Endometrial cell dissemination at diagnostic hysteroscopy: a prospective randomized cross-over comparison of normal saline and carbon dioxide uterine distension

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The incidence of pelvic spreading of endometrial cells at diagnostic hysteroscopy was studied comparing the two distension media carbon dioxide (CO2) and normal saline (N/Saline). Thirty patients requiring laparoscopy and hysteroscopy were included in this study, the main indication for surgery being subfertility. Hysteroscopy was performed using both CO2 and N/Saline distension on each patient, the order of the distension media being randomly allocated. Samples of peritoneal fluid were aspirated from the pouch of Douglas before and after hysteroscopy with each distension medium, and the specimens were investigated cytologically for the presence of endometrial cells. Endometrium was present in 230 (7.6%) peritoneal aspirates before and in 15/60 (25%) collected after the hysteroscopies. There was no major difference between liquid or gaseous distension, transtubal reflux of endometrial cells occurring in 7/30 (23.3%) and in 8/30 (26.7%) hysteroscopies respectively. Positive peritoneal cytology was observed significantly more often in patients who were in the proliferative phase of the menstrual cycle [9/14 (64.3%) versus 0/11, P < 0.004]. In conclusion, transtubal dissemination of endometrium occurs in about one quarter of patients, irrespective whether N/Saline or CO2 is used for uterine distension; there is no advantage to using gaseous distension for hysteroscopy when investigating high-risk cases for endometrial malignancy. Key words: cell dissemination/endometrium/hysteroscopy

Introduction

Due to its higher diagnostic accuracy and suitability for outpatient investigation, hysteroscopy is increasingly replacing dilatation and curettage (D&C) for the evaluation of abnormal uterine bleeding, the single most common reason for gynaecological referrals (Gimpelson and Rappold, 1988; Coulter et al., 1991; Nagele et al., 1996). Hysteroscopy is also becoming routine for the investigation of subfertility and recurrent pregnancy loss (Tulpala et al., 1993; Gaglione et al., 1996). For instance, between 1998 and 1994, the annual number of D&C's performed in England has decreased from 168 404 to 106 146 with a 6-fold increase in the number of diagnostic hysteroscopies (Hospital Episode Statistics, London, 1995). Similarly, there has been a dramatic decline in the D&C rates in the United States over the past decade (National Centre for Health Statistics, United States, 1991). Specially designed for office use, the new generation of small diameter hysteroscopes (3.5 mm) in combination with an atraumatic insertion technique allows success rates of almost 98% for diagnostic hysteroscopy (Wieser et al., 1998; Campo et al., 1999). Therefore, hysteroscopy is now generally acknowledged as the ‘gold standard’ investigation of the uterine cavity.

As the uterine cavity is a potential space, hysteroscopy requires distension of the cavity with a gaseous or liquid medium at a pressure of 50–150 mmHg to allow complete visualization of the fundus and ostial areas. Liquid media used for this purpose include high viscosity fluids such as 32% dextran 70 or low viscosity fluids such as 5% dextrose, Ringer’s solution and normal saline; the gas universally used for diagnostic hysteroscopy is carbon dioxide (CO2) (Baggish et al., 1989). There is evidence from observational studies that liquid distension can be associated with transtubal reflux of endometrial cells and tissue into the peritoneal cavity, particularly with 32% dextran (Siegler and Lindemann, 1984; Bartosik et al., 1986). The two studies looking at CO2 distension presented contradictory results (Beyth et al., 1976; Ranta et al., 1990).

While tubal reflux during menstruation is almost universal and can be considered to be physiological (Halme et al., 1984; van der Linden et al., 1995), endometrial reflux during hysteroscopy is of concern when investigating women complaining of abnormal uterine bleeding who are subsequently found to have endometrial malignancy. Pertinent to this, several investigators have recently reported on retrograde seeding of endometrial carcinoma during hysteroscopy (Romano et al., 1992; Schmitz and Nahhas, 1994; Egarter et al., 1996). Although the clinical implications of such reflux have yet to be determined, in principle it would seem preferable to avoid transtubal dissemination of endometrial tissue at hysteroscopy in high-risk cases. The current evidence suggests that this would be best achieved with gaseous distension. To clarify the influence of the uterine distension medium on tubal reflux, we conducted a prospective randomized comparison of CO2 and normal saline, two of the most common distension media for diagnostic hysteroscopy (Soderstrom, 1992; Nagele et al., 1996).

Materials and methods

Patients

A total of 30 patients requiring laparoscopy and hysteroscopy were included in this study. The main indication for referral was primary
Randomization with respect to the uterine distension medium took place following the initial inspection of the pelvis and the code was installed from a flexible 500 ml bag wrapped in a pressure cuff. Patients were randomly allocated in a ratio of 1:1 to receive hysteroscopy (control sample) and after each hysteroscopy, giving a total of up to 50 ml/min and a pressure not exceeding 100 mmHg was applied, recognizing an epithelial marker present in endometrial epithelium but not in cells of mesothelial origin (De Angelis et al., 1992). In the specimens collected, individual sample cellularities were not reliably assessed, as this would not provide any conclusive additional information.

The data were analysed using the $\chi^2$ test (including Yates correction when appropriate), and a result of $P < 0.05$ was considered statistically significant.

### Results

A total of 36 patients underwent laparoscopies, of whom six were excluded from the study because of macroscopic endometriosis ($n = 3$), peritoneal biopsy showing endometriosis ($n = 1$), or bilateral tubal blockage ($n = 2$). Of the remaining 30 women, 10 had intraperitoneal pathology diagnosed by laparoscopy, including pelvic adhesions ($n = 5$), polycystic ovarian syndrome ($n = 3$) and pelvic inflammatory disease ($n = 2$). Seven patients had abnormal findings at hysteroscopy, namely hyperplastic endometrium ($n = 3$), subseptate uterus ($n = 2$), double uterine cavity ($n = 1$) and submucous leiomyomata ($n = 1$).

The epithelial associated antibody Ber-EP4 was applied in 11 cases to clarify the origin of the cells seen in the peritoneal aspirate. Tubal epithelial cells were found in 31 specimens. Endometrium was present in two control samples and in a total of 15/60 (25%) specimens after the hysteroscopies. Overall, there was no major difference between transtubal dissemination of endometrial cells after CO$_2$ (8/30, 26.7%) or normal saline (7/30, 23.3%) distension [$\chi^2$ (Yates correction) = 0.0000, $P = 1.000$]. The distribution of negative and positive aspirates after the hysteroscopies are summarized in Tables II and III.

### Table I. Patient characteristics

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>30</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>31.6 (20–44)</td>
<td>90</td>
</tr>
<tr>
<td>Nulliparous</td>
<td>27</td>
<td>90</td>
</tr>
<tr>
<td>Phase of menstrual cycle$^a$</td>
<td>prol</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>secre</td>
<td>11</td>
</tr>
<tr>
<td>Pre-operative diagnosis$^b$</td>
<td>primary infertility</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>secondary infertility</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>menstrual abnormalities</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>chronic pelvic pain</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>dyspareunia</td>
<td>3</td>
</tr>
</tbody>
</table>

$^a$Five patients had irregular menstrual cycles.

$^b$Some patients had more than one diagnosis.

and secondary infertility (Table I). Patients with a previous diagnosis of endometriosis or morphological signs at laparoscopy were excluded from the study, peritoneal biopsies being taken in uncertain cases. Further exclusion criteria included menstrual bleeding at the time of surgery and bilateral tubal blockage. All operations were performed irrespective of the phase of the menstrual cycle. The study was approved by the local medical ethics committee and full informed consent was obtained from all participants.

### Laparoscopy, hysteroscopy and fluid collection

Laparoscopy was carried out using a double puncture approach with CO$_2$ distension, and a long Veress needle was employed to aspirate the study samples of peritoneal fluid from the pouch of Douglas. Randomization with respect to the uterine distension medium took place following the initial inspection of the pelvis and the code was kept in sealed envelopes in the operating theatre. Peritoneal biopsies for histological diagnosis were taken from all suspicious lesions, but patients with macroscopic endometriotic implants were excluded from the beginning. Patients were randomly allocated in a ratio of 1:1 to either CO$_2$ distension followed by normal saline or normal saline distension followed by CO$_2$. Hysteroscopies were performed using a standard 4 mm hysteroscope with a 30° fore-oblique lens and 5.5 mm diagnostic sheath. An electronic HAMOU-hysteroflator (manufactured by Karl Storz GmbH, Tuttlingen, Germany) adjusted to a flow rate of up to 50 ml/min and a pressure not exceeding 100 mmHg was used if the uterine cavity was distended with CO$_2$. Normal saline was installed from a flexible 500 ml bag wrapped in a pressure cuff connected to a manometer and pumped up to 100–150 mmHg. All hysteroscopies were carried out by experienced operators and lasted a few minutes.

Peritoneal fluid or washings were collected at the start of the laparoscopy (control sample) and after each hysteroscopy, giving three samples per patient. Each aspiration was followed by copious peritoneal lavage with normal saline to avoid any carry-over effect during the next aspiration. Tubal patency was confirmed after the second hysteroscopy by transcervical injection of 20 ml dilute methylene blue dye through a cervical cannula. At this stage, randomized patients with bilateral tubal blockage were excluded from the study.

### Cytological examination

Cytospin preparations of the three peritoneal fluid specimens were examined at magnifications up to ×900 using Papanicolaou and May–Gränewald–Giemsa stains. Cell populations were assessed morphologically, and endometrial or tubal cells were identified as non-ciliated or ciliated epithelial cell populations respectively. In our experience, epithelial cells of endometrial origin present as three-dimensional clusters of small cells with round, dark nuclei and a thin rim of cytoplasm; in contrast, tubal epithelium present as more irregular to papillary sheets, and cilia are more frequently seen than in endometrial cells. The presence of non-ciliated endometrial cells in the peritoneal washings was regarded as an indication of dissemination by hysteroscopy; in contrast, ciliated tubal cells were considered a routine finding and not linked to the hysteroscopic examination (Sidawy et al., 1987). If the origin of the epithelial cells was uncertain, a monoclonal antibody (Ber-EP4; DAKO Ltd, Bucks, UK) was applied, recognizing an epithelial marker present in endometrial epithelium but not in cells of mesothelial origin (De Angelis et al., 1992). In the specimens collected, individual sample cellularities were not reliably assessed, as this would not provide any conclusive additional information.

The data were analysed using the $\chi^2$ test (including Yates correction when appropriate), and a result of $P < 0.05$ was considered statistically significant.

### Table II. Presence (positive) or absence (negative) of endometrial cells in peritoneal fluid aspirations of 15 patients randomized to have the first hysteroscopy performed with normal saline (N/Saline)

<table>
<thead>
<tr>
<th>N/Saline distension first</th>
<th>CO$_2$-negative</th>
<th>CO$_2$-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/Saline-negative</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>N/Saline-positive</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

### Table III. Presence (positive) or absence (negative) of endometrial cells in peritoneal fluid aspirations of 15 patients randomized to have the first hysteroscopy performed with carbon dioxide (CO$_2$)

<table>
<thead>
<tr>
<th>CO$_2$ distension first</th>
<th>N/Saline-negative</th>
<th>N/Saline-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO$_2$-negative</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>CO$_2$-positive</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>
showing that there was no order effect with respect to the two different distension media. The cytological findings after the two distension media were identical in all but three cases, the latter all in the group randomized to normal saline for the initial hysteroscopy.

The presence of endometrial cells in the fluid samples was significantly influenced by the phase of the menstrual cycle at the time of the investigation. Excluding five patients with irregular cycles, transabdominal spread of endometrial tissue occurred in nine of 14 (64.3%) of patients who were in the proliferative phase (cycle days 5–14) and in none of 11 patients in the secretory phase of the menstrual cycle \( \chi^2 \) (Yates correction) = 8.435, \( P = 0.004 \).

**Discussion**

The results of our pilot study clearly show that CO\(_2\) and normal saline have similar capacities to carry endometrial cells or tissue into the peritoneal cavity via the Fallopian tube, reflux taking place in about a quarter of cases irrespective of the distension medium. The incidence of pelvic dissemination appears to be significantly influenced by the phase of the menstrual cycle, and in our study group only occurred during the proliferative phase. Finally, in a group carefully screened for endometriosis, endometrial cells were found in peritoneal fluid in some cases before any uterine instrumentation.

Comparison with other published reports is made difficult by the use of different study designs. Previous reports were based on observational studies, and to our knowledge, ours is the first prospective randomized trial which compares the incidence of tubal reflux of endometrial cells using different distension media for hysteroscopy, each patient being her own control. We were also careful to exclude women with endometriosis, as they are known to have a higher prevalence of endometrial cells in the peritoneal cavity than controls (Badawy et al., 1984; Halme et al., 1984). Finally, we used a sensitive marker to detect endometrial cells in uncertain cases.

Normal saline has not been studied previously, but Siegler and Lindemann (1984) found endometrial tissue in fluid aspirated from the cul-de-sac after hysteroscopy in 17/30 (57%) of patients using another low viscosity fluid, Ringer’s solution. The use of high viscosity fluid distension with 32% Dextran 70 has been shown to be associated with a 42–100% detection rate of endometrial tissue in the pelvis (Siegler and Lindemann, 1984; Bartosik et al., 1986). Published data about gaseous distension with CO\(_2\) are contradictory. The first study to address this issue reported that endometrial tissue fragments or cells were not detected in the fluid aspirated from the peritoneal cavity either before or after CO\(_2\) hysteroscopy, but the authors do not state the number of patients they investigated or provide any information about CO\(_2\) flow rates or pressure settings used during the hysteroscopies (Beyth et al., 1976). A subsequent study published 14 years later (Ranta et al., 1990) involving 51 patients and standard insufflator settings for CO\(_2\) found that transabdominal seeding of endometrial cells does occur during CO\(_2\) hysteroscopy, two of 16 (12%) cases with and 6/35 (17%) without endometriosis having positive aspirates.

Carbon dioxide is the gas generally used to create a pneumoperitoneum at laparoscopy with intraperitoneal pressure settings being much lower (10–15 mmHg) than the distension pressure normally used at CO\(_2\) hysteroscopy (50–100 mmHg). There is, however, increasing evidence that peritoneal tumour seeding occurs at laparoscopy, and port site recurrences due to subcutaneous tumour growth in patients with intra-abdominal malignancies have been reported (Jacobi et al., 1996; Volz et al., 1998). The most likely explanation for this phenomenon is the potential physical property of circulating CO\(_2\) to seed and disseminate attached original cells to distant sites; our data show that in up to 25% of cases this also happens at hysteroscopy.

Not strictly comparable, because of the use of higher intrauterine distension pressures and longer procedure times, are two recent reports concerning endometrial cell reflux during operative hysteroscopic procedures such as myomectomy and endometrial ablation. Both studies used another low viscosity fluid, 1.5% glycine, for uterine distension. In one study, endometrial cells were not found in any patients before the surgery but in 7/30 (23%) cases after (Benifla et al., 1997). Control samples were not taken in the second report, positive cytology being found in six of 22 (27%) cases following the hysteroscopic surgery (Depypere et al., 1997). The lack of pre-hysteroscopy data makes interpretation of this study difficult as endometrial cells can often be found in peritoneal fluid even in the absence of obvious endometriosis (Badawy et al., 1984; Kruitwagen et al., 1991). Despite this limitation, it has also been found (Depypere et al., 1997) that endometrial reflux was significantly more frequent when hysteroscopy was performed during the first half of the menstrual cycle than the second or in those treated with progestogens or the combined contraceptive pill (5/7 versus 1/6 versus 0/7 cases respectively). The most likely explanation is a tonic effect of progesterone or progestogens on the utero-tubal junction rather than changes in the consistency of the endometrium (Pulkkinen and Jaakkola, 1989).

Most gynaecologists perform the hysteroscopy irrespective of the menstrual cycle, and this situation should be reflected in the present study. Our results show a higher incidence of tubal spread of endometrial cells in patients undergoing hysteroscopy during the proliferative phase. However, performing the hysteroscopy in the second half of the menstrual cycle always bears a potential risk of carrying out the test in the presence of an early pregnancy. As a compromise, it therefore seems logical to schedule the hysteroscopies during the proliferative and secretory phases in subfertile patients with regular cycles and in premenopausal patients who are at risk for endometrial malignancy respectively.

There are practical implications arising from our results. Evidence from this and earlier studies shows that transabdominal peritoneal contamination with endometrial cells can occur with both CO\(_2\) and fluid distension media. The obvious concern is the potential for spread of malignant endometrial cells in women being investigated for abnormal uterine bleeding. Three case reports have addressed this issue specifically, but in two the diagnostic hysteroscopy was performed some time before definitive surgery for endometrial carcinoma so that an association between hysteroscopy and tumour spread could not be confirmed (Romano et al., 1992; Schmitz et al., 1994). The third case report involved a patient with FIGO stage Ia endometrial carcinoma in
whom peritoneal cytology was obtained at the time of laparotomy before and immediately after the hysteroscopy using normal saline; cytology was negative before but positive after the hysteroscopy, making the tumour effectively FIGO stage IIIa (Egarter et al., 1996). It is noteworthy that positive peritoneal cytology has also been reported in patients with stage I endometrial cancer not having undergone hysteroscopy; furthermore, the prognostic impact of positive peritoneal cytology on the 5-year survival in stages I and II of the disease is controversial (Creasman et al., 1981; Hirai et al., 1989; Vecek et al., 1993).

Moreover, there is no evidence that hysterography, which was commonly performed to assess the intrauterine extent and clinical stage of endometrial carcinoma, worsens the prognosis of patients with endometrial carcinoma (Johnsson, 1973; Schwartz et al., 1975).

In conclusion, irrespective of whether gas or liquid is used to distend the uterine cavity, hysteroscopy has the potential to disseminate endometrial cells into the peritoneal cavity, particularly during the first half of the menstrual cycle. While there is understandable concern about such dissemination when investigating women with endometrial carcinoma, there is no convincing evidence that tubal reflux of endometrial tissue has any clinical significance. Indeed, the humble dilatation and curettage, for so long the basis of investigating intrauterine pathology, also leads to transstidal reflux of endometrium (Sampson, 1927, Beyth et al., 1975). For this reason, and the fact that hysteroscopy is the optimum procedure for diagnosing, localizing and staging of early uterine carcinoma (Cicinelli et al., 1993; Taddei et al., 1994; Spiewankiewicz et al., 1995), it should remain the investigation of choice of the uterine cavity even in high-risk cases.

References


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