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POST–HYSTERECTOMY OVARIAN CANCER : HISTOLOGICAL CHARACTERISTICS AND A PREDICTION OF OCCURRENCE OF OVARIAN CANCER BY IMMUNOHISTOLOGICAL INSPECTIONS FOR SPECIMENS IN INITIAL OPERATIONS

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Abstract

Background : The prognosis of ovarian cancer is generally poor. To approach the onset of ovarian cancer, we investigated clinical characteristics and performed pathohistological examinations of organ specimens from ovarian cancer patients who had previously received hysterectomy.

Materials and Methods : This study consisted of a clinical analysis of 192 ovarian cancer patients, of whom 22 had previously received a hysterectomy. Next, we chose three patients who had received hysterectomies for adenomyosis and myoma, and compared the expression of several markers (estrogen receptor, progesterone receptor, Bcl–2, p53 and Ki–67) in their uterine and ovarian specimens.

Results : The rate of endometrioid adenocarcinoma (40.9%, 9/22) in patients with ovarian cancers after hysterectomy was significantly higher than that in women without hysterectomy (13.5%, 23/170). Immunohistochemistry staining showed the expression of Bcl–2 in both uterine and ovarian specimens from a patient with endometrioid adenocarcinoma, but not from a patient with mucinous adenocarcinoma.

Conclusion : In this study, endometrioid adenocarcinoma developed most frequently in patients who had previously undergone a hysterectomy. The onset of ovarian cancer could be predicted in the future by assessing specific marker levels.

Key words : endometrioid ovarian cancer, prior hysterectomy, adenomyosis

Introduction

Because most ovarian cancers are asymptomatic in their early stages, patients with ovarian cancer often present with advanced disease that is classified as clinical stage III or IV. In addition, early diagnostic methods for ovarian cancer are not as well established as they are for cervical cancer and endometrial cancer. Thus, the prognosis of ovarian cancer is often miserable, providing motivation to investigate the emergence and progression of this disease.

The Ministry of Health, Labour and Welfare of Japan[1)] announced that in Japan, (1) the ovarian cancer morbidity in 2000 was 11.5 for a population of 100,000, compared to

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12.5 for cervical cancer ; (2) as many as 8,000 women are diagnosed with ovarian cancer annually ; and (3) 4,400 patients will die from ovarian cancer every year. Moreover, ovarian cancer is expected to become more prevalent as the Japanese population ages in the near future. The recent introduction of paclitaxel into the chemotherapy regimen for ovarian cancer has improved the 5-year survival rate for all clinical stages and decreased the mortality[2]. However, the 5- and 10-year survival rates for patients with advanced-stage disease (III, IV) are 30% and 10%, respectively[3,4]. Generally speaking, the response rate to chemotherapy has improved, but treatment outcomes cannot yet reach satisfactory levels. Thus, there is an urgent need to establish more promising therapies as well as effective and inexpensive methods for mass screening and prophylaxis.

In this study, we performed a retrospective study of ovarian cancers that occurred sporadically after hysterectomies. First, we checked clinical characteristics of 192 ovarian cancer patients, and separated them to two groups, that is, 22 patients who had had a history of hysterectomy and 170 patients who had not. Next, we tried to find some hints for investigating the onset of ovarian cancer.

Furthermore, we examined whether there were differences in the distribution of histologic types between patients who had previously received a hysterectomy and those who had not. Finally, we used immunohistochemistry (IHC) to stain for several markers that can predict the onset of ovarian cancer, comparing ovarian specimens obtained during ovarian cancer surgery with uterine specimens obtained during hysterectomy.

Materials and Methods

Patients and tumor classification

This study enrolled a total of 192 patients who received surgical therapy for ovarian cancer at Akita University School of Medicine over a 14-year period from January 1995 to December 2008. The 192 cases were classified with the following surgical stages based on the system of the International Federation of Gynecology and Obstetrics (*Fédération Internationale de Gynécologie et d'Obstétrique* FIGO). Among the 192 patients, we found 22 cases who had received hysterectomies for uterine myomas and other diseases.

Next, we determined the age at which the above-mentioned 22 patients had received a hysterectomy, and the age at which ovarian cancer was diagnosed. In addition, we evaluated clinical stages of these 22 patients, and examined whether there were differences in the histological types of ovarian cancers between patients who had undergone a hysterectomy and those who had not.

Immunohistochemical analysis

IHC staining for ER, PR, Bcl-2, p53 and Ki-67 was performed on fixed, paraffin-embedded tissue blocks. We used specific antibodies from following two companies (ER, PR, Bcl-2 and p53 ; VENTANA medical systems, Inc., Tuscon, Arizona, USA/Ki-67 ; DAKO Japan, Inc., Iidabashi, Tokyo, Japan)

All IHC blocks were assessed by a single pathologist. Sections (3-4 μm thick) were deparaffinized in xylene, rehydrated through a graded ethanol series into distilled water, and then air-dried. Antigen retrievals were performed as follows. ER, PR ; the slides were incubated in a pressure cooker for 20 minutes. Bcl-2, p53 ; the slides were incubated in 10 mM citrate buffer (pH 3.0) for 15 minutes in a 95-96°C water bath, and were cooled to room temperature for 20 minutes. Ki-67 ; the slides were processed by microwaving to 20 minutes, at 5-minute intervals.

After antigen retrievals, the slides were washed in buffer. Automated IHC staining was performed using BENCHMARK XT immunostainers (VENTANA medical systems). The IHC slides processed by BENCHMARK XT immunostainers were examined using light microscopy. Immunostaining was determined based on a report by Allred *et al.*[5] In Allred score of IHC staining, first, the rate of positive cells (RPC) is counted, and the result is classified as following proportion score (PS). RPC＝0% ; PS＝0, 0%＜RPC＜1% ; PS＝1, 1%≦RPC＜10% ; PS＝2, 10%≦RPC＜1/3 ; PS＝3, 1/3≦RPC＜2/3 ; PS＝4, 2 / 3≦RPC ; PS＝5. And, next, the degree of staining is graded as following intensity score (IS). No staining reaction ; IS＝0, weak staining reaction (weakly positive) ; IS＝1, moderate staining reaction (moderately positive) ; IS＝2, strong staining reaction

(strongly positive) ; IS=3. Finally, the total score (TS) is determined by adding PS and IS, and TS≧3 means a positive result from this procedure. In addition, these phrases (weakly, moderately, and strongly positive) are defined about the intensity of staining and not terms for the TS (the results of Allred score). For briefness and tidiness, we put only proportion scores in the Table 8.

Statistics

Results are expressed as means ± SD. Frequencies between groups were compared using the χ^2-test and Yates correction. Values of $p<0.05$ were considered statistically significant. Statistical analyses were performed using StatView software (SAS Institute, Inc., Cary, North Carolina, USA).

Results

The total of 192 patients were classified by FIGO surgical staging, and the result was as follows. I : 76 (39.6%), II : 13 (6.8%), III : 84 (43.7%), and IV : 19 (9.9%) (Table 1-A). Based on histological analyses, the percentage of epithelial ovarian cancer was 95.8% (184/192). The various histologic types of ovarian cancer included 70 serous (36.4%), 50 clear cell (26.0%), 32 mucinous (16.7%), 32 endometrioid (16.7%), and 8 non-epithelial (4.2%) (Table 1-B). Twenty-two of the patients had undergone hysterectomies without unilateral or bilateral oophorectomies. Patients received hysterectomies for the following diseases : uterine myoma (12 cases), uterine myoma and adenomyosis (4 cases), uterine myoma and ovarian cyst (3 cases), uterine prolapse (1 case), and cervical intraepithelial neoplasia (CIN) 3 (2 cases) (Table 2). This study did not record the family history of ovarian or breast cancers or perform genetic tests for BRCA1 and BRCA2 mutations.

Among the 192 patients, 22 (11.4%) underwent hysterectomies without unilateral or bilateral oophorectomies before they were diagnosed with ovarian cancer.

The patient age distribution at the time of hysterectomy and at the diagnosis of ovarian cancer is shown in Ta-

Table 1. Clinicopathologic characteristics of ovarian cancer diagnosed in 192 patients

A.

FIGO stage (1988)	*n*	%
I	76	39.6
II	13	6.8
III	84	43.7
IV	19	9.9
Total	192	100.0

B.

Histologic type	*n*	%
serous	70	36.4
clear cell	50	26.0
mucinous	32	16.7
endometrioid	32	16.7
Non-epithelial	8	4.2
Total	192	100.0

A, classification of FIGO stages, with numbers and percentages of patients diagnosed with each stage. B, classification of histologic types, with numbers and percentages of patients demonstrating each type.

Table 2. Gynecologic disease at the time of hysterectomy in 22 patients with ovarian cancer

Uterine myoma	12
Uterine myoma & adenomyosis	4
Uterine myoma & ovarian cyst	3
Uterine prolapse	1
Carcinoma in situ	2
Total	22

Table 3. Age distributions at hysterectomy and at diagnosis of ovarian cancer in patients with prior hysterectomy

age	prior hysterectomy	diagnosis of ovarian cancer
-34	2	0
35-39	7	1
40-44	4	2
45-49	7	0
50-54	1	4
55-59	0	7
60-64	0	2
65-69	1	3
70-	0	3
Total	22	22

ble 3. The mean age at the time of ovarian cancer diagnosis was not statistically different between the patients with a previous hysterectomy and those without (57.5 ± 9.7 years for 22 cases with a hysterectomy vs. 53.6 ± 12.3 years in 170 cases without a hysterectomy). The average age at the time of hysterectomy was 43.6 ± 7.3 years, and the ages ranged from 32 to 66 years (Table 4). The time interval from the hysterectomy to the diagnosis of ovarian cancer ranged from 2 to 28 years, with a mean interval of 14.9 ± 8.3 years (Table 4). And, two women developed ovarian cancer two years after their hysterectomy, and one was diagnosed five years later (data not shown). Among women who had previously received a hysterectomy, 13 (59.1%), 0 (0%), 7 (31.8%), and 2 (9.1%) of the ovarian cancers were identified as stages I, II, III and IV, respectively (Table 5). In the 22 patients with ovarian cancer, the histologic types included six (27.3%) serous, three (13.6%) clear cell, four (18.2%) mucinous, and nine (40.9%) endometrioid (Table 6). The frequency of endometrioid adenocarcinoma in patients who had previously undergone a hysterectomy (40.9%, 9/22) was significantly higher than that in women who had not (13.5%, 23/170) (p-value=0.0012, odds ratio=4.42, confidence interval : 1.73-11.28 ; Table 6).

Table 5. Ovarian cancer stage (FIGO 1988) in 22 patients with a history of hysterectomy

Stage	*n* (%)
I ·····	13 (59.1) (Ia ; 7, Ib ; 3, Ic ; 3)
II ·····	0 (0.0)
III ·····	7 (31.8) (IIIa ; 0, IIIb ; 2, IIIc ; 5)
IV ·····	2 (9.1)
Total	22 (100.0)

In addition to the above results, we tried to discover some materials common to both benign uterine lesions and ovarian malignancies by IHC staining, and considered the possibility of making use of such findings as indicators of the emergence of malignancy. After we searched for the patients whose both uterine and ovarian specimens were secured, we could find barely three cases with the support of many medical staffs. Specifically, for these three patients, we assessed the expression of estrogen receptor (ER), progesterone receptor (PR), Bcl-2, p53 and Ki-67 in uterine specimens obtained at the time

Table 4. Mean ages at hysterectomy and at diagnosis of ovarian cancer

Mean age at prior hysterectomy	43.6 ± 7.3	(range ; 32-66)
Mean age at diagnosis of ovarian cancer	57.5 ± 9.7*	(range ; 38-72)
Mean years from prior hysterectomy to diagnosis of ovarian cancer	14.9 ± 8.3	(range ; 2-28)
	(mean ± SD)	

*Mean age at diagnosis of 170 patients with ovarian cancer who did not receive prior hysterectomy was 53.6±12.3. As stated in the text, no statistically significant difference was found between these two groups.

Table 6. Distribution of histologic types in patients with and without prior hysterectomy

Histologic type	Patients with prior hysterectomy *n* (%)	Patients without prior hysterectomy *n* (%)	*P* value
serous	6 (27.3)	64 (37.6)	n.s.
clear cell	3 (13.6)	47 (27.6)	n.s.
mucinous	4 (18.2)	28 (16.5)	n.s.
endometrioid	9 (40.9)	23 (13.5)	0.0012
others	0 (0.0)	8 (4.7)	n.s.
Total	22 (100.0)	170 (100.0)	

Odds ratio=4.42, 95% confidence interval : 1.73-11.28

Table 7. Characteristics patients whose specimens were examined by IHC staining

Patient	FIGO stage	Histologic type	Prior hysterectomy (age)	Ov.ca. diagnosis (age)	Interval (year)
①	I	Mucinous	46	56	10
②	I	endometrioid	42	52	10
③	I	endometrioid	47	56	9

of the hysterectomy as well as ovarian specimens acquired during the operation for ovarian cancer (Table 8). In addition, we showed clinical characteristics of three patients in Table 7.

For the IHC analysis of ER and PR, we obtained results that ranged from moderately positive (30%) to strongly positive (99%) in both the uterine adenomyosis and ovarian cancer specimens in all three patients. From this result, we could not find any distinctive features. For Bcl-2, the patient (①) with mucinous adenocarcinoma had negative results for both uterine and ovarian specimens. In the patient(②) with endometrioid adenocarcinoma, Bcl-2 was negative for the uterus, and weakly positive (10%) for the ovary. The last patient (③) had positive results for both uterus (90%) and ovary (70%) (Table 8, Fig. 1). For p53, only one ovarian specimen from one patient (③) was weakly positive (10%) (Table 8, Fig. 2). For Ki-67, if anything, the percentages

Table 8. Results of IHC staining

Pt.	Site	ER	PR	Bcl-2	p53	Ki-67
①	Ut. adenomyosis	30% (+)	90% (+)	(−)	(−)	(−)
	ov.ca. (Mucinous)	99% (+)	70% (+)	(−)	(−)	10% (+)
②	Ut. adenomyosis	90% (+)	90% (+)	(−)	(−)	(−)
	ov.ca. (Endometrioid)	99% (+)	99% (+)	10% (+)	(−)	20% (+)
③	Ut. adenomyosis	99% (+)	99% (+)	90% (+)	(−)	5% (+)
	ov.ca. (Endometrioid)	50% (+)	50% (+)	70% (+)	10% (+)	8% (+)

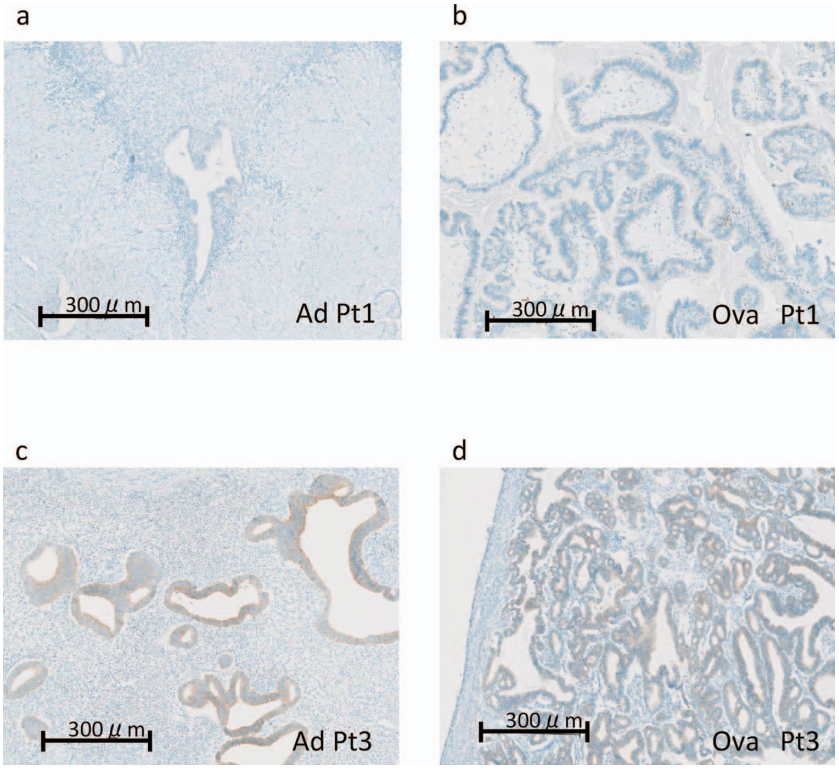

Fig. 1. The light microscopic images showing immunohistochemical (IHC) staining for Bcl-2. Specimens were obtained from two patients with adenomyosis and ovarian cancer. a, IHC staining of uterine adenomyosis sample from patient ①. b, IHC staining of mucinous ovarian cancer sample from patient ①. c, IHC staining of uterine adenomyosis sample from patient ③. d, IHC staining of endometrioid ovarian cancer sample from patient ③. All photos were taken by a microscope with a magnifying power of 100x.

of positive cells in ovarian specimens were higher than that in uterine specimens for all three patients (Table 8).

Discussion

In the present study, we determined that 11.4% (22/192) of women with ovarian cancer had previously received a hysterectomy without a unilateral or bilateral salpingo-oophorectomy, which is similar to recent reports (7.6-14.1%)[6-8]. As shown in Table 3, two individuals underwent hysterectomies at less than 35 years old, seven were between 35 and 39 years old, four were between 40 and 44 years old, and nine were at least 45 years old.

As mentioned in the Materials and Methods, only a few patients underwent a hysterectomy for endometriosis. On the other hand, 9 of the 22 women who underwent a hysterectomy developed endometrioid adenocarcinoma, which is not an insignificant rate. These findings differ slightly from the conventional theory proposed by Sampson and other investigators, which suggests that endometriosis lesions may predispose individuals to endometrioid adenocarcinoma[10-12]. However, we cannot clearly explain why many patients in this study were classified as having endometrioid adenocarcinoma, whereas few women actually underwent a hysterectomy for endometriosis. It is possible that a thorough macroscopic or pathologic examination may detect endometriosis lesions in women who undergo a hysterectomy for pelvic diseases, especially uterine myoma.

We next considered as an index of malignancy the expression of markers that exist on both the uterus and ovary. To determine which biomarkers can warn of the

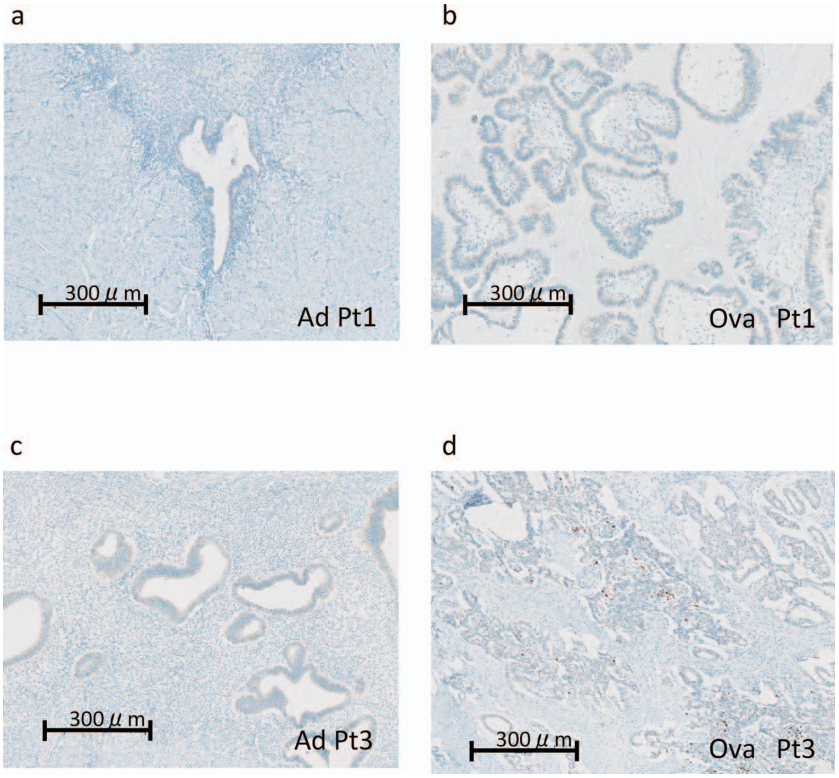

Fig. 2. Light microscopic images showing immunohistochemical (IHC) staining for p53. Specimens were obtained from two patients with adenomyosis and ovarian cancer. a, IHC staining of uterine adenomyosis sample from patient ①. b, IHC staining of mucinous ovarian cancer sample from patient ①. c, IHC staining of uterine adenomyosis sample from patient ③. d, IHC staining of endometrioid ovarian cancer sample from patient ③. All the photos were taken by a microscope with a magnifying power of 100x.

emergence or existence of ovarian cancer, we performed IHC staining on uterine and ovarian specimens from three patients with both ovarian cancer and a history of hysterectomy. The clinical characteristics of the three subjects are presented in Table 7. Specifically, we measured the incidence of five biomarkers (ER, PR, Bcl-2, p53 and Ki-67), with the results shown in Table 8.

According to a report by Del Carmen, the incidence of ER expression in benign atypical ovarian endometriosis was high (100%), but low (23.5%) in endometriosis-associated ovarian cancer[13]. And, the result of PR in Del Carmen's study had a tendency similar to that of ER. This may suggest that as endometriosis progresses, the incidence of ER and PR decrease, and uncontrolled cellular proliferation and metastatic disease increase. In our study, we could meet with such a result that the incidence of ER on benign lesions (uterine adenomyosis) is higher than that on malignancy(ovarian cancer), only in the patient (③). And, in our result of PR expressions, two cases (①, ③) were suited to this tendency, and one cases (②) had a reverse result. As a result, only the patient (③) had a similar staining to that of Del Carmen's study. This may be associated with the fact that the expressions of Bcl-2 on both uterine and ovarian specimens were detected only in the patient (③).

The incidences of Ki-67 in the report by Del Carmen were 37.5% for ovarian endometriosis and 70.5% for ovarian cancer. And, in another report by Ogawa, the data were 9.9% for endometriosis and 23.1% for ovarian cancer[14]. These results on Ki-67 would confirm the hypothesis that the expression of Ki-67 gradually increases, as endometriosis develops into atypical endometriosis

and endometriosis-associated ovarian cancer. In our study, the expression of Ki-67 in uterine adenomyosis samples in all three patients was lower than that in samples of ovarian cancer (Table 8). From this result of Ki-67, we can suggest at least that the capacity of cellular proliferation is higher in ovarian cancer than in adenomyosis. But, it is difficult to regard this fact as an evidence of the sequence from endometriosis to ovarian cancer.

According to a report by Nezhat, the incidences of Bcl-2 and p53 in benign ovarian endometriosis samples were 23% and 0%, respectively, and 67% and 42%, respectively, in endometrioid adenocarcinoma samples[15]. These findings could indicate that there is an association between alterations in Bcl-2 or p53 expression and the progression from an endometriotic ovarian cyst to endometrioid adenocarcinoma. In our study, one patient (③) showed positive expression of Bcl-2 in both uterine adenomyosis and ovarian carcinoma, but the patient (①) with mucinous ovarian cancer had negative results on both organs (Table 8, Fig. 1). Interestingly, only this patient (③) had the same result on the expressions of ER and PR as that of Del Carmen's study. This may suggest that the decreases of ER and PR during the transformation from endometriosis to endometrioid cancer is associated with the decrease of Bcl-2 during the same period. And, we believe in the possibility of Bcl-2 to become one of efficient detectors for endometrioid ovarian cancers with the progression of relevant researches. But, of course, we have to continue our studies, to prove this suggestion to be true, because it is only an example from one patient. For p53, in one patient of endometrioid adenocarcinoma (③), the expression of p53 was detected on only ovarian cancer specimens (Table 8, Fig. 2). But there was no case that had the positive results in both uterine and ovarian specimens, that is, before and after the onset of ovarian cancer.

In summary, IHC staining revealed no definite association between uterine adenomyosis and ovarian cancer. However, we did detect Bcl-2 expression in both uterine and ovarian specimens from a patient (Table 8, Fig. 1). This suggests the possibility that some biomarkers could be established as risk-of-malignancy indexes in future, while we extend the research of materials such as Bcl-2 that exist both before and after the onset of ovarian cancer.

Thus, we suggest that not only intraovarian but also extraovarian endometriosis might be risk factors for endometrioid adenocarcinoma. This hypothesis is supported by the fact that the percentage of endometrioid cancers in women who had previously undergone a hysterectomy was the highest among various histologic types, and significantly higher than that in women who had not previously received a hysterectomy (Table 6).

Finally, we should continue to extend our investigations of potential risk-of-malignancy indexes, and use them for early and exact diagnoses and treatments of ovarian carcinomas that occur following hysterectomies. And, also it is necessary to perform current screening tests such as transvaginal sonography and serum level of CA125 as frequently as possible, especially for the patients with the risk of ovarian cancers that could happen after hysterectomy.

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