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Cytologic Assessment of Tumor Grade in Endometrial Cancer : A Comparison of the Cytologic Scoring System with Fractional Endometrial Curettage

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Abstract To establish a cytological scoring system for the assessment of tumor grade in endometrial cancer, 11 cytological items were examined in 45 patients with this cancer. Four items (the cluster rate, cluster edges, anisokaryosis, and macronucleoli or irregular nucleoli) were shown to have value for determining the tumor grade, and a scoring system was created using these four items. Tumor grading was then performed in 109 patients with endometrial cancer using this cytological score as well as by fractional endometrial curettage, and the results were compared with the pathological tumor grade. The diagnostic accuracy of cytological tumor grading was 88% for grade 1, 63% for grade 2, and 82% for grade 3 tumors, with an overall accuracy of 78%. The diagnostic accuracy of fractional endometrial curettage was 92% for grade 1, 35% for grade 2, and 59% for grade 3 tumors, with an overall accuracy of 66%. Preoperative investigation of the grade of endometrial cancer by endometrial aspiration cytology provides additional information about tumor differentiation and has the potential to replace fractional endometrial curettage.

Introduction

The grade of endometrial cancer is considered to be one of the most significant prognostic indicators[1,2]. It is well known that poorly differentiated tumors are associated with rapid extrauterine spread as well as an unfavorable disease outcome[3,4]. It would be of great value to know the tumor grade precisely before surgery, since patients who are strongly suspected to have poorly differentiated tumors could be candidates for other treatment options, such as more radical surgery or neoadjuvant therapy.

Preoperative determination of the grade of endometrial cancer has relied on fractional endometrial curettage. However, it is not surprising that the diagnostic accuracy of fractional curettage is unsatisfactory[5–8], because only a small portion of the tumor is obtained by this method. Attempts have also been made to determine tumor grade on the basis of endometrial cytology. Several investigators[9–11] have found that the grade of endometrial cancer has an influence on the cytologic features of endometrial smears. However, an overall concept of tumor grading based on endometrial

cytology has not been fully established, and there have been no reports on the use of endometrial aspiration cytology in this context.

In the present study, a new scoring system for the grading of endometrial cancer

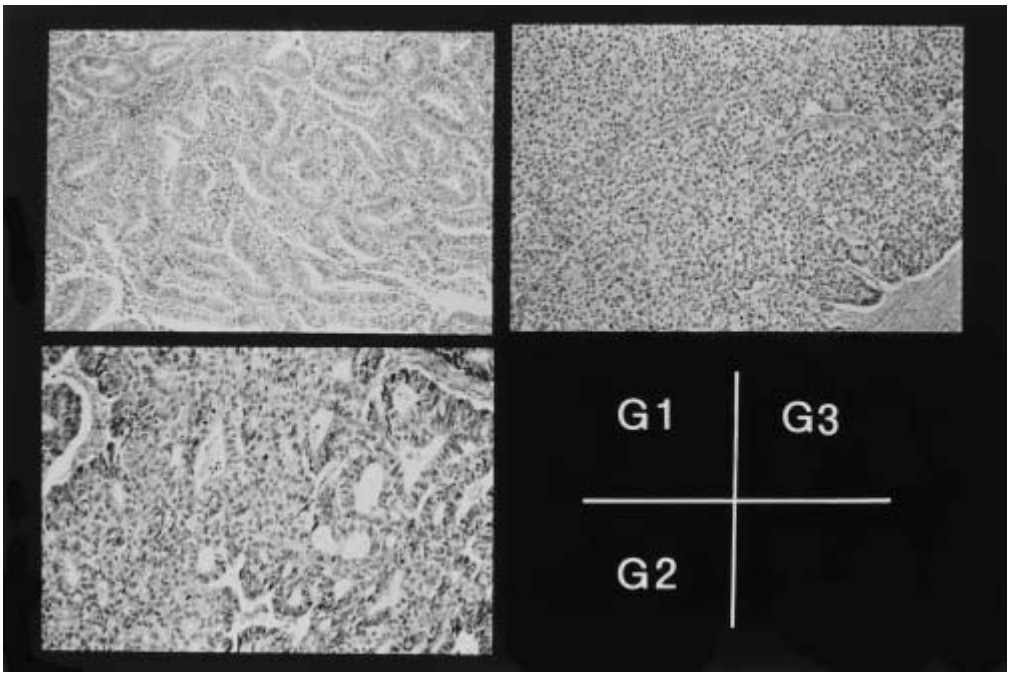

Figure 1. The histologic tumor grade of endometrial cancer. Solid nests of neoplastic cells occupy less than 5% of the lesion in a grade 1 tumor, 5 to 50% in a grade 2 tumor, and more than 50% in a grade 3 tumors. Notable nuclear atypia increases the grade of a grade 1 or 2 tumor by 1 level (Hematoxylin -eosin stain X40).

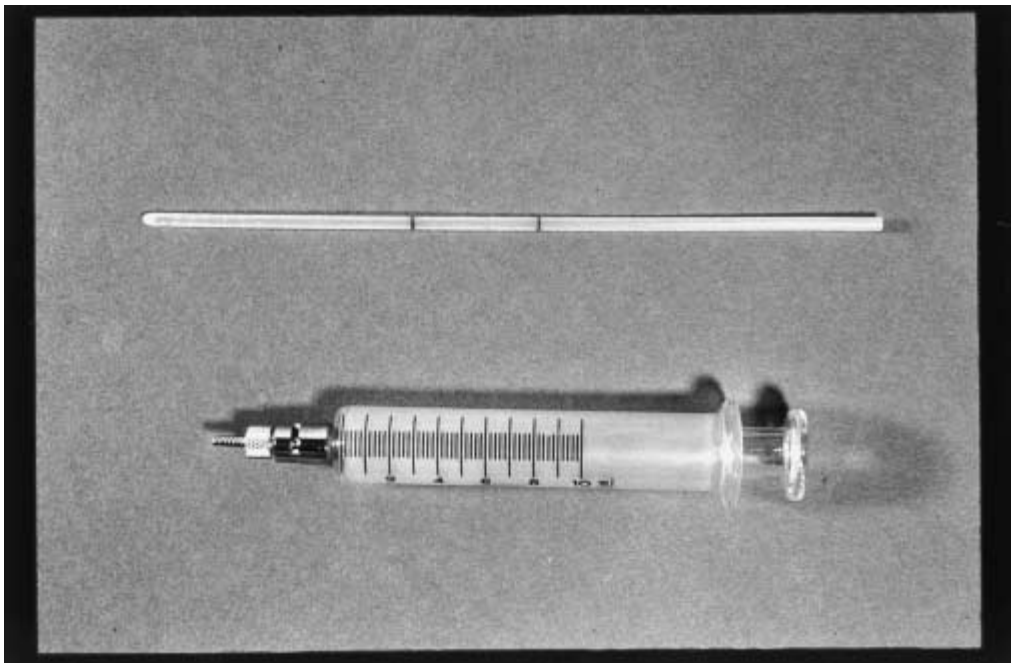

Figure 2. Masubuchi's method of endometrial aspiration cytology. The device is made of a 20-cm polyethylene tube and syringe, with endometrial material being obtained by negative pressure. The tip is inserted through the cervical os as far as the fundus, without dilatation or analgesia. The material obtained from the endometrium and uterine cavity is immediately spread onto two slides.

based on endometrial aspiration cytology was investigated. Using this system, the diagnostic accuracy of tumor grading was compared between endometrial aspiration cytology and conventional fractional curettage.

Materials and Methods

1 Cytological score

Endometrial aspiration cytology was performed preoperatively in 45 patients with endometrial cancer (15 with grade 1, 15 with grade 2, and 15 with grade 3 tumors). The median (range) age was 54 (40-66) years. The final tumor grade was determined by pathological examination of resected materials according to the FIGO criteria[12] (Figure 1). All patients had endometrioid adenocarcinoma, and tumors that were partly or entirely composed of special histologic types (papillary serous, clear cell, villoglandular, or mucinous) were excluded. Fifteen patients were treated at Yamaguchi University School of Medicine and 30 patients were treated at the Cancer Institute Hospital between 1996 and 1999. Endometrial aspiration cytology was performed by Masubuchi's method (Figure 2), using a 20-cm polyethylene tube and a syringe to obtain specimens by negative pressure[13,14]. The specimens obtained from the uterine cavity were stained by the standard Papanicolaou procedure.

To determine the tumor grade on the basis of cytologic features, the following 11 items were examined in each smear: 1) the cluster rate of malignant cells, 2) the appearance of cluster edges, 3) the nuclear size, 4) the nuclear shape, 5) the chromatin distribution 6) anisokaryosis, 7) the number of nucleoli,8) the presence of macronucleoli or irregular nucleoli, 9) tumor diathesis, 10) the presence of histiocytes, and 11) coexisting normal endometrial cells.

The cluster rate of malignant cells was examined in each smear. The appearance of the cluster edge was classified as either

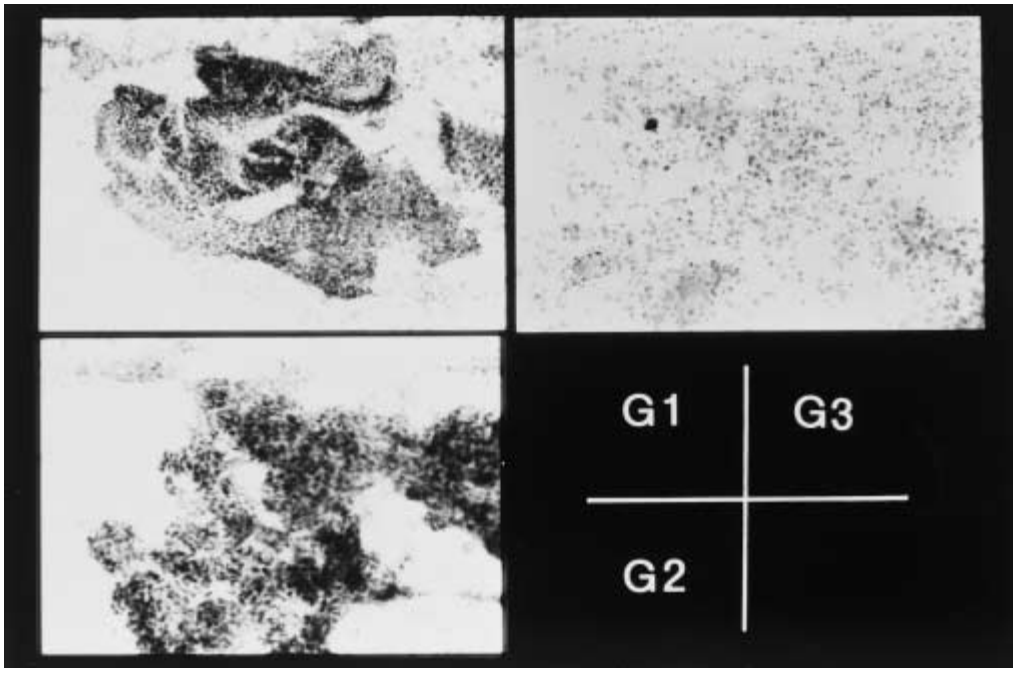

Figure 3. Cytologic features of each grade of endometrial cancer, as shown by endometrial aspiration cytology. Grade 1 tumors show the tight junctions between individual cancer cells, with a cluster rate of more than 75% and well-defined cell clusters. Coversely, grade 3 tumors show loose intercellular connections and many isolated cancer cells, with a cluster rate of under 50% of a clusterization rate and irregular cell clusters. Grade 2 tumors show an intermediate appearance between grade 1 and 3 tumors (Papanicolaou stain, x 40).

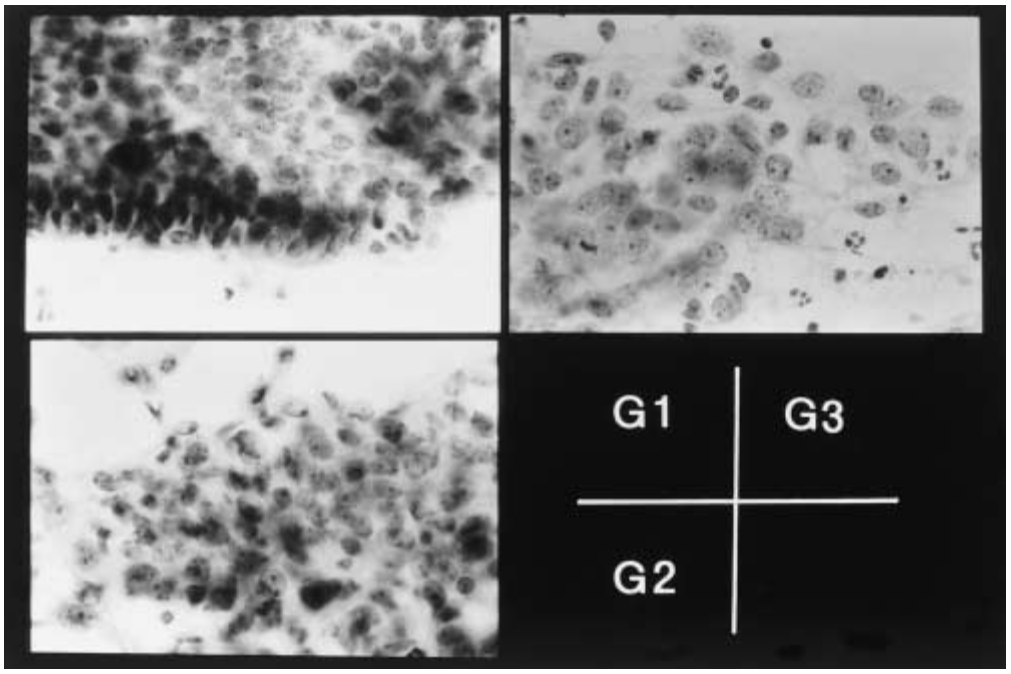

Figure 4. A magnified view of Figure 3 (Papanicolaou stain, x 100).

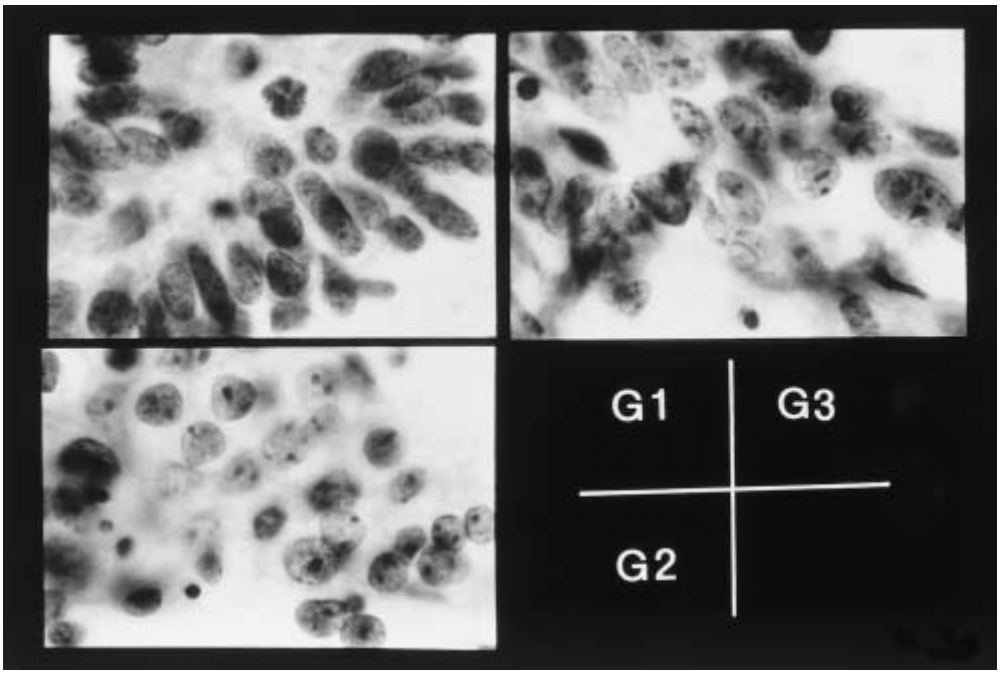

Figure 5. A grade 1 tumor shows minimal anisokariosis and small nucleoli, while a grade 3 tumor shows more than triple anisokariosis and macronucleoli. A grade 2 tumor has an intermediate appearance between the grade 1 and 3 tumors (Papanicolaou stain, x 100).

well-defined or irregular. In well-defined cell clusters, the peripheral borders of the malignant cell clusters showed smooth lines that were contiguous with the cytoplasm. In irregular cell clusters, the peripheral borders of the malignant cell clusters were irregular and frayed, overlapping the cytoplasm or irregular-shaped nuclei of the malignant cells. Isolated malignant cells were often observed around this type of

cluster. In each smear, the ratio of clusters with well-defined edges was examined. For the items concerning the nuclei and nucleoli, 100 malignant cells were examined in each smear. Nuclear size was measured with a Digital Filar Micrometer Eye Piece (OSN-D2, Olympus) and expressed as a mean of the longest and the shortest diameter. Student's t-test was used for this comparison. With respect to nuclear shape and chromatin distribution, the proportion of the cancer cells having irregular nuclei or an irregular distribution of chromatin was examined. The degree of anisokaryosis was classified into less than double, double to triple, and more than triple level. The number of nucleoli and conspicuous nucleoli was counted in each smear. The presence of tumor diathesis, histiocytes, and normal endometrial cells was also examined.

2. Comparison of the diagnostic accuracy of tumor grade between endometrial aspiration cytology and fractional endometrial curettage

One hundred and nine patients with endometrial cancer, all with pure endometrioid type, who were surgically treated between 2000 and 2001 were enrolled in this study (52 with grade 1, 40 with grade 2, and 17 with grade 3 tumors). The median (range) age was 55 (40-70) years. Seven patients were treated at Yamaguchi University School of Medicine and 102 patients were treated at the Cancer Institute Hospital. All patients were preoperatively positive on both endometrial aspiration cytology and fractional endometrial curettage. Each tumor was graded on the basis of the endometrial aspiration cytology findings using the new scoring system as well as from the finding of partial endometrial curettage, and these results were compared with the postoperative tumor grade that was determined from surgical specimens. Fisher's exact test was used for this comparison. A p value of <0.05 was considered to be statistically significant.

Results

1 Cytologic data from the initial 45 patients

(1) Cluster rate

Table 1 shows the cluster rate of malignant cells in each smear. All grade 1 tumors had a cluster rate of more than 50%, whereas 73.3% of grade 3 tumors had a cluster rate of less than 50%.

(2)Appearance of cluster edges

Table 2 shows the appearance of the cluster edges. In all grade 1 tumors, more than 50 % of cell clusters were of the well-defined type, whereas this was the case for only 26.7% of clusters from grade 3 tumors.

(3) Nuclear size

The nuclear size (mean±SD) was 8.50±1.80

Table 1 Cluster rate in relation to tumor grade

	G1	G2	G3
More than 75%	93.3	26.7	0
50−75%	6.7	33.3	26.7
25−50%	0	33.3	33.3
less than 25%	0	6.7	40.0

Table 2 Appearance of cluster edges in relation to grade

Clusters with well-defined edges	G1	G2	G3
More than 75%	93.3	60.0	6.7
50−75%	6.7	33.3	20.0
25−50%	0	6.7	46.7
less than 25%	0	0	26.7

Table 3 Anisokaryosis in relation to tumor grade

Anisokaryosis	G1	G2	G3
More than triple	0	13.3	53.3
Double-triple	20.0	33.3	40.0
Less than double	80.0	53.3	6.7

Table 4 Number of nucleoli in relation to tumor grade

Number of nucleoli	G1	G2	G3
0	13.3	6.7	6.7
1	33.3	66.7	80.0
2	26.7	13.3	13.3
more than 3	26.7	13.3	0

Table 5 Presence of macronucleoli in relation to tumor grade

	G1	G2	G3
More than 75%	0	6.7	33.3
50−75%	0	13.3	26.7
25−50%	26.7	33.3	13.3
less than 25%	73.3	46.7	26.7

μm in grade 1, 9.55±1.66 μm in grade 2, and 9.97±2.83 μm in grade 3 tumors. There was a significant difference between each grade ($P<0.05$).

(4) Nuclear shape

The incidence of more than 50% of the cancer cells having irregular nuclei was 46.7% in grade 1 tumors, 66.7% in grade 2 tumors, and 66.7% in grade 3 tumors.

(5) Chromatin distribution

The incidence of more than 50% of the cancer cells having an irregular distribution of chromatin was 53.3% in grade 1, 80% in grade 2, and 86.7% in grade 3 tumors.

(6) Anisokaryosis

Table 3 shows the cytologic features of anisokaryosis. The incidence of more than triple anisokaryosis was 0% in grade 1, 13.3% in grade 2, and 53.3% in grade 3 tumors.

(7) Number of nucleoli

Table 4 shows the number of nucleoli in relation to tumor grade. A large number of nucleoli were more frequent in well-differentiated tumors.

(8) Conspicuous nucleoli

Table 5 shows the incidence of conspicuous nucleoli in each smear. More than 50% of the cancer cells had macronucleoli in 0% of grade 1, 20% of grade 2, and 60% of grade 3 tumors. Irregular nucleoli were noted in 0% of grade 1, 0% of grade 2, and 13.3% of grade 3 tumors.

(9) Tumor diathesis

The incidence of tumor diathesis was 80% in grade 1, 86.7% in grade 2, and 93.3% in grade 3 tumors.

(10) Histiocytes

Histiocytes were seen along with tumor cells in 46.7% of grade 1, 46.7% of grade 2, and 53.3% of grade 3 tumors.

(11) Coexisting normal endometrial cells

Coexisting normal endometrial cells were found in 66.7% of grade 1, 26.7% of grade 2, and 46.7% grade 3 tumors.

2. Scoring system

Based on these findings, four items (i.e., the cluster rate, appearance of cluster edges, anisokaryosis, and the presence

of macronucleoli or irregular nucleoli) were considered to have discriminatory value for the grade of endometrial cancer. A scoring system based on these four items was developed (Table 6). Although our data suggest that nuclear size, shape or chromatin distribution also have some discriminatory value when determining tumor grade, these items are considered relatively subjective and less practical for clinical work-up. Other items, such as the number of nucleoli or factors associated with the background of the specimen, do not seem to have enough discriminatory property, and some of these items are considered to have an association with tumor size.

In this system, a score of 0-1, 2-3, and 4-6 was assigned to grade 1, 2, and 3, respectively. On re-examination of the 45 patients using this system, 86.7%(13/15) of grade 1 tumors, 80%(12/15) of grade 2 tumors, and 86.7%(13/15) of grade 3 tumors were diagnosed correctly. Figures 3, 4, and 5 show typical cases that were judged as each of the three tumor grades based on the cytological score.

3. Diagnostic accuracy of tumor grading by endometrial aspiration cytology and fractional curettage

The diagnostic accuracy of tumor grading based on the cytological score in 109 patients was 88% for grade 1, 63% for grade 2, and 82% for grade 3 tumors, while it was 78% (85/109) overall. The corresponding figures based on pathologic assessment of fractional endometrial curettage specimens were 92% for grade 1, 35% for grade 2, and 59% for grade 3 tumors, with an overall accuracy of 66% (72/109) (Figure 6). There was a significant difference in agreement with the postoperative grade between endometrial aspiration cytology and partial endometrial curettage for all patients (P=0.035) and for grade 2 tumors (P=0.012), whereas no such difference was found for grade 1 tumors (P=0.37) or grade 3 tumors (P=0.13).

Discussion

The most noteworthy finding of this study was that our proposed cytological scoring system provided more accurate grading of endometrial cancer than conventional fractional curettage. It is assumed that endometrial aspiration cytology may well sample the entire uterine cavity, and representative malignant cells obtained by this method may thus provide more information about tumor differentiation than cells obtained by fractional curettage.

There seem to be three basic cytologic categories to assess for grading endometrial cancer, which are the appearance of malignant cell clusters, the features of the cancer cells themselves, and the nature of the background. Our preliminary analysis of 45 patients suggested that the appearance of malignant cell clusters is the key

Table 6 Scoring system for determining the grade of endometrial cancer from cytology specimens

	0	1	2
Cluser rate	more than 75%	50−75%	less than 50%
Well-defined clusters	more than 50% of malignant clusters	25−50%	less than 25%
Anisokaryosis	less than triple	more than triple	
Conspicuous nucleoli	less than 50% of malignant cells with macronucleoli	more than 50% with macronucleoli (or the presence of irregular nucleoli)	

Grade 1 : 0-1 points, Grade 2 : 2-3, Grade 3 : 4-6

to identifying tumor grade and that the appearance of individual cancer cells is also important, although to a lesser extent. Much attention was paid to cell aggregates in the development of our scoring system, while only the cytologic features of individual cancer cells that are predominant in grade 3 tumors were included in the system. Other studies[9,10] have indicated that features of backgroud, such as the presence of histiocytes, coexisting normal endometrial cells, and the presence of tumor diathesis, are useful to diagnose tumor grade. However, the determination of tumor grade from these items may be criticized as having an association with tumor size or the relationship between tumor tissue and non-cancerous endometrial tissue rather than tumor grade itself. The background of the specimen may not show a tumor diathesis and may be clear or contain a large number of normal endometrial cells if the tumor is focal with minimal or no necrosis and hemorrahage. Conversely, a tumor diathesis is usually present if the cancer is extensive and aggressive with hemorrhage and necrosis. Thus, it seems difficult to determine whether any given manifestation is a reflection of differentiation or extent alone or is related to both factors.

Consequently, only four cytologic features (i.e., the cluster rate, appearance of cluster edges, anisokaryosis, and the presence of macronucleoli or irregular nucleoli) were employed in our scoring system. The former two items are based on the existence of tight junctions between cancer cells in low-grade tumors, while the latter two items are based on the presence of pleomorphism in high-grade tumors. With respect to nucleoli, macronucleoli are uncommon in cells from grade 1 tumors, and their frequency is inversely related to tumor differentiation, while numerous small nucleoli seem to be characteristic of differentiated tumors. An irregular nucleolar configuration was most often observed in cells derived from grade 3 tumors and was almost not seen in more differentiated tumors.

The value of endometrial curettage for the preoperative identification of the grade of endometrial cancer seems to be limited. Udagawa et al[5]. reported that the diagnostic accuracy of partial endometrial

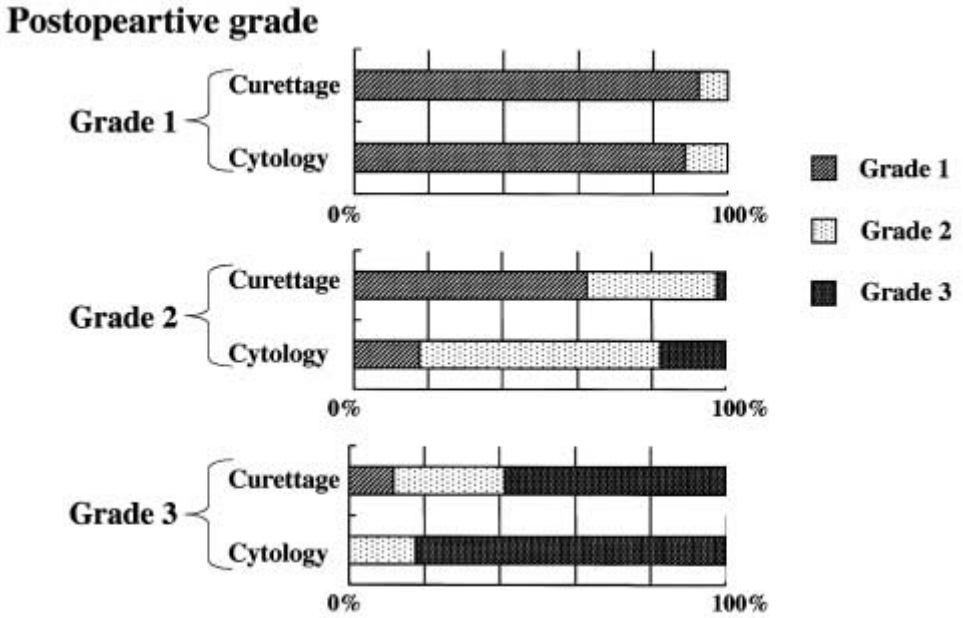

Figure 6. Comparison of the accuracy of tumor grading between the cytological score and fractional endometrial curettage in 109 patients with endometrial cancer. There was a significant difference in agreement with the postoperative grade between endometrial aspiration cytology and partial endometrial curettage for all patients (P=0.035) and for grade 2 tumors (P=0.012), whereas no such difference was found for grade 1 tumors (P=0.37) or grade 3 tumors (P=0.13).

curettage was 66% for grade 1, 53% for grade 2, and 44% for grade 3 tumors, while it was 59%(189/321) for a total. The corresponding figures in this study were 92% for grade 1, 35% for grade 2, and 59% for grade 3 tumors, with a value of 66%(72/109) overall. Soothill et al[6]. found that in 63 out of 117 patients (54%), the tumor grade or type reported from the curettage specimen differed from that shown by examining the hysterectomy specimen. Even with total endometrial curettage, a large discrepancy between the tumor grade obtained and that found at subsequent hysterectomy has still been reported. Obermair et al[7]. reported that 78% of all patients in whom a well differentiated tumor was diagnosed by dilatation and curettage were confirmed to have well-differentiated endometrial carcinoma, whereas 20.4% were upgraded to moderately differentiated tumors after evaluation of the hysterectomy specimen. Daniel and Peters[8] found a 16% discrepancy, with no significant difference between in-hospital dilatation and curettage and office sampling. Histologically, the grade of endometrial cancer has been assigned as an architectural grade based on the proportion of glandular and solid arrays in a tumor. However, studies indicate that the tumor components seen in endometrial biopsy specimens are often different from those of the entire tumor hidden in the myometrium. The inaccuracy of endometrial curettage alone may necessitate further preoperative assessment of tumor grade in order to determine the aggressiveness of an individual tumor.

It must be emphasized that the scoring system proposed in this study is only applicable to endometrial aspiration cytology. Although limited information is available, a difference in cytological features between endometrial aspiration cytology (Masubuchi's method) and the endocyte technique (Laboratoire CCD, Paris, France) was previously reported. Kuramoto et al[11]. reported that round or semi-round clusters are more frequently seen in endometrial cancer patients when aspiration cytology is performed. Conversely, cellular clusters with irregular margins like twigs growing from a tree are a typical cytologic feature of endometrial cancer when the endocyte technique is used. In general, the endocyte technique or other similar sampling devices that come into direct contact with the endometrial tissues provide larger, fresher, and more piled-up cell clusters than aspiration cytology[11,15]. This type of sample is a piece of tissue from the endometrium, so the method is closer to that of fractional endometrial curettage. On the other hand, samples obtained by endometrial aspiration cytology are small and relatively stale, and often show evidence of degeneration. The overlapping of malignant cells is less frequently seen than with endometrial scraping methods[11]. These findings suggest that endometrial aspiration cytology only harvests the surface layers of malignant tissues or desquamated cells floating in the uterine cavity.

It is interesting that endometrial aspiration cytology provides better tumor grading data than fractional endometrial curettage, although the latter extracts malignant cells from the deeper zone of the tumor. Samples obtained by endometrial aspiration cytology are considered to consist of only superficial malignant cells or even pooled degenerated cancer cells from the uterine cavity. Nevertheless, it seems that this type of sample is more readily interpretable and provides more specific information on the degree of tumor differentiation in patients with endometrial carcinoma. This is probably because endometrial aspiration cytology reveals the biological nature of an individual tumor, such as the junctions between cancer cells, rater than directly showing the proportion of glandular and solid components.

In summary, the utility of endometrial aspiration cytology for determining the grade of endometrial cancer has been reported. It seems that the major difference between low-grade and high-grade tumors resides in the appearance of cell clusters and to lesser degree in the cytologic features of the nuclei and nucleoli. This study suggests that preoperative investigation of the grade of endometrial cancer by endometrial cell sampling with aspiration

cytology may provide additional information about tumor differentiation and may also have the potential to replace fractional endometrial curettage.

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