Annals of Diagnostic Pathology 46 (2020) 151503

Contents lists available at ScienceDirect

Annals of Diagnostic Pathology

journal homepage: www.elsevier.com/locate/anndiagpath

Original Contribution

Presence of tumor cells in the vagina during surgical treatment could be the source of vaginal recurrence in patients with endometrial carcinoma — A pilot prospective study

Simona Stolnicu[a,*], Cristina Terinte[b], Nicolae Ioanid[c], Elvio Silva[d]

[a] Department of Pathology, University of Medicine, Pharmacy, Sciences and Technology of Targu Mures, Str Gh Marinescu nr 38, 540139, Romania
[b] Department of Pathology, Regional Institute of Oncology, Str General Henri Mathias Berthelot nr 2-4, Iasi 700483, Romania
[c] Department of Surgery, Regional Institute of Oncology, Str General Henri Mathias Berthelot nr 2-4, Iasi 700483, Romania
[d] Department of Pathology, MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030, USA

ARTICLE INFO

Keywords:
Endometrial cancer
Vaginal recurrence
Surgical treatment

ABSTRACT

Background: The commonest site of recurrence in endometrial cancer (EC) is the vagina, with a rate of 16%. The aim of this study was to determine if vaginal recurrences in EC patients could develop due to contamination of the vagina with glandular tumor cells dropping off on polypoid, large size EC or tumors involving the endocervix, through manipulation of the uterus during surgery.

Methods: This pilot prospective study included 10 consecutive patients with EC, surgically treated with hysterectomy and additional lymphadenectomy according to stage. In every case, 2 proximal vaginal smears were collected before and during the hysterectomy procedure. All smears underwent Papanicolaou staining and the presence of atypical glandular cells in the smears was correlated with clinico-pathological parameters.

Results: Residual tumor was identified on the surgical specimen in the 10 cases; the tumor characteristics were large size (median 6 cm), polypoid type (80%), infiltrating the cervix (70%), and infiltrating more than half of the myometrium (60%). The smears obtained from the vagina showed that five cases (50%) presented tumor cells of glandular type in all smears (before and during the surgery), while in 3 cases (30%) the smears were negative for tumor cells preoperatively, but positive in the perioperative smears.

Conclusions: Our results suggest that the vagina is most often contaminated preoperatively due to bleeding; however, the vaginal wound may also be contaminated perioperatively. We propose a change in the surgical procedure, which is easy to perform and inexpensive compared to postsurgical vaginal radiotherapy.

1. Introduction

Endometrial cancer (EC) is the most common gynecological cancer primarily affecting postmenopausal women worldwide. Annual incidence rates in Western countries range between 15 and 25 per 100,000 women and about 8600 patients are diagnosed each year [1]. The large majority of patients are diagnosed at early FIGO (International Federation of Gynecology and Obstetrics) stage I, due to early occurrence of symptoms. Surgery, consisting of abdominal or laparoscopic, robotic hysterectomy and bilateral salpingo-oophorectomy is the primary treatment for stage I patients with EC.

The potential adverse risk factors are age, stage, histological type and grade, depth of myometrial invasion, and presence of lymphovascular invasion. Based on different clinical studies and data, EC has been classified as low-risk, intermediate-risk, and high-risk for lymph node metastases and early disease spread to the abdominal cavity and distant sites. Low-risk patients are those with stage IA (i.e., with no or superficial { < 50%} myometrial invasion) EC, grade 1 or 2, and endometrioid type histology. High-risk patients are those with stage IB (i.e., with deep { > 50%} myometrial invasion) grade 3 EC, stage II or stage III EC, or non-endometrioid histology (all stages with myometrial invasion). All others are considered to have intermediate-risk EC. The majority of patients with EC have low or intermediate-risk with only 15% having high-risk EC.

A favorable prognosis (low-risk EC) is seen in a large proportion of EC patients with the standard treatment being surgery alone and observation after hysterectomy and bilateral salpingo-oophorectomy, with 95% probability of 5-year relapse-free survival. However, a significant

* Corresponding author at: Department of Pathology, University of Medicine, Pharmacy, Science and Technology of Targu Mures, 38 Gheorghe Marinescu Street, Targu Mures 540139, Romania.
E-mail address: stolnicu@gmx.net (S. Stolnicu).

https://doi.org/10.1016/j.anndiagpath.2020.151503

number of patients do develop local or distant recurrence with the vagina being the most common site of recurrence. According to most clinical trials, adjuvant radiotherapy (RT) for endometrial carcinoma (vaginal brachytherapy or external beam pelvic RT {EBRT}) is a very effective salvage treatment for vaginal relapse in patients not previously irradiated. The use of postoperative RT was initially limited to the group of patients at sufficiently high-risk of locoregional relapse to warrant the risk of treatment-associated morbidity. However, more recently, 4 randomized trials have established the role of EBRT in intermediate-risk endometrial carcinoma even followed by severe complications and side effects [2-5]. Also, because most relapses occur in the vagina, the use of vaginal brachytherapy alone has been advocated by recent international guidelines for patients with low-stage IA grade1, grade2, and grade3 (in association with potential risk factors, considering vaginal brachytherapy if one risk factor is present, and strongly recommended if two risk factors are present) as an alternative to observation alone as well as for stage FIGO IB or more [6]. This treatment approach has become a common practice, but there is no proof that the usual risk factors for endometrial cancer are also found in patients who develop recurrences in the vagina. In fact, in a retrospective study in 2014, the opposite has been proposed [7].

EC patients with vaginal recurrences were found to have the same risk factors as those who did not have recurrences according to a study conducted by Moschiano et al. [7]. Out of 255 low histological grade (FIGO grade I or II) endometrioid adenocarcinoma patients that they studied, clinical follow-up showed that vaginal recurrences developed in 40 (16%) patients, with 3 disease-related deaths. These 3 patients with vaginal recurrence developed subsequent extravaginal recurrence before death. Patients who had vaginal recurrence showed increased cervical tumor involvement but lacked other risk factors associated with recurrent disease at other sites. There were no deaths among patients with isolated vaginal recurrence, suggesting that vaginal recurrence is not a marker of aggressive tumor biology [7]. Treating these low-/intermediate-risk EC patients with RT for the improvement of local vaginal control and overall survival is unnecessary and could be avoided.

The aim of this study was to determine if glandular tumor cells dropping off polypoid, large diameter EC specimen are present in the vaginal wound after the surgical manipulation. These cells could be a potential source of vaginal recurrence in EC patients.

2. Patients and methods

This prospective study involved 10 consecutive patients previously diagnosed with endometrial carcinoma based on a Pipelle biopsy and with residual tumor in the uterus at the time of surgery. In cases with cervical involvement, immunohistochemical stains were done to rule out a primary cervical carcinoma. Consent was obtained from all patients and the Ethical Committee of the Regional Institute of Oncology, Iasi approved the study (protocol no. 15/2019). All patients were surgically treated with hysterectomy, bilateral salpingo-oophorectomy with abdominal extra fascial nerve sparing and additional pelvic lymphadenectomy in stage IB and II regardless of degree of differentiation and para-aortic lymphadenectomy for high-risk patients. In every case, 2 smears were collected 30 min preoperatively, before washing the vagina with betadine. The patient was placed in the gynecological position, and using two different brushes, 1 sample was collected from the proximal vagina around the cervix (bottom of the posterior vaginal sac) and 1 from the ectocervix. For the intraoperative mobilization of the uterus, for every case included in the study, 2 Kocher pins were used, each placed at the level of each uterine horn. As much as possible, mobilization and palpation of the uterus were avoided in order to prevent the passage of tumor cells from the uterine cavity to the cervix.

Two additional smears were collected during surgery, before incising the upper part of the vagina to remove the uterus (1 sample collected from the proximal vagina around the cervix and 1 from the ectocervix). Colpotomy was performed using the monopolar device, the vaginal cuff being correlated with each type of hysterectomy. After collecting the samples of 4 smears per case, conventional smear slides were prepared and labeled for accurate identification, fixed in 96% ethyl alcohol for 15 min and then dried, and sent to the pathology laboratory for Papanicolaou staining.

The presence of atypical glandular cells in all the smears was correlated with the age of the patient, FIGO stage of the tumor, presence of residual tumor, histological subtype and grade of the tumor, depth of myometrial invasion, presence of lympho-vascular invasion, and the presence of cervical involvement in every case by an experienced gynecological pathologist, after sampling the surgical specimen. Histological slides were prepared from (i) the uterine tumor (with a mean of 5 slides/case, in correlation with the tumor diameter) as well as from the uterine wall to assess the depth of myometrial invasion, (ii) the cervix in order to identify the cervical involvement, and (iii) all the other organs that were surgically removed, including the lymph nodes.

3. Results

Ten patients (age range 47–79 years, mean 63.4 years) with endometrial carcinoma were selected for the study. Five of the patients were FIGO stage II, four were stage I, and only one patient was stage III.

In all cases, a residual tumor was found on sampling the surgical specimen. Macroscopically, 8 out of 10 tumors were of polypoid type, with 2 diffuse type tumors. The tumor diameter varied between 3 and 8 cm (median 5 cm), and in 7 out of 10 cases (70%), there was macroscopic as well as microscopic infiltration of the endocervix by the tumor.

In 9 cases the tumor was of endometrioid type with/without squamous metaplasia, while in 1 case the tumor was of clear cell type. Microscopically, there were 3 cases of FIGO grade 1, 4 cases of FIGO grade 2, and 3 cases of FIGO grade 3. Myometrial involvement was present in all the cases (100%) with 6 of the cases showing infiltration of $> 50\%$ of the myometrium (60%). In 80% of the cases, the presence of lympho-vascular invasion was identified. None of the cases had tumor involvement of the ovaries, fallopian tubes, and vaginal cuff, and only one case had tumor associated with lymph node metastases (10%). Five cases (50%) presented glandular tumor cells in all smears (before and during the surgery), while in 3 cases (30%), the smears were negative for glandular tumor cells before surgery, but positive in the smears taken during surgery. Atypical glandular cells were not identified in any of the smears in 2 cases (20%). Of these 2 cases with negative smears, one had a 3.2 cm diameter tumor involving more than half of the myometrium; cervical involvement was not identified in any of them (Figs. 1, 2) (Table 1).

4. Discussion

Our results show that vaginal contamination with glandular tumor cells in EC patients mostly occurs either before or during the surgical procedure. This could be avoided by washing the vagina before and after the surgical procedure as well as by using a special device to prevent tumor cells to contaminate the vagina during surgery. These 3 steps might prevent the contamination of the vaginal wound by glandular tumor cells and the development of recurrences in the vagina.

Surgical treatment, consisting of abdominal or laparoscopic, robotic hysterectomy, and bilateral salpingo-oophorectomy is the primary standard treatment for patients with EC. Depending on the associated risk factors, pelvic and para-aortic lymphadenectomy is also performed during surgery. Prognosis of EC is related to various risk factors such as age, advanced clinical stage, tumor histological subtype (FIGO grade 3 endometrioid, serous, clear cell adenocarcinoma, undifferentiated carcinoma, and carcinosarcoma) and FIGO histological grade, presence of lympho-vascular involvement, and deep infiltration of the myometrium.

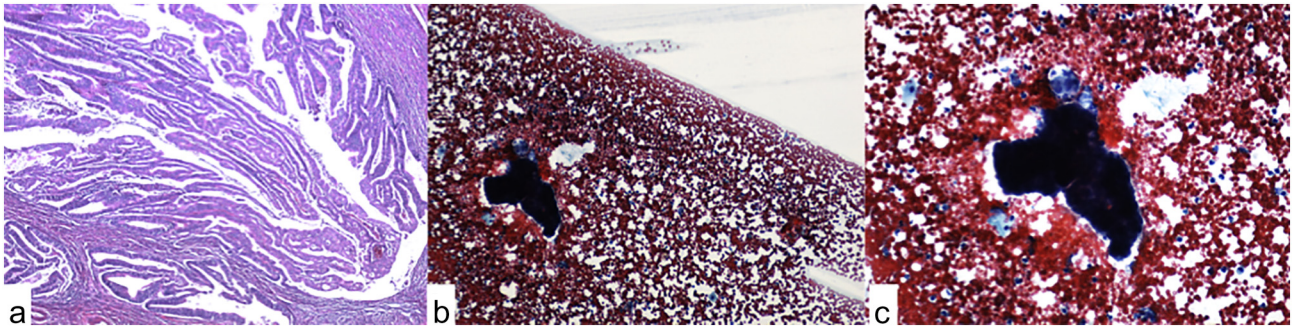

Fig. 1. Invasive endometrioid type of endometrial adenocarcinoma (surgical specimen). (a); Presence of atypical tumor cells in smear obtained during surgery (b, c); (Papanicolaou stain).

EC patients may develop vaginal and/or extravaginal recurrences with the former being more common (16% of all patients diagnosed with EC). To treat or prevent the development of vaginal recurrences, vaginal brachytherapy is routinely recommended for these patients even if the EC is of low/intermediate-risk prognosis. Previous studies have shown, however, that risk factors like histological grade, deep (more than half) myometrial invasion, and presence of lympho-vascular invasion are not related to vaginal recurrence [7]. Moreover, vaginal recurrence is not a marker of aggressive tumor biology; however, following a vaginal recurrence, a low-grade EC might transform into an aggressive neoplasm [7].

In the past, low-dose radiation brachytherapy with radium-226 was used in combination with EBRT [8,9] while more recently, low-dose radiation brachytherapy has been replaced by high-dose radiation brachytherapy with iridium-192 after loading [10,11]. The PORTEC-2 clinical trial showed excellent and equivalent vaginal and pelvic control rates with both adjuvant radiation approaches and no difference in overall survival [12]. Recently, results were published from a long-term follow-up study (median 20.5 years) of 568 patients with early-stage EC [13]. The study compared long-term outcomes in women who received vaginal brachytherapy plus EBRT versus vaginal brachytherapy alone. The findings suggested no statistical difference in overall survival between the study groups, and in this cohort, patients younger than 60 years of age who received EBRT had increased incidence of secondary cancers and subsequent higher mortality rates [13].

The use of adjuvant radiotherapy did not improve overall survival in any of the clinical trials [6]. Vaginal brachytherapy is however associated with morbidity and increased severity in acute complications (vaginal inflammation, irritation, dryness, discharge, soreness, swelling, fungal infection, vault laceration, vaginal laceration, deep vein thrombosis, fever, bladder perforation, and bowel obstruction) as well as late complications (tiredness, diarrhea or nausea, urinary complications, vaginal complications such as vaginal discharge, dryness, itching, bleeding, fibrosis, telangiectasis, stenosis, short or narrow vagina, dyspareunia, and cardiac complications) that develop in 2 up to 27% of the patients [14-17]. Moreover, vaginal brachytherapy is expensive.

One of the reasons for the presence of glandular tumor cells in the vagina of patients with EC is that, in polypoid tumors, or in those involving the cervix, the tumor cells may detach and drop into the vagina while the tumor is bleeding. As a consequence, cleaning the vagina before the surgery can prevent vaginal contamination. Another cause for the presence of tumor cells in the vagina is manipulation of the uterus by pressing the fundus during surgery.

The data from this pilot study demonstrates that, in most cases, the vagina is contaminated before surgery (due to bleeding) or during surgery, especially if the endometrial tumor is large, polypoid, and infiltrates the cervix, and this can be avoided. Based on this pilot study, a larger group of patients can be evaluated by applying some changes in the surgical procedure, which are easy to perform and less expensive than postsurgical vaginal RT.

We are proposing using a device, such as a contraceptive diaphragm or a specifically designed more sophisticated device, to protect vaginal contamination during surgery. Also, we propose that the vagina should be additionally washed after the uterus has been removed. These measures applied before and during hysterectomy for EC might prevent the contamination of the vaginal wound by glandular tumor cells and the development of recurrences in the vagina. Consequently, these patients may not need vaginal brachytherapy, an oncological procedure that is sometimes associated with severe early and late complications as well as a high cost for the patients.

Compliance with ethical standards

This manuscript meets the ethical standards.

Funding

None.

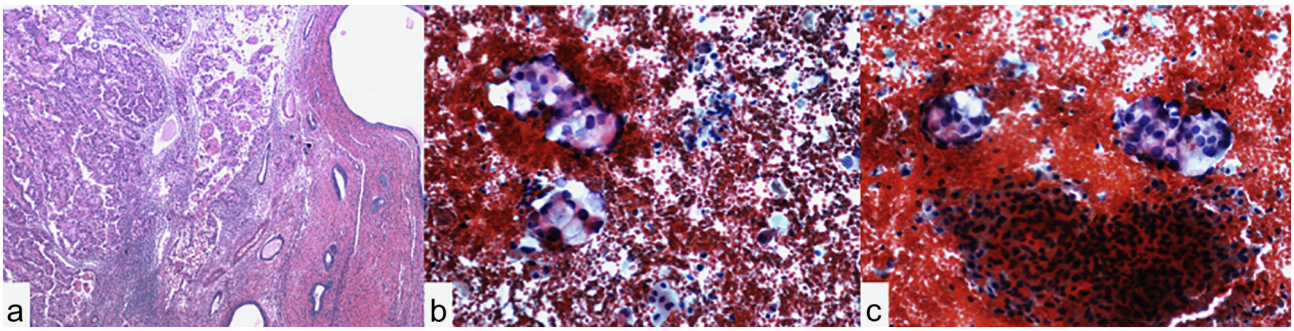

Fig. 2. Invasive clear cell type of endometrial carcinoma developed in a postmenopausal patient (surgical specimen). (a); Presence of multiple clusters of atypical tumor cells in smears obtained preoperatively and (b); during surgery (c); (Papanicolaou stain).

Table 1
Clinical-pathological parameters of the cases included in the study.

Case number	Age	FIGO stage	Tumor diameter/ tumor shape	Residual tumor	Histological subtype	Histological FIGO grade	Lympho-vascular invasion	Cervical involvement	Atypical glandular cells in preoperative Pap smear	Atypical glandular cells in Pap smear during surgery
1	47	II	7.8 cm/Diffuse	Present	Adenocarcinoma of endometrioid type with squamous metaplasia; > 1/2 myometrial involvement	3	Yes	Present	Yes	Yes
2	72	IA	8.5 cm/Polypoid	Present	Adenocarcinoma clear cell; < 1/2 myometrial involvement	3	Yes	Present	Yes	Yes
3	61	IA	3.2 cm/Polypoid	Present	Adenocarcinoma of endometrioid type with squamous metaplasia; < 1/2 myometrial involvement	1	No	Absent	Yes	Yes
4	63	IIIC1	4 cm/Polypoid	Present	Adenocarcinoma of endometrioid type with squamous metaplasia; > 1/2 myometrial involvement	2	Yes	Present	No	Yes
5	60	II	3.5 cm/Polypoid	Present	Adenocarcinoma of endometrioid type with squamous metaplasia; > 1/2 myometrial involvement	2	Yes	Present	No	Yes
6	79	IB	6.4 cm/Polypoid	Present	Adenocarcinoma of endometrioid type with squamous metaplasia; > 1/2 myometrial involvement	2	Yes	Absent	No	No
7	69	II	3.3 cm/Polypoid	Present	Adenocarcinoma of endometrioid type with squamous metaplasia; > 1/2 myometrial involvement	2	Yes	Present	No	Yes
8	69	IA	3.2 cm/Polypoid	Present	Adenocarcinoma of endometrioid type with squamous metaplasia; < 1/2 myometrial involvement	1	No	Absent	No	No
9	57	II	7 cm/Diffuse	Present	Adenocarcinoma of endometrioid type with squamous metaplasia; 1/2 myometrial involvement	1	Yes	Present	Yes	Yes
10	57	II	4.2 cm/Polypoid	Present	Adenocarcinoma of endometrioid type; > 1/2 myometrial involvement	3	Yes	Present	Yes	Yes

Authors contribution

SS- drafting of the manuscript, literature review, reading the slides; CT- reading the slides; NI- collecting the cases; ES- conception, drafting of the manuscript, literature review.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Acknowledgements

None.

Informed consent if the research involved human participants

Obtained for each patient involved in the present study.
This research did not involve animal.

References

[1] Anon. Projection d'incidence et de mortalité des cancers en. http://lesdonnees.e-cancer.fr/Themes/epidemiologie/Incidence-mortalite-nationale/Projection-d-incidence-et-de-mortalite-des-cancers-en-2017#ind25896; 2017, Accessed date: June 2019.
[2] Keys HM, Roberts JA, Brunetto VL, et al. A phase III trial of surgery with or without adjunctive external pelvic radiation therapy in intermediate risk endometrial adenocarcinoma: a gynecologic oncology group study. Gynecol Oncol 2004;92:744–51.
[3] Creutzberg CL, van Putten WL, Koper PC, et al. Surgery and postoperative radiotherapy versus surgery alone for patients with stage-1 endometrial carcinoma: multicentre randomised trial. PORTEC Study Group Post Operative Radiation Therapy in Endometrial Carcinoma Lancet 2000;355:1404–11.
[4] Aalders J, Abeler V, Kolstad P, et al. Postoperative external irradiation and prognostic parameters in stage I endometrial carcinoma: clinical and histopathologic study of 540 patients. Obstet Gynecol 1980;56:419–27.
[5] Blake P, Swart AM, Orton J, et al. Adjuvant external beam radiotherapy in the treatment of endometrial cancer (MRC ASTEC and NCIC CTG EN.5 randomised trials): pooled trial results, systematic review, and meta-analysis. Lancet 2009;373:137–46.
[6] NCCN. Uterine neoplasms. www.nccn.org, Accessed date: June 2019.
[7] Moschiano EJ, Barbuto DA, Walsch C, et al. Risk factors for recurrence and prognosis of low-grade endometrial adenocarcinoma; vaginal versus other sites. Int J Gynecol Pathol 2014;33:268–73.
[8] Chao CK, Grigsby PW, Perez CA, et al. Brachytherapy-related complications for medically inoperable stage I endometrial carcinoma. Int J Radiat Oncol Biol Phys 1995;31:37–42.
[9] Kucera H, Sagl R, Skodler W, Weghaupt K. Afterloading short-term irradiation of inoperable uterine cancer. Geburtshilfe Frauenheilkd 1986;46:515–9.
[10] Nag S, Erickson B, Thomadsen B, Orton C, Dermanes JD, Petereit D. The American brachytherapy society recommendations for high-dose-rate brachytherapy for carcinoma of the cervix. Int J Radiat Oncol Biol Phys 2000;48:201–11.
[11] Schwarz JK, Beriwal S, Esthappan J, et al. Consensus statement for brachytherapy for the treatment of medically inoperable endometrial cancer. Brachytherapy 2015;14:587–99.
[12] Nout RA, Smit VT, Putter H, et al. Vaginal brachytherapy versus pelvic external beam radiotherapy for patients with endometrial cancer of high-intermediate risk (PORTEC-2): an open-label, non-inferiority, randomised trial. Lancet 2010;375:816–23.
[13] Onsrud M, Cvancarova M, Hellebust TP, Trope CG, Kristensen GB, Lindemann K. Long-term outcomes after pelvic radiation for early-stage endometrial cancer. J Clin Oncol 2013;31:3951–6.
[14] Gupta P, Aich RK, Deb AR. Acute complications following intracavitary high-dose-rate brachytherapy in uterine cancer. J Contemp Brachytherapy 2014;6:276–81.
[15] Valduvieco I, Rovirosa Á, Herreros A, et al. Three or four fractions per week in postoperative high-dose-rate brachytherapy for endometrial carcinoma. The long-term results on vaginal relapses and toxicity. Clin Transl Oncol 2013;15(8):602–7.
[16] Ríos I, Rovirosa A, Ascaso C, et al. Vaginal-cuff control and toxicity results of a daily HDR brachytherapy schedule in endometrial cancer patients. Clin Transl Oncol 2016;18(9):925–30.
[17] Delishaj D, Barcellini A, D'Amico R, et al. Vaginal toxicity after high-dose-rate endovaginal brachytherapy: 20 years of results. J Contemp Brachytherapy 2018;10(6):559–66.