*Int J Gynecol Cancer* 2007, 17, 182–187

# Liquid-based endometrial cytology: its possible value in postmenopausal asymptomatic women

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**Abstract.** Buccoliero AM, Castiglione F, Gheri CF, Garbini F, Fambrini M, Bargelli G, Pappalardo S, Scarselli G, Marchionni M, Taddei GL. Liquid-based endometrial cytology: its possible value in postmenopausal asymptomatic women. *Int J Gynecol Cancer* 2007;17:182–187.

**The incidence of endometrial adenocarcinoma in asymptomatic women is low. Nevertheless, some of these women might require endometrial surveillance. In this study, we evaluated the accuracy of liquid-based endometrial cytology compared to biopsy in asymptomatic postmenopausal women. Three hundred twenty women scheduled for hysteroscopy were enrolled for this study. After hysteroscopy, patients were submitted to endometrial cytology and to biopsy. Two hundred ninety-three (92%) women had sonographically thickened endometrium (>5 mm), 53 (17%) were on tamoxifen, and 16 (5%) were on hormonal substitutive treatment. The evaluation of the biopsies determined that six (2%) women had adenocarcinoma, one (<1%) had adenomatous atypical hyperplasia, and eight (3%) had simple nonatypical hyperplasia. Endometrial cytology evidenced 5 (2%) neoplastic cases, 2 (<1%) hyperplastic with atypia cases, and 25 (8%) hyperplastic without atypia cases. Two hundred twenty-two biopsies (69%) and 17 (5%) cytologies were inadequate. One adenocarcinoma and one simple nonatypical hyperplasia were underrated by cytology resulting, respectively, as atypical hyperplasia and as negative. Four cases were false positive (simple nonatypical hyperplasias on cytology, negative on biopsy). The sensitivity and specificity were estimated, respectively, at 94% and 95%; the positive and negative predictive value were estimated, respectively, at 80% and 99%. Endometrial cytology provided sufficient material more often than biopsy ($P < 0.01$). We suggest to introduce liquid-based endometrial cytology in the management of some subpopulations of asymptomatic postmenopausal women. Particularly, the combination of liquid-based endometrial cytology and transvaginal sonography may improve their diagnostic accuracy and reduce unnecessary more invasive and expensive procedures.**

KEYWORDS: adenocarcinoma, asymptomatic, endometrial cytology, liquid based, thin layer.

Endometrial carcinoma is the most common malignancy of the female genital tract in developed countries. Nowadays, more than 1 in 20 female malignances in Europe are of the endometrium[1]. Endometrial cancer more frequently occurs in postmenopausal women over 50 years of age with abnormal uterine bleeding. This early presenting symptom makes diagnosis possible during the first stages in the majority of cases[2,3]. Consequently, mortality from this neoplasm is low with the exception of some more aggressive histotypes, ie, serous papillary and clear-cell carcinoma. However, abnormal uterine bleeding is a frequent and unspecific symptom. It is one of the most common reasons for postmenopausal women to visit a gynecologist[4,5]. Many pathologic conditions, ie, hormonal dysfunction, myomas, genital inflammation, or polyps, may cause uterine bleeding[6]. In contrast, the most common cause of this symptom in postmenopausal women is endometrial atrophy[7,8]. It has been estimated that only 10% of women with postmenopausal bleeding are affected with endometrial cancer[7,9]. Iatrakis *et al.*[7] referred that in a group of 628 symptomatic patients with a median age of 52 years atrophic endometrium was found in 83% of

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doi:10.1111/j.1525-1438.2006.00757.x

the cases, whereas endometrial carcinoma in 11%. Despite all of this, there is general agreement that all abnormal uterine bleeding, particularly during the postmenopausal age, should arouse suspicion of neoplasia and consequently should be promptly investigated.

Only a small number of endometrial carcinomas, ranging between 0.07% and 0.6%, are incidentally diagnosed in asymptomatic patients[10–13]. This percentage is higher in women with risk factors such as tamoxifen and unopposed exogenous estrogens therapy, obesity, low parity, hypertension, diabetes, and autosomal dominantly inherited hereditary nonpolyposis colorectal cancer. For this reason, some high-risk asymptomatic subpopulations might require a surveillance for endometrial carcinoma[14].

Several diagnostic procedures are available in the investigation of the endometrium. Transvaginal sonography (TVS), hysteroscopy, sonohysterography, endometrial biopsy, and dilatation and curettage are believed to be efficacious diagnostic methods. The measurement of the endometrial stripe by TVS is frequently used in the endometrial investigation due to the low cost and very good tolerability. However, its specificity is low. Often TVS does not provide definitive diagnoses particularly due to its high false-positive rate. Consequently, a large number of women with echographically thick endometrium, both symptomatic and asymptomatic, are scheduled for further exams[15].

Endometrial cytology has been hampered in its dissemination because of the common presence of excess blood and overlapping cells. In addition, nonpathologists may experience difficulty in the interpretation of the endometrial cytology: the endometrium is a hormone-dependent organ undergoing various important morphologic changes in women depending on their age and upon the cyclical ovarian hormonal stimulation or the assumption of hormonal therapy. Liquid-based cytology, because of its capacity to reduce obscuring factors and to provide thin-layer specimens, represents an opportunity to reevaluate endometrial cytology.

The aim of this study is to asses the utility of liquid-based method in the endometrial diagnosis in asymptomatic postmenopausal women. We evaluated its accuracy compared to biopsy in a group of 320 consecutive women scheduled for hysteroscopy.

## Materials and methods

Three hundred twenty consecutive postmenopausal asymptomatic women between 50 and 89 years old (median 61) were accepted for this study. After providing informed consent, all women were submitted first to hysteroscopy, then to endometrial cytology, and last to biopsy. Two hundred ninety-three (92%) women had thickened endometrium (>5 mm) as evaluated by TVS, 53 (17%) were on tamoxifen, and 16 (5%) were on hormonal substitutive treatment.

All patients underwent diagnostic hysteroscopy with a 3.5-mm sheath, and carbon dioxide as the distension medium. Endometrial hysteroscopic features such as polyps, myomas, suspected hyperplasias, and suspected neoplasia were recorded.

Cytologic sampling was performed by *brushing* using the Endoflower device (RI-MOS, Mirandola, Modena, Italy). It measures 3 mm in diameter and consists of a mandrel with a shaped tip containing microholes on curved thin arms that slide inside an outer sheath. After the endometrial sampling, the tip of the device was immersed in the Cytolyt® (Cytyc Corporation, Boxborough, MA) vial where it was vigorously stirred in order to facilitate the cell releasing.

The samples were centrifuged and the pellet containing the cells was transferred into a vial containing PreservCyt® (Cytyc Corporation). Blood and mucus were eliminated by washing through the succession of centrifugation and resuspension in N-Acetil-L-Cisteine (mucolysis; before the fixation in PreservCyt®) and/or acetic acid (hemolysis; after the fixation in PreservCyt®). The vial was processed by the ThinPrep 2000 automated slide processor (Cytyc Corporation). The slides were stained with routine Papanicolau stain.

Histologic sampling was performed using the Endoram device (RI-MOS) measuring 3.8 mm in diameter. It consists of an outer sheath, which covers a plunger having a rhomboidal shape at the tip. Endometrial samples were routinely fixed in neutral buffered formol, embedded in paraffin, and stained with hematoxylin and eosin.

Cytologic and histologic diagnoses were executed blindly by two cyto-histo-gynecopathologists (A.M.B. and G.L.T.). In case of discordant diagnosis, both pathologists reviewed the case together and reached an agreement on the diagnosis. The slides were considered unsatisfactory when there were fewer than five endometrial clusters (endometrial cytology) or severe fragmentation or scarcity of the endometrial tissue (endometrial biopsy).

The cytologic diagnosis was given according to criteria that we had previously reported[16]. We considered four categories, negative, nonatypical hyperplasia, atypical hyperplasia, and carcinoma, on the basis of the architectural and cytologic features considering all anamnestic and clinical information (Figs. 1–4).

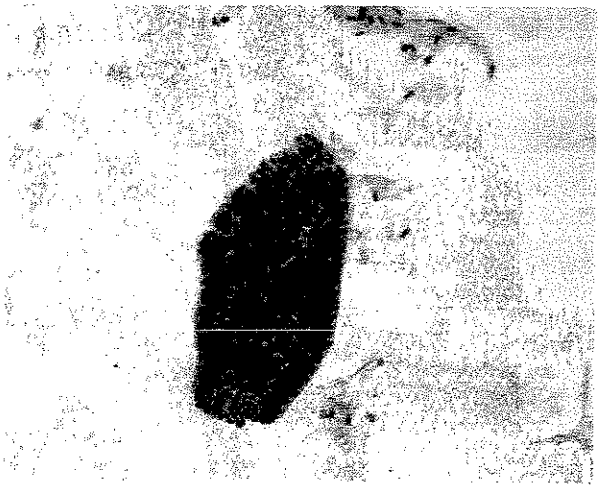

**Figure 1.** Atrophic endometrium: small three-dimensional endometrial cluster formed by isomorphic cells, with scant cytoplasm and iperchromatic regular nuclei. Original magnification ×200, hematoxylin–eosin.

**Figure 3.** Atypical hyperplasia: three-dimensional endometrial cluster formed by pleomorphic cells showing architectural disorder. Original magnification ×200, hematoxylin–eosin.

The histologic diagnosis was given according to the World Health Organization criteria[17].

Because of the small number of pathologic specimens, statistical analyses were performed categorizing the cases as nonpathologic and pathologic (hyperplasia, atypical hyperplasia, carcinoma). The sensitivity was calculated by dividing the true pathologic cases diagnosed by endometrial cytology by the total number of patients with endometrial pathology and multiplying by 100; the specificity was calculated by dividing the number of true-negative cases diagnosed by endometrial cytology by the total number of women who did not have endometrial disease and multiplying by 100; the positive predictive value was calculated by dividing the true pathologic cases by the total of both true-positive cytologic specimens and false-positive cases and multiplying by 100; the negative predictive value was calculated by dividing the true-negative cases by the total of both true-negative and false-negative cytologic specimens and multiplying by 100. The Chi-square test was used to compare the number of unsatisfactory cytologic specimens with that of endometrial biopsies. Statistical significance was judged as $P < 0.05$. Data analysis was performed using the Glaz SA 3.03 Version statistical package (Mc Graw-Hill, New York, NY).

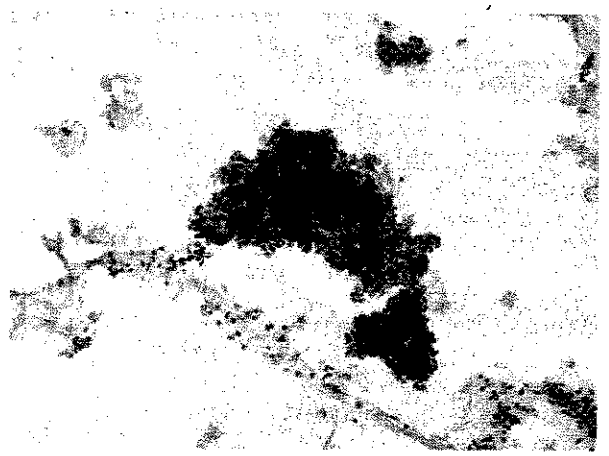

**Figure 2.** Nonatypical hyperplasia: three-dimensional endometrial clusters formed by isomorphic cells with scant cytoplasm and iperchromatic nuclei; cellular crowding and architectural disorder are prominent. Original magnification ×100, hematoxylin–eosin.

## Results

Hysteroscopy evidenced the presence of endometrial polyp in 177 cases (55%), of submucosal myoma in 21 cases (7%), of endometrial hyperplasia in 18 cases (6%), and of adenocarcinoma in 4 cases (1%).

The evaluation of the biopsies determined that six (2%) women had adenocarcinoma, one (<1%) had adenomatous atypical hyperplasia, and eight (3%) had simple nonatypical hyperplasia. Endometrial cytology evidenced 5 (2%) neoplastic cases (Fig. 2), 2 (<1%) hyperplastic with atypia cases, and 25 (8%) hyperplastic without atypia cases (Table 1).

Two hundred twenty-two biopsies (69%) and 17 (5%) cytologies were inadequate. In 4 cases, the cytology was inadequate and the biopsy was adequate; in 209 cases, the biopsy was inadequate and the cytology was adequate; and in 13 cases, both the cytology and the biopsy were inadequate. Cyto-histologic correlation was possible in the remaining 94 (29%) cases.

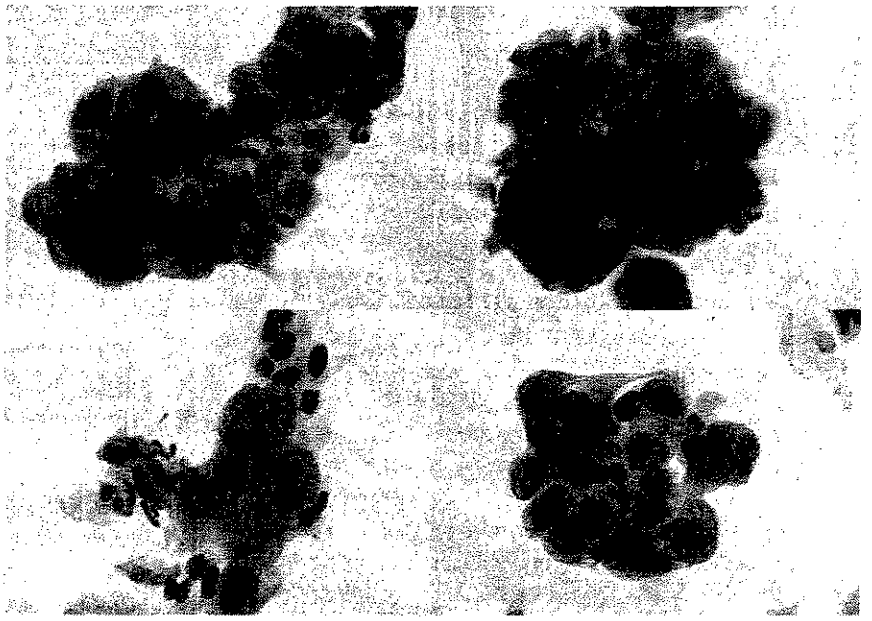

**Figure 4.** Endometrial adenocarcinoma: endometrial clusters with variable dimension, architectural disorder, and presence of macronucleoli. Original magnification ×200, hematoxylin–eosin.

In the series of 94 cases in which both endometrial biopsy and cytology were adequate, endometrial cytology recognized 5 neoplastic cases, 2 hyperplastic with atypia cases, and 13 hyperplastic without atypia cases. One endometrial adenocarcinoma and one simple nonatypical hyperplasia were underrated by endometrial cytology resulting, respectively, as atypical hyperplasia and as negative. Four cases were false positive (nonatypical hyperplasias on cytology, negative on biopsy) (Table 2).

When we categorized the cases studied as nonpathologic and pathologic, endometrial cytology showed the sensitivity estimated at 94%, the specificity at 95%, the positive predictive value at 80%, and the negative predictive value at 99%.

Endometrial cytology provided sufficient material for the diagnosis more often than endometrial biopsy ($P < 0.01$).

## Discussion

Endometrial cancer manifests early with uterine bleeding[18]. On this basis, the American Cancer Society guidelines affirmed that there is no proven role for screening asymptomatic women who are at an average or somewhat increased risk for endometrial malignancies[19]. However, some conditions, ie, a long-time administration of tamoxifen in patients older than 50 years, may cause anxiety both in the women and in the clinicians. Moreover, the dissemination of TVS, because of its high false-positive rate, produced a large number of women who required further exams. In such situations, an effective and low-cost exam could certainly reassure women and protect the clinician.

The improvement of the diagnostic accuracy of endometrial cytology related to the introduction of the liquid-based method suggests a concrete introduction of this test in the endometrial diagnosis. The main characteristics of this method are the reduction of obscuring factors, the distribution of cells in a thin layer, and the possibility to obtain more than one slide available to further investigation, ie, immunohistochemistry. Some recent studies emphasized the diagnostic potential of endometrial liquid–based cytology[20–23]: Garcia *et al.*[20] in 2003 performed a prospective study of 103 symptomatic women and

**Table 1.** Cytologic and histologic results (320 women)

| | Cytology, *n* (%) | Histology, *n* (%) |
|---|---|---|
| Nonpathologic | 271[a] (85) | 83[a] (26) |
| Pathologic | 32 (10) (5 adenocarcinomas, 2 atypical hyperplasias, 25 non atypical hyperplasias) | 15 (5) (6 adenocarcinomas, 1 atypical adenomatous hyperplasia, 8 simple non atypical hyperplasias) |
| Inadequate | 17 (5) | 222 (69) |
| Total | 320 (100) | 320 (100) |

[a]Nonhyperplastic or neoplastic cases were grouped.

**Table 2.** Clinical and pathologic findings in the 21 positive cases when both cytology and biopsy were adequate

| | | Hysteroscopy | | | |
|---|---|---|---|---|---|
| Case | Age (years) | Indication | Features | Cytology | Biopsy |
| 1 | 63 | EET | EP | EC | EC |
| 2 | 71 | EET | EP | EC | EC |
| 3 | 72 | EET | EC | EC | EC |
| 4 | 83 | EET | EC | EC | EC |
| 5 | 65 | EET | EC | EC | EC |
| 6 | 75 | EET | EC | HGH | EC |
| 7 | 55 | EET | EH | HGH | HGH |
| 8 | 52 | EET | EP | LGH | NEG |
| 9 | 73 | EET | EP | LGH | LGH |
| 10 | 79 | EET | EP | LGH | LGH |
| 11 | 57 | EET | EP | LGH | LGH |
| 12 | 61 | EET | EP | LGH | LGH |
| 13 | 83 | EET/TAM | EP | LGH | LGH |
| 14 | 55 | EET | EP | LGH | NEG |
| 15 | 56 | EET | EP | LGH | NEG |
| 16 | 56 | EET | EP | LGH | LGH |
| 17 | 57 | EET | EP | LGH | LGH |
| 18 | 62 | EP | EP | LGH | LGH |
| 19 | 52 | EET | EP | LGH | NEG |
| 20 | 53 | EET | EH | LGH | LGH |
| 21 | 52 | EET | EH | NEG | LGH |

EET, ecographic endometrial thickness; TAM, tamoxifen; EP, endometrial polyp; EC, endometrial carcinoma; EH, endometrial hyperplasia; HGH, high-grade endometrial hyperplasia; LGH, low-grade endometrial hyperplasia; NEG, nonpathologic.

reported a very good specificity (96%), a good (78%) sensibility, and a low (15%) inadequate rate (lower than endometrial biopsy calculated as 26%); in the same year, our group[21], in a population of 162 women, documented a cyto-histologic concordance of 98% and a low unsatisfactory rate of 18%; in 2005, Papaefthimiou *et al.*[22] referred that the liquid-based endometrial cytology allows for the applications of the common diagnostic criteria making possible a nearly perfect interobserver and intraobserver agreement. Moreover, it has recently been suggested that cell block preparation obtained from liquid-based cytologic sampling, when necessary, might further increase the diagnostic accuracy of thin-layer endometrial cytology[23]. The low percentage of unsatisfactory specimens, the high sensitivity, positive and negative predictive values, and the good specificity seen in our present study support these findings. The high inadequate rate of endometrial biopsies in our collection may be related to the prevalence of nonneoplastic or hyperplastic cases. A further cause of the high rate of inadequate biopsies could be related to the sequence of the endometrial sampling: first the cytology and then the biopsy. The first sampling might impair the success of the second sampling.

Our results demonstrate that in some subpopulations of asymptomatic women, endometrial cytology could be useful in endometrial surveillance. In our collection of asymptomatic women, who nearly always had thickened endometrium and/or were on tamoxifen, the prevalence of carcinomas and hyperplasias was 2% and 4%, respectively. In these categories of women, the risk of cancer increases on the sonographic threshold, which separates normal from abnormal endometrial thickness as well as on the duration of the tamoxifen administration and on the woman's age. In 2004, Smith–Bindman *et al.*[24] calculated that with a cutoff of 11 mm, the frequency of endometrial adenocarcinoma is approximately 6.7%. The reported relative risk for uterine malignancies from tamoxifen reaches 15.2 in elderly long-term users[25].

This current study provides the first specific evaluation of the effectiveness of liquid-based endometrial cytology for endometrial surveillance in some selected asymptomatic patients. Although further studies are necessary, our results suggest the possibility of liquid-based endometrial cytology as a useful diagnostic step. In particular, the combination of TVS and endometrial cytology may improve their diagnostic accuracy, which would reduce more invasive and expensive procedures.

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*Accepted for publication July 10, 2006*