in patients with vulvar cancer [4,5] and to a lesser extent with uterine cervical cancer [6]. Phase III studies of sentinel node detection in vulvar cancer are currently in progress in the USA and in Europe. However, only three reports [7–9] of sentinel node detection utilizing blue dye in endometrial carcinoma have been published, and these studies did not establish the feasibility of the method for these cases.

The aim of the present study is to examine the feasibility of sentinel lymph node detection using preoperative lymphoscintigraphy and an intraoperative gamma probe for patients with endometrial cancer with the goal of avoiding unnecessary lymph node resection.

Materials and methods

Patients

Between June 2001 and January 2003, consecutive patients undergoing laparotomy for endometrial cancer at Tohoku University Hospital were enrolled in this study. The study design was explained to the patients, and only patients who provided written informed consent participated in the study. However, excluded were patients with obvious cervical invasion and obvious extrauterine spread at their preoperative evaluation with MRI, CT, and transvaginal ultrasonography. The lymph node spread pattern from the uterine cervix was taken into consideration in this evaluation. The patients were scheduled for total abdominal hysterectomy, bilateral salpingo-oophorectomy, total pelvic lymphadenectomy, and paraaortic lymphadenectomy to the level of renal veins.

Lymphoscintigraphy with 99m-Technetium

On the day before surgery, the patient was carried to the clinic of Nuclear Medicine and Radiology Department, and preoperative lymphoscintigraphy was performed by injection into the endometrium of 2.0 ml of fluid containing 38–70 MBq 99m-Technetium (^{99m}Tc)-labeled phytate (DRL, Tokyo, Japan) dissolved with patent blue as the followings. In this study, we used blue dye not to detect sentinel nodes, but to ensure the injection under the endometrium without leakage. After the uterine cervix was dilated with laminaria for 15 h, a hysteroscope, 5.5 mm in diameter, was inserted into the uterine cavity with physiological saline. Tumors in every patient were observed through the hysteroscopy. We only judged cases with apparently focal tumorous lesion as focal. These cases could be reconfirmed by postoperative histopathological examination. Visually directed injection of [^{99m}Tc] phytate with blue dye under the hysteroscopic observation was performed with a 21-gauge needle at four sites under the endometrium around the tumor. During the procedure, no anesthesia was requested. For patients with multiple or diffuse tumors in the uterine cavity, ^{99m}Tc-radiocolloid was injected into the following five sites: the fundus, the right mid-lateral wall, the left mid-lateral wall, and the mid-anterior or mid-posterior wall. Dynamic lymphoscintigraphy was performed, and hot spots, indicating sentinel lymph nodes, were identified within 10 min in most cases. The first lymphoscintigram was taken at this time, and the second lymphoscintigram was taken the next morning just before the patient entered the operating room. Phytate is 200–1000 nm in diameter and half-life of ^{99m}Tc is short at 6 h. Total radioactivity used for one person was 2 mCi, which is much less than used for the standard bone scintigram at 20–50 mCi. Even if one operator performed the present procedure of sentinel node detection 500 times, the operator's total exposure would be much less than 50 mSv per year, which is the maximum allowable exposure per year as proposed by International Commission on Radiological Protection (ICRP) 1977. The irrigation fluid used through the hysteroscopic procedure was collected in the clinic and disposed of according to the laws for the disposal of radioactive waste in Japan.

Intraoperative lymphatic mapping and sentinel lymph node identification

Before starting lymphadenectomy, the radioactive lymph node was located by using a gamma-detecting probe (Navigator GPS, RMD: Watertown, MA). After lymphadenectomy, the area of lymphadenectomy was surveyed with the probe to confirm that no radioactive tissue remained. When the gamma-detecting probe registered counts over 10-fold above background radiation levels, the node was considered radioactive. All radioactive nodes were considered sentinel lymph nodes. All surgically removed lymph nodes were reexamined with the gamma-detecting probe ex vivo.

Pathology

All surgically removed lymph nodes, including the sentinel lymph nodes, were examined histopathologically using routine hematoxylin and eosin (H&E) staining. At least one section from each lymph node divided at the maximal diameter was reviewed by two independent pathologists. Lymph nodes that were diagnosed as negative for metastasis by routine H&E staining were immunostained with an anti-cytokeratin antibody (MNF116, DAKO, Japan) to detect cytokeratin, which is characteristic of micrometastatic cancer cells.

Results

Patient characteristics

The characteristics of 28 patients enrolled on the study are summarized in Table 1. Patient ages ranged from 29

Table 1
Patient characteristics

Case	Age	Stage	Histology	Myometrial invasion	Tumor distribution	Washing cytology
1	57	IIIC	G1	>1/2	not focal	–
2	57	IA	s/p	<1/2	focal	–
3	60	IB	G2	<1/2	focal	–
4	49	IIIA	G1	<1/2	not focal	+
5	65	IC	G1	>1/2	not focal	–
6	71	IA	G1	<1/2	focal	–
7	30	IA	G1	<1/2	focal	–
8	37	IIB	G1	<1/2	focal	–
9	53	IA	G1	<1/2	focal	–
10	58	IB	G2	<1/2	not focal	–
11	63	IIIC	G3	>1/2	not focal	+
12	59	IC	G1	>1/2	not focal	–
13	48	IC	G1	>1/2	not focal	–
14	52	IB	G2	<1/2	focal	–
15	29	IB	G1	<1/2	focal	–
16	48	IB	G1	<1/2	not focal	–
17	67	IIA	G2	<1/2	focal	–
18	53	IB	G1	<1/2	focal	–
19	50	IIA	G1	<1/2	not focal	–
20	49	IB	G1	<1/2	focal	–
21	47	IA	G1	<1/2	not focal	–
22	70	IA	s/p	<1/2	focal	–
23	59	IB	G1	<1/2	focal	–
24	49	IA	G1	<1/2	focal	–
25	56	IB	G1	<1/2	focal	–
26	63	IC	G1	>1/2	not focal	–
27	60	IB	G1	<1/2	focal	–
28	56	IB	G2	<1/2	not focal	–

s/p: serouspapillary.

to 71 years (median 56 years). The mean number of lymph nodes removed was 42.9 (range: 22–75) for pelvic lymph nodes and 27.9 (range: 6–46) for paraaortic lymph nodes.

Detection rates and sites of sentinel lymph nodes

Preoperative lymphoscintigraphy detected at least one hot spot indicating a sentinel lymph node in 19 of 28 patients (68%) (Fig. 1). For four of the remaining nine patients, an intraoperative or ex vivo survey with the gamma probe for hot spots identified sentinel lymph nodes. Altogether, the detection rate for sentinel lymph nodes was 82% (23 of 28). Among 22 patients with a superficial myometrial invasion, sentinel node identification was missed in only one case. On the other hand, among six patients with deep myometrial invasion, four patients were missed. The detection rate for the former group was significantly higher than that for the latter group: 95% (21/22) versus 33% (2/6) ($P = 0.003$, Fisher's Exact Test). The mean number of sentinel nodes detected was 3.1 (range: 1–9).

The location and number of sentinel nodes detected are summarized in Table 2. The paraaortic region was a critical site for sentinel nodes: paraaortic nodes (18 patients), external iliac nodes (11 patients), and obturator basin (10 patients). Three patients had sentinel nodes only in the paraaortic area, five patients had them only in the pelvic area, and fifteen patients had nodes in both areas. Four patients had sentinel nodes in the right side of the paraaortic area above the inferior mesenteric artery and eight patients had sentinel nodes in the area below the inferior mesenteric artery. Eight patients had sentinel nodes in the left side of the paraaortic area above the inferior mesenteric artery where it was near left renal vessels and five patients had sentinel nodes in the area below the inferior mesenteric artery.

Sensitivity and specificity of SLN for detecting lymph node metastasis

Two patients were diagnosed as having lymph node metastasis after the routine H&E staining. Lymph nodes judged to be metastasis-negative according to the routine H&E staining were stained immunohistochemically with an anti-cytokeratin antibody to detect micrometastases, but no positive antibody signals were detected in any of these lymph nodes.

Among 23 patients with at least one SLN, only 1 patient (case 11) had metastatic lymph nodes. This patient had seven metastatic lymph nodes, one of which was at the external iliac basin and was successfully detected as an SLN. Among the other 22 patients with at least one SLN, all of the SLNs were metastasis-negative and all of the other lymph nodes were metastasis-negative. These results thus indicated that SLN detection gave 100% sensitivity (1/1)

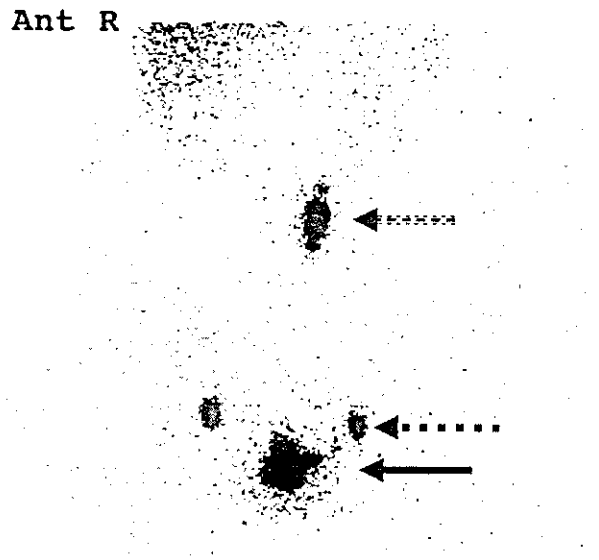

Fig 1. Preoperative lymphoscintigram for case 7. Hot nodes are visible in the pelvic and paraaortic areas. ◀---: paraaortic lymph node ◀····: pelvic lymph node ◀—: injection site in the uterus.

Table 2
Location and number of sentinel nodes

Case	Paraaortic				Common iliac		Sacral		External iliac		Internal iliac		Obturator		Supra-inguinal		Total
	Upper		Lower														
	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	1[a]	0	0	0	0	0	0	0	0	0	0	0	0	0	1
3	0	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	3
4	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	3
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	3
7	0	3	0	1	0	0	0	0	1	1	0	0	0	0	0	0	6
8	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
9	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
10	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
11	0	0	0	0	0	0	0	0	0	1[a,b]	0	0	0	0	0	0	1
12	0	1[a]	0	0	0	0	0	0	0	1[a]	0	0	0	0	0	0	2
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	1	0	0	0	0	0	1	1	0	0	1	0	0	0	4
15	0	1	1	0	1	0	0	0	0	0	0	1	0	0	0	0	4
16	1	0	1	0	0	0	0	0	0	0	1	0	1	2	0	0	6
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	3
19	0	0	2	0	0	0	0	0	0	1	1	0	0	0	0	0	4
20	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	3
21	0	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	3
22	0	0	0	0	0	0	0	0	0	1[a]	0	0	0	0	0	0	1
23	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	3
24	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	3
25	1	0	0	3	1	0	0	0	1	2	0	0	1	0	0	0	9
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
28	0	0	1	0	1	0	0	0	0	1[a]	0	0	1	0	0	0	4
Total	4	10	9	7	4	0	1	0	6	12	2	1	7	7	0	1	71

The landmark separating upper and lower is inferior mesenteric artery.
Case 1: this patient had three swelling and palpable lymph nodes with high suspicion of metastasis upon inspection.
[a] Lymph node detected only by gamma probe.
[b] Metastasis-positive lymph node.

and 100% specificity (22/22) in detecting lymph node metastases.

Among the remaining five patients in whom an SLN was not detected, one patient (case 1) had three metastatic lymph nodes, which were swollen and palpable during lymphadenectomy. These were strongly suspected to be metastatic even on inspection during the surgery.

Discussion

We identified SLN for endometrial cancer with a success rate of 82%, which is comparable to the SLN detection rates for vulvar cancer [5] (86%) and cervical cancer [6] (85%). In the 22 patients whose SLNs were metastasis-negative, all the other lymph nodes were also metastasis-negative, indicating a sensitivity of 100%. In previous reports of SLN in endometrial cancer, the detection rates of SLN were not satisfactory. Echt et al. [8] reported that no sentinel nodes were identified using the isosulfan blue dye method for eight patients with endometrial cancer. They injected the dye directly into the subserosal myometrium after the abdominal cavity was opened. Holub et al. [9] reported that sentinel nodes could be identified with patent blue dye, resulting in a 72% detection rate (18/25). They also injected the dye into the subserosal myometrium and the cervix subserosal myometrium intraoperatively. However, since they did not seem to be concerned with the paraaortic lymph nodes, no information about paraaortic nodes was available from their report. Burke et al. [7] used the isosulfan blue dye method, injecting it into the subserosal myometrium intraoperatively. They detected SLN with a 67% success rate (10/25). Their detection rate was lower than that seen in the present study, but they also detected SLNs at paraaortic sites.

Our higher detection rate for SLNs were possibly due to the following factors. Firstly, we used a radioisotope, which could remain at the SLN much longer than dyes. In our preliminary studies with injection of blue dye through a hysteroscope, it was difficult to survey all sentinel nodes with the blue dye alone in the broad retroperitoneal lymph node area from the level of renal vessels to the level of bilateral inguinal ligaments, and to the bottom of the pelvic

cavity through the bilateral internal iliac vessels. We noticed that the blue dye sometimes passed through the SLN within 15 min and became invisible during our search of the broad retroperitoneal space. Since the method using blue dye alone was limited to use as the intraoperative procedure, we chose a combination of preoperative lymphatic mapping with intraoperative probe detection. However, for future laparoscopic lymphadenectomies, the combination of ^{99m}Tc and blue dye would make it easier to detect sentinel nodes because it was visible intraoperatively.

Secondly, we injected radioisotope dissolved with the blue dye for hysteroscopic guidance, which allowed us to confirm the precise points of injection into the endometrium. The drainage of radioisotope injected into the endometrium mimics the natural lymphatic drainage of cancer cells arising in the endometrium. With this technique, we identified sentinel nodes both in the pelvic and paraaortic areas, as observed by Burke et al. [7]. Eighteen of the 23 patients with SLN had hot spots in the paraaortic region. Also, only the paraaortic lymph nodes were detected as SLN in three patients. It thus appears that the paraaortic basin is a very important primary site for lymphatic drainage and that both paraaortic lymphadenectomy and pelvic lymphadenectomy are important in management of women with endometrial cancer.

Thirdly, we think the intraoperative survey with a gamma probe is more sensitive for the detection of SLNs than preoperative lymphoscintigraphy. Four patients who were found intraoperatively to have a sentinel node were not detected on preoperative lymphoscintigraphy.

Moreover, it would clearly be very useful to locate the SLNs preoperatively by scintigraphy. If considering omission of systematic lymph node resection for endometrial carcinoma, the information obtained preoperatively for the area surrounding the lymph node biopsy would be very useful for preparing the approach to the lymph nodes. For example, this information would be of great use in preparing for procedures such as skin incision, choosing laparotomy or laparoscopy, making decisions on operation time and surgical position, preparing instruments, etc.

The location and incidence of SLNs identified in this report appear very similar to those found for lymph node metastasis of endometrial cancer in another report. Among patients with lymph node metastases, approximately 50% had lymph node metastasis in the pelvic area, 30% in both the pelvic and paraaortic areas, and 20% in the paraaortic area alone [2]. In the pelvic nodes, the obturator basin and the external iliac basin were common sites of SLN. This was consistent with a previous study of the incidence and location of lymph node metastases in endometrial cancer, as determined by performing systematic lymphadenectomy [10].

We suspect that the location of carcinoma in the uterine cavity is also related to that of sentinel nodes. By injecting five sites in the uterine cavity, we may detect more sentinel nodes than true sentinel nodes. However, we believe that the sentinel nodes we do detect include all true sentinel nodes. Lymphatic mapping will make it possible to biopsy more precisely and more easily in the broad regional lymph node area even in such cases.

SLNs were not identified in five patients, and this detection failure seemed to be related to the depth of myometrial invasion. In six patients with deep myometrial invasion (>1/2), two had a radioactive node, and one patient had multiple lymph node metastases, and nodes were swollen and palpable intraoperatively. In the cases of deep invasion, the lymphatic flow may be disturbed, or the involved lymph nodes may no longer filter lymph, as described previously [11]. Alternatively, for patients who fail SLN detection, lymphatic mapping may not be necessary.

Using our method, most of the SLNs were identified by the first lymphoscintigram immediately after injection. However, for four patients, sentinel lymph nodes were detected for the first time 19 h after ^{99m}Tc injection, at the time of the second scintigram. Since lymphatic drainage from the endometrium seems complex and variable among the patients; not only one scintigram but also a second scintigram should be taken and evaluated to identify SLNs according to our method.

Our data suggest that the combination of preoperative lymphoscintigraphy with intraoperative probe detection may be useful in identifying sentinel nodes in early endometrial cancer. We hope that the value of the sentinel node concept will be proven in endometrial cancer by additional larger studies applying this technique. Moreover, this technique may also be applicable to laparoscopic surgery. We currently require a long skin incision for this surgery, but if we survey sentinel nodes with a laparoscopic gamma probe, we may be able to reduce the length of the skin incision, even if sentinel nodes are detected in both the paraaortic and pelvic areas. In order to take adequate and quick samplings of SLNs from such a broad area under the laparoscopic procedure, it is critically important to predict where SLNs are before surgery is begun. We believe that our ^{99m}Tc method makes this possible.

It is further hoped that this method of SLN detection will improve the management of the large majority of women with 'early' endometrial cancers by sparing them unnecessary and potentially harmful total lymphadenectomies.

Acknowledgments

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Reply to Barranger and Darai

(1) Barranger et al. asked a very reasonable question about inadequate visualization of the endometrial cavity if the blue dye with the isotope was injected into the endometrium. If the injected dye leaks from the endometrium, it may well interfere with successful visual acuity of the inspection. It takes a little experience to be familiar to the procedure, with the accumulation of our experience. The depth of injection was approximately 4–5 mm from the endometrial surface.

All the patients we selected were considered to have only focal lesions rather than intensive widespread tumor in the cavity. The pathology specimens of these cases confirmed our hysteroscopic observation. To the widespread tumor in the cavity, five sites injection as described should be selected.

(2) The average time of the hysteroscopic surgery was about 10 min. Normal saline was used to expand the uterine cavity simply with gravity without any additional pressure. The amount of saline used for each case was about 1000 ml. Compared with previously reported studies, the positive cytology of peritoneal washing was seen in only two cases

in our study (7.1). It seems that hysteroscopic inspection of the endometrial cavity without undue pressure did not affect the incidence of positive cytology [1].

(3) We believe firmly that it is very useful to identify SLN prior to the operation for endometrial cancer. Without previous isotope mapping, it is very difficult to identify SLN with simple dye injection just prior to the operation. We believe that if only blue dye is used, it rapidly flows away from the wide pelvic area so that it does not provide us adequate time to detect these nodes during the surgery.

If the radioisotope was injected into the cervix, we believe that the lymphatic spread of the endometrial cancer is different from the way cervical cancer spread into the pelvic lymphatic system.

With the knowledge of SLN sites obtained from hysteroscopic isotope injection, and combined with blue dye injection just prior to the operation, we may increase the detection rate of SLN for endometrial cancer. Eventually, this technique may provide us less invasive laparoscopic approach and avoid total pelvic or paraaortic lymph node dissection.

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Hitoshi Niikura
Nobuo Yaegashi*
Department of Obstetrics and Gynecology, Tohoku University School of Medicine, Sendai 980-8574, Japan
E-mail address: yaegashi@mail.cc.tohoku.ac.jp

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* Department of Obstetrics and Gynecology, Tohoku University School of Medicine, 1-1 Seiryo-machi, Sendai 980-8574, Japan. Fax: +81-22-717-7258.

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Expression of copper-transporting P-type adenosine triphosphatase (ATP7B) as a prognostic factor in human endometrial carcinoma

Takeshi Aida[a,b], Yuji Takebayashi[a,*], Takao Shimizu[b], Chikako Okamura[b], Masashi Higasimoto[a], Atsuko Kanzaki[a], Kentaro Nakayama[c], Kunihiko Terada[d], Toshihiro Sugiyama[d], Kohji Miyazaki[c], Kiyoshi Ito[b], Seiichi Takenoshita[a], Nobuo Yaegashi[b]

[a]*Department of Surgery II, Fukushima Medical University, Fukushima, Hikarigaoka 1, 960-1295, Japan*
[b]*Department of Obstetrics and Gynecology, Tohoku University School of Medicine, Seiryou-Machi 1-1, Aoba-ku, Sendai 980-8570, Japan*
[c]*Department of Obstetrics and Gynecology, Shimane Medical University, Izumo 693-8501, Japan*
[d]*Department of Biochemistry, Akita University School of Medicine, Akita 010-8543, Japan*

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Abstract

Objective. Copper-transporting P-type adenosine triphosphate (ATP7B) has been reported to be associated with cisplatin resistance in vitro. However, the clinical significance of this transporter has not previously been addressed in endometrial carcinoma. Our goal was to investigate if ATP7B is expressed in endometrial carcinoma and whether its expression correlates with prognosis.

Methods. We performed immunohistochemical analysis of ATP7B using a monoclonal antibody against ATP7B in 51 endometrial endometrioid adenocarcinomas. 27 of 51 patients were treated with cisplatin-based chemotherapy after surgery.

Results. Cytoplasmic staining of tumor cells was observed in 37.3% (19/51 cases) of the analyzed carcinomas and no staining was observed in adjacent non-neoplastic cells. ATP7B positivity in the degree of differentiation of G2 and G3 carcinoma was significantly higher than that of G1 carcinoma ($P = 0.019$). The patients with ATP7B-positive tumors had a worse prognosis than that with ATP7B-negative tumors in overall survival and disease-free survival, respectively ($P < 0.01$).

Conclusions. These findings suggest that overexpression of ATP7B expression in endometrial carcinoma is correlated with unfavorable clinical outcome in patients treated with cisplatin-based chemotherapy. ATP7B expression could be considered as a prognostic factor in patients with endometrial carcinoma.

Keywords: ATP7B; Cisplatin; Endometrial carcinoma; Chemoresistance

Introduction

Endometrial carcinoma is one of the most common gynecological malignancies [1] and the incidence has increased in Japan. Although the treatment with cisplatin-based malignancy after reductive surgery has improved prognosis of patients with this carcinoma, one of the most important clinical problems in its treatment is the intrinsic/acquired resistance to cisplatin-based chemotherapy. Knowledge of the active mechanism of drug resistance may lead to new treatment strategies and may allow selection of those patients for specific treatment modalities.

Resistance to cisplatin includes decreased drug accumulation, enhanced detoxification, and increased DNA repair efficiency. Multidrug resistance (MDR) has been noted to be an important mechanism of drug resistance. Several genes including *MDR1*, *MRP*, and *LRP* have been identified [2–5]. *MDR1* and *MRP1* function as a drug efflux pump and are classified in the ABC transporter gene family [4,5] and are expressed in both human solid tumors

* Corresponding author. Fax: +81 24 548 3249.
E-mail address: tyuji@fmu.ac.jp (Y. Takebayashi).

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and hematological malignancies [6,7]. The 110-kd LRP, the major vault protein, is frequently overexpressed in multidrug resistance cells and plays an important role in transport of drugs from nuclei to cytoplasm and confers to multidrug resistance in vitro [3]. *BCRP* (*MXR/ABCP*) gene, another member of the ABC transporter family, has been examined in breast, colon, gastric, and fibrosarcoma cell lines [8–11]. However, evidence that this molecule is involved in cisplatin resistance in vitro and in vivo has not been found. To sum up, the mechanism of cisplatin transport and its significance to drug resistance.

ATP7B is member of a class of heavy metal-transporting P-type ATPases that pump copper, cadmium, zinc, silver, or lead [13–17]. Copper is an essential trace element that is integrated into many enzymatic reactions. Excess copper is transported to the extracellular environment by an energy-dependent system [13], and alteration in copper homeostasis can cause severe problems. For example, Wilson disease (WND), an autosomal recessive disorder in copper transport, is characterized by chronic liver and/or neurological disorder, sometimes accompanied by kidney damage [18]. Detailed understanding of ATP7B is therefore crucial in several diseases including cancer. The fact that this transporter can also transport small molecule drugs is intriguing, and could potentially have significant value in the clinic.

Recently, copper-transporting P-type adenosine triphosphatase (ATP7B) was found to be associated with cisplatin resistance in vitro [12]. The *ATP7B* gene is induced by exposure to cisplatin in human prostate cells, and the *ATP7B*-transfected cells showed dramatic decrease of cisplatin concentration in cytoplasms [12]. Although an active efflux pump for cisplatin has yet to be identified, it is likely that ATP7B functions as efflux for cisplatin from some carcinoma cells. Furthermore, the expression of ATP7B was demonstrated as a cisplatin-based chemoresistance marker in ovarian cancer [19]. The aim of this retrospective study was to investigate the expression of ATP7B and to determine whether its expression was predictive of survival of patients with endometrial carcinoma.

Materials and methods

Patients and samples

Specimens of endometrial adenocarcinoma were collected from patients who underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy, and pelvic and paraaortic lymphadenectomy at Tohoku University Hospital and Shimane Medical University Hospital between 1994 and 2001. Diagnostic verification and tumor subtyping and grading were conducted by gynecological pathologist using permanent pathologic specimens. All samples were embedded in O.C.T. compound (Sakura Finetechnical Co., Ltd., Tokyo) and immediately stored at −80°C until use. Surgical staging for primary endometrial carcinoma was performed according to guidelines of the International Federation of Obstetrics and Gynecology. The clinicopathologic variables such as age, degree of differentiation, and clinical stages are shown in Table 1. The patients with other histological variants of endometrial carcinoma were excluded—i.e., only pure endometrioid adenocarcinoma specimens were selected for this study. However, samplings from surgical specimens were avoided if the lesion in the uterine cavity was small in order to give priority to accurate pathological diagnosis. Among the 51 patients selected, 27 patients with risk factors were primarily treated with reductive surgery and three to six courses of postoperative chemotherapy, consisting of the cisplatin-based combination regimen CAP (cisplatin 60–70 mg/m^2, doxorubicin 40 mg/m^2, and cyclophosphamide 500 mg/body). The remaining 24 without clinicopathologic risk factors did not undergo the adjuvant chemotherapy. A signed informed consent, approved by the Institutional Review Board of Tohoku University Hospital and Shimane Medical University Hospital, was obtained from each patient before the surgery. After examining histopathological features of the sections stained with hematoxylin and eosin, the sections including more than 60% carcinoma cells were used.

Tissue staining and evaluation of stained sections

A 5-μm section of each submitted frozen block was stained with hematoxylin and eosin to verify the histopathologic diagnosis and the quality of fixation for immunohistochemical analysis. Immunostaining was performed on cryostat sections using immunoperoxidase procedure (Vectastain Elite ABC kit, Vector, Burlingame, CA). After recovering from O.C.T. compound, the sections were fixed in 10% neutral buffered formalin, incubated in 0.03% H2O2 in absolute methanol for 30 min at room temperature, and blocked in 3% skim milk in PBS for 30 min at room temperature. The sections were then incubated with 100-fold diluted monoclonal antibody against the

Table 1
Relationship between ATP7B expression and clinicopathologic variables

	ATP7B expression		
	Negative	Positive	
Total	32	19	
Age[a]	57.4 (26–79)	59.2 (37–76)	n.s.[b]
Grade			
G1	24	8	
G2/G3	8	11	$P = 0.019$[c]
Stage			
I	25	11	
II	2	3	
III	4	5	
IV	1	0	n.s.[c]

n.s.: not significant.
[a] Median (ranges).
[b] Mann–Whitney *U* test.
[c] Chi-square test.

NH2-terminal region of ATP7B, which included six copper-binding domains (amino acid number from 21 to 623; see Ref. [20]) for 15 h at 4°C. After rinsing with PBS, the sections were incubated in biotinylated horse anti-mouse IgG at 1:200 in 1.5% normal horse serum for 40 min at room temperature. Sections were then rinsed in PBS and incubated for 50 min at room temperature in the avidin–biotin horseradish peroxidase macromolecular complex. After rinsing with PBS, the sections were incubated for 7 min in 0.03% diaminobenzidine in PBS with 0.003% H2O2. The slides were counterstained with hematoxylin, dehydrated, and mounted. The serial sections were routinely incubated with irrelevant mouse IgG as negative control.

The slides were examined and scored independently by two observers (T.A. and Y. T.) without knowledge of clinical information of the patients. If more than 10% of the tumor cells were stained, the samples were considered to be ATP7B-positive carcinomas. The 10% cutoff level was specified for the following reasons: (1) a 10% positive cells was considered the lowest level of expression that could be most consistently detected in cryostat sections; (2) Cban et al. demonstrated that a small percentage of cells positive for multidrug-resistance-related proteins (i.e., P-gp) could have clinical significance [21]; and (3) we evaluated validation of this cutoff in our previous studies, which examined ATP7B expression in ovarian carcinoma [19,22]. When the two observers' reports differed from each other, they evaluated together the images of stained sections on a TV-captured station.

Statistical analysis

Data analysis was performed using Statview Version 5 statistical software package. Continuous variables were analyzed with Mann–Whitney *U* test, and categorical variables were analyzed with chi-square test. Overall survival and disease-free survival were determined with the Kaplan–Meier method, and differences in survival between subgroups were compared with log-rank test. We estimated relative risks and 95% confidence interval of survivals, using Cox's proportional-hazards model with adjustment for risk factors. Less than 0.05 of *P* values was hypothesized to be significant. All reported *P* values were two sided.

Results

Expression of ATP7B protein in human endometrial carcinoma

We used 51 primary endometrial carcinoma tissues for the detection of ATP7B by immunohistochemistry using ATP7B monoclonal antibody. This antibody specifically reacted with ATP7B by immunoblotting analysis [19]. A granular staining of ATP7B was observed in cytoplasm of endometrial carcinoma cells (Fig. 1A) and some of carcinoma had no staining of ATP7B (Fig. 1B). In adjacent non-neoplastic cells, ATP7B expression was not detected (Fig. 1C). The immunostaining results were summarized in

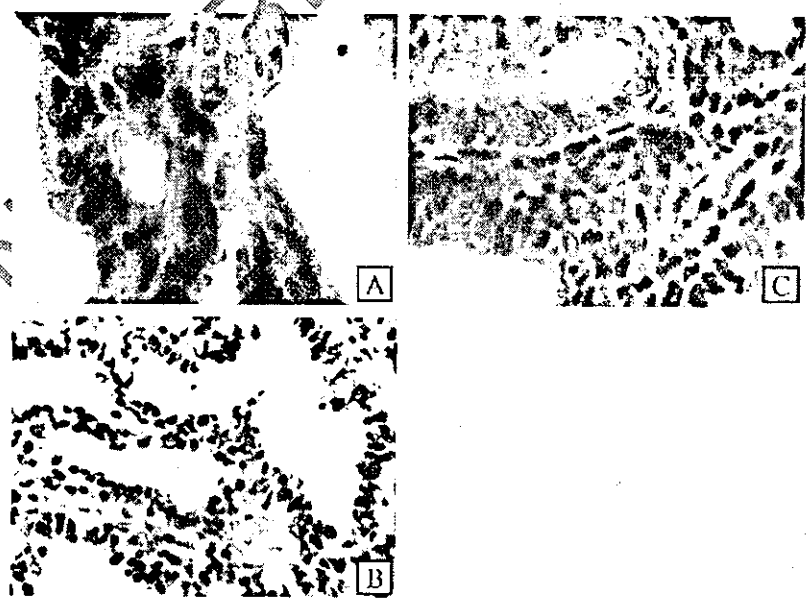

Fig. 1. Immunohistochemical staining of endometrial carcinoma specimens using antibodies to ATP7B in cryostat sections. (A) ATP7B-positive tumor stained with anti-ATP7B monoclonal antibody. Note the distinct cytoplasmic staining in endometrial carcinoma cells (immunoperoxidase stain, original magnification, ×400). (B) ATP7B-negative tumor (immunoperoxidase stain, original magnification, ×400). (C) Normal epithelium cells in the serial section (magnification, ×400).

Table 1. A variable degree of cytoplasmic staining of tumor cells was observed and 37.3% (19/51 cases) of the analyzed tumors were evaluated to be positive, following the criteria described in Materials and methods.

Relationship between clinicopathologic findings and ATP7B expression

Table 1 summarizes the relationship between clinicopathologic features and ATP7B expression in endometrial carcinomas. No significant association was found between ATP7B expression and age, FIGO stage, or histopathologic subtypes (Table 1). However, ATP7B positivity was correlated with tumor grades, i.e., ATP7B positivities in the degree of G2 and G3 carcinoma were significantly higher than that in G1 carcinoma with chi-square test ($P = 0.019$).

Prognostic relevance of ATP7B expression

Kaplan–Meier estimates of disease-free or overall survival were plotted in Figs. 2A and B. The patients with ATP7B-positive carcinomas had poorer disease-free survival and overall survival than those with ATP7B-negative tumors with log-rank test ($P < 0.01$).We performed analysis using Cox's proportional hazards model with the adjustment of age, stage, and grade. In univariate analysis, relative risks of overall survival were 1.06 (0.98–1.16 $P = 0.17$) with age, 8.13 (2.11–31.3, $P < 0.01$) with grade, 2.78 (1.18–6.54, $P = 0.02$) with stage, and 14.31 (1.56–130.9, $P = 0.019$) with ATP7B expression. With multivariate analysis, we found significant relation between overall survival and grade: RR 10.03 (1.22–82.2, $P = 0.032$). With regard to ATP7B expression, marginal relation between overall survival and ATP7B expression was found but it was not statistically significant; RR 47.1 (0.69–320.8, $P = 0.074$).

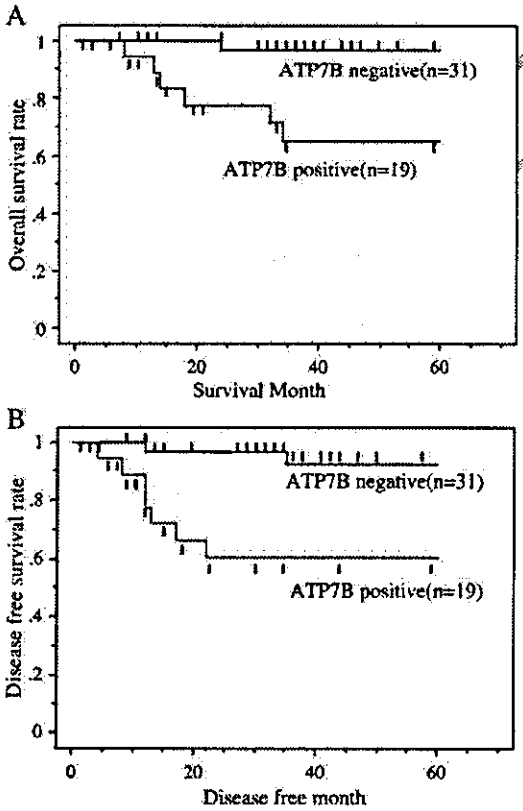

Fig. 2. Kaplan–Meier survival curves of patients with endometrial carcinoma. (A) Overall survival curve. (B) Disease-free survival curve. Comparison of survival curve for patients whose tumors were positive for ATP7B with that negative for ATP7B expression. The differences were analyzed with log-rank test.

Discussion

Several important findings are presented in this report. First of all, ATP7B was expressed in human endometrial carcinoma as assessed by immunohistochemistry (Fig. 1). ATP7B immunoreactivity in tissues was detected as granular cytoplasmic staining. In agreement with this observation, ATP7B has been reported to be abundant in the Golgi apparatus [18]. These findings are the first evidence(s) that ATP7B is expressed in endometrial carcinoma. Secondly, ATP7B expression in the degree of differentiation of G2 and G3 endometrial carcinoma was more frequent than that of G1 carcinoma. Thirdly, no expression of ATP7B gene and protein could be detected in adjacent non-neoplastic tissues. The fact that the expression level of ATP7B was not detected in normal endometrial tissues raised the possibility that ATP7B might be involved in transformation of a normal cell to a malignant tumor cell and/or differentiation of carcinoma cell.

ATP7B expression assessed by immunohistochemistry has the potential to become a prognostic factor for survival in patients with endometrial carcinoma treated with cisplatin-based chemotherapy. In univariate analysis, ATP7B expression was significant relation with survival. However, this relation was marginal in multivariate analysis. This seemed to be due to the strong correlation between ATP7B and grade, which is of special clinical interest because undifferentiated carcinomas is usually more refractory to therapy. The same relationship with respect to ATP7B and endometrial cancer has also been found for ovarian carcinoma [19,22]. A priori knowledge of the ATP7B expression may be important in the choice of therapy. It will be necessary to study comparison of clinical response to cisplatin-based chemotherapy with ATP7B expression in endometrial carcinoma. In the future, drugs targeting ATP7B may be useful in combination with cisplatin-based chemotherapy for the improvement of survival rate of gynecologic cancers.

Acknowledgments

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Precis
This study investigated the role of ATP7B expression in endometrial carcinoma using 51 patients.

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Effectiveness of Mass Screening for Endometrial Cancer

Chikako Nakagawa-Okamura, M.D., **Shinji Sato**, M.D., **Ichiro Tsuji**, M.D., **Hiroyuki Kuramoto**, M.D., F.I.A.C., **Yoshitaka Tsubono**, M.D., **Daisuke Aoki**, M.D., **Toshiko Jobo**, M.D., F.I.A.C., **Mineo Oomura**, M.D., **Shigeru Hisamichi**, M.D., and **Akira Yajima**, M.D.

OBJECTIVE: To investigate the effectiveness of mass screening for endometrial cancer using Endocyte (Laboratoire CCD, Paris, France) endometrial smears.

STUDY DESIGN: The study subjects were consecutive patients with documented endometrial cancer diagnosed between January 1, 1989, and December 31, 1997, at 22 hospitals in Japan. One hundred twenty-six cases were detected by mass screening and 1,069 diagnosed in outpatient clinics. We compared the stage of cancer at diagnosis and survival rate of patients in the two groups.

Screening with endometrial smears has the potential to reduce mortality from endometrial cancer.

RESULTS: Early stage was significantly more frequent in the screening group ($P < .001$); stage I comprised 88.1% of the screening group as compared with 65.3% of the outpatient group. Well-differentiated adenocarcinoma was significantly more frequent in the screening group ($P < .01$); grade 1 constituted 74.7% of the screening group as compared with 61.0% of the outpatient group. The five-year survival rate was significantly higher in the screening group than

From the Departments of Obstetrics and Gynecology and of Public Health, Tohoku University School of Medicine, Sendai; Departments of Obstetrics and Gynecology, Keio University School of Medicine, Tokyo; and Kitasato University School of Medicine, Kanagawa, Japan.

Dr. Nakagawa-Okamura is Lecturer, Department of Obstetrics and Gynecology, Tohoku University School of Medicine.

Dr. Sato is Associate Professor, Department of Obstetrics and Gynecology, Tohoku University School of Medicine.

Dr. Tsuji is Associate Professor, Department of Public Health, Tohoku University School of Medicine.

Dr. Tsubono is Assistant Professor, Department of Public Health, Tohoku University School of Medicine.

Dr. Aoki is Assistant Professor, Department of Obstetrics and Gynecology, Keio University School of Medicine.

Dr. Jobo is Assistant Professor, Department of Obstetrics and Gynecology, Kitasato University School of Medicine.

Dr. Kuramoto is Professor, Department of Obstetrics and Gynecology, Kitasato University School of Medicine.

Dr. Oomura is Director, Tokyo Gan Kenshin Center.

Dr. Hisamichi is Professor, Department of Public Health, Tohoku University School of Medicine.

Dr. Yajima is Professor, Department of Obstetrics and Gynecology, Tohoku University School of Medicine.

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Address reprint requests to: Chikako Nakagawa-Okamura, M.D., Department of Obstetrics and Gynecology, Tohoku University School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai, 980-8574, Japan (cnakagawa@ob-gy.med.tohoku.ac.jp).

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in the outpatient group (94.0% vs. 84.3%, P = .041). The crude hazard ratio (HR) of dying of endometrial cancer for the screening group as compared to the outpatient group was .47 (95% CI .22–.99, P = .048). HR became .96 (95% CI .45–2.08, P = .925) after adjustment for age, study area and cancer stage.
CONCLUSION: The results suggest that an endometrial cancer screening program would lead to early detection and improved survival among women with endometrial cancer. (Acta Cytol 2002;46:277–283)

Keywords: mass screening, endometrial neoplasms, survival rate, survival analysis, Endocyte.

The incidence of endometrial cancer in Japan has increased markedly in recent years and is expected to keep increasing. The age-adjusted incidence rate per 100,000 (30–74 years of age) increased from 2.6 in 1970 to 7.3 in 1985 in Miyagi prefecture, Japan.[1]

The five-year survival rate in the screening group (94.7%) was significantly higher than that in the outpatient group (85.7%).

Mass screening for endometrial cancer has been conducted in Japan since 1987 under the Health and Medical Service Law for the Aged. Among the participants in cervical cancer screening, gynecologists select subjects at high risk for endometrial cancer according to an interview and invite them to participate in screening for endometrial cancer. Endocyte (Laboratoire CCD, Paris, France) endometrial smears are used for screening, and cytodiagnostic findings are classified as positive, suspicious or negative. In positive or suspicious cases, close examination of tissues is performed by fractional curettage. According to the Ministry of Health and Welfare, Japan, 3,843,482 women participated in cervical cancer screening in 1995. Of these, 217,827 participated in endometrial cancer screening, 4,219 (1.94%) were referred for a diagnostic work-up, and 238 (0.1%) were diagnosed as having endometrial cancer.[2]

Past studies of endometrial cancer screening were aimed at estimating prevalence and incidence of endometrial cancer[3-5] and sensitivity and specificity of the screening test.[6-10] To our knowledge, there has been no epidemiologic study before evaluating the effectiveness of endometrial cancer screening. In this study, we compared the stage of cancer and the survival rate of endometrial cancer cases detected by screening (screening group) with those diagnosed at outpatient clinics (outpatient group).

Materials and Methods

Screening Program in Japan

Screening for endometrial cancer has been conducted throughout Japan since 1987 under the Health and Medical Service Law for the Aged. According to the law, gynecologists interview participants in the cervical cancer screening program to select candidates for endometrial cancer screening. Women are recommended to participate in endometrial cancer screening if they have had abnormal genital bleeding within the previous six months and meet at least one of the following three conditions: (1) age ≥ 50 years, (2) postmenopausal, or (3) nulligravid, with irregular menstruation. Endometrial cancer screening is done with cytodiagnostic findings. The subjects with positive or suspicious results are referred for a diagnostic workup by fractional curettage.

Study Subjects

The study subjects were consecutive patients with documented endometrial cancer diagnosed between January 1, 1989, and December 31, 1997, at 22 hospitals in Japan. These included 15 hospitals in Miyagi prefecture and three hospitals in the metropolitan area. The hospitals in Miyagi prefecture, ranging from community hospitals to the university hospital, covered all endometrial cancer cases in that prefecture; thus, the cases in Miyagi were population based. The three hospitals in the metropolitan area were Kitasato University Hospital, Keio University Hospital and Tokyo Gan Kenshin Center. Those are specialized medical centers; thus, the cases in the metropolitan area were not population based. In Miyagi prefecture there were 697 subjects. In Tokyo there were 729. There was no difference in mean age, stage distribution or survival rate between the cases in Miyagi prefecture and those in the metropolitan area; therefore, we combined the two groups for this study.

We collected 1,426 cases. Of these, 167 were detected by a screening program under the Health and Medical Service Law for the Aged, 1,122 were

identified in outpatient clinics, and 137 were detected at health checkups other than those provided though the Health and Medical Service Law. We excluded the subjects of the last group because they were screened for endometrial cancer irrespective of symptoms or risk factors; that is, their eligibility for participating in endometrial cancer screening was different from that under the Health and Medical Service Law for the Aged.

The following information was recorded for each subject: name of hospital, name of patient, date of birth, date of established histologic diagnosis, awareness of symptoms, history of participating in mass screening, treatment used (surgery, chemotherapy, radiation, etc.), cytology and histology of the tumor, stage of the disease according to the International Federation of Gynecology and Obstetrics system (FIGO) (1988)[11] and either the last date of confirmed survival, or the date and cause of death.

In the screening group, 41 subjects were excluded from the analysis, 31 because they were diagnosed with hyperplasia and 10 because there was a lack of medical records. In the outpatient group, 53 were excluded: 7 because of hyperplasia and 46 for lack of medical records. Thus, the total number of study subjects was 126 for the screening group and 1,069 for the outpatient group.

Prognosis

The subjects were followed from the date of established diagnosis until either the date of death or December 31, 1997. The survival status of the subjects on December 31, 1997, was confirmed by chart review or telephone call.

In this study, 150 subjects (12.6%) were lost to follow-up because they moved away without notification of the new address before December 31, 1997. We regarded these subjects as censored at the date of the last visit documented in the medical records. There was no difference in the mean age, ratio of screening/outpatient group or distribution of cancer stage between the censored cases (n = 150) and the others (n = 1,045).

Cancer cases at earlier stages were significantly more frequent in the screening group.

The underlying cause of death was determined by reviewing the medical records. The subjects (n = 19) who died of causes other than endometrial cancer were censored at their date of death.

Data Analysis

We compared survival after diagnosis of endometrial cancer between the screening and outpatient groups using the Kaplan-Meier method and tested its statistical significance with the log-rank test. We estimated hazard ratios (HRs) and their 95% CIs

Table 1 *Age and Clinical Characteristics of Subjects in the Screening and Outpatient Groups*

	No. of subjects (%)				
	Screening group (n = 126)		Outpatient group (n = 1,069)		
Characteristic	No.	(%)	No.	(%)	*P* value
Mean age (yr)	55.2 ± 8.2		56.1 ± 10.7		
Stage	No.	(%)	No.	(%)	
I	111	(88.1)	698	(65.3)	
II	4	(3.2)	98	(9.2)	.001[a]
III	8	(6.3)	220	(20.6)	
IV	3	(2.4)	53	(5.0)	
Histology					
Endometrioid adenocarcinoma	115	(91.3)	910	(85.1)	
Endometrioid adenocarcinoma with squamous differentiation	5	(4.0)	64	(6.0)	
Endometrial stromal sarcoma	0	(0)	10	(0.9)	.48[a]
Carcinoma	2	(1.6)	33	(3.1)	
Other	4	(3.2)	52	(4.8)	

[a]Tested with χ^2 test.

Table II *Grade Distribution of the Subjects with Adenocarcinoma in the Screening and Outpatient Groups*

	No. of subjects (%)				
	Screening group (n=115)		Outpatient group (n=910)		
Grade	No.	(%)	No.	(%)	*P* value
1	86	(74.7)	556	(61.0)	
2	21	(18.2)	249	(27.4)	.01[a]
3	8	(6.1)	105	(11.5)	

[a]Tested with χ^2 test.

using the Cox proportional hazard model. We estimated crude HR and HR after adjustment for age at diagnosis, study area (Miyagi vs. Tokyo) and stage of endometrial cancer. PROC PHREG with Statistical Analysis System software (Cary, North Carolina, U.S.A.) was used for analysis. We considered $P < .05$ the level of statistical significance.

Results

Characteristics of the Screening and Control Groups

Table I shows the characteristics of the two groups. The median period of observation tended to be shorter in the screening group (38.9 ± 5.2 months) than in the outpatient group (43.5 ± 8.7 months), although it was not statistically significant. The mean age at diagnosis was the same for the screening and outpatient groups.

Early stages were significantly more frequent in the screening group ($P < .001$). Stage I was found in 88.1% of subjects in the screening group as compared with 65.3% in the outpatient group. The rates of stages II–IV were higher in the outpatient group. The distribution of histologic types was the same for both groups. Endometrial adenocarcinoma was the most common type in both groups: 91.3% in the screening group and 85.1% in the outpatient group (Table I).

Table II shows the grade distribution of subjects with adenocarcinoma for the two groups, ranging from grade 1, the most differentiated, to grade 3, the most anaplastic (FIGO, 1988). There was a significant difference in the grade distribution between the groups ($P = .01$) (Table II). Well-differentiated adenocarcinoma was significantly more frequent in the screening group.

Survival Analysis

Figure 1 shows Kaplan-Meier survival curves for all the subjects according to stage. The five-year survival rate was 96.5% for stage I, 82.6% for stage II, 72.4% for stage III and 14.5% for stage IV.

Figure 2 shows Kaplan-Meier survival curves for the screening and outpatient groups. The five-year survival rate was 94.7% for the screening group and 85.7% for the outpatient group, and this difference was statistically significant ($P = .041$).

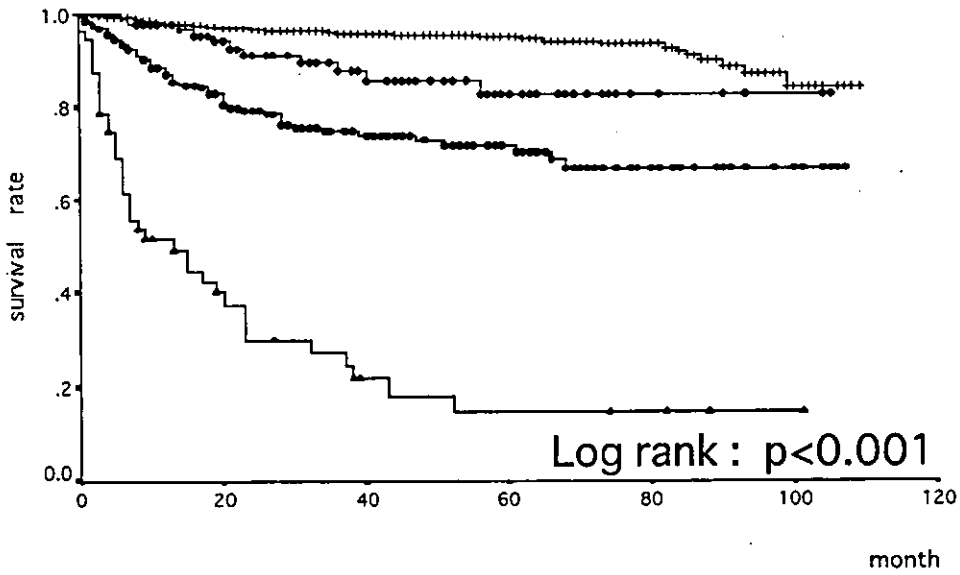

Figure 1 Overall survival curves according to FIGO stage by Kaplan-Meier method. + = Subjects at stage I (n = 809), five year = 96.5%, ◊ = subjects at stage II (n = 102), five year = 82.6%, ● = subjects at stage III (n = 228), five year = 72.4%, ▲ = subjects at stage IV (n = 56), five year = 14.5%. Log rank: $P = .001$.

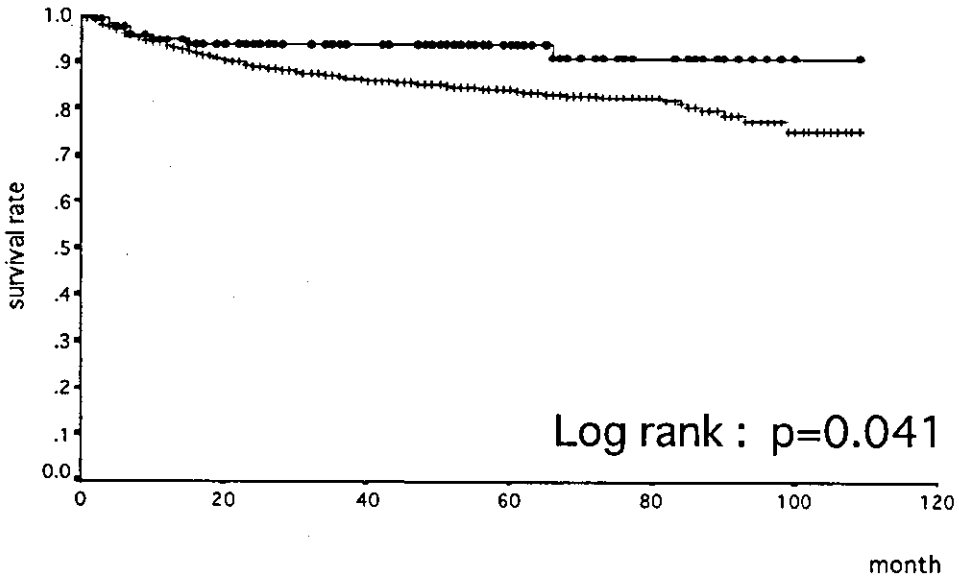

Figure 2 Comparison of survival curves between screening and outpatient groups by Kaplan-Meier method. Survival rates for subjects with endometrial cancer were compared between cases that were detected by screening and those diagnosed in the hospital during the same period. ● = Subjects in screening group (n = 126), five year = .94, + = Subjects in outpatient group (n = 1,069), five year = .84. Log rank: $P = .041$.

Table III shows the results of the Cox proportional hazard model with adjustment of the differing covariates. With no adjustment, the detection method (screening vs. outpatient) showed significant effects on survival. The crude HR of dying of endometrial cancer, for the screening group as compared to the outpatient group, was .47 (95% CI .22–.99). HR did not change much after adjustment for age at diagnosis and area (.49 [.23–1.05]), but the statistical significance disappeared. However, after adjustment for the stage of endometrial cancer, HR changed markedly toward the null: .96 (.45–2.08). Thus, it appears that the survival benefit for the screening group, as determined by the Kaplan-Meier survival analysis and crude HR, was due largely to the fact that the screening program detected cancer cases at earlier stages.

Discussion

Recommendations regarding screening for endometrial cancer have long been controversial in many countries. The American Cancer Society recommends screening at menopause in high-risk women (those with a history of infertility, obesity, failure to ovulate, abnormal uterine bleeding or estrogen therapy).[12] However, the U.S. Preventive Services Task Force has not decided whether to recommend screening.[13] In Japan, the government subsidizes endometrial cancer screening under the Health and Medical Services Law for the Aged.[14] The controversy is partly responsible for the fact that no epidemiologic studies have examined the effectiveness of endometrial cancer screening. To our knowledge, this study was the first to attempt to evaluate the effectiveness of endometrial cancer

Table III *Multivariate Analysis of Factors in the Cox Proportional Hazard Model for Subjects in the Screening and Outpatient Groups*

	Factor					
Detected method[a]	Age[b]	Area[c]	Stage[d]	HR[e]	95% CI	*P* value
+	–	–	–	0.47	0.22–0.9	.048
+	+	+	–	0.49	0.23–1.05	.068
+	+	+	+	0.96	0.45–2.08	.925

[a]Screening and not screening.
[b]Age at diagnosis (yr).
[c]Miyagi and metropolitan area.
[d]FIGO stages I–IV.
[e]HR (outpatient detected group/screening detected group).

screening. The results indicate a survival benefit for patients in the screening group.

Before interpreting the result, it would be valuable to discuss the validity of our data. First, the study subjects were derived from different population types. Subjects in Miyagi prefecture were population based, and those in metropolitan Tokyo were hospital based. However, there was no significant difference in distribution of age, stage of cancer and survival rate between the subjects from those areas. Therefore, we combined these two groups. Second, we were able to follow only 1,045 (87.4%) of the 1,195 eligible cases. However, there was no difference in age or stage distribution between those who were followed (n = 1,045) and those who were lost to follow-up (n = 150). Therefore, bias associated with follow-up status seems unlikely. Third, stage distribution of endometrial cancer among the subjects in the outpatient group was quite consistent with the national average in Japan. According to a report by the Japan Society of Obstetrics and Gynecology, stage I has been found in 68%, stage II in 18.8%, stage III in 7.8% and stage IV in 4.5% of endometrial cancer cases.[15] This finding was similar to the stage distribution in our outpatient group. Thus, selection bias regarding our study subjects seems unlikely. Fourth, grade distribution of endometrioid adenocarcinoma among the subjects in the outpatient group was consistent with the national average in Japan. The Japan Society of Obstetrics and Gynecology reported that grade 1 was found in 57.9%, grade 2 in 26.9% and grade 3 in 9.4% of adenocarcinoma cases.[15] Selection bias seems unlikely. Fifth, survival rates according to stage of endometrial cancer in our subjects (Figure 1) were higher than the national average in Japan. According to the report of the Japan Society of Obstetrics and Gynecology, the five-year survival rate is 79.1% in stage I, 65.9% in stage II, 47.1% in stage III and 18.6% in stage IV.[15] In our study, both the screening and outpatient groups showed better survival than the national average.

The participants in endometrial cancer screening were in a high-risk group as designated under the law. We used Endocyte endometrial smears in the cytodiagnosis of endometrial cancer, they are the most popular detection method in Japan. Shinohara reported that the sensitivity was 83%.[6]

In our study, the five-year survival rate in the screening group (94.7%) was significantly higher than that in the outpatient group (85.7%). This survival benefit was confirmed by a Cox proportional hazard model. The crude HR of dying of endometrial cancer was .47 for the screening group as compared to the outpatient group. Cancer cases at earlier stages were significantly more frequent in the screening group. After adjustment for age, study area and cancer stage, HR shifted markedly toward the null: .96. Thus, it seems that the survival benefit for the screening group, as determined by the Kaplan-Meier survival analysis and crude Cox proportional hazard model, is largely due to the effect of detecting cancer cases at an earlier stage.

The comparison of survival rates between the screening and outpatient groups is not free from lead-time bias, length bias or other factors. Therefore, we require more valid evidence to evaluate the effectiveness of endometrial cancer screening.[16] However, our study was the first to suggest that screening with endometrial smears has the potential to reduce mortality from endometrial cancer. Further investigation on the effectiveness of endometrial cancer screening is warranted.

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