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Research Report

Recurrence risk of occult micrometastases and isolated tumor cells in early stage endometrial cancer: A case control study

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ABSTRACT

Objectives: To determine whether previously undetected occult micrometastasis (MM) or isolated tumor cells (ITC) is associated with increased recurrence odds in stage I-II endometrioid adenocarcinoma.

Methods: Women with recurrent stage I/II EC who had complete pelvic and para-aortic were identified as the outcome of interest. A case-control study was designed with the exposure defined as occult MM/ITC not seen on original nodal pathology. Controls were found by frequency-matching in a 1:2 case control ratio. Original nodal slides were re-reviewed, stained and tested with immunohistochemical to detect occult MM/ITC and the odds of associated recurrence was calculated.

Results: Of 153 included, 50 with and 103 without recurrence, there was no difference in age ($p = 0.46$), race ($p = 0.24$), stage ($p = 0.75$), FIGO grade ($p = 0.64$), lymphovascular space invasion (LVSI); $p = 1.00$, or GOG 99 high-intermediate risk (HIR) criteria ($p = 0.35$). A total of 18 ITC (11.8%) and 3 MM (2.0%) not previously identified were found in 19 patients. Finding occult MM/ITC was not associated with more lymph nodes (LN) removed ($p = 0.67$) or tumor grade ($p = 0.48$) but was significantly associated with stage ($p < 0.01$). LVSI ($p = 0.09$) and meeting high-intermediate risk criteria ($p = 0.09$), were closely associated but not statistically significant. Isolated ITC were not associated with increased odds for recurrence (OR 0.71, CL: 0.20 – 2.22, $p = 0.57$), recurrence free survival (RFS) ($p = 0.85$) or overall survival (OS) ($p = 0.92$).

Conclusions: In early-stage EC, identification of occult MM or ITC is uncommon and associated with stage. The presence of ITC was not associated with increased odds of recurrence. Adjusting stage or treatment may avoided based on ITC alone. Isolated MM were rare in our population, and further investigation is warranted.

1. Introduction

Endometrial cancer (EC) is the most common gynecologic malignancy in the United States. Prognostic factors include stage, histology, tumor grade, LVSI, and lymph node (LN) metastasis (Boronow et al., 1984; Creasman et al., 1987; DiSaia et al., 1985). Based on these factors, recurrence risk is stratified from low- to high-intermediate risk (HIR) and guides adjuvant treatment including external beam pelvic radiation (EBRT) or vaginal cuff brachytherapy (VCB) (Keys et al., 2004; Nout et al., 2010; de Boer et al., 2018). Full pelvic and para-aortic lymphadenectomy (PPLND) in clinical stage I EC is gradually being replaced by the adoption of sentinel lymph node biopsy (SLNB) with ultra-staging that frequently identifies occult MM/ITC without evidence of macro-metastatic disease (Ballester et al., 2011; Rossi et al., 2017). MM are defined as metastases>0.2 mm and<2.0 mm, while ITC are small tumor deposits < 0.2 mm in largest diameter. In breast cancer, SLNB has been a standard procedure worldwide for decades. Several published studies serve as guidelines on management and prognostication based on the presence of occult MM/ITC in axillary SLNB (Weaver et al., 2011; de Boer et al., 2010; Giuliano et al., 2011; Donker et al., 2014). Increased use of SLNB and pathological ultra-staging has increasingly identified regional LN involvement in EC (Backes et al., 2019). However, the absolute clinical risk of recurrence associated with finding MM and ITC in the absence of macro-metastatic (>2mm) LN in EC is not yet established.

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Borrowing from breast and colorectal cancer data (Weaver et al., 2011; de Boer et al., 2010; Sloothaak et al., 2014), the International Federation of Obstetrics and Gynecology (FIGO) 2009 EC staging and the American Joint Committee on Cancer (AJCC) both currently upstage clinical stage I EC to a stage IIIC based on the findings of lymph node MMs, but not ITC. The AJCC designated a new category pN0(i +) based on the finding of ITC detected in ultra-staged LN, which leaves the door open for increased adjuvant treatment based on degree of clinical concern (Olawaiye and Mutch, 2018). Macrometastasis is an important diagnostic factor in prescription of adjuvant treatment of EC (Milgrom et al., 2014). However, there is no consensus on the benefit of adjuvant treatment with the finding of occult MM/ITC in EC. Current practices vary amongst physicians and institutions, particularly as SLNB have become standard practice for many. Our study sought to determine if the presence of occult MM/ITC conferred an increased odd of recurrence in otherwise usually treated EC.

2. Materials and methods

Study Design: We designed an IRB approved (OU-IRB#9601) case-control study to determine the effect that occult MM/ITC has on the odds of recurrence. We included women diagnosed with stage I/II endometrioid EC following hysterectomy and PPLND from July 2008 to July 2018 with pathology-reported LN nodes at time of surgery. Women with non-endometrioid histology, positive LN, stage III or IV disease, or incomplete PPLND were excluded. Our institution did not routinely perform SLNB for endometrial cancer patients until after the study period, thus, PPLND was standard practice during this time. The outcome of interest was clinically confirmed EC recurrence; these patients were defined as cases. The control group was defined as those without recurrence $\geq$ 24 month (m) following surgery. Cases and controls were frequency-matched in a 1:2 case to control ratio based on established prognostic factors including age, FIGO stage, grade, and LVSI. The exposure was previously undetected low volume metastases (LVM), including both occult MM or ITC on original LN pathology. Baseline demographic, surgical, pathologic, and outcome data were collected. To analyze potential differences in treatment that could affect odds ratio (OR) for recurrence, we collected detailed adjuvant treatment information for all cases and controls.

Pathological methods: H&E stained glass slides from each case were reviewed to verify all LN sections. Paraffin blocks were recut and stained using pan-cytokeratin (AE1/AE3, Ventana) immunohistochemical (IHC) stain, Fig. 1. Following staining, slides were reviewed using conventional light microscopy by a blinded gynecologic pathologist. Slides were scored as "positive" for occult malignant cells if any tumor specific IHC panCK positive cells were identified, or "negative" if no such tumor cells were identified. Cases which were scored as positive were then re-examined by routine H&E to determine if the occult positive cells were detectable by regular staining (false-negative by initial report) or truly occult and undetectable by routine sampling. Positive cases were further sub-classified as ITC or MM.

Statistical Analysis: Demographic, clinicopathologic, treatment and survival factors were collected and tested for differences. Categorical characteristics were compared using chi-squared tests or Fisher's exact test. Continuous variables were compared with two-sample *t*-tests, or Wilcoxon rank-sum tests. Ordinal and rank-based variables were compared using Wilcoxon rank-sum tests. The effect of LVM on presence or absence of recurrence was modeled using logistic regression, adjusting for any variables found to be significantly associated with case/control status in univariate analyses, as well as all variables used in the frequency matching between cases and controls. Time to recurrence was modeled using Cox proportional-hazards models, with similar adjustment terms. To evaluate whether recurrence was attributed to adjuvant treatment, patients meeting HIR from each cohort were compared by treatment appropriateness to test for differences. Likewise, we determined whether the presence of MM/ITC was associated with greater odds of receiving treatment before and after adjusting for GOG99 high-risk status. It is important to note that all cases and controls were

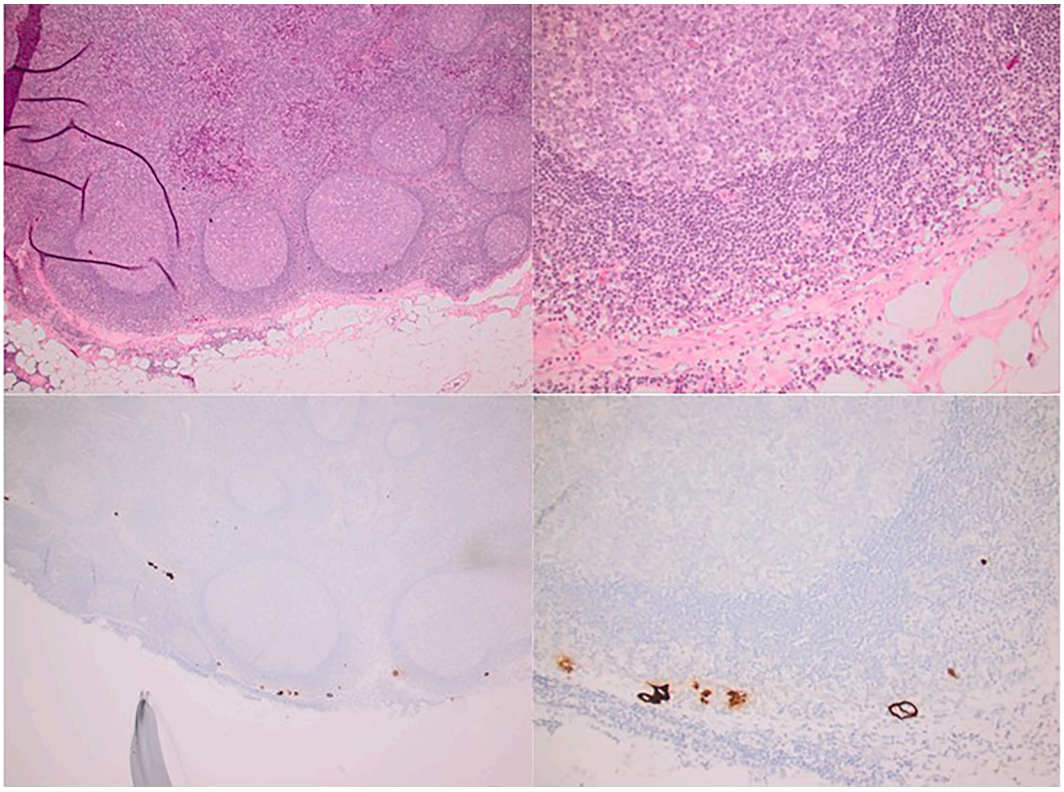

Fig. 1. Possible isolated tumor cells on PanCK at 4x and 20x magnification, not visualized on H&E 4x and 20x.

dispositioned to adjuvant treatment agnostic to SLNB status, but rather as fully staged, stage I or II EC. In accordance with the journal's guidelines, we will provide our data for the reproducibility of this study in other centers if such is requested.

3. Results

All University of Oklahoma patient scheduled for surgical staging hysterectomy with full PPLND from 2008 to 2018 (n = 1024) were screened. Of those, 437 (42.7%) met inclusion criteria. Whereas 45 (4.4%) had incomplete surgical pathology, 181 (17.7%) were missing PPLND for evaluation and 128 (12.5%) were benign or pre-invasive. A total of 134 patients had non-endometrioid histology (13.1%), and among the endometrioid subtypes, 87 (8.5%) had stage III disease, and of those 60 (5.9%) had macrometastasis to their LN, making them IIIC disease, and 12 (1.2%) had stage IV disease (*SupFig1*).

Of the 437 subjects meeting inclusion criteria, 52 (11.9%) had a confirmed recurrence and 50 had complete demographic and clinicopathologic data and served as study cases. Based on these 50 cases, controls were identified from the remaining pool of eligible subjects and frequency matched at an approximate ratio of 1:2 based on tumor grade, tumor stage (equal frequencies of stage IA, IB and stage II), median age per stage group, and presence of LVSI.

Of the 153 cases and controls identified, 152 pathologic cases were reviewed; one control was excluded due to slide degradation, this is represented by 2219 slides (average 14.6 slides per case/control, 3.4 slides per dissection site). A total of 162 (7.3%) blocks belonging to the series were not available for sectioning, leaving 2057 panCK slides for evaluation. Where slide quality was inadequate, repeat staining was requested. Final slide review identified 18 previously unseen ITC and 3 MM among 19 cases and controls (12.5%); two patients had both MM and ITC, leaving 16 cases of ITC alone (*SupFig 2)*. Of the 2057 IHC slides examined, 36 contained IHC positive cells determined to be most likely tumor cells, with an average of 2.25 slides per positive subject (Fig. 1). Two of the MM cases were found on retrospective review to be false negatives and were able to be identified on routine H&E.

A total of 153 participants were included for analysis, 50 cases of recurrence and 103 without recurrence at a minimum of 24 m. At time of pathology evaluation, one control was non-evaluable. Table 1 shows the demographics. The majority (77.8%) of patients had minimally invasive surgery for predominately stage IA disease (46.4%). Grade 2 was most frequently observed, as was the absence of LVSI. Upon final pathology reporting, most patients (64.1%) met HIR criteria according to GOG 99; this is expected, as this was a population matched for the outcome of recurrence, thus enriched for HIR patients. Cases and controls were well balanced according to BMI (p = 0.20), race (p = 0.27), performance status (p = 0.21), surgical type (p = 0.80) and frequency of positive intraperitoneal washings 6.0% vs 5.9% (p = 1.00), table 1. However, interestingly, we found that there were significantly more LN removed in the recurrent cases compared to controls (22 vs 19; p < 0.05). Median follow-up between cases and controls was 53.3 versus 62.3 m, respectively, but was not significantly different, p = 0.06. For the controlled characteristics, there was no significant difference in age, (p = 0.46), stages IA vs IB vs II (p = 0.75), FIGO grade (p = 0.64), presence of LVSI, p = 1.00, or meeting HIR criteria by GOG-99, p = 0.36 (table 1).

Due to the relative rarity of MM alone, we looked at those with any occult LVM (MM and/or ITC) and those with ITC alone. Between cases and controls, there were 6 (12.0%) and 13 (12.7%) cases of occult LVM, respectively, which was not different between cohorts, p = 1.00. When looking at the presence of ITC alone, there remained no significant difference between cases and controls, p = 1.00. There were also no significant differences in those with and without ITC alone for demographic factors, surgical approach, or number of LN removed (Table 2). ITC positive status tended towards association with presence of LVSI (p = 0.07), however, this was not statistically significant. Presence of occult ITC was found to be significantly associated with tumor stage and depth of invasion (DOI), and tumor size, 5.3 cm vs 3.8 cm, all p < 0.05. Finding occult ITC was marginally associated with being GOG-99 HIR but this was not statistically significant (p = 0.07).

In the cohort with occult MM/ITC (n = 19) there were 6 recurrences, compared to 44 in those with no occult MM/ITC (n = 133), representing 31.6% vs 33.1% of patients, respectively. After adjusting for total LN removed, presence of MM/ITC was not associated with recurrence (OR 1.11, CL: 0.40–3.37; p = 0.85), Table 3. The unadjusted effect of occult MM/ITC on OS and RFS was modeled and plotted with Kaplan-Meier curves, and neither RFS (p = 0.87) nor OS (p = 0.89) was associated with the presence of occult LVM (Supplementary figure 3). After adjusting for total LN, this was unchanged. When investigating the effect of finding occult ITC alone, we found that neither RFS (p = 0.85) nor OS (p = 0.92) differed when compared to ITC negative populations (Supplementary figure 3).

To assess the potential for confounding, differences in treatment according to recurrence and MM/ITC status were examined. A total of 29 cases (58.0%) of recurrence met GOG-99 HIR criteria, whereas n = 69

Table 1
Baseline characteristics for cases and controls.

Characteristic	All N = 153	Cases n = 50	Controls n = 103	p-value
Age *Median [25%, 75%]*	64.0 [59.0, 72.0]	62.5 [57.0, 71.8]	64.0 [59.0, 72.5]	0.46
Race/Ethnicity				0.27
AI/PI	9 (5.9%)	3 (6.0%)	6 (5.8%)	
Asian/Middle-Eastern	5 (3.3%)	0	5 (4.9%)	
Black/non-Hispanic	6 (3.9%)	1 (2.0%)	5 (4.9%)	
Hispanic, LatinX	4 (2.6%)	1 (2.0%)	3 (2.9%)	
White/non-Hispanic	129 (84.3%)	45 (90.0%)	84 (81.6%)	
Payer status				0.22
IHS	5 (3.3%)	4 (8.2%)	1 (1.0%)	
Medicaid	1 (0.7%)	0 (0.0%)	1 (1.0%)	
Medicare/Medicare combo	88 (58.3%)	24 (49.0%)	64 (62.7%)	
Not insured/sooner care	10 (6.6%)	2 (4.1%)	8 (7.8%)	
private	47 (31.1%)	19 (38.8%)	28 (27.5%)	
N missing	2	1	1	
BMI *Median [25%, 75%]*	33.0 [27.7, 38.9]	34.0 [28.1, 41.8]	31.4 [27.6, 37.7]	0.21
Performance status				0.21
0	141 (92.2%)	44 (88.0%)	97 (94.2%)	
1–2	12 (7.8%)	6 (12.0%)	6 (5.8%)	
Diabetes	45 (29.4%)	18 (36.0%)	27 (26.2%)	0.29
Cardiovascular Disease	31 (20.3%)	10 (20.0%)	21 (20.4%)	1.00
Surgery Type				0.80
Minimally Invasive	119 (77.8%)	40 (80.0%)	79 (76.7%)	
Laparotomy	34 (22.2%)	10 (20.0%)	24 (23.3%)	
Stage				0.75
Ia	71 (46.4%)	22 (44.0%)	49 (47.6%)	
Ib	66 (43.1%)	23 (46.0%)	43 (41.7%)	
II	16 (10.5%)	5 (10.0%)	11 (10.7%)	
Grade				0.64
1	26 (17.0%)	8 (16.0%)	18 (17.5%)	
2	79 (51.6%)	25 (50.0%)	54 (52.4%)	
3	48 (31.4%)	17 (34.0%)	31 (30.1%)	
LVSI	50 (32.7%)	16 (32.0%)	34 (33.0%)	1.00
DOI *(%) Mean*	44.1 ± 30.4	47.1 ± 32.3	42.7 ± 29.4	0.40
Tumor size (cm)	4.0 ± 2.7	4.3 ± 2.7	3.8 ± 2.7	0.30
Washings +	9 (5.92%)	3 (6.00%)	6 (5.88%)	1.00
Nodes removed *Mean*	19.8 ± 7.8	21.9 ± 9.2	18.9 ± 7.0	**<0.05**
GOG-99 High Risk	98 (64.1%)	29 (58.0%)	69 (67.0%)	0.36
MM/ITC+	19 (12.4%)	6 (12.0%)	13 (12.7%)	1.00
ITC + alone	16 (10.5%)	5 (10.0%)	11 (10.8%)	1.00
FU *(m) Median [25%, 75%]*	59.6 [43.6, 79.9]	53.3 [28.5, 76.5]	62.3 [48.1, 84.4]	0.06

*PI (Pacific Islander), AI (American Indian), HIS (Indian Health Service), BMI (body mass index), CV (cardiovascular), LVSI (lymphovascular space invasion), DOI (depth of invasion), FU (length of follow-up)
p-values unadjusted for matching factors in case-control design

Table 2
Factors associated with occult low volume metastases.

Characteristic (N = 153)	MM/ITC(+) n = 19	MM/ITC(-) n = 133	p-value	ITC only(+) n = 16	ITC only(-) n = 136	p-value
Age *Median [25%, 75%]*	63.0 [58.0, 67.5]	64.0 [59.0, 73.0]	0.50	63.5 [59.0, 67.2]	64.0 [58.0, 73.0]	0.73
Race/Ethnicity			0.74			0.47(2)
AI/PI	1 (5.3%)	8 (6.0%)		0 (0.0%)	9 (6.6%)	
Asian/Middle-Eastern	0 (0.0%)	5 (3.8%)		0 (0.0%)	5 (3.7%)	
Black/non-Hispanic	0 (0.0%)	6 (4.5%)		0 (0.0%)	6 (4.4%)	
Hispanic, LatinX	1 (5.3%)	3 (2.3%)		1 (6.2%)	3 (2.2%)	
White/non-Hispanic	17 (89.5%)	111 (83.5%)		15 (93.8%)	113 (83.1%)	
BMI *Median [25%, 75%]*	34.8 [30.0, 42.3]	32.7 [27.7, 37.9]	0.07	33.5 [28.3, 43.1]	33.1 [27.7, 38.0]	0.14
Diabetes	7 (36.8%)	38 (28.6%)	0.64	7 (43.8%)	38 (27.9%)	0.25
Surgery Type			1.00			0.76
Minimally Invasive	15 (78.9%)	103 (77.4%)		12 (75.0%)	106 (77.9%)	
Laparotomy	4 (21.1%)	30 (22.6%)		4 (25.0%)	30 (22.1%)	
Stage			**<0.01**			**<0.01**
Ia	3 (15.8%)	67 (50.4%)		2 (12.5%)	68 (50.0%)	
Ib	10 (52.6%)	56 (42.1%)		8 (50.0%)	58 (42.6%)	
II	6 (31.6%)	10 (7.5%)		6 (37.5%)	10 (7.4%)	
Grade			0.48			0.58
1	2 (10.5%)	24 (18.0%)		2 (12.5%)	24 (17.6%)	
2	14 (73.7%)	65 (48.9%)		11 (68.8%)	68 (50.0%)	
3	3 (15.8%)	44 (33.1%)		3 (18.8%)	44 (32.4%)	
LVSI	10 (52.6%)	40 (30.1%)	0.09	9 (56.2%)	41 (30.1%)	0.07
DOI *(%) Mean*	66.3 ± 21.4	41.0 ± 30.3	**<0.01**	66.3 ± 20.8	41.5 ± 30.4	**<0.01**
Tumor size (cm)	5.3 ± 2.7	3.8 ± 2.7	**0.02**			
Washings +	1 (5.26%)	8 (6.06%)	1.00	1 (6.25%)	8 (5.93%)	1.0
Nodes removed *Mean*	20.5 ± 6.2	19.7 ± 8.1	0.67	20.9 ± 6.0	19.7 ± 8.1	0.55
GOG-99 High Risk	16 (84.2%)	81 (60.9%)	0.09	14 (87.5%)	83 (61.0%)	0.07

* PI (Pacific Islander), AI (American Indian), BMI (body mass index), LVSI (lymphovascular space invasion), DOI (depth of invasion), MM (micrometastases), ITC (isolated tumor cells)
(2) P-value computed for White vs others

Table 3
a Logistic regression for recurrence predicted by MM/ITC. **b:** Logistic regression for recurrence predicted by ITC.

Recurrence	OR	95% LCL	95% UCL
a.			
Yes vs. No	0.87	0.27	2.59
b.			
Yes vs. No	0.71	0.20	2.22

The effect of MM/ITCs on recurrence was modeled using logistic regression, adjusting for total LNs removed and characteristics used for frequency matching (age, stage, grade, and LVSI). Adjusting for these factors, presence of MM/ITCs was not significantly associated with recurrence (p = 0.81).
The effect of ITCs on recurrence was modeled using logistic regression, adjusting for total LNs removed and characteristics used for frequency matching (age, stage, grade, and LVSI). Adjusting for these factors, presence of ITCs was not significantly associated with recurrence (p = 0.57).
*Micrometastases/Isolated tumor cell (MM/ITC), Lower confidence limit (LCL), Upper confidence limit (UCL)

(67.0%) of controls met criteria, p = 0.36. We found no significant difference in surgery type (p = 0.80), receipt of adjuvant treatment (p = 0.66), or receipt of adjuvant treatment according to GOG-99 HIR criteria between cases and controls (78.0% vs 82.5%; p = 0.65). In the subgroup of HIR patients, n = 98, 64.1%, we found no difference in receipt of adjuvant treatment according to GOG99 criteria between those of HIR that recurred compared to those that did not, p = 0.88 (*SupTab1*). The receipt of any radiation (VCB and/or EBRT) was found to be significantly higher in the + MM/ITC cohort. However, when adjusting for GOG99 high-risk criteria, presence of MM/ITC was not significantly associated with patients receiving more radiation therapy ($p = 0.33$) but was marginally associated with higher odds of receiving systemic chemotherapy (OR = 2.52 [0.8, 7.4]; $p = 0.10$). It is important to note, that at our institution, during this study period we were enrolling widely into clinical trials that evaluated combination chemoradiation for high risk early stage EC. For additional information on adjuvant treatment, see table 4.

For those that did recur there were no significant differences in the patterns of recurrence according to MM/ITC status. There was a larger proportion of vaginal recurrences in those without MM/ITC compared to those with (5.3% vs 11.3%, p = 0.67). Pelvic and nodal recurrences were relatively similar according to MM/ITC status as were rates of upper abdominal and/or distant metastases (*SupTab2*).

Table 4
Adjuvant treatment by occult ITC/MM status.

Treatment	MM/ITC (+) n = 19	MM/ ITC (-) (n = 133)	*p*-value	*p*-value adjusted for GOG-99 HIR criteria
High Risk (GOG99 criteria)	**16 (84.2%)**	**81 (60.9%)**	**0.09**	NA
Receipt of adjuvant treatmentaccording to HIR criteria	17 (89.5%)	106 (79.7%)	0.53	0.53
Clinical trial enrollment	7 (53.8%)	26 (37.7%)	0.43	0.39
GOG 249	*0*	*7*		
RTOG 1203	*3*	*13*		
RTOG-092	*1*	*1*		
IIT of T/C × 3cycles + VCB	*3*	*5*		
Systemic chemo	5 (26.3%)	20 (15.0%)	0.16	0.10
VCB	8 (42.1%)	45 (33.8%)	0.61	0.87
EBRT	7 (36.8%)	26 (19.5%)	0.13	0.25
Any Radiation	14 (73.7%)	66 (49.6%)	**0.05**	0.33
CisRT	2 (10.5%)	4 (3.0%)	0.16	0.20

*Gynecologic Oncology group (GOG), High intermediate risk (HIR), Radiation therapy oncology group (RTOG), Investigator-initiated trial (IIT), Vaginal cuff brachytherapy (VCB), External beam radiation therapy (EBRT), Radiation therapy(RT), cisplatin (cis)

4. Discussion

Summary of main results: In our study, finding occult MM/ITC in usually treated EC patients did not negatively impact odds of recurrence, RFS or OS. Our data affirms that occult MM/ITC are uncommon. MM not previously detected is even more rare and likely due to a false negative H&E examination. Accepting that ex post facto ultra-staging introduces confounding and Our data suggests that in the setting of ITC alone found through ultrastaging, patients may be safely offered adjuvant treatment according to risk stratification based on uterine factors (i.e. GOG99 criteria) and potentially spared systemic chemotherapy if ITC is their only concerning feature.

Results in the context of published literature: Adoption of SLNB for EC is popular as it offers decreased morbidity while allowing a sensitive evaluation for otherwise unapparent nodal disease. In fact, a recent survey of SGO members found that nearly 70% of respondents were using SLNB for EC and that > 50% of those using SLNB for EC use it regardless of tumor grade or histology (Chambers et al., 2019). However, the increased detection of LVM provides more prognostic information but also mounts clinical uncertainty.

A beneficial outcome of patients randomized to systematic LND in the Benedetti and ASTEC trials is that they received less adjuvant treatment (ASTEC study group, Kitchener H, Swart AM, Qian Q, Amos C, Parmar MK. , 2009; Panici et al., 2008). As current practice shifts to SLNB, some women prescribed less treatment in these original EC trials may increasingly be offered adjuvant treatment based on increased detection of LVM. It has been repeatedly demonstrated that SLNB and ultrastaging increases detection of nodal disease by 40–50% and that nearly half of those detected are occult MM/ITCs (de Boer et al., 2010; Holloway et al., 2016; Kim et al., 2013).

Holloway et al, evaluated the performance of SLNB compared to full LND and found that the patients who underwent SLN mapping were more likely to undergo combination radiotherapy and chemotherapy (28.6% vs. 16.3%; $p < 0.01$) (Holloway et al., 2016). Plante et al. reported the prognosis of ITCs in EC; at median follow-up of 29 months, the PFS of ITC and MM was 95.5%, 85.5%, respectively, which was comparable to the node negative group cohort of 87.6% (Plante et al., 2017). Of note, in this study, compared to Holloway et al, those with ITC alone received significantly less chemotherapy and pelvic RT with similar 3-year PFS outcomes as those that were node negative (Plante et al., 2017). Pineda et al demonstrated PFS was also significantly worse in the macrometastasis group (61.1%) compared to the LVM group (71.4%) and negative lymph nodes (83.2%, $p < 0.05$), though those with LVM did receive more adjuvant therapy (García Pineda et al., 2020;9 (6):1999.). Backes et al performed a prospective trial to determine the detection rate of SLNB in clinically early-stage EC and prospectively assessed occult MM/ITC in the ultrastaged cohort. For 10 patients with occult MM/ITC, 5 were treated with adjuvant therapy based on clinical/uterine factors alone and at the time of reporting, no recurrences were noted (Backes et al., 2019). At the practice level, it remains unanswered whether occult MM/ITC may be associated with additional metastases in distal LN, though Multuni, et al, described distant occult LVM in 30% of those with presumed isolated *para*-aortic nodes, 2.5% of their population (Multinu et al., 2019). Holloway found that 4 of the 12 with SLN positive ITC also had positive non SLN, whereas Backes et al found that none of the 10 pts with ITC had non SLN metastases. Similarly, in the study by Plante et al, none of the patients with LVM had other positive non-SLN (Backes et al., 2019; Holloway et al., 2016; Plante et al., 2017). Low volume disease is more likely to be detected in patients with low grade endometrioid endometrial cancer (Bogani et al., 2019). As the prevalence of ITC is low in high risk EC, this potentially explains why ITC is less likely to impact outcomes; leading to further questions as to who confers benefit from increased adjuvant treatment of occult MM/ITC.

Strengths and Limitations: Though this is a retrospective, single-institution study with the inherent risk for bias and non-randomization, we minimized selection bias and detection bias by controlling for known prognostic factors in EC recurrence risk and by blinding our pathologist to case or control status. Lastly, the relatively racially and ethnically homogenous study population weakens generalizability. Strengths of our study include use of a case-control design which is best suited to investigate associations with rare outcomes such as recurrent early-stage EC and studying a population in which full nodal dissections were completed and presumed negative to minimize confounding treatment effect. Additionally, we were able to review a high number of slides with dedicated specialized gynecologic pathologists.

Implications for practice and future research: Many gynecologic oncologists are already adjusting treatment based on MM and ITC. In the survey of gynecologic oncology surgeons, 77.2% and 21.3% of respondents reported that MM and ITC should be treated as node positive disease, respectively (Chambers et al., 2019), which makes investigation of this topic relevant and timely. Outside of a well-funded, cooperative group trial, definitive recommendation for adjuvant treatments based on the presence of ultrastaged occult MM/ITC cannot be made. However, solace can be found in the consistent reporting that occult MM/ITC follows well established uterine high-risk factors such as DOI, LVSI, tumor grade and size. As LVM are very likely molecularly driven, it is important that we begin to understand EC according not only to histologic risk factors but within the context of molecular risk stratification. As such, when deciding upon adjuvant treatment recommendations for patients, the presence of occult MM/ITC should not be viewed in a vacuum but rather in context with other clinicopathologic and molecular risk factors.

***Conclusions*:** In an early stage, usually treated EC population, identification of occult MM or ITC is uncommon and associated with stage, DOI, tumor size and closely associated with the presence of LVSI and meeting HIR GOG-99 criteria. The presence of ITC was not associated with increased odds for recurrence, RFS, or OS.

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Precis: This is a timely case-control study that evaluates whether the presence of previously undetected MM or ITC in otherwise standardly treated early-stage endometrial cancer is associated with an increased odd of recurrence.

CRediT authorship contribution statement

Tara Castellano: Conceptualization, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **Lewis Hassell:** Methodology, Supervision. **Rachel Conrad:** Methodology, Supervision. **Conner S. Davey:** Investigation, Data curation. **Sunam Husain:** Methodology, Supervision. **Justin D. Dvorak:** . **Kai DING:** . **Camille Gunderson Jackson:** Conceptualization, Investigation, Supervision.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [Author Jackson reports the following disclosures: Consulting: Clovis, LEAP, Cordgenics, Agenus, GSK/Tesaro; Research Funding: Lilly, Genentech, Clovis].

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gore.2021.100846.

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