Cytology

PF-01

Fixation of serous effusions – to do or not to do

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Background:
Three pathology departments will be united in a few years. Currently, serous effusions are received non-fixated in one department and fixated in the other departments.
The purpose of this study was to find the optimal morphology of cells in serous effusions by using either fixated or non-fixated specimens.

Material:
Each of 8 different non-fixated pleural effusions was split in 5 different tubes:

1. Non-fixated, prepared immediately
2. Non-fixated, prepared after 3 days (kept refrigerated)
3. Fixated in 70 % ethanol
4. Fixated in Sure Path fixative
5. Fixated in CytoRich Red Preservative

3-5 were fixated immediately and prepared in 1-3 days.

Method:
All specimens were centrifuged for 5 min. From the cell pellet, 3-5 drops were applied to a SurePath vial for Liquid Based Cytology (LBC) and set for preparation at the Multiprocessor. SurePath Papanicolau (PAP) was performed at a Slide Prep Processor. The remaining cell pellet was either prepared by the Plasma-Thrombin method (non-fixated specimens) or centrifuged in tubes with a formaldehyde-ethanol mixture for a clot. Both kinds were then embedded as a cell block.
The PAP stained specimens and the Hematoxylin/ Eosin-stained sections from the cell block was blinded, and assessment was performed by two experienced cytotechnologists.
Assessment were made by scoring the following parameters: Chromatin structure, sharpness of the nuclear membrane, contrast between nucleus and cytoplasm, sharpness of the nucleolus, quality of the cytoplasmic dye, +/- clear background and density of the cells in the cell block.

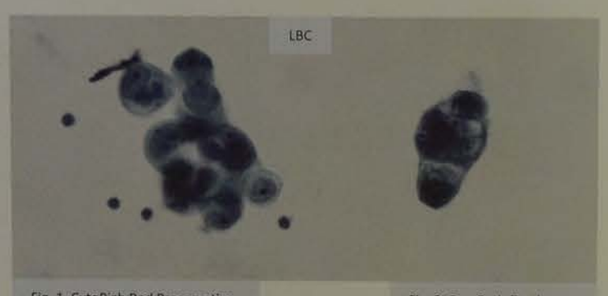

Fig. 1. CytoRich Red Preservative

Fig. 2. SurePath fixative

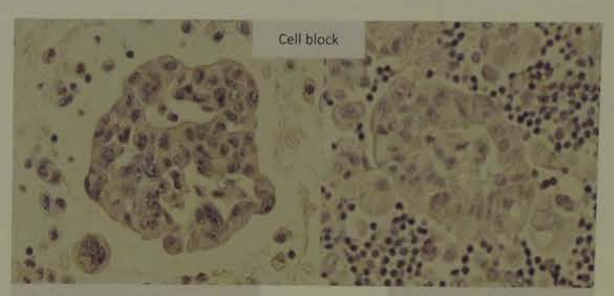

Fig. 3. CytoRich Red Preservative

Fig. 4. Non fixated, 3 days

Results:
Tumor cells were found in only one of the 8 specimens. The tumor cells had the best morphology when fixated in CytoRich Red Preservative. (Fig. 1 & 3). This fixation was also best in 3 of the other cases, where the assessment were based on mesothelial cells, and/or inflammatory cells.
In the residual 4 cases, there was neither a clear difference between the fixated specimens nor the specific fixative. Fig. 2 & 4 show the worst cases.

Discussion/conclusion:
Primarily the tumor cells should be well prepared for diagnosis, secondary the mesothelial cells. The morphology of the inflammatory cells were of minor concern. The limited number of specimens, and especially the fact, that only one of them contained tumor cells, made it difficult to conclude. Although, this study suggests, that **the optimal cell morphology in LBC and the cell block is obtained by fixation in Cytorich Red Preservative**.

Perspective:
The next step is to get more residual material containing tumor cells, in order to perform immunocytochemistry in non-fixated specimens and specimens in CytoRich Red Preservative respectively. These results, compared with those above, will be the basis for deciding, whether or not to use fixated specimens in all three departments.

Acknowledgement to the staff from Department of Pathology, Næstved.

REGION SJÆLLAND

Cytology

PF-02

A case of primary prostate neuroendocrine tumors appeared in the urine

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Introduction

The origins of neuroendocrine tumor are rare, the cells are unusually seen in the diagnosis, they appeared in the urine cells of human waste. Our primary report is about the neuroendocrine prostate tumor. Our hospital encountered neuroendocrine prostate tumor, that was shown in the urine cytology.

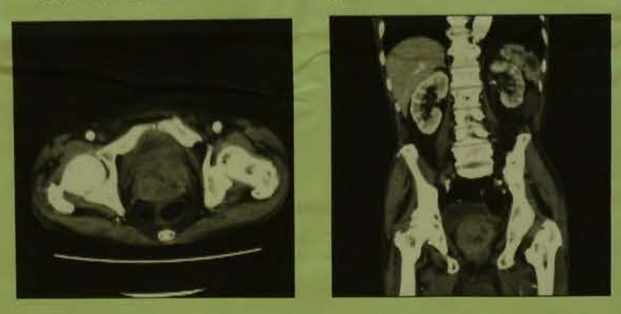
Case

This is a case of an 82 year old male patient, highlighted with urine cytology in Class V.
CT scan also recognized a non-uniform of the prostate, which indicates malignancy. Medical history reveals that the patient underwent gastrectomy, angina and a suspected gastric cancer when he was 72 years old . The patient was referred to our hospital's urology department for further evaluation such as prostate TUR and biopsy.

Laboratory deta

GOT	31 U/L	BUN	14.9 mg/dl	Ly	18.4 %
GPT	12 U/L	Cr	1.23 mg/dl	Mo	5.3 %
LDH	290 U/L	CK	166 U/L	Eo	0.2 %
ALP	248 U/L	Fe	46 μg/dl	Ba	0.2 %
γ-GTP	25 U/L	BS	128 mg/dl	NSE	8.8 ng/ml
T-Bil	0.4 mg/dl	WBC	6.4 10*3/μL	ProGRP	39.1 pg/mL
TP	6.4 g/dl	RBC	2.64 10*6/μL	HS-PSA	3.36 ng/ml
ALB	2.6 mg/dl	Hb	8.6 g/dl	OB	(3+)
S-AMY	123 U/L	Ht	26.2 %	UL	(2+)
UA	6.1 mg/dl	Neu	75.9 %	UPr	(3+)

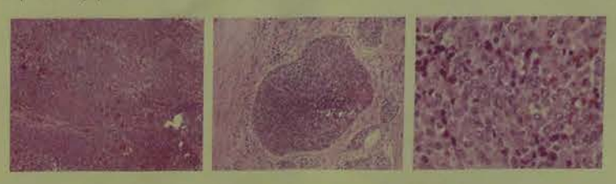
TP and ALB admitted low value, the anemia in biochemical tests. It showed an increase of LDH and creatinine and blood sugar.



Recognized a non-uniform swelling of the prostate, bladder wall showed a thickening of the entire circumference of at CT.

Histological findings

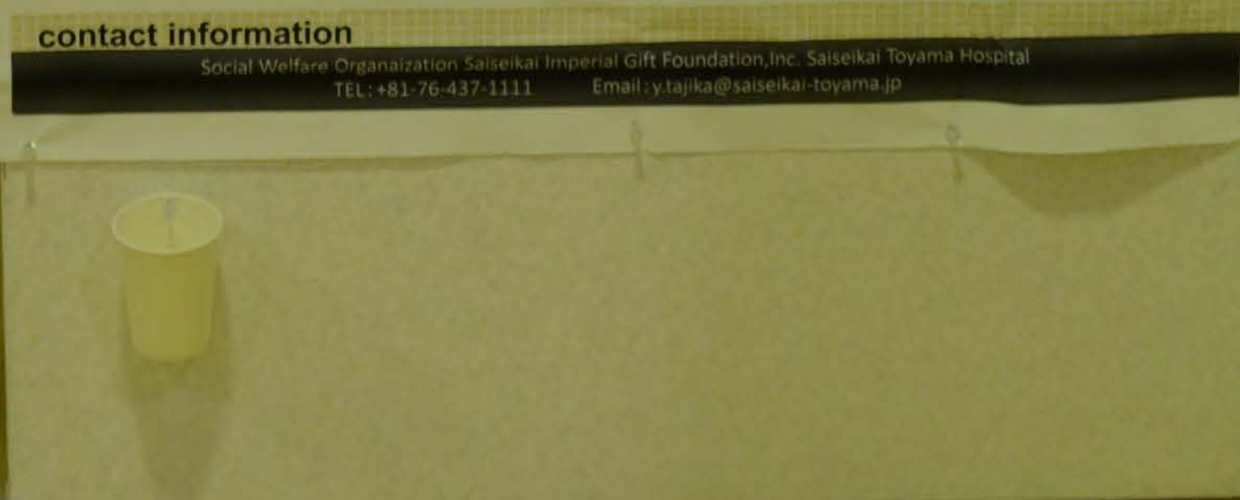
P504S of prostate markers are positive, Synaptophysin also have the properties of both will be positive is a nervous system marker. CD56 is negative in the tumor, CK7 was also negative. Synaptophysin becomes positive neuroendocrine marker, it was diagnosed than histology and immunohistochemistry results and primary prostate neuroendocrine tumor.



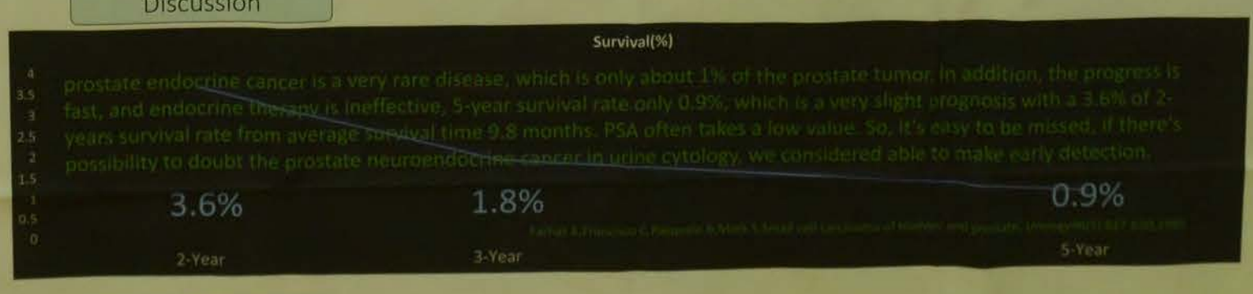
Tumor cells is poor in ductal formation, it recognized the solid alveolar and comedones necrosis, were also observed fence-like sequences in the marginal while obscure.
Admitted undifferentiated cells with a circular-like nucleus, during the specimen was found here and there also mitosis.

Cytological findings

The Cytology showed atypical flat cells with high N / C ratio circular kind. Cells are relatively large, chromatin has been admitted there's 1-2 in the nucleolus by thin granular. In addition, it shows if the separated small cells appeared in the glandular cavity-like sequence in small clumps.



Discussion



Conclusion

We has reported a case of primary prostate neuroendocrine tumor that appeared in the urine. In cytology it's suspected as urothelial cancer, but the tumor cells in the biopsy is to mark neuroendocrine showed positive, which diagnosed with prostate primary Neuroendocrine cell carcinoma.

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Cytology

PF-03

Liquid based cytology (LBC) preparation method in Routine Work at our Laboratory

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Introduction

Our laboratory changed the way to make fluid specimen preparation from the conventional method to the LBC method (Becton Dickinson Co., LTD.) two years ago. Now the fraction of gynecological specimens treated with the LBC method has increased from 1/5 to 4/5. Cytological preparations have been made more efficient through the introduction of the LBC method. The preparation technique before and after to starting of LBC method will be compared.

Effusions & Urine specimens

The number of preparation slides for fluid and urine specimens has decreased from 3 to 1 with this method and has led to saving time for cytotechnologists to observe them(Fig.1). In our attached satellite laboratory, by adding fixative solution for sediment enables not only cellular morphology but also reduce a fixation work at night(Fig.2). This method is unsuitable for Giemsa's stain but applicable to mucinous stain or immunocytological stain instead (Fig.3a, 3b).

Aspiration & curetting specimens

Medical doctors put the specimen sampling brush and puncture needle into the container filled with fixative solution. Thus combination of conventional preparation method and LBC can collect more amounts of cells (Fig4a,4b).

Modified slide rack

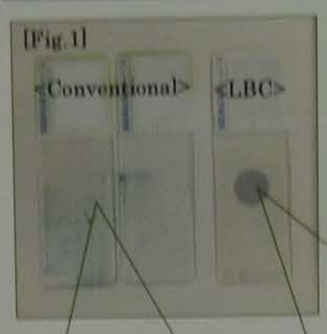
We use a modified slide rack (Fig.5)with which two spots of smear can be placed on one slide. This helps to collect more amounts of cells, too. Specifically, we can prepare endocervix and endometrium specimens on one slide (Fig.6). The equipment is useful for immunocytological stain in which two or more types of antibodies are used (Fig.7).

Conclusion

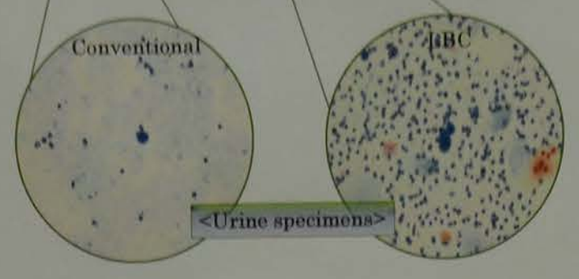
LBC method is effective to keep cellular morphology or cellular condition. Also, the method improves efficiency at work, and shortens time for examination with an optical microscope and describing medical reports. Furthermore our full-time staff's skill for LBC work reaches an almost required level, so that a cytotechnologist can have enough time for examination of prepared slides.

Figures

[Fig.1]

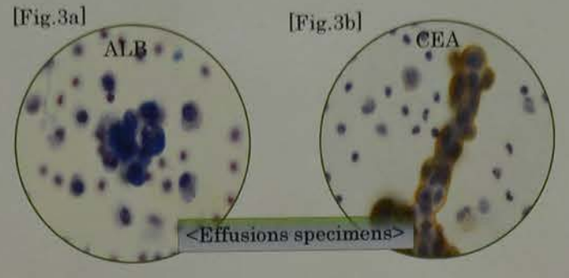


[Fig.2]



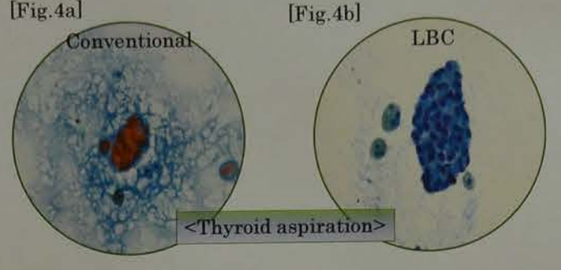
[Fig.3a]

[Fig.3b]

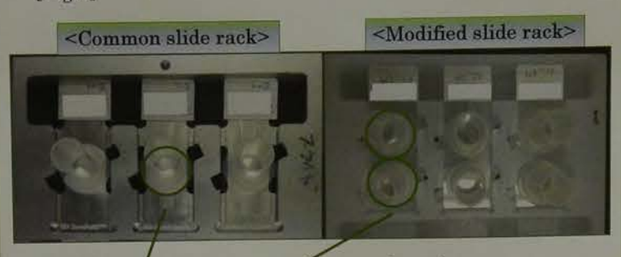


[Fig.4a]

[Fig.4b]

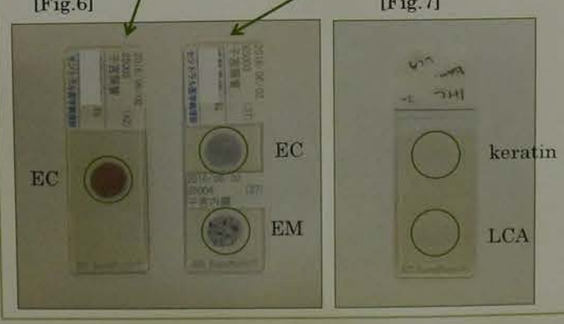


[Fig.5]



[Fig.6]

[Fig.7]



ogy

05

The utility of imprint (a touch smear) cytol ovarian tumor during an operation

Shiho Azami, Yuji Aoki, Mizuki Iino, Asumi Sakaguchi, Toshiharu Matsumoto

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] to gain the histological type of an ovarian tumor on. Therefore, a frozen section diagnosis during s important to make decision of an operative a treatment policy. However, there are many ich are not able to perform frozen section cause of understaffed and/or poor facilities. the utility of imprint (a touch smear) cytology eration , and compared with that of a frozen osis and pathological diagnosis.

d Methods]

55 cases of ovarian tumors in which frozen nosis were conducted in our hospital from o September 2015. Imprint cytology was om several parts of a tumor which were ly different and did Papanicolaou stain . We hem blindly in clinical information, and the compared with those of the frozen section the final pathological diagnosis.

e utility of imprint cytology about diagnostic ly, including histological type.

accuracy (Table 1)

mples, 15 were benign. 8 were borderline and nant. It was possible to diagnose benign and nors by a frozen section diagnosis and imprint t was difficult to distinguish borderline tumors alignant tumors especially imprint cytology. agreement between imprint cytology and d diagnosis (Table 2)

nors

gy was possible to diagnose about histological A frozen section diagnosis was possible to t it in 93.3%, both were approximately similar

umors

gy was possible to diagnose about histological . A frozen section diagnosis was possible to t it in 59.4%. It was the result that imprint better than a frozen section diagnosis. rface epithelial - stromal tumors tended to accuracy(Figure 1).

tumors

gy was possible to diagnose about histological A frozen section diagnosis was possible to t it in 50%. Especially, mucinous borderline ifficult to diagnose with accuracy.

a]

o factors that it is difficult to distinguish erline tumors from another mucinous tumors. place, mucinous tumors contain various the same tumors. In 4 cases that we weren't ose with accuracy, when we observed the des, not only mucinous borderline tumor but adenoma was seen. In a touch smear cytology, d that imprint cytology were gathered from oma(Figure 2). In the second place, the cells mors show a middle image of benign and ors. So, it is difficult to distinguish adenoma noma. According to literatures, especially derline tumors tend to underdiagnosis in ors. In cytological findings, clusters with accumulate and papillary clusters appear ors. Necrosis, isolated cells and cell atypia alignant tumors(Table 3). If we get the ell image from imprint cytology , we think le to diagnose with accuracy.

gy of an ovarian tumor during an operation is thod that distinguish benign tumors from nors. If we get the characteristic cell image cytology, we think that it is possible to improve curacy. In actual use, the mutual understanding ician is necessary about diagnosis and its

gnostic accuracy

	A frozen section diagnosis (%)	Imprint cytology (%)
	15 (100)	15 (100)
	5 (62.5)	2 (25)
	32 (100)	32 (100)
	52 (94.5)	49 (89.1)

Table 2. Diagnostic agreement between imprint pathological diagnosis

Histological type	Pathological diagnosis	Number cases
Benign (n=15)	Mucinous tumor	3
	Endometriotic cysts	1
	Mature teratoma	2
	Struma ovarii	2
	Fibrothecoma	5
	Serous cystadenofibroma	1
	Fibrothecoma admixed with serous cystadenoma	1
Borderline (n=8)	Serous tumor	1
	Mucinous tumor	5
	Endometrioid tumor	1
	Mixed-epitherial papillary cystadenoma	1
Malignant (n=32)	Serous tumor	5
	Mucinous tumor	3
	Endometrioid tumor	6
	Clear cell tumor	10
	Mixed-epitherial tumor	5
	Metastatic adenocarrinoma	1
	Undifferentiated carcinoma	1
	Juvenile granulosa cell tumor	1

Table 3. Cytological findings of mucinous tumors

KANAHARA [1]	Benign	Mucin	Sheet, Pseudo-cribriform pattern	+ (10%)
	Borderline	Mucin	Sheet, overlapping, Pseudo-cribriform pattern	+ (28%)
	Malignant	Necrosis	Papillary	+ (100%)
SHIMIZU [2]	Benign	Clear	Sheet, Papillary	ND
	Borderline	Relatively clear	Sheet, Papillary, Ball	ND
	Malignant	Necrosis	Sheet, Papillary, Ball	ND

References 1) J. Jpn. Soc. clin. Cyto

2) J. Jpn. Soc. clin. Cyto

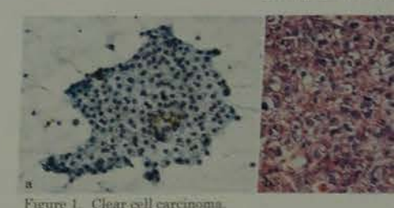

Figure 1. Clear cell carcinoma.
a) Imprint cytological findings(Pap, ×20). Sheet-like cell composed of cells with clear cytoplasm and conspicuous n
b) Histological findings(HE, ×20). It was same as imprint

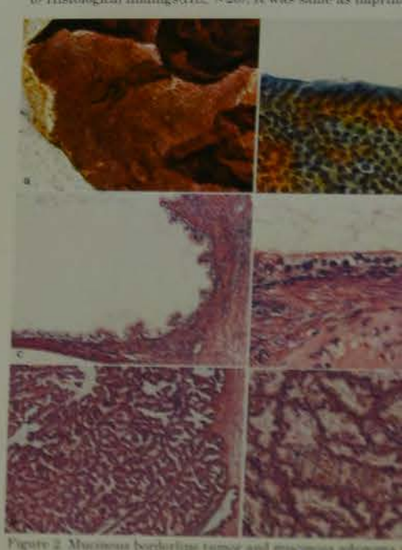

Figure 2. Mucinous borderline tumor and mucinous adenoma some tumor.
a,b) Imprint cytological findings(Pap, a: × 10, b: × 40). Sheet-like overlapping clusters composed of cells with mucin. It resembles mucinous adenoma. c,d,e,f) Histological findings(HE, c: × 10, d: c × 4, e, f × 20). Mucinous borderline and adenoma were admixed

Cytology

PF-04

A cytological study of ALK-positive lung cancer

Department of Clinical Laboratory Gunma Prefectural Cancer Center
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Objective

Since characteristic histological findings of ALK-positive lung cancer have been reported, we investigated cytological findings.

Study Design

Using 48 samples from 46 patients with pulmonary adenocarcinoma in whom the ALK fusion gene was searched for and the investigation of cytology preparations was possible, cytological findings were compared between ALK-positive and –negative groups.

Clinical characteristics of the 46 patients

		ALK +		ALK -
Cytological specimen		TBLB	5	TBLB	24
		PL	3	CNB	11
		PE	1	PL	5
Age (median SD)		54±9		68±8	
Sex		Male	2	Male	24
		Female	5	Female	11
Smoking		+	5	+	25
		-	2	-	10

TBLB: transbronchial lung biopsy
CNB: CT-guided transdermal lung biopsy
PL: pleural effusion
PE: pericardial effusion

Comparison of cytological findings between ALK-positive and ALK-negative lung cancer

		ALK +	ALK -	p value
Appearance form	Aggregates	5	25	0.31
	Scattered	0	10	
Mucus	+	4	7	0.02
	-	1	28	
Nuclear form	mild irregularity	4	14	0.16
	> moderate irregularity	1	21	
Nucleoli	Small	4	13	0.14
	Moderate	1	22	

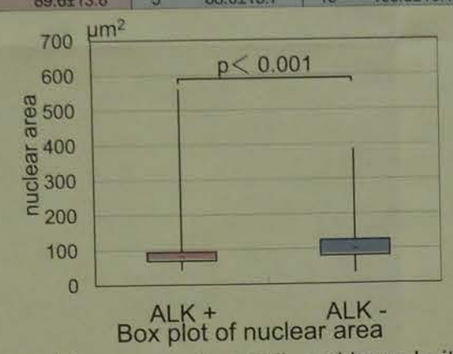
A cribriform pattern was noted in atypical cell aggregates in impression preparations of a patient in the ALK-positive group. (Pap. staining , x 20)

Signet-ring cells were mixed in aggregates in impression preparations of a patient in the ALK-positive group. (Pap. staining , x 40)

In the ALK-positive group, cells were present in aggregates in impression preparations, and cells containing mucus in the cytoplasm and signet-ring cells were observed.

Average nuclear area (μm²)

ALK +		ALK -													
case	nuclear area	case	nuclear area	case	nuclear area	case	nuclear area	case	nuclear area	case	nuclear area	case	nuclear area	case	nuclear area
1	70.1±14.0	1	101.1±18.9	6	148.0±49.4	11	111.0±38.3	16	94.9±15.2	21	107.9±54.4	26	123.3±35.4	31	86.5±18.5
2	77.6±15.5	2	132.5±41.8	7	103.9±32.1	12	107.2±27.4	17	128.9±54.6	22	77.9±14.6	27	106.5±34.0	32	48.0±6.8
3	95.5±76.0	3	111.2±32.3	8	82.4±29.0	13	80.8±23.7	18	122.1±31.1	23	92.2±18.6	28	89.8±26.0	33	92.5±23.6
4	96.2±54.4	4	109.0±22.6	9	142.1±49.0	14	78.0±18.6	19	90.3±26.7	24	111.1±23.0	29	120.9±32.0	34	170.4±32.6
5	89.6±13.6	5	88.0±15.7	10	106.8±16.1	15	88.1±15.0	20	102.1±21.6	25	98.7±28.4	30	116.8±19.3	35	160.6±41.0



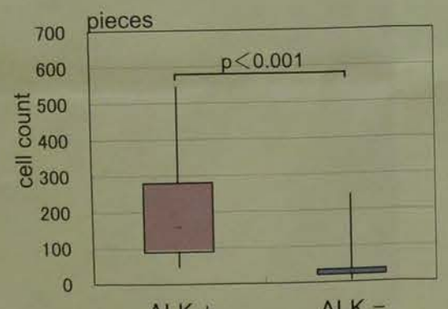
Box plot of nuclear area

In impression preparations of 2 patients in the ALK-positive group, relatively homogenous small atypical cells were mainly noted and a small number of large atypical cells were mixed. (Pap. staining , x 40)

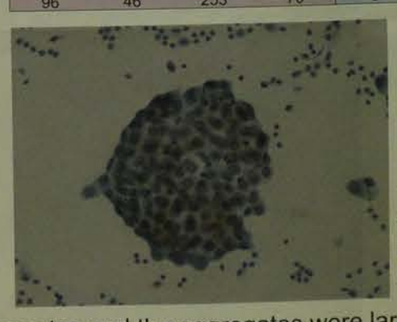
The nuclei were mostly small, and irregularity of the nuclear shape was mild to moderate, but a small number of large cells were mixed.

Cell count of cell aggregates (pieces)

ALK +				ALK -				
case 1	case 2	case 3	case 4	case 1	case 2	case 3	case 4	case 5
343	133	546	234	21	29	53	244	35
296	57	334	182	13	29	31	236	25
191	53	315	136	13	23	27	222	23
125	52	275	116	6	23	24	212	22
96	46	253	70	5	22	14	115	16



Box plot of the cell count of cell aggregates



Tumor cells formed large aggregates in pleural fluid of ALK-positive patients. (Pap. staining , x 20)

In celomic fluid preparations, many cells formed aggregates and the aggregates were large in the ALK-positive group.

Conclusion

It is difficult to judge the presence or absence of the ALK fusion gene based on cytological findings alone, but when small atypical cells containing a clear nucleolus are mainly present in addition to mucus-producing cells and signet-ring cells in impression preparations with mixed large atypical cells, or when large cell aggregates are present in a celomic fluid preparation, it may be necessary to consider the possibility of ALK-positive lung cancer.

The 32nd World Congress of Biomedical Laboratory Science
COI Disclosure
Harumi Kamiyama
The author have no financial conflicts of interest to disclose concerning the presentation.

Cytology

PF-05

The utility of imprint (a touch smear) cytology of an ovarian tumor during an operation

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【Introduction】

It is difficult to gain the histological type of an ovarian tumor before operation. Therefore, a frozen section diagnosis during an operation is important to make decision of an operative method and a treatment policy. However, there are many hospitals which are not able to perform frozen section diagnosis because of understaffed and/or poor facilities. So we report the utility of imprint (a touch smear) cytology during an operation , and compared with that of a frozen section diagnosis and pathological diagnosis.

【Materials and Methods】

We studied 55 cases of ovarian tumors in which frozen section diagnosis were conducted in our hospital from June 2013 to September 2015. Imprint cytology was performed from several parts of a tumor which were macroscopically different and did Papanicolaou stain . We investigated them blindly in clinical information, and the results were compared with those of the frozen section diagnosis and the final pathological diagnosis.

We report the utility of imprint cytology about diagnostic accuracy mainly, including histological type.

【Results】

➢ Diagnostic accuracy (Table 1)

Among 55 examples, 15 were benign, 8 were borderline and 32 were malignant. It was possible to diagnose benign and malignant tumors by a frozen section diagnosis and imprint cytology. But it was difficult to distinguish borderline tumors from benign/malignant tumors especially imprint cytology.

➢ Diagnostic agreement between imprint cytology and pathological diagnosis (Table 2)

≪Benign tumors≫

Imprint cytology was possible to diagnose about histological type in 86.7%. A frozen section diagnosis was possible to diagnose about it in 93.3%, both were approximately similar results.

≪Malignant tumors≫

Imprint cytology was possible to diagnose about histological type in 84.4%. A frozen section diagnosis was possible to diagnose about it in 59.4%. It was the result that imprint cytology had better than a frozen section diagnosis.
Especially, surface epithelial - stromal tumors tended to diagnose with accuracy(Figure 1).

. ≪Borderline tumors≫

Imprint cytology was possible to diagnose about histological type in 20%. A frozen section diagnosis was possible to diagnose about it in 50%. Especially, mucinous borderline tumors were difficult to diagnose with accuracy.

【Consideration】

We have two factors that it is difficult to distinguish mucinous borderline tumors from another mucinous tumors. In the first place, mucinous tumors contain various component in the same tumors. In 4 cases that we weren't able to diagnose with accuracy, when we observed the histological slides, not only mucinous borderline tumor but also mucinous adenoma was seen. In a touch smear cytology, it was suggested that imprint cytology were gathered from mucinous adenoma(Figure 2). In the second place, the cells of borderline tumors show a middle image of benign and malignant tumors. So, it is difficult to distinguish adenoma and adenocarcinoma. According to literatures, especially mucinous borderline tumors tend to underdiagnosis in borderline tumors. In cytological findings, clusters with the tendency to accumulate and papillary clusters appear at benign tumors. Necrosis, isolated cells and cell atypia appear at malignant tumors(Table 3). If we get the characteristic cell image from imprint cytology , we think that it is possible to diagnose with accuracy.

【Conclusion】

Imprint cytology of an ovarian tumor during an operation is the useful method that distinguish benign tumors from malignant tumors. If we get the characteristic cell image from imprint cytology, we think that it is possible to improve diagnostic accuracy. In actual use, the mutual understanding ...e clinician is necessary about diagnosis and its ...ng.

...e 1. Diagnostic accuracy

...thological diagnosis	A frozen section diagnosis (%)	Imprint cytology (%)
Benign (n=15)	15 (100)	15 (100)
Borderline (n=8)	5 (62.5)	2 (25)
Malignant (n=32)	32 (100)	32 (100)
Total (n=55)	52 (94.5)	49 (89.1)

Table 2. Diagnostic agreement between imprint cytology and pathological diagnosis

Histological type	Pathological diagnosis	Number of cases	Diagnostic accuracy Number of cases (%)
Benign (n=15)	Mucinous tumor	3	3 (100)
	Endometriotic cysts	1	1 (100)
	Mature teratoma	2	2 (100)
	Struma ovarii	2	2 (100)
	Fibrothecoma	5	5 (100)
	Serous cystadenofibroma	1	0 (0)
	Fibrothecoma admixed with serous cystadenoma	1	0 (0)
Borderline (n=8)	Serous tumor	1	1 (100)
	Mucinous tumor	5	1 (20)
	Endometrioid tumor	1	0 (0)
	Mixed-epitherial papillary cystadenoma	1	0 (0)
Malignant (n=32)	Serous tumor	5	3 (60)
	Mucinous tumor	3	3 (100)
	Endometrioid tumor	6	6 (100)
	Clear cell tumor	10	10 (100)
	Mixed-epitherial tumor	5	4 (80)
	Metastatic adenocarcinoma	1	1 (100)
	Undifferentiated carcinoma	1	0 (0)
	Juvenile granulosa cell tumor	1	0 (0)

Table 3. Cytological findings of mucinous tumors of ovary

	Histology	Background	Architecture	Isolated cells	N/C ratio	Hyperchromasia
KANAHARA[1]	Benign	Mucin	Sheet, Pseudo-cribriform pattern	+ (10%)	ND	ND
	Borderline	Mucin	Sheet, overlapping, Pseudo-cribriform pattern	+ (25%)	ND	ND
	Malignant	Necrosis	Papillary	+ (100%)	ND	ND
SHIMIZU[2]	Benign	Clear	Sheet, Papillary	ND	Preserved	Mild
	Borderline	Relatively clear	Sheet, Papillary, Ball	ND	Relatively preserved	Mild to moderate
	Malignant	Necrotic	Sheet, Papillary, Ball	ND	Increase	Moderate to severe

References : 1)J. Jpn. Soc. clin. Cytol. 1998;37(6):577～582

2)J. Jpn. Soc. clin. Cytol. 1998;37(6):583～590

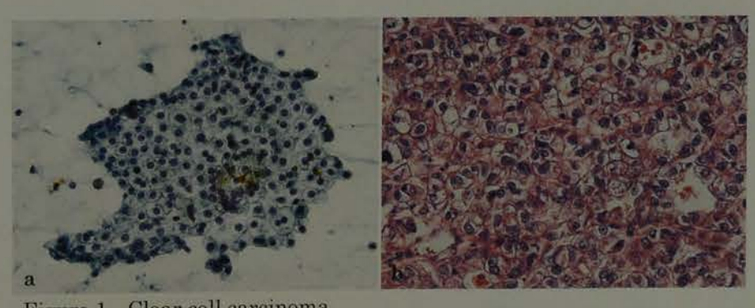

Figure 1. Clear cell carcinoma.
a) Imprint cytological findings(Pap, ×20). Sheet-like cell clusters composed of cells with clear cytoplasm and conspicuous nucleoli.
b) Histological findings(HE, ×20). It was same as imprint cytology.

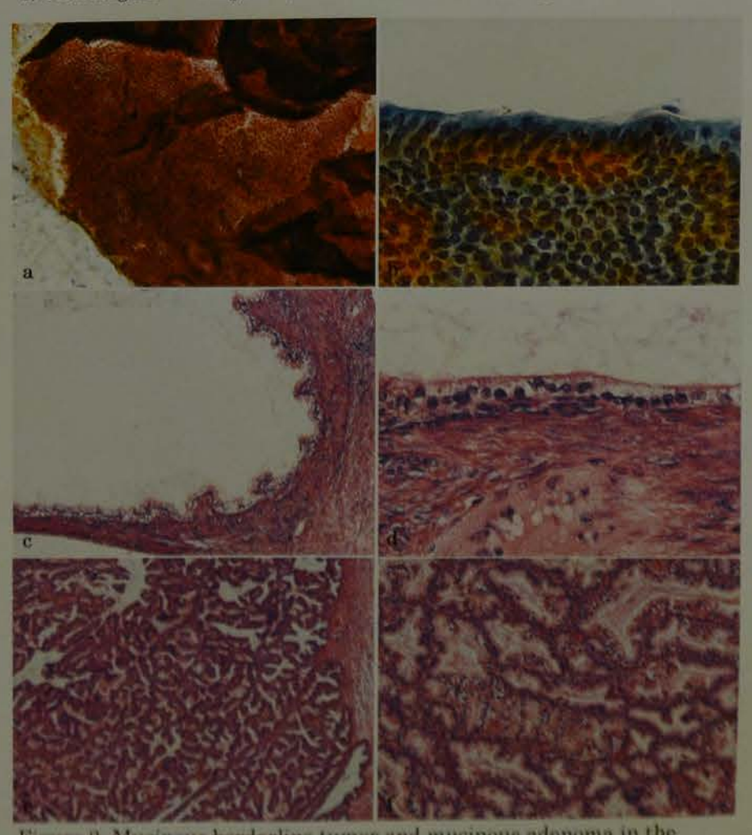

Figure 2. Mucinous borderline tumor and mucinous adenoma in the same tumor.
a,b) Imprint cytological findings(Pap, a:× 10, b:× 40). Sheet-like or overlapping clusters composed of cells with mucin. It resembled mucinous adenoma.c,d,e,f) Histological findings(HE, c:× 10, d:× 40, e:× 4, f:× 20). Mucinous boderline and adenoma were admixed.

A-1

Cytology

PF-06

Endometrial carcinoma associated with endometrial polyp: Clinicopathological and cytological analysis

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Introduction

Endometrial (EM) polyp harbors a potential risk where EM carcinomas represented by serous carcinoma, including serous endometrial intraepithelial carcinoma (SEIC), tend to occur especially in elderly women.

Materials

The examined EM carcinoma specimens were taken from the 27 patients who underwent total hysterectomy with/without adnexectomy and lymph node dissection. In these patients, the most part of EM carcinomas were found in their EM polyp.

Table1. clinicopathological presentation

		n	%
age, median (range)		63 (49-78)	
menopause age, median		51	
presenting symptoms	postmenopausal bleeding	12	44
	abnormal cytology	8	30
	abnormal imaging	1	4
	ascites	3	11
	incidental	1	4
	medical check	2	7
history of breast cancer		6	22
Em cytology	positive	23	96
	negative	1	4
	ND	3	
biopsy*	positive	16	66
	negative	8	34
	ND	3	
FIGO stage	IA**	22	81
	IIIC	1	4
	IVB	4	15
peritoneal cytology	positive	7	32
	negative	15	68
	ND	5	
outcome	NED	22	81
	recurrent	1	4
	DOD	4	15
treatment	NAC, TAH+BSO	3	11
	polypectomy, TAH+BSO	3	11
	TAH+BSO	14	52
	TAH+BSO, AC	7	26

*polypectomy: 2cases **double cancer: 1 case with ovarian cancer at IA
NAC: neoadjuvant chemotherapy, AC: adjuvant chemotherapy, TAH: total abdominal hysterectomy
ND: not done NED: not evidence disease DOD: death of disease

Table 2. pathological characteristics

			n	%
polyp	number	single	23	85
		multiple	4	15
	size/median (mm) (range)		17 5-55	
	location	fundus	12	44
		left	9	33
		right	1	4
		posterior	3	11
		ithmus	2	7
histology		serous*	17	63
		endometrioid	5	18
		clear	1	4
		NOS	4	14
carcinoma location		EM polyp only	13	48
		EM polyp + endmetrium	14	52
atrophic background		absent	3	11
		present	24	89
extrauterine desease		absent	23	85
		present	4	15
myometrial invasion		-	18	67
		<1/2	9	33
		≥1/2	0	0
stromal invasion		-	5	19
		+	22	81
ER expression		-	22	88
		+	3	12
p53 expression		-	9	35
		+	17	65

*SEIC alone: 5 cases

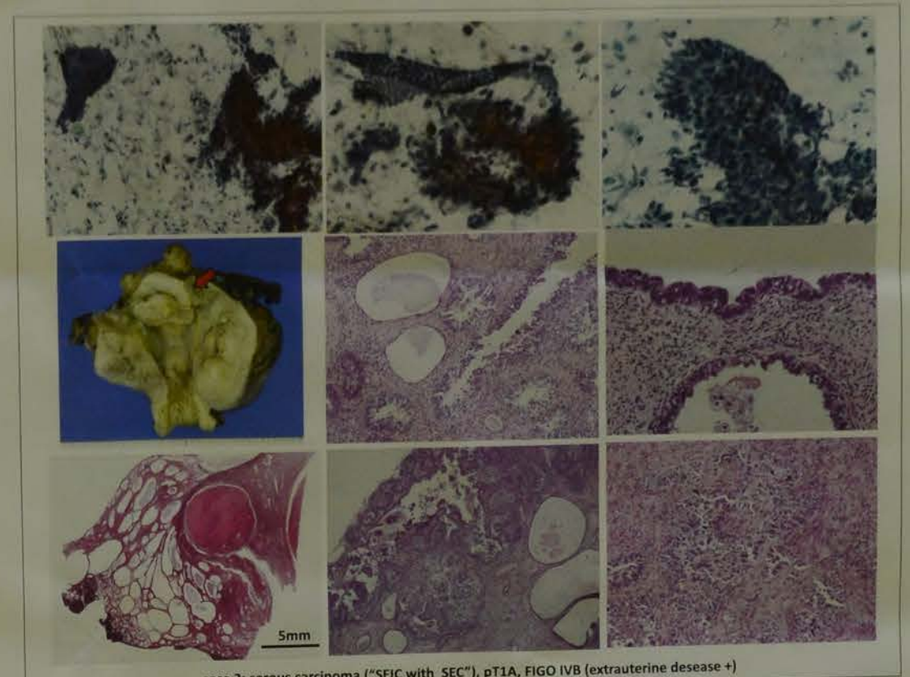
Conclusion

EM cytology is more helpful than biopsy in detection of the EM carcinomas at early stage, i.e., limited to EM polyp and/or atrophic endometrium in the background. It is supposed that the EM carcinoma cells are easily designated as malignancy in EM cytology on the comparison with atrophic benign endometrial cells. It should be noted that some of EM carcinomas at early stage, may develop extra-uterine extent, which can be detected by peritoneal cytology.

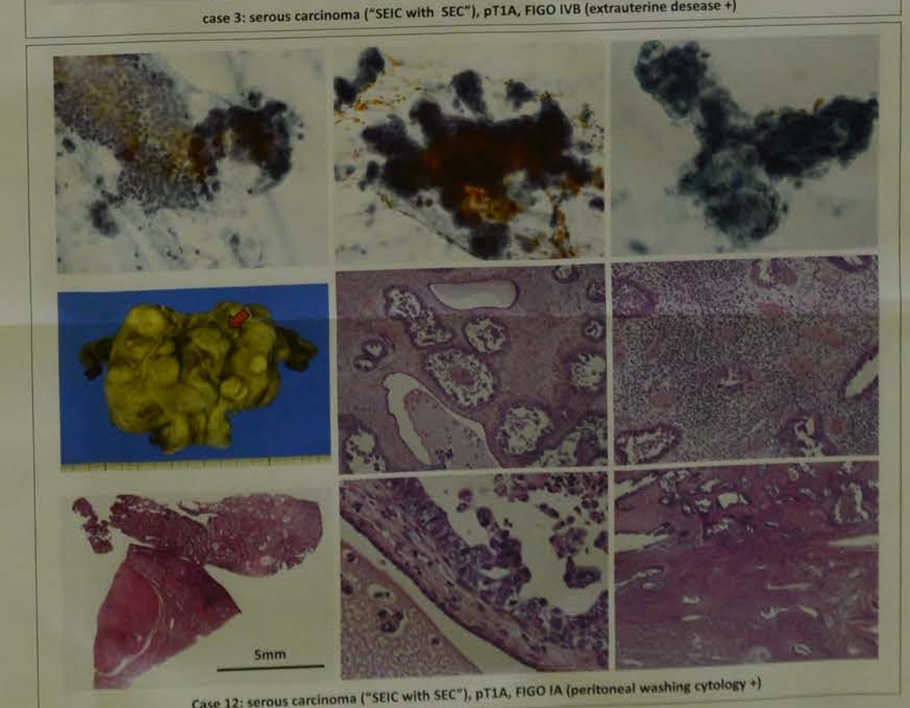
Table 3. cytological characteristics

case	age	Em cytology	histology	size of polyp (mm)	cytological characteristics: background	atrophy	cluster	relative size	others
1	49	+	EMC>SEC	15	watery, mucus	++	micropapillary	2.1	
2	55	+	SEC	55	watery, necrosis	+	individual	NA	
3	57	+	SEC	55	inf	+	tubular > papillary	2.0	
4	59	+	EMC	15	inf	+	crowded	1.4	a
5	59	ND	SEC	5	ND	ND	ND	ND	
6	60	+	SEC	15	watery	+	micropapillary	2.3	
7	61	+	CCC	15	watery	++	tubular,sheet like	2.2	b
8	61	+	EMC/G1	32	mucus	+	tubular	1.8	
9	61	+	SEC	10	watery	+	papillary > tubular	2.3	
10	61	+	SEC	22	bloody	+	micropapillary > tubular	2.4	
11	62	+	SEC	17	watery	+	individual > micropapillary	2.2	
12	62	+	SEC	50	bloody	+	micropapillary > tubular	2.3	
13	62	ND	EMC/G1	20	ND	ND	ND	ND	
14	63	+	SEC	8	watery	++	micropapillary > tubular	2.1	
15	63	+	SEC	15	necrosis	+	tubular > micropapillary	2.7	
16	64	+	SEC	30	necrosis	+	papillary > tubular	2.2	
17	65	+	SEC & EMC	8	bloody > necrosis	+	tubular > papillary	2.3	
18	65	-	EMC	27	ND	ND	ND	ND	
19	65	+	SEC	20	watery	++	tubular	2.8	
20	67	+	SEC	5	bloody	+ (a few)	papillary >> tubular	3.0	
21	69	+	SEC	17	bloody>necrosis	-	micropapillary > tubular	ND	
22	69	+	SEC > EMC	45	inf > watery	+	micropapillary	2.0	c
23	71	+	EMC > SEC	15	bloody>mucus	+	tubular	1.7	
24	75	+	EMC/G2	15	watery > inf	+	crowded	NA	
25	76	+	SEC	30	watery	++	tubular>micropapillary	1.7	
26	76	+	SEC	35	bloody	+	tubular	2.1	
27	78	ND	SEC > EMC	36	ND	ND	ND	ND	

Inf: inflammation a: metaplastic change, b: hyalinous stroma, c: emperipolesis atrophy: atrophic endometrium ND: not done
relative size: compared to normal nucleus EMC: endometrioid carcinoma SEC: serous carcinoma CCC: clear cell carcinoma



case 3: serous carcinoma ("SEIC with SEC"), pT1A, FIGO IVB (extrauterine desease +)



Case 12: serous carcinoma ("SEIC with SEC"), pT1A, FIGO IA (peritoneal washing cytology +)

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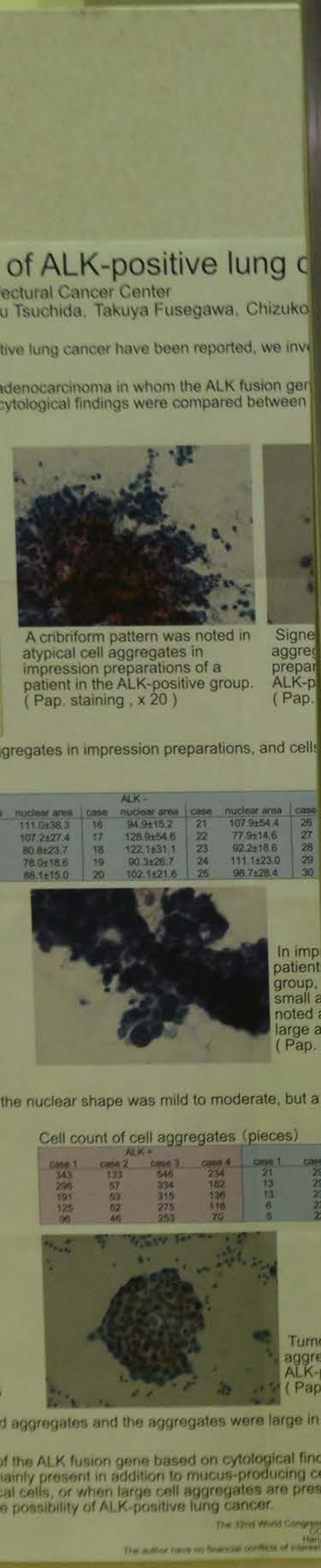

Cytology

PF-07

The examination of washing fluid, washing cytology of urinary tract

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Objectives

The washing cytology from urinary tract is useful method for diagnosis of tumor localization and spread. However, the cell morphology was degenerated because of washing by physiological saline. Therefore, it is difficult to observe the cell morphology, and we often experience the cases to rack its brains about differentiation of benign or malignancy for microscopic examination.
We report that performed the examination about using infusion preparation as washing fluid of urinary tract.

Materials and Methods

The materials were organs removed surgically in 2013(Table 1). It was washed by infusion preparation(Table 2), and prepared a cytodiagnosis specimen from a cell suspension. We observed it mainly on nuclear findings of the cell and evaluated the cytopathic degree and examined suitable infusion preparation as washing fluid of the urinary tract(Fig.1).

Table 1 Materials

Case No.	Gender / Age	Pathological diagnosis	Sample: Lymph node	Sample: Tumor	Sample: Non-tumor
1	F / 57	IDC(breast)	●		
2	F / 69	IDC(breast)	●		
3	F / 66	IDC(breast)	●		
4	F / 37	IDC(breast)	●		
5	F / 40	IDC(breast)	●		
6	M / 84	Adenocarcinoma_tub1(Colon)	●		
7	M / 71	Adenocarcinoma_tub2(Colon)	●		
8	M / 69	Adenocarcinoma_tub2(Colon)	●		
9	M / 80	Adenocarcinoma_tub2(Colon)	●		
10	F / 64	Adenocarcinoma_tub2(Colon)	●		
11	M / 82	UC,HG(Renal pelvis)		●	●
12	M / 67	UC,HG(Ureter)		●	●
13	M / 72	UC,LG(Renal pelvis)		●	●
14	F / 78	UC,LG(Ureter)		●	

Table 2 Infusion preparations

Category	Name	Component: NaCl	KCl	$CaCl_2$	Other	pH	Osmotic pressure (approx.)	Use
Physiological saline	Physiological saline	●				4.5～8.0	1	Supplement and revision of the cell external solution
Acetic acid Ringer's solution	Veen-F Inj.	●	●	●	Sodium acetate	6.5～7.5	1	Supplement of the cell external solution Metabolic acidotic revision
	Veen-D Inj.	●	●	●	Sodium acetate Glucose	4.0～6.5	2	Supplement of the cell external solution Metabolic acidotic revision Supply of the energy
Lactic acid Ringer's solution	Lactec Inj.	●	●	●	Sodium lactate	6.7	0.9	Supplement of the cell external solution Metabolic acidotic revision
	Lactec D Inj.	●	●	●	Sodium lactate Glucose	4.9	2	Supplement of the cell external solution Metabolic acidotic revision Supply of the energy
Electrolyte solution	Solita-T No.1	●			Sodium lactate Glucose	3.5～6.5	1	Supply of water and the electrolyte
	Solita-T No.3	●	●		Sodium lactate Glucose	3.5～6.5	1	Supply and maintenance of water and the electrolyte
Plasma-substitute	Hespander fluid	●	●	●	Sodium lactate Glucose Hydroxyethylated starch	7.4	0.9	Blood wash in the extracorporeal circulation

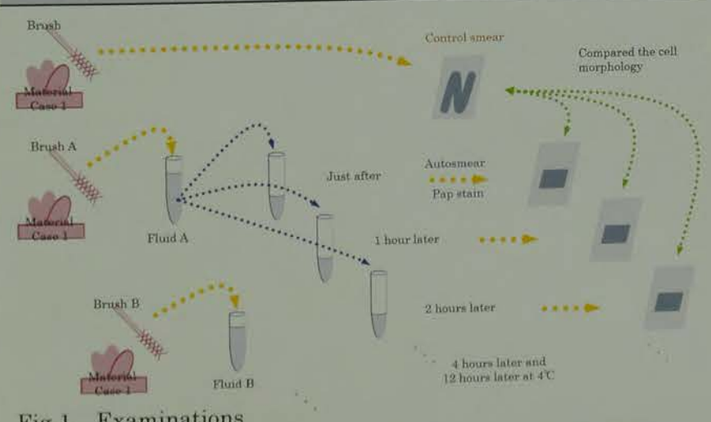


Fig.1 Examinations

Evaluation

1. Evaluation of Nuclear Area (case 1-10)
The nuclear area of lymphocytes was measured by image analysis software, and made the scatter chart for the distribution of the nuclear area(Fig.2).

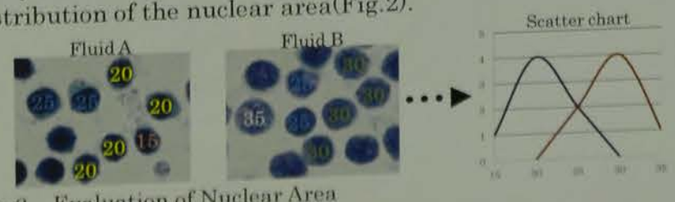


Fig.2 Evaluation of Nuclear Area

2. Evaluation of Nuclear Findings (case 11-14)
We observed nuclear findings in comparison with the cells of the control smear, and classified cells in three types from the cytopathic degree. We made a distribution map from the classification data (Fig.3).

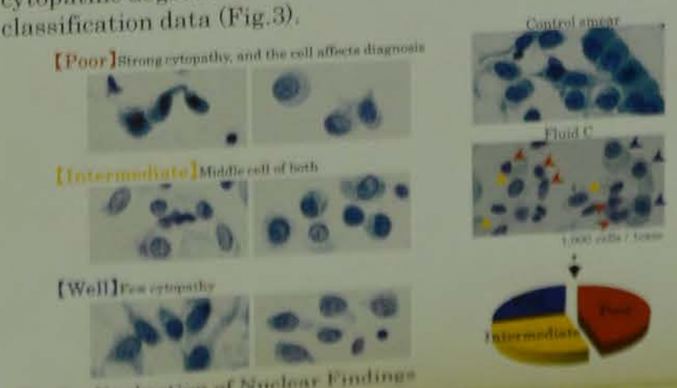
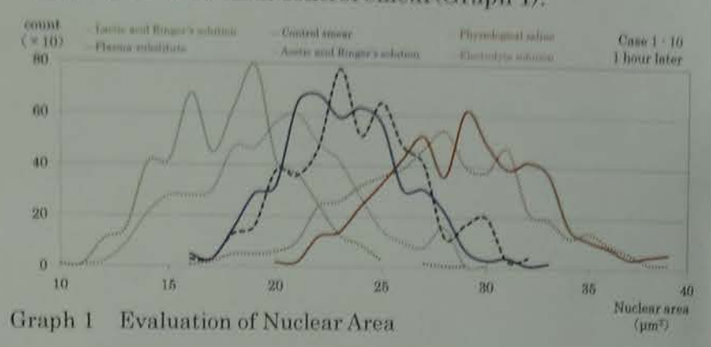


Fig.3 Evaluation of Nuclear Findings

Results

1. Evaluation of Nuclear Area
In the acetic acid Ringer's solution, the nuclear area was approximately equal to control smear. The physiological saline and electrolyte solutions, a nuclear area became large.
The lactic acid Ringer's solution and plasma-substitute tended to become smaller than control smear(Graph 1).



Graph 1 Evaluation of Nuclear Area

2-1). Evaluation of Nuclear Findings
In the control smear and the acetic acid Ringer's solution, a nucleolus is clear, and chromatin is form of granule. The physiological saline and electrolyte solution, chromatin structure is indistinct. As for the lactic acid Ringer's solution and the plasma-substitute, nuclear form is irregular, and chromatin structure is indistinct(Fig.4 & Graph 1).

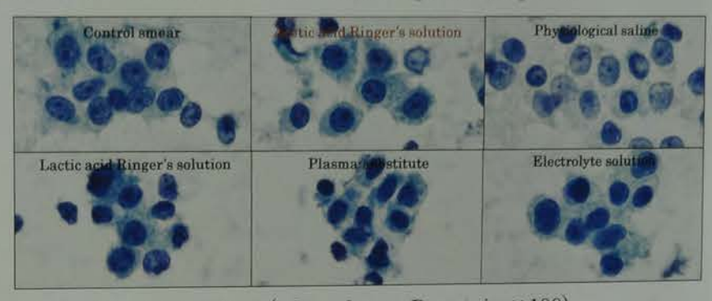
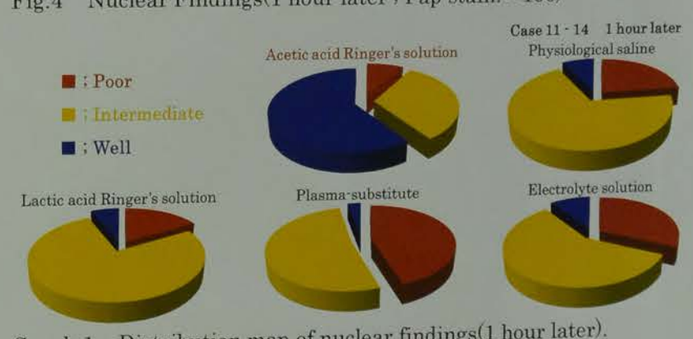


Fig.4 Nuclear Findings(1 hour later ; Pap stain. × 100)



Graph 1 Distribution map of nuclear findings(1 hour later).

2-2). The cell morphologic change with time
In two kinds of different acetic acid Ringer's solutions of the glucose addition, there was cytomorphologic change by the progress time. In the Veen-F, a diagnosis became slightly difficult in 3 hours specimen because the cytopathy was strong. But, in the Veen-D, there is little cytopathy in 3 hours at room temperature, and a diagnosis did not include the trouble.
In 4 degrees Celsius, 12 hours, it was a diagnosable specimen(Fig.5 & Graph 2).

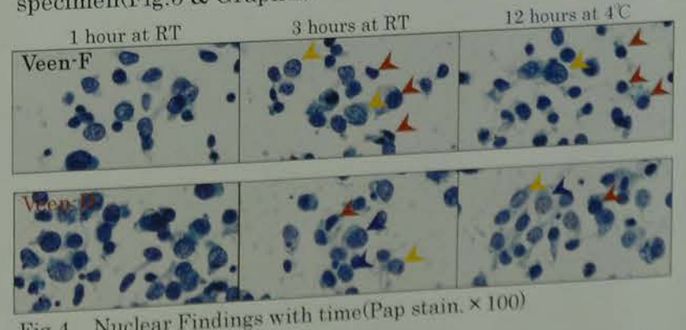
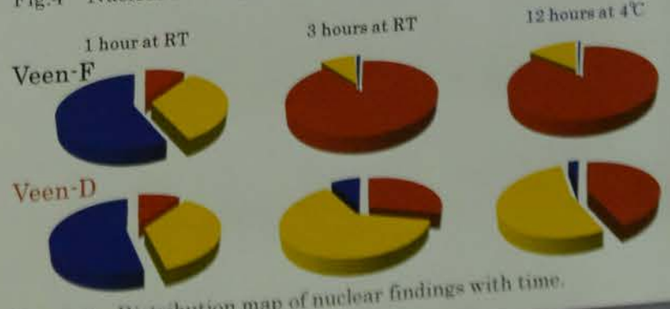


Fig.4 Nuclear Findings with time(Pap stain. × 100)



Graph 2 Distribution map of nuclear findings with time.

Conclusion

The infusion preparation which had good cell shape was an acetic acid Ringer's solutions. It was possible to prepare the specimen for a diagnosis without an influence. We regard the use of the acetic acid Ringer's solution(Veen-D) to urinary tract washing cytodiagnosis as a valuable method.

Cytology

PF-08

Study on the effectiveness of on site diagnosis and sample production in EUS-FNA

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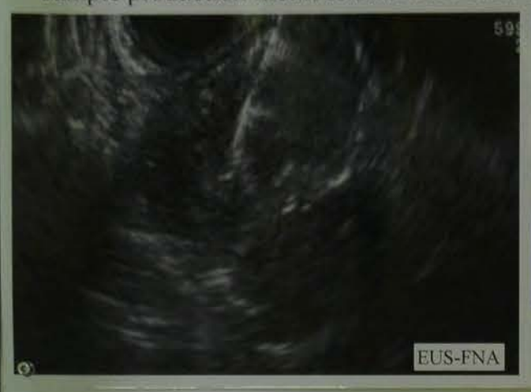
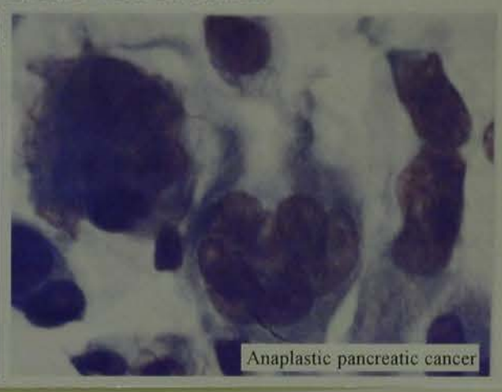
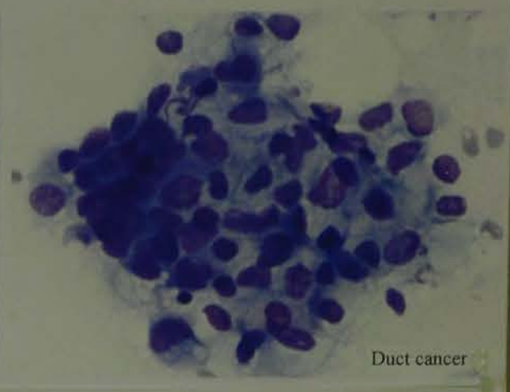
Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) was applied to the diagnosis for confirming a duodenal or pancreatic tumor, and it now takes a central role in this field. At present, many facilities conduct the so-called on site diagnosis, in which a cytotechnologist carries out cytoscreening and sample production at the on site of a patient in an endoscopic room for EUS-FNA. In this study, we discussed the cell forms of a pancreatic tumor and the sample production methods for Diff-Quik stained samples and immunohistochemically stained samples.

II .Subjects and Methods

We studied 10 cases of pancreatic duct cancer, 4 cases of pancreatic endocrine tumor, 1 case of acinar cell cancer, 2 cases of STPT, and 1 case of anaplastic pancreatic cancer for which EUS-FNA was conducted in a period from January 2013 to December 2015. The Diff-Quick stained cell forms in each tumor (chromatin increase, nucleoli, glandular cavity formation, mucus production, trichorrhexis, polarity, background, formation of multiple nuclei, acinar structure, vessel axis, etc.) were studied, and sample production methods suited for each disease were discussed.

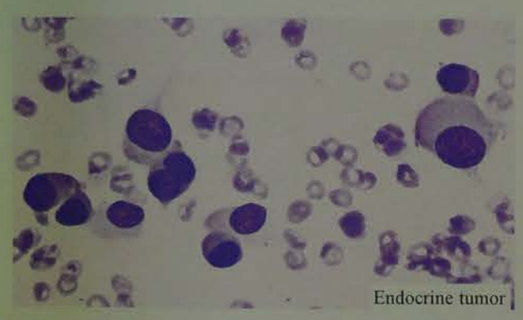

EUS-FNA

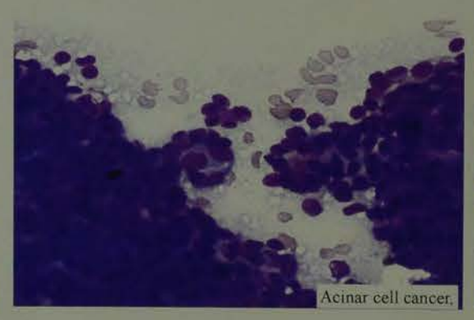

Anaplastic pancreatic cancer

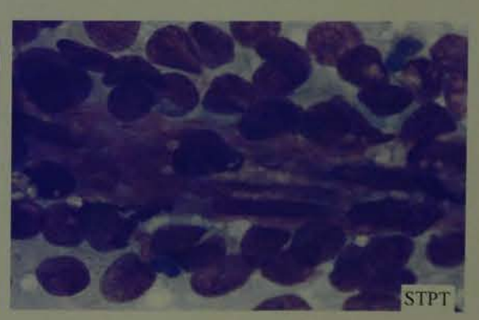

Duct cancer

III.Results

Chromatin increase, clear nucleoli, trichorrhexis, and polarity disturbance were observed in all cases. Glandular cavity formation was detected in 5 cases of pancreatic duct cancer, 4 cases of mucus production, 4 cases of poor background, and 1 case of the formation of multiple nuclei. For anaplastic pancreatic cancer, all of them were observed. Salt-and-pepper-like chromatin, acinar structure, and spindle cells were found in all cases of pancreatic endocrine tumor, STPT, and acinar cell cancer. The vessel axis was detected in the cases of pancreatic endocrine tumor and STPT.

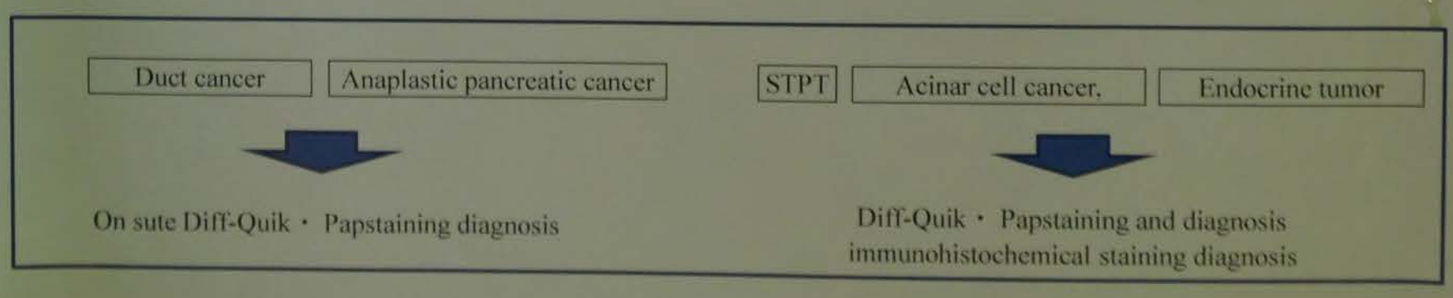

Endocrine tumor


Acinar cell cancer,


STPT

IV.Discussion

Since mucus production whose cellular atypism is high, polarity disturbance, and glandular cavity formation were observed in many cases of pancreatic duct cancer and anaplastic pancreatic cancer, it is considered that the on sitr Diff-Quik staining diagnosis is effective. It was considered difficult to identify pancreatic endocrine tumor, STPT, and acinar cell cancer with the on site Diff-Quik staining diagnosis, although it can be confirmed with this diagnosis that the disease is not pancreatic duct cancer or anaplastic pancreatic cancer. If a patient is suspected of having a disease other than pancreatic duct cancer as a result of the on site Diff-Quik staining, it will be necessary to conduct immunohistochemical staining to identify the disease. Therefore, it was considered necessary to produce LBC and cell blocks.



V .Conclusion

On site Diff-Quik staining diagnosis by cytotechnologists in EUS-FNA is expected to enable sample production suited for each disease and reduce the number of times of puncture and the burden on patients.

Cytology

PF-09

A Case of Mammary Analogue Secretory Carcinoma which Diagnosis was Supported by Cell Block Preparation.

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Background

Mammary analogue secretory carcinoma (MASC) is a recently recognized salivary gland tumor which harbors t(12;15)(p13;q25) ETV6-NTRK3 translocation[1)] . Histological features share with the secretory carcinoma of the breast. We report a case of MASC of the upper lip of which diagnosis was supported by cell block preparation from fine needle aspiration biopsy.

Clinical history

A 76-years-old woman noticed a tumor on the left upper lip. She had a history of invasive carcinoma of the right breast 18 years ago. Mucus retention cyst was thought and fine needle aspiration cytology (FNA) was performed. After cytological diagnosis was obtained, tumor excision was done.

Cytological findings

The specimen was viscous liquid. First, smear with Papanicolaou and Giemsa staining was prepared from the specimen. Then, from the remaining specimen, cell block was made (figure1) .

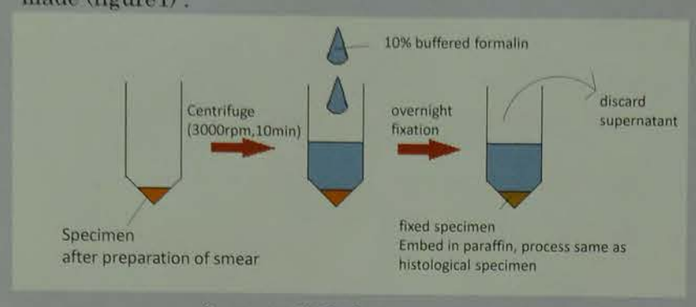

figure 1 cell block preparation

In pap smear, tumor cells displayed various pattern. They were composed of papillary, microcystic, and aggregation of signet ring cell like cells structures (fig. 2a, b, c). Mucus like material showed metachromasia in the Giemsa stain (fig. 2d).

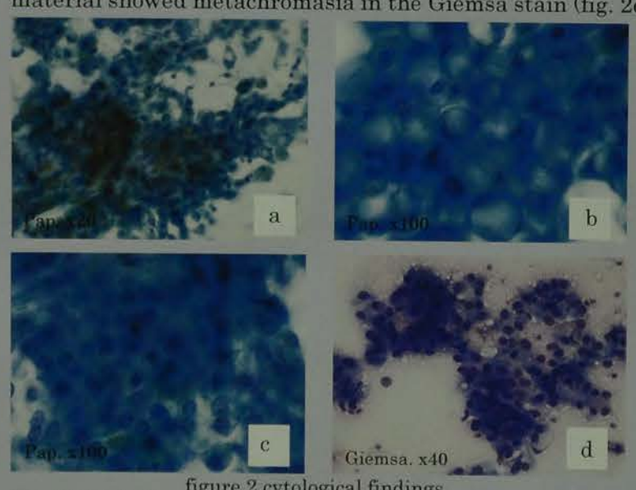

figure 2 cytological findings

The Cell block

Histopathology of cell block showed papillary cell clusters and aggregation of signet ring cell like cells in the mucus-like material (fig. 3a, b), likely to be corresponding the cytological findings. Panels of immunohistochemistry were done (fig. 3c, 3d, table1).

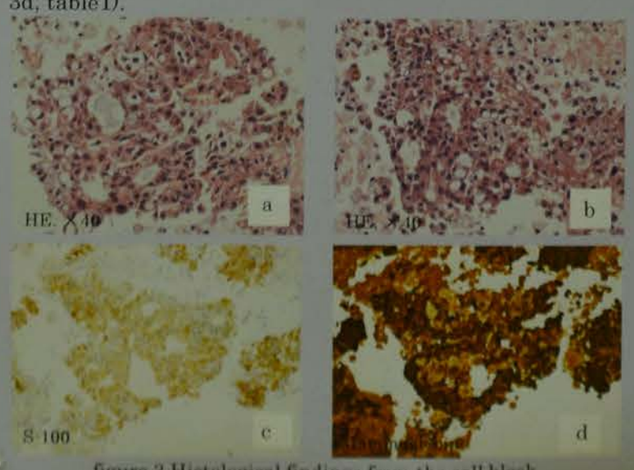

figure 3 Histological findings from the cell block

Positive	Equivocal	Negative
S-100	Ck5/14	CDX2
Mammaglobin	α-SMA	Villin
SOX-10	P40	TTF-1
GCDFP15 (focal)		PAX8
GATA3		ER
Ck7		
Ck AE1/AE3		

Table.1 Immunohistochemistry of the cell block

Histological findings

Tumor was measured 12x10 mm. Grossly, fibrous capsule of the tumor was not observed. Histologically, tumor was invading into adjacent skeletal muscle and connective tissue (fig. 4a, b). Histopathology of the tumor cells was the identical to the cell block preparation (fig. 4c, d). Immunohistochemical features were identical to those of cell block.

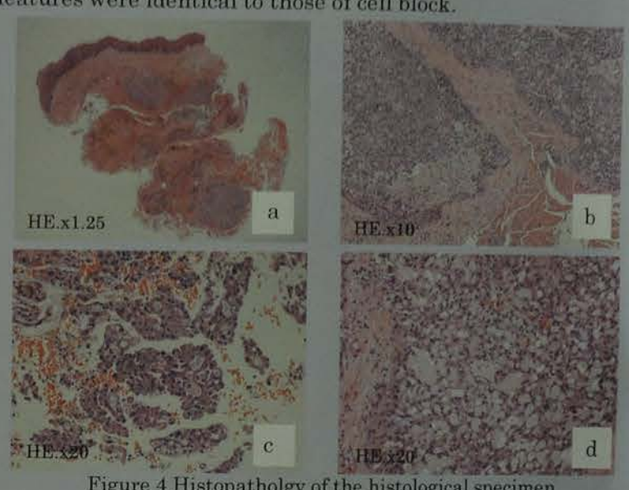

Figure 4 Histopatholgy of the histological specimen

RT-PCR analysis

RT-PCR for ETV-NTRK3 fusion transcript was performed by Dr. Toshitaka Nagao at Department of Pathology, Tokyo Medical University, as previously described in the literature[1)]. RNA was extracted from histological section. Size of the PCR product is 110bp.

M: DNA Marker (100, 200, 300bp from the bottom)
Lane 1: Negative control
Lane 2: This case
Lane 3: This case (duplicate)
Lane 4: Positive control

Figure 5 RT-PCR for ETV6-NTRK3

Diagnosis

Mammary analogue secretory carcinoma of the upper lip.

Discussion

In this case, there was excess specimen which made possible to process the cell block. The cytological findings seem to be typical for MASC, however, the differential diagnosis of acinic cell carcinoma remains[2,3)]. Preparation of the cell block allowed us to perform immunohistochemisty, resulted in diffuse S-100 and mammaglobin staining of tumor cells which indicated the possibility of MASC[4)]. Literally, the utility of cell block for ETV6 FISH analysis is described[4)]. From this case, we regard the cell block preparation from FNA specimen would contribute to diagnosis for MASC not only by molecular analysis, but also by histological and immunohistochemical examination. Thus the preparation of the cell block is recommended in the FNA specimen of the salivary gland.

Acknowledgement

We thank Dr. Toshitaka Nagao, Department of Pathology, Tokyo Medical University for giving us histopathological advice and performing RT-PCR analysis.

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2) Cancer Cytopathol 2013;121:228-233.
3) Cancer Cytopathol 2013;121:234-241.
4) Mod Pathol 2014;27:30-37.

Cytology

PF-10

Diagnosis of metastatic pancreatic leiomyosarcoma by EUS-FNA; a case report

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【Introduction】

Primary and metastatic malignant mesenchymal tumors of the pancreas are rare, and a few reports about the metastatic pancreatic leiomyosarcoma diagnosed by EUS-FNA have been published. Herein, we present a case of metastatic pancreatic leiomyosarcoma diagnosed by EUS-FNA.

【Case history】

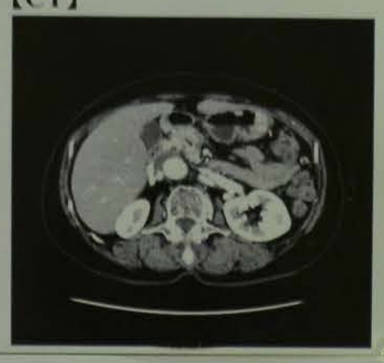
A 70-year-old woman was admitted to our hospital for evaluation of the pulmonary multiple nodular lesions. Computed tomography (CT) - guided needle biopsy of the pulmonary tumoral nodules were performed, and diagnosed with the leiomyosarcoma. The patient received segmentectomy and radiofrequency ablation (RFA). During follow up, the lesion of pancreas tail was detected by CT. EUS-FNA was performed.

【Clinical laboratory】

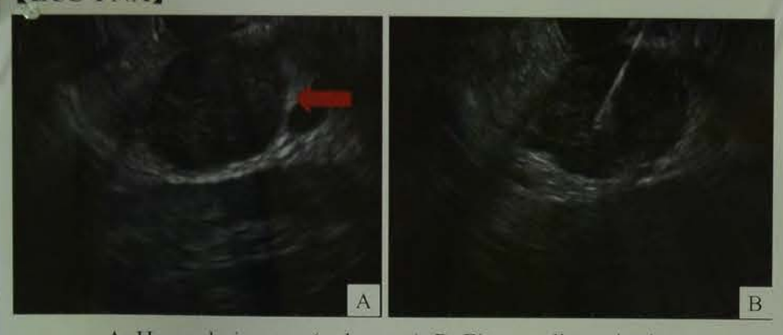
WBC	9104	/μl	BUN	14	mg/dl
RBC	3.96×10⁴	/μl	Cre	0.53	mg/dl
Hb	11.6	g/dl	Glu	102	mg/dl
Ht	34.7	%	Na	139	mEq/l
Plt	9.8×10⁴	/μl	K	4.8	mEq/l
			Cl	109	mEq/l
TP	6.0	g/dl	CRP	0.28	mg/dl
Alb	3.3	g/dl	T-Chol	176	mg/dl
T-Bil	1.3	mg/dl	TG	77	mg/dl
AST	33	IU/l	ChE	261	IU/l
ALT	14	IU/l	CEA	1.3	ng/ml
LDH	382	IU/l	CA19-9	5.4	U/ml
ALP	36	IU/l	SPAN-1	<1.0	ng/dl
g-GTP	20	IU/l	DUPAN-2	<25	U/ml
AMY	79	IU/l			
Lipase	58	U/l			

*Red; high, Blue; low

【CT】

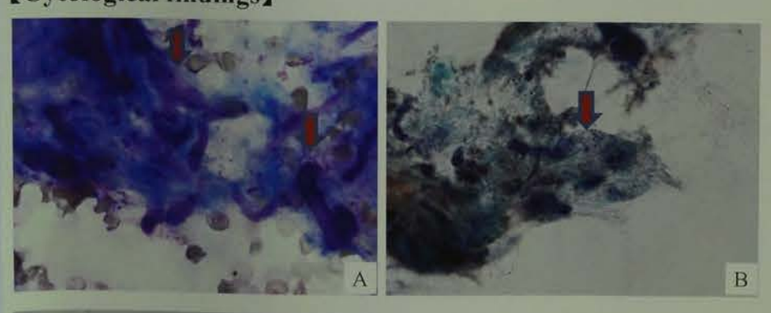


【EUS-FNA】



A: Hypoechoic mass (red arrow), B: Fine needle aspiration

【Cytological findings】

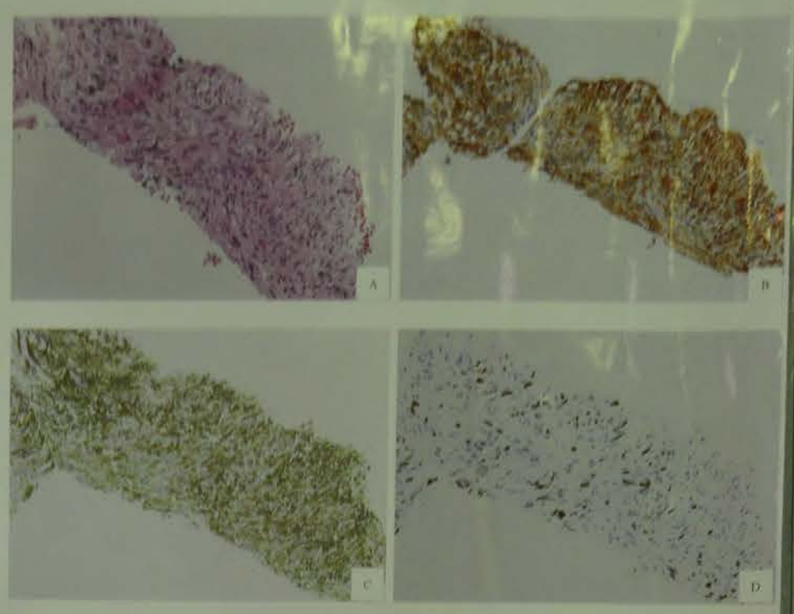


A: Scattered spindle cells with large nuclei (red arrow) (Diff-Quik. ×40)

B: Tumor cells with multiple small nucleoli (red arrow) (Pap. ×40)

C: Loose arrangement of tumor cells with blunt-end nuclei (red arrow) (Pap. ×40)

【Histological findings; cell block】



A: Tumor cells showed fascicles of spindle cells. (HE. ×20)

B: Positive for α-SMA, immunohistochemistry (×20)

C: Negative for c-kit, immunohistochemistry (×20)

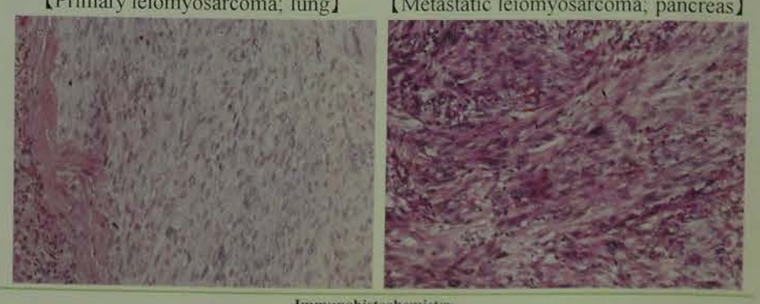
D: MIB-1 was indicated 20%; immunohistochemistry (×20)

【Surgical resections】

A: Smooth mass with elastic and hard in pancreatic tail (red arrow)
B: Membrane-covered grayish white solid mass
C: : Tumor cells showed growth of spindle cells.(HE. ×20)
Immunohistochemistry
α-SMA (+), desmin (+), CD34 (-), c-kit (-), s-100 (-)

【Comparison of histological findings and immunohistochemistry】

【Primary leiomyosarcoma; lung】 【Metastatic leiomyosarcoma; pancreas】



Immunohistochemistry	
【Primary leiomyosarcoma; lung】	【Metastatic leiomyosarcoma; pancreas】
αSMA(+), desmin(+)	αSMA(+), desmin(+)
MIB-1 index 20%	MIB-1 index 20%
CD34(-), c-kit(-), s100(±)	CD34(-), c-kit(-), s-100(-)

【Discussion】

Pancreatic metastasis of malignant tumors are 2-3% of the pancreatic tumor. Pancreatic metastasis of leiomyosarcoma is extremely rare. In this case, EUS-FNA allowed the differential diagnosis with other mesenchymal tumors, such as gastrointestinal stromal tumor, schwannoma. In addition, our case demonstrated that a metastatic pancreatic leiomyosarcoma can yield diagnostic lesional material by EUS-FNA with a small needle.

We presented a case of metastatic pancreatic leiomyosarcoma diagnosed by EUS-FNA. EUS-FNA is useful in the diagnosis of metastatic pancreatic leiomyosarcoma.

【Reference】

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Cytology

PF-11

The breast pleomorphic lobular carcinoma with eosinophilic cytoplasm: A case report.

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【Introduction】

Pleomorphic lobular carcinoma (PLC) of a breast has variants that showed apocrine or histiocytoid differentiation and composed of signet ring cells. They are worse prognosis than classical invasive lobular carcinoma. Therefore, early diagnosis is required. We report the differentiation points from other tumors with apocrine metaplasia including review of our case.

【Clinical findings】

- A 51-year-old woman noticed the mass on her right breast.
- 3 years later, she found that the mass was enlarged. A core needle biopsy (CNB) was performed and reported as invasive lobular carcinoma at the former hospital.
- Incidentally, another mass was found on her left breast during the examination of the right breast tumor.
- She underwent fine needle aspiration biopsy (FNAB) and CNB of her left breast at our hospital.
- Partial resection of left breast was done.

Diagnosis on FNAB :

"indeterminate"

Diagnosis on CNB :

"Pleomorphic lobular carcinoma in situ"

【Cytological findings】

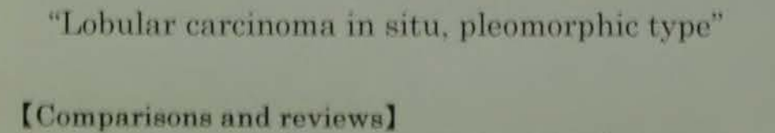

Figure 1 A, The atypical cells were consisted of small clusters and isolated cells (Pap,x10). B, The tumor cells were poorly cohesive (Pap,x20). C, The atypical cells had eosinophilic granular cytoplasm and enlarged nucleoli like apocrine differentiation. The nuclei showed mild irregularities and hyperchromatic (Pap,x100).

- Benign tumors were suspected for the diagnosis because of the mild cellular atypia and apocrine differentiation.
- However, it was difficult to completely deny apocrine carcinoma because of hyperchromatic and poorly cohesive.

Differential diagnosis on FNAB:

"Ductal adenoma" or "Apocrine carcinoma"

【Pathological findings】

Figure 2. A, Lobular units expanded by the tumor cells (HE,x4). B, The tumor cells had eosinophilic cytoplasm and enlarged nucleoli (HE,x40). C, The tumor cells were negative (E-cadherin,x20). D, The tumor cells were positive (GCDFP-15,x20).

- The tumor cells with eosinophilic cytoplasm and enlarged nucleoli like apocrine differentiation proliferated intraductally.
- Immunohistochemically the tumor cells were negative for E-cadherin, positive for GCDFP-15.

Final diagnosis :

"Lobular carcinoma in situ, pleomorphic type"

【Comparisons and reviews】

In our case, we studied about these points : 1) cell appearance pattern, 2) cell morphology, 3) nuclei findings, 4) other findings, in order to identify as lobular carcinoma (Figure 4,5,6,7). In addition, we considered the differentiation points from other tumors with apocrine mataplasia (Table 1and 2, Figure 8).

1) Cell appearance pattern

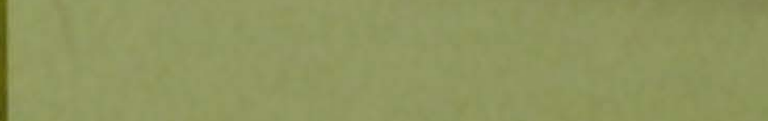

Figure 4. A, The tumor cells appeared small clusters and isolated cells (Pap,x10). B, The tumor cells appeared mild layers clusters and isolated cells (Pap,x10). C, The tumor cells appeared three dimensional clusters and isolated cells (Pap,x10).

2) Cell morphology

Figure 5. A, The tumor cells were small. The cytoplasm was round and relatively abundant (Pap,x40). B, The tumor cells were large. The cytoplasm was polygon and abundant (Pap,x40). C, The tumor cells were large. The cytoplasm was polygon and abundant (Pap,x40).

3) Nuclei findings

Figure 6. A, The nuclei showed mild irregular. The chromatin showed finely granular to granular (Pap,x100). B, The nuclei were enlarged. It had prominent nucleoli and granular chromatin (Pap,x100). C, The nuclei showed irregular. The chromatin was granular (Pap,x100).

4) Other findings

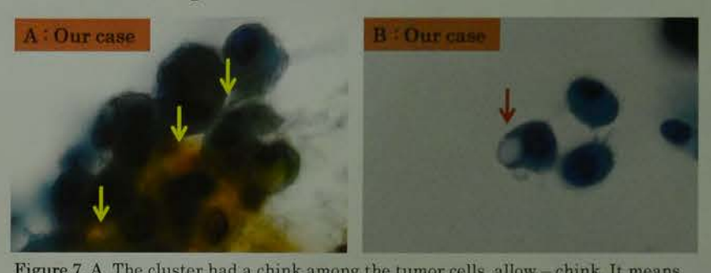

Figure 7. A, The cluster had a chink among the tumor cells, allow – chink. It means poorly cohesive (Pap,x100). B, Some of the tumor cells had intracytoplasmic lumina (ICL), allow – ICL (Pap,x100).

Table 1 The differentiation points

	Cell appearance	Cell morphology		Nuclei findings		Other findings
		Shape	Size	Atypia	Chromatin	
Our case	Small clusters to isolated cells	Round	Small	+	Finely granular to granular	A chunk among the tumor cells : ICL
PLC apocrine differentiation[1]	Isolated cells	Round		+	Finely granular	ICL
Ductal adenoma	Mild layers clusters to isolated cells	Polygon	Large	+	Granular	
Apocrine carcinoma	Three-dimensional clusters to isolated cells	Polygon	Large	+	Granular	

1) T.Tashiro et al:Acta Cytol.2003;47:265-269

Table 2 Immunohistochemistry

		E-cadherin	GCDFP-15	ER	PR	HER2	MIB-1 index
Our case		–	+	–	–	–[2]	8%
PLC apocrine differentiation	Mamatha C[3]	– (12/12)		+ (11/12)	+ (6/12)	+ (3/12)	Moderate to high (11/12)
	T.Tashiro[4]	–	+				
Ductal adenoma		+	+				
Apocrine carcinoma		+[5]	+[6]	–[6]	–[6]	+[6]	

2) Reference level from pleomorphic lobular carcinoma in situ
3) Am J Surg Pathol.2008;32:1721-1726
4) Acta Cytol.2003;47:265-269
5) Arch Pathol Lab Med.2013;137:1688-1692
6) WHO Classification of Tumours of the Breast 4th Edition

Figure 8. E-cadherin is useful for differentiation of PLC and apocrine carcinoma. A. The tumor cells were negative (E-cadherin,x40). B. The tumor cells were positive (E-cadherin,x40).

- The tumor cells of our case was characterized by small round cells showed poorly cohesive. In addition, the tumor cells had intracytoplasmic lumina (ICL) and showed negative for E-cadherin.
- We considered that these findings seen our case were useful as the differentiation points from other tumors with apocrine metaplasia.

【Conclusions】

- The tumor cells of PLC were characterized by eosinophilic cytoplasm like apocrine metaplasia and prominent nucleoli.
- The differentiation from other tumors with apocrine metaplasia is necessary.
- Immunocytochemistry may help the diagnosis. Because, the diagnosis of PLC is difficult only in cytological findings.
- Therefore, it is necessary to leave diagnosis in "indeterminate".

Cytology

PF-12

Small Cell Carcinoma Combined with Urothelial Carcinoma and Adenocarcinoma of the Urinary Bladder

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I. Background

Small cell carcinoma of the bladder (SCCB) is very rare and only 0.7% of all tumors of the urinary bladder. SCCB must be differentiated from urothelial carcinoma(UC) or malignant lymphoma. We report a case of SCCB coexisted with other types of carcinomas.

II. Case

A 73-year-old male was admitted to the Okayama Saiseikai General Hospital with asymptomatic gross hematuria. An urethrocystoscopy revealed a broad-based papillary mass, 3 cm in diameter on the right wall of bladder.

The cytology of voided urine specimen showed pleomorphic cells with coarsely granular chromatin, and thick chromophilic light-green cytoplasm, characteristic for UC (Figure 1, 3). However, the histology of the tumor removed by trans-urethral resection (TUR) showed high grade UC, coexisted with small cell carcinoma, and also adenocarcinoma (Figure 4).

Immunohistochemical study of the resected tumor showed that small cells were CD56 (+), synaptophysin (+), chromogranin A (partially +), and Ki-67 labeling index was over 80 % (Figure 5). In retrospect, the cytology of the urine specimen revealed the presence of atypical small cells (Figure 1, 2). Adenocarcinoma could not be identified in cytology, possibly because of the morphological resemblance to UC cells.

The patient received a drug regimen for his cancer. However, 13 months after surgery, metastatic tumor was found in his lung. Also, 37 months later, recurrent tumor was identified in the urinary bladder, and the histology of this tumor showed only high grade UC. After that, extensive metastases developed in the brain, lung and ilium. He expired 47 months after the surgery.

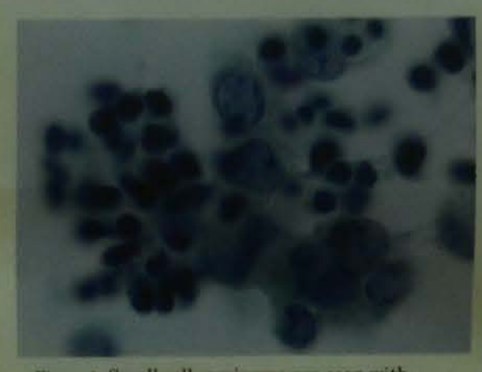

Figure.1 Small cell carcinoma are seen with high grade UC in the cytology of voided urine specimen (Pap. Stain, ×100).

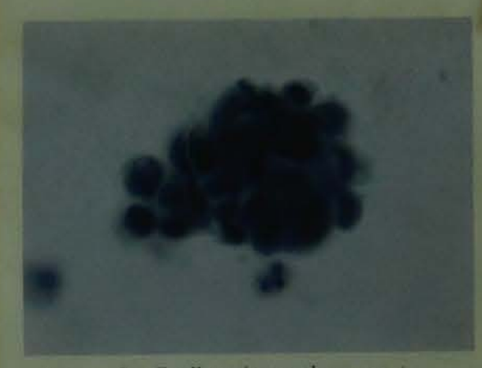

Figure. 2 Small cell carcinoma shows scant cytoplasm, nuclear molding, and fine chromatin (Pap.Stain, ×100).

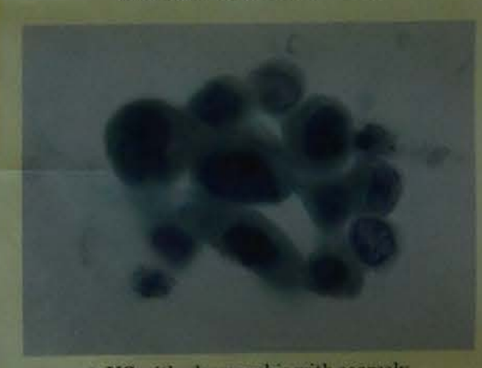

Figure.3 UC with pleomorphic with coarsely granular chromatin, and thick chromophilic light-green cytoplasm (Pap.Stain, ×100).

Figure.4 The histology of the specimen by TUR showed high grade UC, together with small cell carcinoma, and also adenocarcinoma. (HE. Stain).

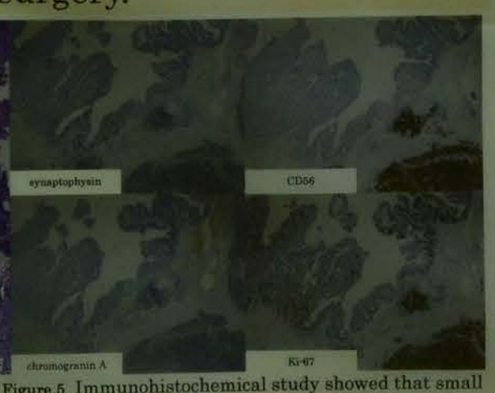

Figure.5 Immunohistochemical study showed that small cells were synaptophysin (+), CD56(+), chromograninA (partially +), and Ki-67 labeling index was over 80 % .

III. Discussion

Several hypotheses have been reported as for the origin of SCCB. Podesta[2] suggested the possibility of divergent differentiation of multipotential tumor cell. Other theories include malignant change of neuroendocrine cells[3], or urothelial cell metaplasia[4]. Since our case had the coexistence of three types of cancer, divergent differentiation is most likely.

The presence of SCCB coexisted with UC were found in 68%, while SCCB coexisted with UC and adenocarcinoma were found only in 4.7%[5]. SCCB is much more aggressive and likely to show extensive metastasis than UC. Median survival of patients with SCCB is around 20 months[6].

In our case, terminal metastases to various organs were possibly due to SCCB, although not confirmed. Since the small cell carcinoma can coexist in 0.7 % of UC, we must be careful not to overlook SCCB in urinary specimens, because the prognosis of such patient will be quite serious.

IV. Conclusions

A case of SCCB coexisted with UC and adenocarcinoma in the urinary bladder was reported. In cytology smears of voided urine, the presence of atypical small cells with other types of tumor requires careful consideration, because the prognosis of SCCB coexisted with UC is very poor than pure UC.

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Cytology

PF-13

Usefulness of intraoperative rapid immunocytochemistry

- A case of pineal germinoma diagnosed by intraoperative histology and cytology -

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[2)]Dept. of Integrative Pathology of Nippon Medical School

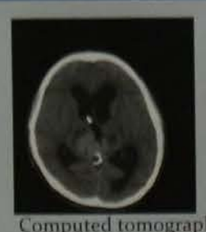
【 Introduction 】

Intraoperative rapid diagnosis of various brain tumors has become a routine practice in pathology departments and often proves to be challenging when only a frozen histological specimen is available. On the contrary, cytology specimens are basically free from freezing artifacts, which are often observed in frozen histology specimens, enabling detailed observation of individual cells.
We herein report a case of pineal gland germinoma diagnosed by intraoperative combined analysis on histology and cytology with rapid immunocytochemistry and introduce briefly our intraoperative immunocytochemical staining system.

【 Case 】

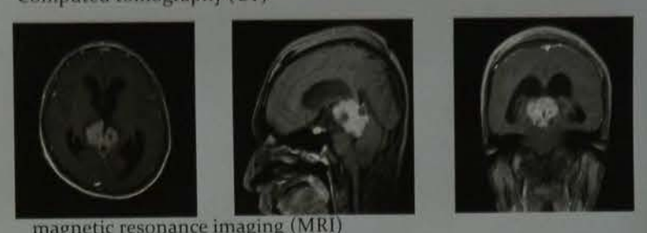
An 18-year-old Japanese male consulted a local hospital with chief complaints of headache and anorexia. Brain CT revealed a pineal tumor with a diameter of 4 cm causing severe obstructive hydrocephalus when he was referred to our hospital. An operation was performed for primary treatment and pathological diagnosis.

【 Imaging 】



Computed tomography (CT) of the head showed a 44mm hyperdense pineal gland mass, suggesting Pineal germinoma, Pinealocystic tumor

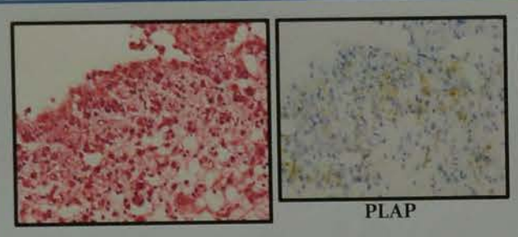
Computed tomography (CT)



magnetic resonance imaging (MRI)

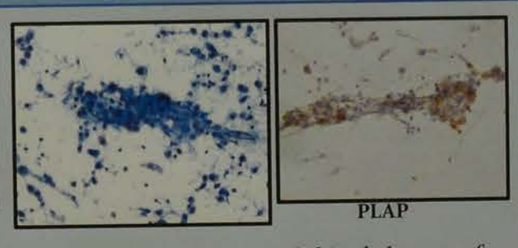
MRI of the head showed hydrocephalus with expanded lateral and third ventricles.
Also cyst with mass were hyperintensity on T2, suggesting Pineal germinoma, hydrocephalus

【 Intraoperative histo-cytological findings 】



PLAP

A rapid H-E histology specimen of frozen section revealed a sheet-like proliferation of atypical polygonal cells with mature small lymphocytes forming a “two-cell” pattern.
LCA(-), PLAP(weakly+)

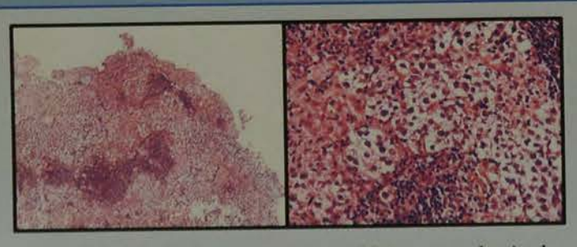


PLAP

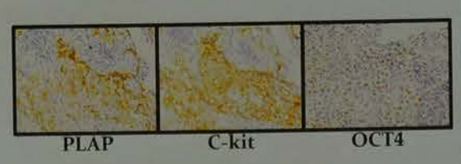
Intraoperative cytology exhibited clusters of ovoid cells with prominent nucleoli in the background of mature small lymphocytes. Scattered polygonal cells showing loose cohesiveness were also observed.
LCA(-), PLAP(+)

Intraoperative diagnosis : **Germinoma**

【 Postoperative permanent pathological diagnosis 】 【Immunochemistry 】



In addition to above-mentioned histo-cytological findings, epithelioid cell granulomas were also observed in the vicinity of the tumor cells.
With a diagnostic finding of Oct-4 positivity in formalin-fixed permanent section, our final diagnosis was pineal germinoma.



PLAP C-kit OCT4

Positive	Negative
PLAP	HCG
C-kit	ALP
OCT4	CEA
AE1/AE3	
EMA（a part）	

【 Rapid immunostaining Protocol 】

1 Fixation.
1) Cytological samples; 95% ethanol, 1 minute.
2) Frozen sections; mixture of formalin (90ml), 100% ethanol (10ml), and acetic acid (1ml), 1 minute.
After fixation, rinse gently with distilled water with a wash bottle.
Place in fresh buffer bath.

2 Apply primary antibody for 3 minutes.
(Negative control in buffer bath.)
Rinse gently with buffer from a wash bottle.

3 Apply Polymer/HRP for 3 minutes.
Rinse slides as Step 5.

4 Color development with diaminobenzidine for 1 minutes.
Rinse gently with distilled water.

5 Counterstaining with hematoxylin.

6 Mounting and judgment, following dehydration and remounting.

✱ Buffer: Tris buffer saline with Tween-20

The time required,
it is **only ten minutes.**

Doesn't need a special tools!!

【 Conclusions 】

- We have presented a case of pineal germinoma diagnosed by both histology and cytology using intraoperative rapid immunocytochemistry.
- Immunocytochemistry has usually a higher sensitivity than that of immunohistochemistry and often provides valuable information required for a correct diagnosis.
- The methodology of intraoperative immunocytochemistry is neither extremely difficult nor complicated as will be exhibited in our poster presentation.
- Intraoperative combined analysis on histology and cytology with rapid immunocytochemistry, therefore, is regarded as an effective and practical tool for a routine intraoperative rapid diagnosis.

The 32nd World Congress of Biomedical Laboratory Science
COI Disclosure
Name of First Author: Yumi Yanagida

The author has no conflict of interest to disclose with respect to this presentation

Cytology

PF-14

A Study on Pregnancy Rate and Miscarriage Rate According to Morphology of Transferred Blastocysts in Single Frozen Blastocyst Transfer.

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【Introduction】

For the purpose of improvement of the pregnancy rate, prevention of the multiple pregnancy and ovarian hyperstimulation syndrome, we ordinary conduct a single frozen blastocyst transfer.

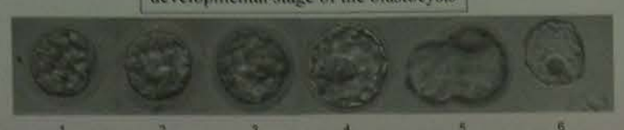
We examined the pregnancy rate(PR) and the miscarriage rate(MCR) in single frozen blastocyst transfer according to the morphology of the transferred blastocysts.

【Materials and Methods】

During the period of January 2007 to December 2015,a total of 1,404 single frozen blastocyst transfer cycles were subjected to this study. Blastocysts were graded before transfer according to Gardner's grading criteria[developmental stage of the blastocysts(Grade1～6),grade of inner cell mass(ICM) and trophectderm cell(TE)(A,B,C)]. In this study, we ruled out developmental stage Grade1 and 2 brastocysts because there were no pregnancy cases.

Gardner's grading criteria

developmental stage of the blastocysts



1 2 3 4 5 6

developmental stage	Blastocyst development and stage status
1	Blastocoel cavity less than half the volume of the embryo
2	Blastocoel cavity more than half the volume of the embryo
3	Full blastocyst,cavity completely filling the embryo
4	Expanded blastocyst,cavity larger than the embryo,with thinning of the shell
5	Hatching out of the shell
6	Hatched out of the shell

grade of inner cell mass(ICM)

A B C

ICM grade	Inner cell mass quality
A	Many cells,tightly packed
B	Several cells,loosely grouped
C	Very few cells

grade of trophectderm cell(TE)

A B C

TE grade	Trophectoderm quality
A	Many cells,forming a cohesive layer
B	Few cells,forming a loose epithelium
C	Very few large cells

【 Results 】

Table 1 : Pregnancy Rate and Miscarriage Rate according to developmental stage of the blastocyst

developmental stage	No. SFBT cycles	No. pregnancy cycles	No. miscarriage cycles	PR(%)	MCR(%)
G2	10	0	0	0.0	0.0
G3	126	42	9	33.3	21.4
G4	266	116	21	43.6	18.1
G5	893	386	86	43.2	22.3
G6	109	60	13	55.0	21.7
total	1404	604	129	43.0	21.4

single frozen blastocyst transfer:SFBT、pregnancy rate:PR、miscarriage rate:MCR

Fig.1 : Pregnancy Rate according to developmental stage of the blastocyst

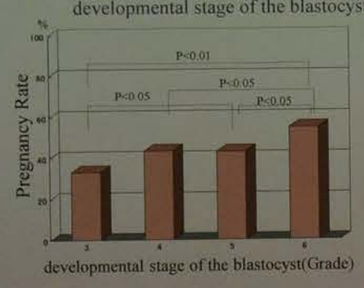
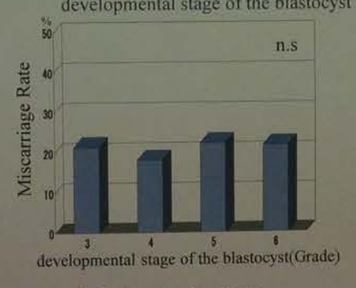


Fig.2 : Miscarriage Rate according to developmental stage of the blastocyst



The PR was significantly higher in Grade 5～6 than in Grade3 ,in Grade6 than in Grade 4, in Grade6 than in Grade 5.
For the MCR, there were no significant differences among each developmental stage.（Table 1、Fig.1、Fig.2）

Table 2 : Pregnancy Rate and Miscarriage Rate according to ICM Grade

ICM Grade	No. SFBT cycles	No. pregnancy cycles	No. miscarriage cycles	PR(%)	MCR(%)
A	192	90	13	46.9	14.4
B	981	436	101	44.4	23.2
C	231	78	15	33.8	19.2
total	1404	604	129	43.0	21.4

single frozen blastocyst transfer:SFBT、pregnancy rate:PR、miscarriage rate:MCR

Fig.3 : Pregnancy Rate according to ICM Grade

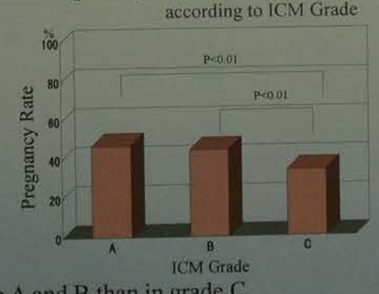
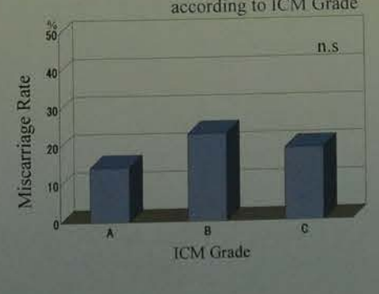


Fig.4 : Miscarriage Rate according to ICM Grade



The PR was significantly higher in ICM grade A and B than in grade C.
For the MCR, there were no significant differences among each ICM grade.（Table 2、Fig.3、Fig.4）

Table 3 : Pregnancy Rate and Miscarriage Rate according to TE Grade

TE Grade	No. SFBT cycles	No. pregnancy cycles	No. miscarriage cycles	PR(%)	MCR(%)
A	172	97	19	56.4	19.6
B	884	396	84	44.8	21.2
C	348	111	26	31.9	23.4
total	1404	604	129	43.0	21.4

single frozen blastocyst transfer:SFBT、pregnancy rate:PR、miscarriage rate:MCR

Fig.5 : Pregnancy Rate according to TE Grade

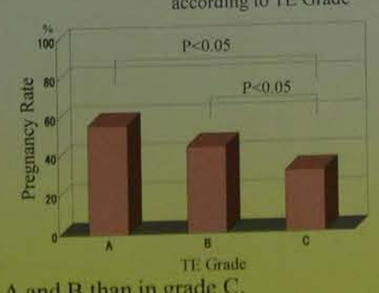
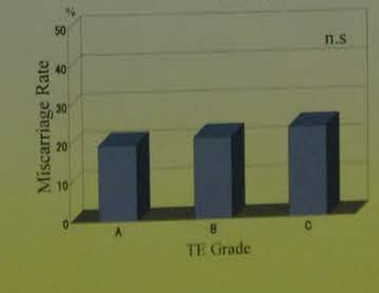


Fig.6 : Miscarriage Rate according to TE Grade



The PR was significantly higher in TE grade A and B than in grade C.
For the MCR, there were no significant differences among each TE grade.（Table 3、Fig.5、Fig.6）

【 Discussion 】

It is a well-known fact that the PR of high grade brastocysts is higher than low grade brastocysts.In this study,the results have the same tendency as the fact.

In other study,the results suggest that the MCR depend on ICM or TE grade.But in this study ,the results suggest that morphology of the transferred blastocysts bear no relation to pregnancy prognosis.

【 Conclusion 】

The results show that the PR are closely related to developmental stage, grade of ICM and TE. They suggest that morphology of the transferred blastocysts bear no relation to pregnancy prognosis.

Cytology

PF-15

Sperm cryopreservation for patient with malignant or non-malignant diseases in ART center

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【Introduction】

Modern cancer therapy has improved survival rates in men, however chemotherapy, radiotherapy and surgical treatment may lead to male infertility thus sperm cryopreservation is advanced for QOL after treatment.

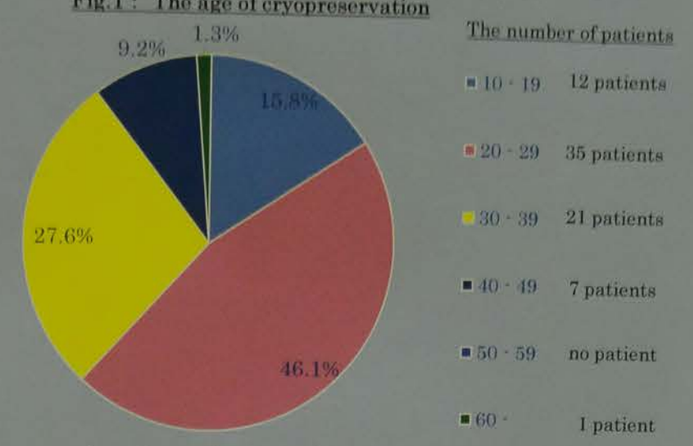
We reviewed cases of sperm cryopreservation in malignant and non-malignant diseases.

【Materials & Methods】

Seventy-six cases from January 2000 to June 2015 were included in this study. The clinical records were reviewed retrospectively.

① The age of cryopreservation (Fig.1)
② Marriage status (Fig.2)
③ Original diseases (Fig.3)
④ The timing of cryopreservation (Fig.4)
⑤ Implementation of cryopreservation (Fig.5)
⑥ Semen quality (Fig.6)
⑦ The state after cryopreservation (Fig.7)
⑧ Treatment in ART center (Table 1)

Fig.1： The age of cryopreservation



Age 13～64 (median 29)

Fig.2： marriage status

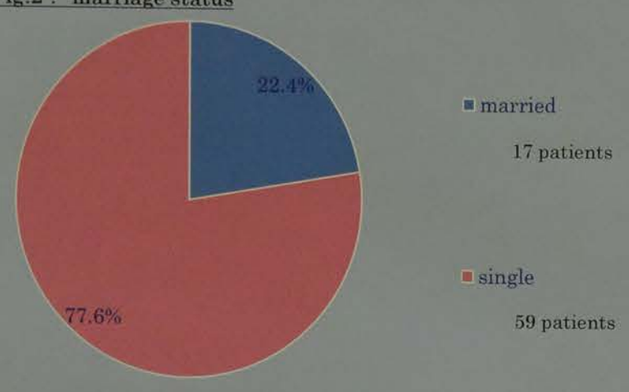


Fig.3： Original diseases

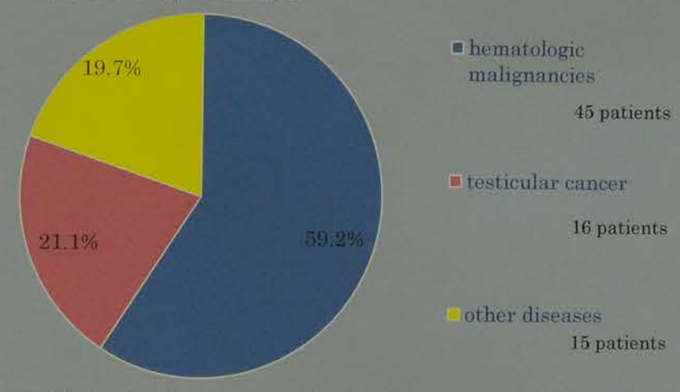


Fig.4： The timing of cryopreservation

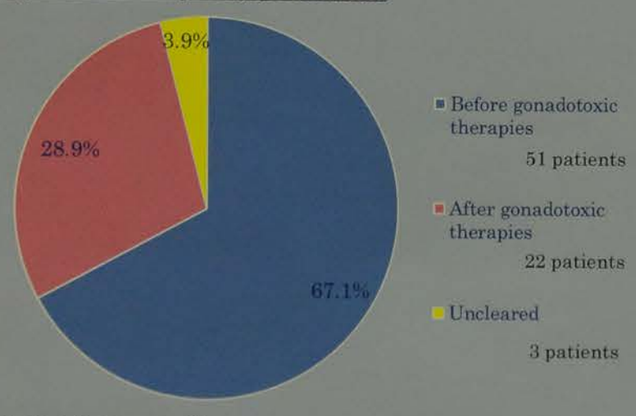


Fig.5： Implementation of cryopreservation

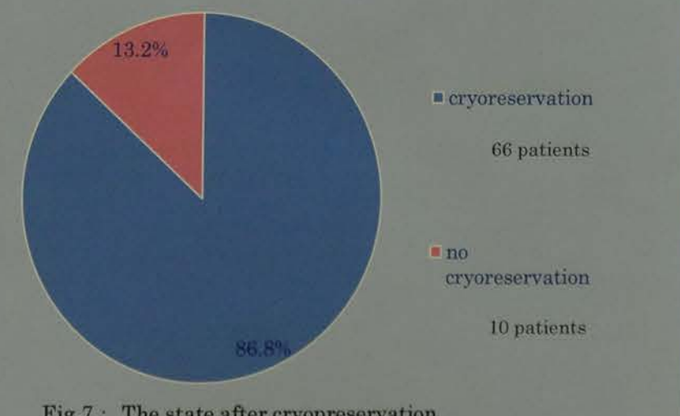


Fig.6： Semen quality

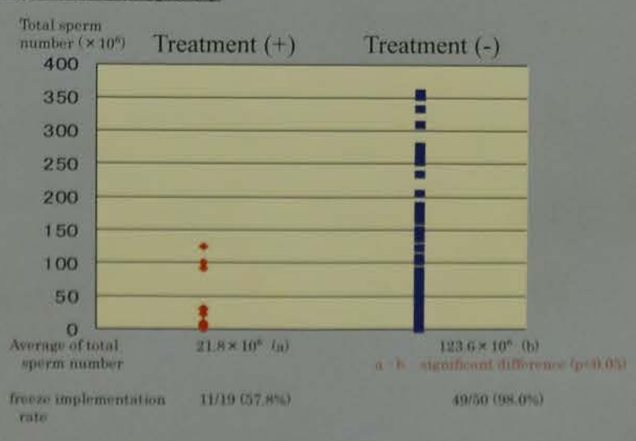


Fig.7： The state after cryopreservation

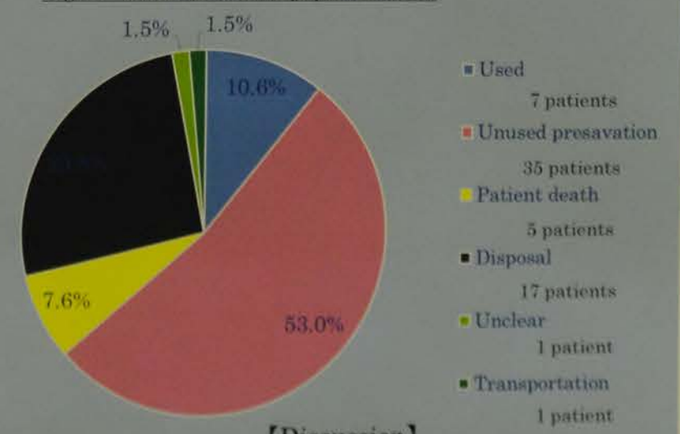


Table 1： Treatment in ART center

case	The age of cryopreservation	Original diseases	Cryopreservation period	The age of partner	ART treatment	Result
1	31	Rectum cancer	11 months	33	IUSI cryo ET	[illegible]
2	43	Malignant lymphoma	18 months	38	IUSI cryo	Treatment stop
3	28	ALL	35 months	32	IUSI cryo RET	[illegible]
4	34	Myeloma	34 months	36	IUSI cryo DET	[illegible]
5	40	CML	39 months	46	IUSI	Treatment continuation
6	39	Mantle cell lymphoma	15 months	35	IUSI cryo DET	[illegible]
7	40	Thyroid cancer	11 months	45	IUSI	Treatment continuation

【Discussion】

The average age of the patient was 29 years old and the 77.6% was single.

There were a lot of hematologic malignant diseases and testicular malignant tumors for a target disease of a cryopreservation.

The number of sperm after original diseases treatment decreased significantly. ($p<0.05$)

In sixty-six cases, sperm cryopreservation to a patient with a possibility of the spermatogenic dysfunction and the disappearance is aggressively being conducted.

In ART center, four out of seven cases (57.1%) achieved successful pregnancies by treatment using frozen sperm.

【Conclusion】

After treatment of malignant diseases, progress was seen due to advanced medical technology, and sperm cryopreservation is advanced for QOL.

After chemotherapy, azoospermia occurs thus the number of sperm decreases.

Freezing a sperm before chemotherapy is considered important for fertility preservation.