervical cancer	453	443 (97.8)	10 (2.2)	
Ovarian cancer	322	312 (96.9)	10 (3.1)	
Other malignancies	66	64 (97.0)	2 (3.0)	
Benign diseases	836	825 (98.7)	11 (1.3)	
Malignant tumor				0.0332*
−	839	828 (98.7)	11 (1.3)	
+	1268	1234 (97.3)	34 (2.7)	
Operation time				0.0055*
<180 min	1087	1073 (98.7)	14 (1.3)	
≥180 min	1020	989 (97.0)	31 (3.0)	
Surgical position				0.0052*
Gynecologic supine position	1799	1763 (98.0)	36 (2.0)	
General surgical supine position	176	167 (94.9)	9 (5.1)	
Lithotomy position	132	132 (100.0)	0 (0.0)	
Lymphadenectomy				0.1602
−	1061	1043 (98.3)	18 (1.7)	
+	1046	1019 (97.4)	27 (2.6)	
Age, mean (SD), yr		50.5 (12.7)	60.6 (13.5)	<0.0001*
BMI, mean (SD), kg/m^2		22.4 (3.5)	25.4 (3.6)	<0.0001*
Blood loss, mean (SD), g		350.9 (438.8)	528.5 (522.5)	0.0827

Ovarian cancer includes ovarian cancer, borderline tumor, tubal cancer, and peritoneal cancer.
*Significant according to the univariate analysis ($P < 0.05$).

TABLE 6. Univariate analyses of the major medical complications and prophylaxis

Type of Complication and Prophylaxis		Total (n = 2107), n	Without PE (n = 2062), n (%)	With PE (n = 45), n (%)	*P*
Complication or concomitant disease					
Hypertension	−	1799	1769 (98.3)	30 (1.7)	0.0003*
	+	308	293 (95.1)	15 (4.9)	
DM including IGT	−	1997	1960 (98.2)	37 (1.8)	0.0001*
	+	110	102 (92.7)†	8 (7.3)‡	
Asthma	−	2064	2019 (97.8)	45 (2.2)	0.3277
	+	43	43 (100.0)	0 (0.0)	
Heart diseases	−	2067	2023 (97.9)	44 (2.1)	0.8722
	+	40	39 (97.5)	1 (2.5)	
Prophylaxis					
GCS	−	990	960 (97.0)	30 (3.0)	0.0075*
	+	1117	1102 (98.7)	15 (1.3)	
Antithrombotic drugs	−	1421	1388 (97.7)	33 (2.3)	0.3939
	+	686	674 (98.3)	12 (1.7)	
Intraoperative IPC device	−	1825	1787 (97.9)	38 (2.1)	0.6654
	+	282	275 (97.5)	7 (2.5)	
VFP	−	1945	1908 (98.1)	37 (1.9)	0.0102*
	+	162	154 (95.1)	8 (4.9)	
Perioperative IPC device	−	1432	1391 (97.1)	41 (2.9)	0.0008*
	+	675	671 (99.4)	4 (0.6)	

The perioperative IPC device was used during the operation to the postoperative state, and it was removed after the first walking.
*Significant according to a univariate analysis ($P < 0.05$).
†Ten of 102 cases were IGT.
‡Three of 8 cases were IGT.

the lower limbs; and none (0/132) of the patients in the lithotomy position in which the lower limbs were lifted up ($P = 0.0052$). The incidence of PE among the patients treated in the lithotomy position was significantly lower than the others (Table 5).

A lymphadenectomy was performed in approximately half of all the cases. The incidence of PE in the lymphadenectomy cases increased, but the difference was not statistically significant (Table 5).

The mean age and the mean BMI were significantly higher among the PE population than in the non-PE population ($P < 0.0001$ in both comparisons). The mean blood loss was larger among the PE population, but it was not significant (Table 5).

The main complications or concomitant diseases were hypertension, DM including IGT, asthma, and heart diseases. A univariate analysis showed that hypertension ($P = 0.0003$) and DM including IGT ($P = 0.0001$; Table 6) were significantly associated with PE. Among the DM including IGT cases, insulin was only used in one case with PE on the day of the operation.

The prophylaxis measures for postoperative PE were also analyzed, and the GCS and the perioperative IPC device significantly reduced the incidence of PE ($P = 0.0075$ and

TABLE 7. Multivariate analysis of the variables influencing the incidence of PE

Variables	Comparison Category	Reference	Odds Ratio	95% CI	*P*
Age, yr	By 10 years		1.957	1.497–2.559	<0.0001*
Operation time	≥180 min	<180 min	1.664	1.180–2.346	0.0037*
BMI	By 5 kg/m^2		2.457	1.735–3.479	<0.0001*
Surgical position	Other positions	Lithotomy position	2.253	1.468–3.458	0.0002*
Perioperative IPC device	+	−	0.389	0.229–0.659	0.0005*

*Significant at 0.05.

P = 0.0008, respectively). In contrast, VFP increased the PE incidence (P = 0.0102; Table 6). The incidence of PE was 23 (2.43%) of 946 cases from 1996 to 2000 when no prophylaxis was used. The incident rate has decreased to 0.5% or lower per year since the perioperative IPC device started to be used.

A univariate analysis showed that malignant tumors, operation time, surgical position, age, BMI, hypertension, DM including IGT, GCS use, VFP use, and perioperative IPC device use were associated with PE (Tables 5 and 6).

The 10 variables significantly associated with PE based on a univariate analysis were evaluated using a multivariate analysis. A multivariate analysis demonstrated that PE was associated with age in the derivation set (odds ratio [OR], 1.957; 95% confidence interval [CI], 1.497–2.559), operation time (OR, 1.664; 95% CI, 1.180–2.346), BMI (OR, 2.457; 95% CI, 1.735–3.479), surgical position (OR 2.253; 95% CI 1.468 to 3.458), and perioperative IPC device use (OR, 0.389; 95% CI, 0.229–0.659: Table 7).

DISCUSSION

The current concepts regarding the initial clinical features of VTE and PE are too narrow, and they also tend to be biased toward symptomatic cases. Pulmonary embolism may also be asymptomatic or associated with mild clinical symptoms, but when they become extensive, they produce a serious clinical manifestation as Roderick et al[10] suggested. The monitoring of SpO_2 level is considered to be useful for the detection of PE at an asymptomatic or a mild symptomatic stage. These unrecognized or occult PE were included in the current study.

Kucher et al[15] reported that the 90-day mortality rates were 52.4% and 14.7% of the patients with massive and the patients with nonmassive PE, respectively. There was no patient death caused by PE in the current series. A substantial number of the PE cases were detected during the asymptomatic state, and the general condition of the patients was good except for the one massive PE case. The detection of hypoxia before the symptoms occur might be a key to avoid a massive PE during the perioperative state.

Elliott et al[16] reported that acute PE was diagnosed in 17% of the patients more than 1 week after symptom onset and in 5% of the patients more than 3 weeks after symptom onset. In contrast, PE was diagnosed in 68.9% of the patients in the present series on either the day of or the day after either the onset of symptoms or hypoxia detection. The longest interval between the onset of symptoms or hypoxia detection and the PE diagnosis was 7 days. Carson et al[17] concluded that PE is an unusual cause of death when correctly diagnosed and treated. The mortality rate was 2.5% in their study.

Nijkeuter et al[18] found that a recurrent thromboembolic event presenting as a recurrent PE occurred in 2.1% of the patients with PE and was fatal in the majority (79%) of these patients, usually occurring in the first week of follow-up. There were 2 recurrent PE cases (4.4%) in the current study, but there was no recurrent case within 1 month after the first episode. Either a history of gynecologic surgery or immobilization due to such surgery was found to be a risk factor of DVT.[1] The effort of early ambulation of the PE patients might have led to the lack of recurrence of PE soon after the first episode, and no patient died.

It is difficult to predict which individuals will develop a clinically important thromboembolic event.[1] Martino et al[19] investigated the postoperative PE after gynecologic oncology surgery and identified a cancer diagnosis and an age of more than 60 years to be risk factors based on univariate logistic regressions. The current study found that increasing age, an operation time of 3 hours or longer, and obesity were identified to be risk factors of PE after gynecologic oncology surgery based on univariate and multivariate analyses. It might be helpful to identify the high-risk patients having gynecologic oncology surgery.

A malignant tumor is a risk factor of VTE,[1,6,7] and among symptomatic VTE, it is a risk factor of fatal PE.[9] Univariate logistic regressions reveal that it is also a risk factor of PE in gynecologic surgery.[19] Malignancy was a significant risk factor of PE according to a univariate analysis in the current study, but it was not significant based on a multivariate analysis. This was probably because of the high percentage of malignant diseases in the study population. Therefore, the presence of a malignant tumor is still a strong risk factor for PE.

Clarke-Pearson et al[20,21] reported that the IPC device significantly reduces the incidence of postoperative venous thrombosis when applied during surgery and for 5 days postoperatively, and that the IPC device, when used only in the perioperative period, seems to be of no benefit in reducing the incidence of postoperative VTE. In fact, 37 (82.2%) of 45 PE cases were diagnosed from 2 to 4 days after operation in the present study. However, a multivariate analysis showed that when the IPC device was used in the perioperative period, a significantly reduced PE was observed. The compression sleeves were removed after the patients first began to walk. Most of the patients began to walk on the day after surgery. Multiple prophylactic methods were included in this study, and the perioperative-type IPC device was used with GCS, and dalteparin was also used in most of the obese patients or the patients with malignant diseases. The present study focused on PE not DVT; however, according to the results of a multivariate analysis, the perioperative use of IPC device might also be effective in multimodal prophylactic conditions to reduce the incidence of postoperative DVT and PE. There were few symptomatic DVT cases in our series. It might imply that most of our PE cases originated from asymptomatic DVT.

The IPC device clearly reduces the incidence of postoperative DVT,[20] but no mechanical thromboprophylaxis option has been studied in a large-enough sample to determine if there is a reduction in the risk of death or PE[1,22] at least in the gynecologic field.[10] An additive effect to improve the prophylactic ability of postoperative PE has been demonstrated in patients who underwent cardiac surgery when the IPC device was used in combination with anticoagulant medication.[22,23] The current study sample was not very large, and the study was retrospective, but the results