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Significance of Noninvasive Diagnosis of Prostate Cancer with Cytologic Examination of Prostatic Fluid

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Abstract

Objectives: While reassessing the value of exfoliative cytologic examination of prostatic fluid (PF) for the diagnosis of prostate cancer, we found that PF is easily obtained with transrectal ultrasonography during prostate biopsy and that cytologic examination of PF is useful for the diagnosis of prostate cancer.

Methods: The cohort included 53 consecutive patients who underwent transrectal prostate biopsy from May through September 2005. Patient age was 66.7 ± 7.24 years, and the mean concentration of prostate-specific antigen (PSA) was 15.1 ± 25.8 ng/m*l*. The PF for cytologic examination was obtained before biopsy, and Papanicolaou's staining was performed. The results of cytologic examination are expressed as class 1 to 5. Results of cytologic examination and prostate tissue pathologic examination were analyzed. Patient age, PSA levels, total prostate volume (TPV), and PF volume were compared with cytologic class by means of analysis of variance.

Results: The mean PF volume was 378.4 ± 245.3 μ*l*, and the mean TPV was 38.0 ± 18.8 m*l*. The numbers of patient in classes 1 to 5 were 1 (1.9%), 37 (69.8%), 11 (20.7%), 1 (1.9%), and 3 (5.7%), respectively. Pathologic examination showed 23 (43.4%) cases of cancer, 27 (50.9%) cases of benign prostatic hyperplasia, and 3 (5.7%) cases of high-grade prostatic intraepithelial neoplasia. All three patients with class 5 results had prostate cancer (Gleason score, 7 to 10). All 9 patients with a PSA level greater than 16 ng/m*l* had biopsy-proven cancer, and 3 of these 9 patients (33.3%) were in cytology class 5. Therefore, PF cytologic examination showed a specificity of 100% and a sensitivity of 33.3% in patients with PSA levels higher than 16 ng/m*l*. The cytologic classes differed in PSA levels (F=8.271, P=0.000) but not in patient age, TPV, or PF volume.

Conclusions: Exfoliative cytologic examination of PF is a valuable, noninvasive method for detecting prostate cancer, especially in patients with high PSA levels.
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Key words: prostatic fluid, prostate cancer, diagnosis, cytology

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Introduction

Prostate cancer is the third most commonly diagnosed cancer in the male population worldwide[1] and remains the most frequently diagnosed malignancy and the second leading cause of cancer mortality in Western men. There were 230,110 new cases and 29,900 deaths due to prostate cancer in the United States in 2004[2]. Prostate cancer shows an increasing incidence with advancing age. The probability of the diagnosis of prostate cancer is 1 in 19,299 for men younger than 40 years, 1 in 45 for men aged 40 to 59 years, and 1 in 7 for men aged 60 to 79 years[3].

With this tendency toward prostate cancer prevalence, diagnosis during the early stage is extremely important because the cancer may be cured by radical prostatectomy when it is confined to the prostate. However, the absence of clinical symptoms makes early diagnosis difficult. The discovery and application of prostate-specific antigen (PSA) was a significant contribution to detecting the development and postoperative recurrence of prostate cancer. However, measurement of serum PSA levels lack specificity, because PSA is also elevated in men with benign prostatic hyperplasia (BPH), prostatitis, and other nonmalignant conditions[4]. Urologists are seeking cost-effective, noninvasive, simple, and specific methods and tumor markers to replace PSA for the early diagnosis of prostate cancer.

Exfoliative cytologic examination is a mature method that is extensively used in clinical practice to diagnose and differentiate numerous benign and malignant diseases. In the urinary system, urine exfoliative cytologic examination is commonly performed to detect urinary transitional epithelial cancer. In the prostate, exfoliative prostatic gland cells enter the glandular lumen and become a component of the prostatic fluid (PF). Thus, PF might be examined for exfoliated cells to detect prostate cancer, as is done with urine.

However few articles have reported the detection of prostate cancer using cytologic examination of PF, because of the difficulty of obtaining PF[5]. Recently we found that PF could be easily obtained during screening transrectal ultrasound (TRUS) of the prostate just before prostate biopsy[6]. Therefore, we asssessed the usefulness of exfoliative cytologic examination of PF for detection of prostate cancer.

Materials and Methods

The cohort included 53 consecutive patients who underwent prostate biopsy because of elevated serum PSA levels or hard nodular prostatic surface palpated on digital rectal examination or both from May 2005 through September 2005. Mean patient age (±SD) was 66.7 ± 7.24 years (range, 51 to 84 years), and the mean PSA level (±SD) was 15.1 ± 25.8 ng/m*l* (range, 1.1 to 150.0 ng/m*l*).

After general anesthesia was induced, the patient was placed in the lithotomy position. First, TRUS was performed. The ultrasound probe was placed firmly against the rectal wall behind the prostate, and a thorough examination was performed. The probe was moved from proximal to distal, and from left to right, after which digital massage was performed as usual to obtain PF.

PF was stored in a clean Eppendorf tube. After the volume was measured, the PF was sent to the pathology department as quickly as possible. The total prostate volume (TPV) was calculated with the formula $\pi/6 \times a \times b \times c$, where a, b, and c represent the three diameters of the prostate measured before biopsy under TRUS. The patients' clinical characteristics are shown in **Table 1**.

Tissue was obtained with standard sextant biopsy in the peripheral zone and additional sextant biopsy in the bilateral lobes of the transitional zone of the prostate. The sections of biopsy tissue were stained with hematoxylin and eosin (HE) and examined by one pathologist (G. K.).

The PF sample was centrifuged at 1,500 *g* for 5 min, after which the supernatant was discharged. Then, 1 m*l* of fixation fluid of YM solution (Mutou Chemical Company. Tokyo, Japan) was added to the sediment and mixed thoroughly. The above mixture was then centrifuged at 1,500 *g* for 5 min. The supernatant was discharged, and 0.2 m*l* of 95% ethanol was added and mixed with the sediment

Table 1 Characteristics of all patients

No	Age	PSA	TPV	PF volume	Pathology	Cytologic class
1	59	9.5	55.3	451	BPH	2
2	69	14.0	56.6	865	BPH	2
3	62	4.3	35.6	212	Adenocarcinoma	2
4	73	14.0	58.1	141	BPH	2
5	70	10.0	53.2	283	HGPIN	2
6	64	18.0	19.5	140	Adenocarcinoma	2
7	62	7.7	35.9	860	BPH	1
8	80	18.0	20.7	172	Adenocarcinoma	2
9	67	7.3	31.5	170	Adenocarcinoma	2
10	68	7.0	32.9	622	BPH	2
11	61	8.8	42.1	663	BPH	3
12	65	4.3	26.5	1,012	Adenocarcinoma	3
13	68	4.4	96.5	310	BPH	3
14	66	73.58	22.4	62	Adenocarcinoma	5
15	62	4.6	35.4	940	BPH	2
16	56	66.0	38.3	228	Adenocarcinoma	2
17	76	5.4	51.8	263	Adenocarcinoma	2
18	65	6.9	106.5	642	BPH	2
19	80	13.0	17.8	558	Adenocarcinoma	3
20	84	10.0	21.8	174	Adenocarcinoma	2
21	62	8.1	39.1	240	HGPIN	2
22	77	7.4	61.4	512	BPH	3
23	66	17.0	33.2	202	Adenocarcinoma	3
24	68	3.3	25.0	302	BPH	2
25	60	7.8	16.9	460	Adenocarcinoma	3
26	66	4.9	15.9	376	Adenocarcinoma	2
27	56	1.9	22.3	184	BPH	2
28	58	5.4	32.3	781	BPH	2
29	76	4.2	72.2	250	BPH	3
30	73	100.0	37.7	104	Adenocarcinoma	3
31	58	5.2	21.7	112	BPH	2
32	80	150.0	33.5	193	Adenocarcinoma	5
33	69	10.0	36.3	231	BPH	2
34	58	11.0	43.2	580	BPH	2
35	61	5.1	28.8	78	Adenocarcinoma	2
36	75	6.9	43.5	362	BPH	2
37	64	4.1	37.4	424	BPH	2
38	64	1.1	20.9	548	BPH	2
39	65	4.8	35.0	412	BPH	2
40	71	6.2	43.3	291	BPH	4
41	51	11.0	44.4	228	BPH	2
42	61	7.3	19.4	216	Adenocarcinoma	2
43	75	9.6	39.5	482	HGPIN	2
44	66	5.2	41.1	220	BPH	2
45	71	4.9	35.8	131	Adenocarcinoma	2
46	75	26.0	18.7	782	Adenocarcinoma	2
47	69	8.4	25.4	252	Adenocarcinoma	2
48	64	19.0	18.6	284	Adenocarcinoma	5
49	63	7.1	51.1	738	BPH	2
50	54	8.3	21.9	596	Adenocarcinoma	2
51	71	8.2	61.1	302	BPH	2
52	69	8.8	60.7	336	BPH	3
53	61	6.8	16.6	80	Adenocarcinoma	3

Table 2 Cytologic results of all patients

	Class 1	Class 2	Class 3	Class 4	Class 5	Total
Cancer	0	14	6	0	3	23
Noncancer	1	23	5	1	0	30
Total	1	37	11	1	3	53

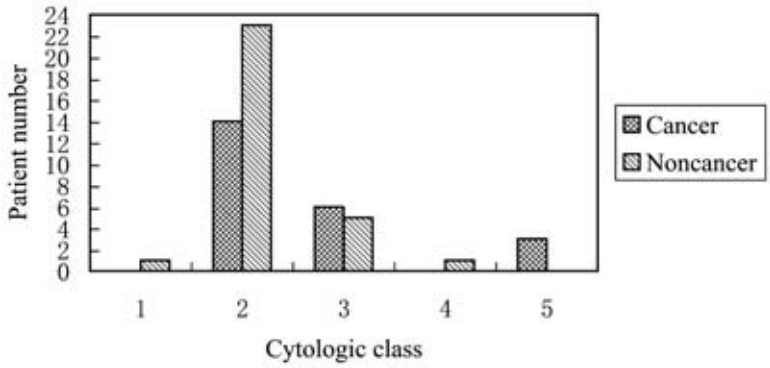

Fig. 1 Cytologic results of all patients

Table 3 Cancer distribution

Cytologic class	PZ cancer	TZ cancer	PZ+TZ cancer
1–2	4	4	6
3–5	2	1	6

PZ: peripheral zone of prostate
TZ: transitional zone of prostate

overnight. On the second day, a smear was made with the sediment mixture dried in air, and then Papanicolaou's staining was performed. All procedures were performed at room temperature. The cell morphology was studied by one pathologist (Y. S.).

The cytologic results were expressed with the Papanicolaou classification[7]. Classes 1 to 5 were defined as follows: class 1: no evidence of a malignant neoplasm and no atypical cells; class 2: atypical cells present but no evidence of malignant neoplasm; class 3: cells present suggesting malignant neoplasm; class 4: fairly conclusive evidence of malignant neoplasm, and class 5: conclusive evidence of malignant neoplasm.

We analyzed the results of cytologic examination of the PF and the pathologic results of prostate biopsy, and also analyzed the difference of patient age, PSA, TPV, and PF volume among cytologic classes by means of analysis of variance (ANOVA), using the Statistical Package for Social Science, version 10.0 software (SPSS, Inc., Chicago, IL, USA). Differences with P values less than 0.05 were considered significant.

Informed consent for obtaining PF and for using PF and biopsy specimens for possible investigation was obtained from each patient before biopsy.

Results

PF was obtained from the 53 patients, the mean volume of PF (±SD) was 378.4 ± 245.3 μ*l* (range, 62.0 to 1,012.0 μ*l*), and the mean TPV (±SD) was 38.0 ± 18.8 m*l* (range, 15.9 to 106.5 m*l*). (**Table 1**)

The numbers of patients with cytologic classes 1 to 5 were 1 (1.9%), 37 (69.8%), 11 (20.7%), 1 (1.9%), and 3 (5.7%), respectively (**Table 2** and **Fig. 1**).

Pathologic examination showed 23 (43.4%) cases of prostate cancer, 27 (50.9%) cases of BPH, and 3 (5.7%) cases of high-grade prostatic intraepithelial neoplasia (HGPIN). Cancers detected with biopsy were in the peripheral zone, the transitional zone, and in both zones in 6, 5, and 12 patients, respectively (**Table 3**).

The percentage of cancers detected with cytologic examination of PF was calculated using the tissue

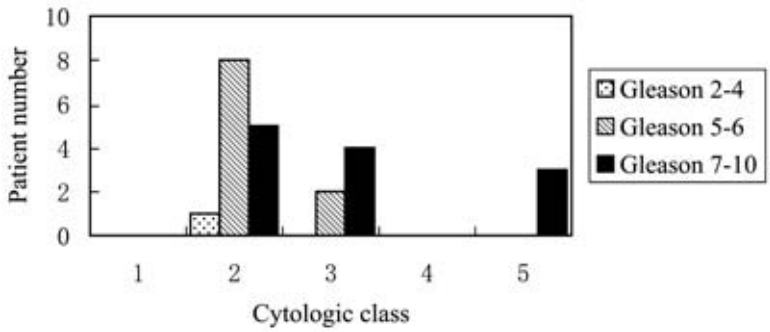

Fig. 2 Relationship between cytologic class and Gleason score in cancer patients

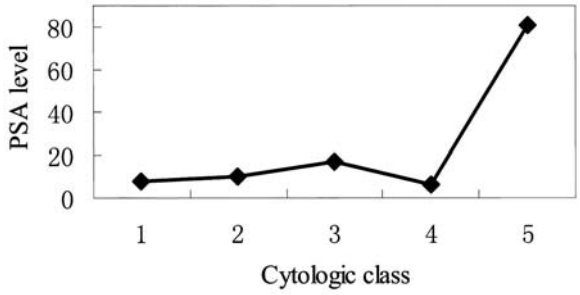

Fig. 3 Relationship between cytologic class and PSA level

pathologic results of prostate biopsy. Of the 23 patients with cancer, 9 (39.1%) showed a cytologic class higher than 3, but only 3 showed class 5 found with prostate biopsy. Of the 9 cancer patients with PSA levels greater than 16 ng/m*l*, 3 (33.3%) showed cytologic class 5. Therefore, cytologic examination of PF showed a specificity of 100% and a sensitivity of 33.3% in patients with PSA levels greater than 16 ng/m*l*.

The relationship between the cytologic class and the Gleason score of prostate cancer is shown in **Figure 2**. Patients with a low Gleason score showed a low cytologic class. That is, one patients with Gleason scores of 2 to 4 showed cytologic class 2, whereas all three patients with cytologic class 5 showed Gleason scores of 7 to 10 **(Fig. 2)**.

All three patients with HGPIN showed cytologic class 2.

Cytologic classes differed significantly in PSA levels but not in patient age, TPV, or PF volume. The F values of ANOVA analysis for group PSA levels, patient age, TPV, and PF volume were 8.271 (P=0.000), 0.687 (P=0.605), 0.644 (P=0.634), and 1.600 (P=0.190), respectively. Because there was only one patient with class 1 and one patient with class 4, the PSA levels did not correctly represent the true levels in these two classes. However, we could observe a tendency of the PSA levels in the other three classes **(Fig. 3)**.

Discussion

Cytologic examination of PF was first used in the diagnosis of prostate malignancy by Mulholland in 1931[8]. Thereafter, researchers reported controversial findings regarding the sensitivity of the cytologic examination of PF[9,10]. Since it is difficult for urologists to obtain PF for research, Ichijo et al. have reported that they successfully collected PF with a specially designed catheter through a rather painful procedure[11]. However, the rapid development of prostate biopsy under TRUS has made it the gold standard for diagnosing prostate cancer. Together, these circumstances hindered the development of cytologic examination of PF as an effective tool in the diagnosis of prostate cancer. Some researchers have used urine after prostate massage instead of PF[12,13], but urine specimens include exfoliative cells from the urinary system but few exfoliative cells from the prostate. Inclusion of exfoliative cells from the urinary tract might produce misleading results. Recently we found that it was relatively easy to obtain PF before prostate biopsy by massage with the index figure after massage with ultrasound probe examination[6]. Therefore, we reassessed the usefulness of cytologic examination of PF for the diagnosis of prostate cancer.

Our data show that the specificity of cytologic examination of PF is 100% in the diagnosis of prostate cancer. Even if positive results of cytologic examination of PF showed a low rate of cancer detection among all patients (13.0%, 3 of 23 patients), it showed a higher detection rate in patients with PSA levels greater than 16 ng/m*l*. Positive results of cytologic examination of PF were obtained in 33.3% of patients with a PSA level greater than 16 ng/m*l* in our study. Statistical analysis showed that PSA levels differed significantly between cytologic classes ($p=0.000$). This result is consistent with the fact that the higher are the PSA levels the greater is the percentage of prostate cancer. Therefore, we could screen patients with a high PSA levels by cytologic examination of PF before prostate biopsy, and the patient would avoid the biopsy if the cytologic results were positive.

Possible reasons that some cancer patients had negative results of cytologic examination of PF are that earlier-stage cancer was scattered or confined to a small region of the prostate and that the PF obtained did not contain fluid from the glandular alveolus with malignant change. Sometimes repeated prostate biopsy with TRUS also provides negative results for these patients. Obtaining PF specimens repeatedly and massaging thoroughly might increase the positivity rate. All three patients with cytologic class 5 had more than 1 core (3 of 12, 5 of 12, and 6 of 12, respectively) positive result on tissue biopsy in this study. This result indicates that cytologic examination of PF is likely to be positive if cancer is located in multiple sites in the prostate.

A second possible reason for negative result of cytologic examination of PF in cancer patients is that the orifice of the glandular cavity is obstructed by cancer tissue and PF containing exfoliative cells could not be pressed out from the cancer lesion. A third possible reason for negative result is an association between cancer and chronic prostatitis. We and other researchers have found inflammation in most tissue in cases of BPH and prostate cancer[14,15]. Chronic inflammation can cause the prostate to become small and hard, making it is difficult to obtain an adequate PF sample.

Although prostate biopsy is a relatively noninvasive examination, it does carry some risk of complications such as hematuria, infection, and bleeding[16]. The noninvasive cytologic examination of PF has many advantages. It is painless, inexpensive, rapid, can be performed in the outpatient clinic, and can be performed repeatedly. The most important advantage is that it does not carry any risk of complications. The PF also contains many biochemical constituents, seems to reflect the metabolic status of the prostatic epithelium, and might provide a means for detecting prostate cancer through biochemical and molecular biological processes[17].

Cytologic examination of urine has been used in screening and monitoring the recurrence of uroepithelial carcinoma for many years, even though its overall sensitivity ranges from 40% to 60% and findings are dependent on both tumor grade and the operator[18]. Cytologic examination of urine is not replaced by tissue biopsy. Furthermore, researchers have increased the positive predictive value to a certain extent by interpreting findings in combination with other biomarkers using molecular biological techniques, such as measurements of bladder tumor antigen (BTA), mucin, nuclear matrix protein-22 (NMP22), matrix metalloproteinase-9, and telomerase[19]. Cytologic examination of PF may be considered under similar circumstances. We realized that cytologic examination of PF can not be replaced by prostate biopsy as urine cytology dose for bladder cancer. However, if cytologic examination of PF is used with some new biomarkers, the positive predictive value may improve.

In this study, 37 of 53 patients (69.8%) showed cytologic class 2. However, 14 (38%) of these patients had cancer. Thus, the findings of cytologic examination of PF should be interpreted with clinic findings and other new markers. Newly reported biomarkers for detecting prostate cancer include glutathione S-transferase-1, DD3, and telomerase[20]. Theoretically, these markers can be used with cytologic examination of PF for the diagnosis of prostate cancer. We are now performing further trials to increase the positive predictive value using both cytologic examination of PF and the aforementioned new biomarkers.

Conclusions

Exfoliative cytologic examination of PF is a useful method for detecting prostate cancer especially in patients with high PSA levels, and this examination has many advantages, such as being less invasive than prostate biopsy.

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