Uterobrush Method in the Detection of Endometrial Pathology

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Abstract. *Background: Endometrial brush cytology is a widely accepted method for the detection of endometrial lesions. The aim of this study was to evaluate the role of cytological sampling using Uterobrush in the screening of endometrial pathology. Patients and Methods: This is a prospective double-blind study evaluating the efficacy of the Uterobrush method (Cooper Surgical, Trumbull, USA) in the detection of endometrial abnormalities. Endometrial cytology was performed during the period January 2009 to April 2010 in all symptomatic patients that underwent dilatation and curettage. The collected samples were firstly smeared directly onto a glassslide and consequently into Thin-Prep buffer. Cytologic features were evaluated according to the criteria of Tao. The main objective was to evaluate the efficacy of Uterobrush method comparing the results of cytologic and histopathologic examination. Results: The sample of the study consisted of 100 women aged 55.8 years (range 38-78 years) with recorded data regarding Uterobrush test and classic histologic examination. Fifty-five patients were postmenopausal. A total of 92% of the samplings were performed by trainees. Endometrial carcinoma was cytologically diagnosed in 8/9 patients, whereas endometrial polyps were diagnosed in 5/34 patients (14.7%). All the patients with simple hyperplasia were correctly diagnosed with the Uterobrush method, whereas the diagnosis of complex hyperplasia with or without atypia was correct in 85.7% and 100% of patients, respectively. Regarding endometrial carcinoma, the sensitivity, specificity, positive and negative predictive values were 88.9%, 100%, 100% and 98.9%, respectively. On the other hand, regarding endometrial polyps, the sensitivity, specificity, positive and negative predictive values were 14.7%, 100%, 100% and 69.5%, respectively. Conclusion: Uterobrush is a reliable direct intrauterine sampling for detecting endometrial abnormalities especially endometrial carcinoma and hyperplasia, but not endometrial polyps. It is a well-tolerated, easy to use method, which provides generous endometrial sampling without contamination from the endocervix or the vagina.*

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Hippocrates in his treatise on "Airs, Waters and Places" defined the concept of prevention as an aspect of medicine. Abnormal uterine bleeding can be caused by fibroids, polyps, hyperplasia, cancer, atrophy or hormonal changes. Moreover, endometrial cancer is the most common invasive neoplasm of the female genital tract with its incidence rising in recent years (1). No screening test is available for the early detection of endometrial carcinoma and its precursors. At present, evaluation of women with abnormal uterine bleeding includes transvaginal ultrasound scanning, hysteroscopy, endometrial biopsy, dilatation and curettage.

George Papanicolaou proposed cervical cytology in the screening of cervical cancer in 1943. Since then, the incidence of cervical carcinoma has been decreasing due to its early detection. The first cytologic description of endometrial carcinoma was performed by Jordan *et al*. in 1956 (2). Johnson and Stormby in 1968 were the first to use a cytological brushing for detection of endometrial pathology (3). Since then, a variety of cytologic devices have been developed for detection of endometrial pathology such as Endocyte, Endopap, Mi-Mark helix, Isaacs' Endometrial Cell Sampler, Gravlee Jet Washer, and Taobrush. Among them, the Uterobrush was firstly used under the name Endobrush in 1987 (4). The first case series of endometrial cytology using Uterobrush was reported by Sato *et al*. (5).

The aim of our study was to evaluate the feasibility and reliability of cytological endometrial sampling using Uterobrush in the detection of endometrial pathology. For this reason, we compared the results of cytologic and histopathologic examination of patients with endometrial abnormalities.

Patients and Methods

This is a prospective double-blind study evaluating the efficacy of the Uterobrush method (processed both by the conventional and the Thin-Prep method) in detection of malignancy and atypical hyperplasia of the endometrium, which was organized by the collaboration of Second Department of Obstetrics and Gynecology, University of Athens, Aretaieion Hospital and the Department of Gynecology, Metaxa Memorial Hospital, Piraeus, Greece during the period February 2009 to April 2010. The Ethics Committee of Metaxa Memorial Cancer Hospital approved the study.

Cytological sampling. Cytological sampling of the endometrium was performed by using Uterobrush (Cooper Surgical, Trumbull, USA). Uterobrush is a presterilised device consisting of a polypropylene sheath 2.5 mm in diameter and 175 mm in length, a thin wire 0.4 mm in diameter and a handle 25 mm in length. The brush bristles are made of nylon and are 6 mm in length and 20 mm in width. Uterobrush allows endometrial sampling without excessive manipulation after a 360 endocavity rotation, an outer sheath that inhibits sample contamination from the endocervix and vagina, and a smooth and rounded end in order to avoid uterine wall injuries.

The brush sampler was retracted completely into the outer sheath. It was gently inserted to the level of the uterine fundus. The outer sheath was then pulled back and the brush was rotated 360 clockwise and then counterclockwise. Then the outer sheath was pushed again and the device was removed. After the collection of endometrial sample, the Uterobrush was withdrawn. The sample was smeared by the conventional method and consequently placed into Thin-Prep buffer. The tip of the brush was placed on a glass slide and immediate fixation followed and Papanicolaou stain was performed. The brush was then plunged into a vial of fixative solution (Thin-Prep) as a liquid-based cytological (Thin-Prep) method was also used in the evaluation of endometrial cytology. The method was followed by dilatation and curettage. The histological findings were compared to the cytological ones either by conventional or Thin-Prep method.

Cytological diagnoses were rendered by one cytologist without knowledge of the tissue section diagnoses, whereas histological diagnoses were rendered by one pathologist without knowledge of the cytological diagnoses, respectively. Histopathologic findings were used as the gold standard for determining the cytologic characteristics. Cytologic features were evaluated according to the previously described criteria of Tao (6). Adequacy of the smears was assessed as satisfactory when sufficient cellular material was present to make a diagnosis or to exclude a pathological process with confidence. Clinical information was collected using the patients case report forms.

Sample size calculations. Considering that the comparison of diagnostic procedures require specific methods, we used receiver operating characteristics (ROC) curves. The main objective was to evaluate the efficacy of Uterobrush method, in terms of area under the curve (AUC) in ROC curves. The power level of the comparison was set to 80% and the level of the type I error (level of significance) was 5%. Using different scenarios in AUC differences, we tried to evaluate the ability of each sample size in detecting differences in AUC (*i.e.* differences in performance) and we analyzed several performance combinations (AUC pairs comparing performance of the two methods). We derived a sample size of 100 individuals. With this sample size the comparison could detect statistically significant differences in performance from 11% to 16% at a 5% significance level with a power level of 80%. Of course, differences greater than 16% are also detectable, since the greater the difference, the easier the detection, using a standard sample size.

The selected sample size allows comparison with results with similar studies since a minimum level of power and reliability in detecting differences in diagnostic ability of the method was achieved.

Statistical methods. Descriptive statistics (*e.g.* mean, standard deviation) were used to present numeric variables (*e.g.* age). Absolute and relative frequencies were used for categorical variables. To explore the relation between categorical variables, we used contingency tables along with Fisher's exact test (for 2×2 tables) or Pearson's Chi-square test (for tables of higher dimensions than 2×2). In order to evaluate the validity of Uterobrush test results in agreement between cytologic and histologic diagnosis, we used the kappa coefficient for agreement. To find any 'direction' in case of 'disagreement' in the result of the two methods, we used Mc-Nemar's test for direction. Odds ratios were also calculated for 2×2 contingency tables to estimate the relative risk. Kolmogorov-Smirnov test was used to check the normality assumption of numeric variables. To investigate differences in mean values of numeric variables between levels of categorical variables we used independent *t*-test (for categorical data with 2 levels) or ANOVA (for categorical variables with 3 or more levels). Additionally Mann-Whintey and Kruskall-Wallis non parametric test result are presented to cover any normality deviation problems. Finally, the logistic regression model was fitted for accuracy of diagnosis of polyps. For all statistical methods, a significance level of 5% was used.

Results

The sample of the study consisted of 100 women aged 55.8±1.05 (mean±s.e.) years, (range 38-78 years) with recorded data regarding Uterobrush method and classic histology examination used as the gold standard for determining the performance characteristics of cytology. Fifty-five patients were postmenopausal, 14 nulliparous and 63 obese. 92% of the samplings were performed by trainees. The demographic data of our study are presented in Figure 1.

Our study evaluated the feasibility and reliability of endometrial cytology using Uterobrush. The method was easily performed in 97/100 of cases. It was not possible to be obtain a sample in 3/100 patients because of atrophy, nulliparity or cervical stenosis. A total of 8/100 collected smears were inadequate, containing insufficient cells for interpretation. None of the procedures was complicated by either hemorrhage or infection and the smears were easily collected. There was little bloody cervical discharge after the procedure which did not require any treatment.

Endometrial carcinoma was diagnosed cytologically in 8/9 patients, whereas endometrial polyps were diagnosed in 5/34 patients (14.7%). All the patients with simple hyperplasia were correctly diagnosed with Uterobrush method, whereas

the diagnosis of complex hyperplasia with or without atypia was correct in 6/7 (85.7%) and 4/4 (100%) of patients, respectively. Regarding endometrial carcinoma the sensitivity, specificity, positive and negative predictive values were 88.9%, 100%, 100% and 98.9%, respectively. On the other hand, regarding endometrial polyps, the sensitivity, specificity, positive and negative predictive values were 14.7%, 100%, 100% and 69.5%, respectively (Figure 2). Similar results were identified with the conventional and with Thin-Prep cytologic techniques. It should be mentioned that when gynecologists and cytologists were more familiar with the method, the results became optimal.

Discussion

In our study, we tried to clarify the role of endometrial cytology in comparison to the traditional methods of histologic detection of endometrial pathology. Norimatsu *et al*. proposed that diagnosis of endometrial cytology should be based on awareness of the cytoarchitectural characteristics (7). Our diagnostic categories were benign endometrium, atrophic endometrium, polyps, hyperplasia, and cancer (Figures 3-5).

Endometrial cancer is the most common gynecologic malignancy. It is divided into two types: cancer associated with hyperplasia and cancer not associated with hyperplasia. Type 1 cancer is of low grade, estrogen-related endometrioid and has precursor hyperplasia. Type 2 is higher grade papillary serous or clear cell and non estrogen-related. Koss *et al*. proposed that endometrial cancer develops as a focal event in an atrophic or only focally hyperplastic endometrium (8). Ambros *et al*. believed that endometrial carcinoma develops from atypical hyperplasia, whereas serous carcinoma arises from *in situ* endometrial adenocarcinoma (9). It is known that over 40% of cases of hyperplasia with nuclear atypia progress to cancer compared to 2% without atypia (6, 10-12). For this reason, the Uterobrush method could be used as a screening test for the early detection of endometrial cancer or precancerous lesions.

The advantages of cytologic detection of endometrial abnormalities vary. Uterobrush is an outpatient clinic procedure which could be performed without anesthesia. Although, we used the method under general anaesthesia and we did not evaluate patient's pain or discomfort, it is known from the literature that the procedure is generally comfortable. Furthermore, more than one slide can be prepared as a decent smear is obtained. Cytoblocks can also be prepared for histological examination, while diagnosis can even be made the same day. Regarding the cost of the procedure, a day case dilatation and curettage is estimated to cost around 600 euros whilst a Uterobrush costs 7.20 euros in Greece. As known from the literature, endometrial cytology is very effective when performed in conjunction with liquid fixation. In our study, both liquid-based cytology and conventional methods were performed. No differences were found between the two different cytologic techniques. The advantages of this method are that a smaller area is required for screening to identify endometrial cells, the quality of cell presentation is superior and the cells can easily be preserved (13). Thin-layer endometrial cytology has similar high specificity, sensitivity, positive and negative predictive value to those of conventional method for the detection of endometrial pathology similarly to previous studies (14).

A disadvantage of the method was that well-differentiated adenocarcinoma is similar to endometrial hyperplasia as it is characterized by good cellular cohesion and mild atypia. In addition, around 10% of endometrial samples do not provide adequate smears (15). In our study, only 8% were not adequate samples. The detection of endometrial pathology by Uterobrush depends on the size and type of the lesion, its location in the uterine cavity, the sampling and preparation methods. Another disadvantage of the procedure is that it is painful for postmenopausal nulliparous women with a narrow cervical canal. In our study, we did not manage to penetrate the cervical canal in 3/100 patients who were all postmenopausal, nulliparous women. Hence, nulliparity was significantly associated with insertion failure.

Uterobrush is a safe, noninvasive procedure which is easy to use and well tolerated by patients. It allows an adequate, representative sample to be taken without contamination from endocervical or vaginal cells. However, in our study, 24/100 samples were contaminated with cervical cells. It should be mentioned that in these cases, the cytologists did not face problems in diagnosis as they are familiar with cervical cytology. Similarly to previous studies, the method in our study had the disadvantage of being unable to detect benign polyps (1). However, compared to previous studies (1, 16), our cytologists did not refer difficulty in the differential diagnosis of simple hyperplasia and proliferative endometrium.

Endometrial sampling using the Uterobrush device produces adequate specimens which contain cellular material for the identification of endometrial pathology. For this reason, it could be applied as screening of patients with endometrial pathology (*e.g*. hyperplasia or carcinoma). Regarding our data, they are similar to previously published results in which the sensitivity and specificity ranged from 95.5% to 100%, while both sensitivity and specificity of endometrial hyperplasia detection were 100% (11, 17-20). Koss *et al*. proposed that all women over 50 years old should have endometrial cytology screening at least once per their lifetime (8). It should be mentioned that in a meta-analysis (21) of endometrial sampling with various techniques in pre- and post-menopausal patients, the detection of endometrial cancer was higher in postmenopausal women; this finding was also confirmed in

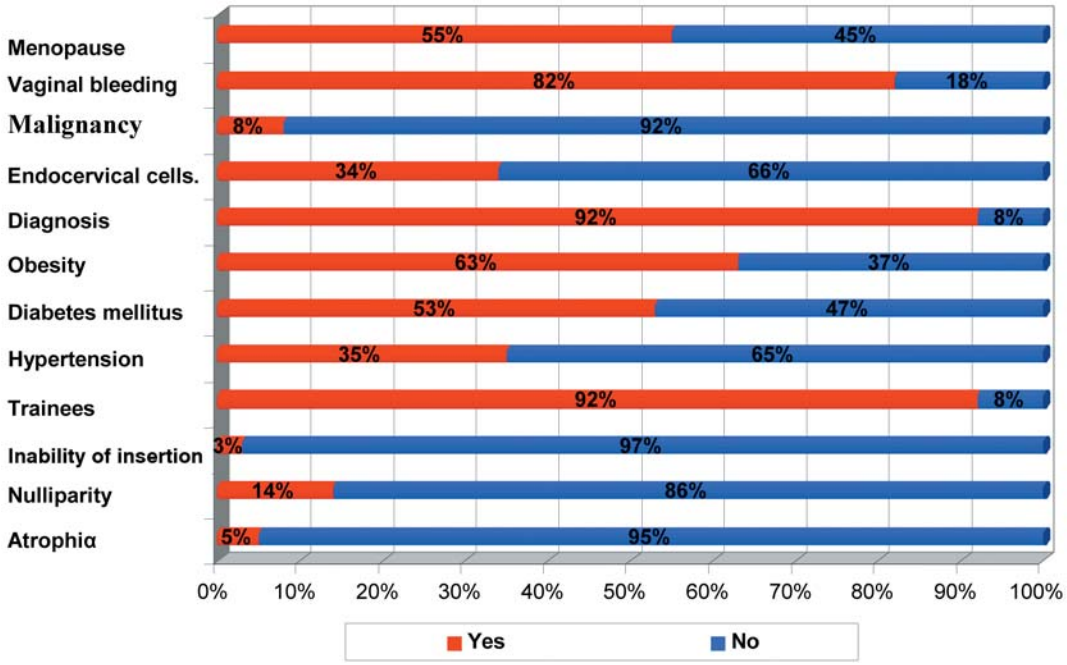

Figure 1. *Demographic characteristics of the study.*

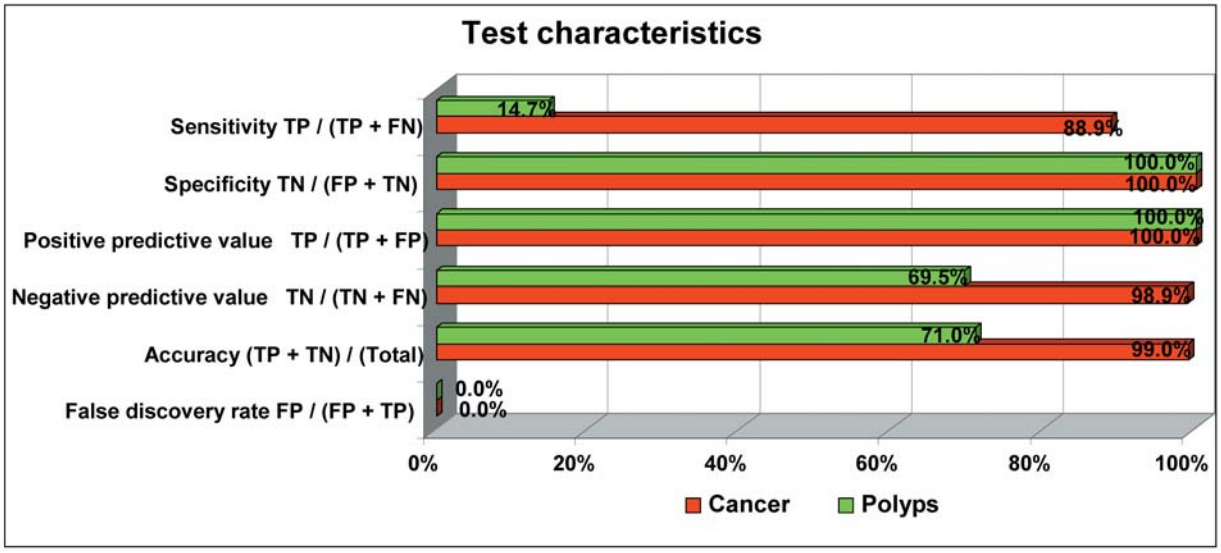

Figure 2. *Statistical results regarding detection of endometrial cancer and polyps by using the Uterobrush method. TP: True positive, TN: true negative, FP: false positive, FN: false negative.*

our study. Furthermore, cytomorphologic appearances of normal endometrial cells during different menstrual phases can be used as an approach for endometrial dating (22). The method could be used for patients with irregular bleeding who are using hormonal replacement therapy or tamoxifen. It could also be used in patients with abnormal uterine bleeding, pathological transvaginal ultrasound, in need of microbiologic studies, or with suspicious Pap smear. One of the advantages of using the Uterobrush is that it can be used in women with atrophic endometrium. The method could also be used in elderly patients with serious comorbidity as the optimal approach of endometrial pathology.

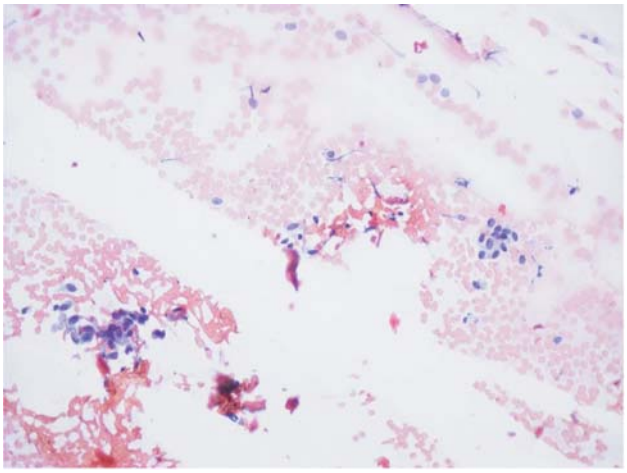

Figure 3. *The cytology of normal endometrium comprises flat sheets, uniform glands, stroma, vessels and banal-shaped nuclei. Benign endometrium is characterized by homogeneous narrow, straight uniform glands and cohesive flat sheets (proliferative endometrium). The nuclei are banal-to-cigar-shaped. The stroma is dense and edematous (×25).*

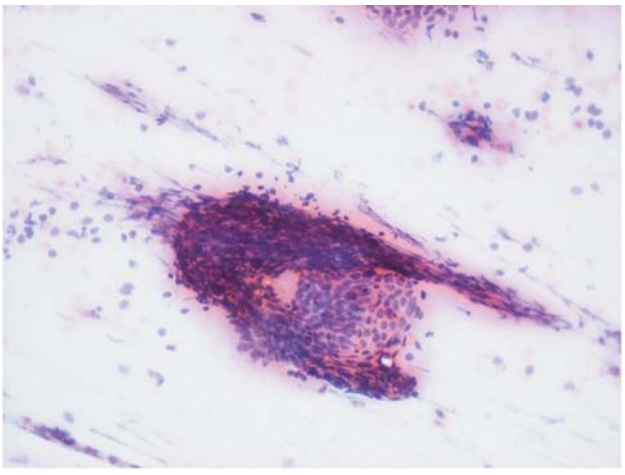

Figure 4. *Secretory endometrium has more cytoplasm and larger nuclei, which are rounded and vesicular with small nucleoli (×120).*

On the other hand, many could argue that the use of the Uterobrush could have an increased incidence of pain and cramping, as well as the inability to pass through the cervical canal into the endometrial cavity. In our study, we faced these problems in 3/100 patients. Furthermore, others could state that the Uterobrush may collect less tissue than other sampling techniques, such as the Pipelle. For this reason, further studies are needed comparing the Uterobrush to Pipelle, with dilatation and curettage to confirm the diagnosis in order to compare the efficacy of the two methods.

The Uterobrush is a minimally invasive alternative technique for obtaining an adequate endometrial sample for cytologic examination, with a high sensitivity and specificity for detection of hyperplasia and malignancy. It is characterized by negligible patient discomfort. In conclusion, the use of Uterobrush endometrial cytology in patients with abnormal uterine bleeding and endometrial thickness >5 mm could avoid the need for hysteroscopy or dilatation and curettage. However, histologic examination is still used as the gold standard for exploring endometrial pathology. Further studies are necessary in order to clarify the role of endometrial cytology in order to achieve better tolerance of gynecologists and cytologists to this method.

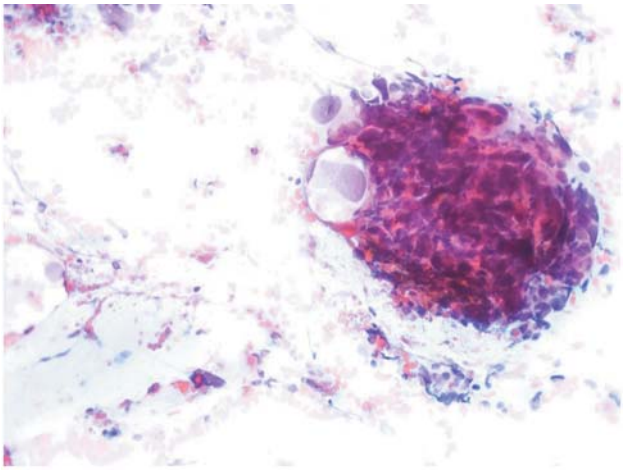

Figure 5. *Endometrial carcinoma is characterized by malignant cells which replace endometrial surface epithelium. Endometrial carcinoma is also characterized by anisonucleosis, poikilonucleosis, molding of the nuclear envelope, vesiculation of the nucleoplasm with coarse chromatin, granularity, hyperchromasia and nucleolar prominence. Depending on its grade, it is characterized by either tumor diathesis, stromal foam cells, necrosis, neutrophilic emperipolesis (low grade), or anaplasia, dyshesion and dedfferentiation (high grade) (×120).*

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