

# The incidence and clinical significance of lymph node micrometastases determined by immunohistochemical staining in stage I - lymph node negative endometrial cancer

Amy McCoy<sup>1</sup>, Michael A. Finan<sup>1</sup>, F.T. Boudreaux<sup>2</sup>, J. Alan Tucker<sup>3</sup>,  
John J. Lazarchick<sup>2</sup>, Robert M. Donnell<sup>2</sup> and Rodney P. Rocconi<sup>1</sup>

<sup>1</sup>University of South Alabama, Mitchell Cancer Institute, <sup>2</sup>Mobile Infirmary Medical Center

Pathology Department and <sup>3</sup>University of South Alabama, Department of Pathology, South Alabama, USA

**Summary.** Objective: Determine the incidence and clinical relevance of lymph node micrometastases found with immunohistochemical (IHC) staining in patients diagnosed with stage I lymph node-negative endometrial adenocarcinoma. Methods: Eligible patients with endometrioid-type histology and negative lymph nodes by H&E were identified by a computerized database. After histologic confirmation, all paraffin-embedded pathologic specimens were freshly sliced and stained with IHC stains for pancytokeratin. Slides were interpreted by two pathologists and positive IHC staining for micrometastases was defined as positive staining of cells <2 mm in greatest dimension. Patient demographics, clinicopathologic factors, and follow-up data were abstracted. Results: Fifty-one patients were included in our study. Most patients had stage IA (84%) tumors of grade 2/3 histology (51%), and 11 patients (22%) received adjuvant therapy. Mean number of lymph nodes was 12.2 per patient. Of 151 lymph node paraffin blocks evaluated for pancytokeratin, only two (1.3%) had IHC-positive micrometastases. The two lymph node-positive results occurred in separate patients, leading to 3.9% of all patients in our cohort. Both micrometastatic lymph node-positive patients had adjuvant radiation therapy for uterine high-risk factors and are currently without evidence of disease at 15 and 16 months, respectively. Three lymph node-negative

patients (6.1%) have developed recurrences within a median follow-up of 15 months. Conclusion: The incidence of IHC stain-positive micrometastases in H&E-negative lymph nodes is low in surgically staged endometrial cancer and does not justify routine IHC staining. Additionally, as little evidence exists to support the clinical significance of IHC-stained micrometastases in endometrial cancer, further study is warranted.

**Key words:** Endometrial cancer, Lymph nodes, Micrometastases, Immunohistochemistry

## Introduction

Endometrial cancer is the most common cancer of the female reproductive tract, accounting for 42,160 new cases and 7,780 deaths in 2009 in the United States alone (Jemal et al., 2009). Fortunately, the majority of patients with endometrial cancer present with early stage I disease with portends a favorable prognosis.

Accepted risk factors for extrauterine spread and recurrence are cancer grade, histologic subtype, depth of invasion, lower uterine segment/cervical involvement, lymph-vascular space involvement, and tumor size (Boronow et al., 1984; Morrow et al., 1991). Surgical staging demonstrates that 5-10% of patients with clinical stage I disease will have microscopic involvement of pelvic LN. In clinical stage I endometrial cancer with deeply invasive grade 3 cancers, the incidence of pelvic and para-aortic lymph node metastasis ranges up to 24-34%, respectively (Creasman et al., 1987).

Offprint requests to: Rodney P. Rocconi, MD, Assistant Professor in Gynecologic Oncology, Abraham A. Mitchell Cancer Research Scholar, University of South Alabama, Mitchell Cancer Institute 1660 Springhill Avenue, Mobile, AL 36604, USA e-mail: [rocconi@usouthal.edu](mailto:rocconi@usouthal.edu)

Documented lymph node involvement significantly worsens overall survival with 5-year survival being approximately 50%. Accordingly, the proper surgical staging and identification of patients that would benefit from adjuvant therapy is paramount to this prognosis. Current standard for detection of endometrial lymph node metastasis involves hematoxylin and eosin (H&E) staining. Despite a favorable prognosis in patients with negative lymph nodes of metastasis, up to 15% of patients experience a recurrence of disease. One hypothesis to this recurrence is the false negative rates with current pathologic lymph node assessments or presence of micrometastasis (MM). As such, authors have investigated numerous ways to enhance detection of lymph node metastasis such as serial sectioning (Reich et al., 1996), immunohistochemical staining, molecular testing, alone or in combination (Delpech et al., 2008; Bezu et al., 2010). Using these combination techniques in FIGO stage I-IV cancers demonstrated a detection rate of MM in endometrial cancer between 0-20% (Bezu et al., 2010). Using immunohistochemistry techniques for anticytokeratin antibodies has been studied in other cancer types including breast (Breast Cancer Study Group, 1980), colon (Greenson et al., 1994), gastric (Maehara et al., 1996), esophageal (Izbicki et al., 1997), and lung (Cote et al., 1998). Most studies detected micrometastasis at a higher rate than H&E, however, most did not see an improvement in clinical outcomes. Interestingly, most breast cancer patients with MM had other high risk factors necessitating radiation therapy prior to the cytokeratin identification (de Mascarel et al., 1992).

Gonzalez Bosquet et al. evaluated cytokeratin IHC staining of resected lymph nodes in 16 endometrial cancer patients in order to determine if IHC was more sensitive than traditional H&E staining for MM (Gonzalez Bosquet et al., 2003). Nearly 25% of patients were stage II and only 2 of 16 (12.5%) demonstrated MM in previously negative H&E lymph nodes. One of IHC positive MM experienced a recurrence and died of disease, while the other was without evidence of disease. Importantly, 2 other patients negative for IHC MM experienced a recurrence and died of disease. Despite their conclusion that their study “strongly suggests that MM are clinically significant”, their small numbers and the fact that twice as many patients without IHC MM recurred and died compared to those with positive MM precluded any definitive conclusions.

NCCN guidelines recommend adjuvant therapy in all stage II-IV endometrial cancer patients. Additionally, a trend has been seen with increasing use of adjuvant therapy for stage I/II disease. Stage I disease has been an intense area of prospective studies in order to determine which patients should receive adjuvant therapy as well as which modality is superior, chemotherapy versus radiation therapy. Considering that most stage II-IV patients and many stage I/II patients will receive adjuvant therapy, the cohort of focus remains identifying those patients with surgical stage I disease that will benefit from the “ultrastaging” of lymph nodes via IHC.

Assumptions to this rationale are two-fold: 1) The incidence of micrometastasis in surgical stage I patients warrants evaluation and 2) detection of micrometastasis in patients with previously unrecognized metastasis by H&E improves survival with subsequent treatment.

To date, no data exists that focuses entirely on the detection of surgical stage I endometrial cancer MM. As such our objective was to determine the rate of MM as well as the impact on treatment and survival in patients with previous lymph node negative stage I endometrial cancer using IHC stains for pancytokeratin.

## **Materials and methods**

### *Study design*

After obtaining institutional review board approval, we identified patients with surgical Stage I endometrial cancer using a tumor registry database. All patients underwent total hysterectomy, bilateral salpingo-oophorectomy, and surgical lymphadenectomy by a gynecologic oncologist for endometrial cancer. Only patients with endometrioid-type histology and negative lymph nodes by hematoxylin and eosin stain (H&E) were eligible. Exclusion criteria included inadequate lymph node paraffin blocked specimens or lack of post-operative follow-up. Stage, myometrial invasion, and tumor grade were defined according to the International Federation of Gynecology and Obstetrics (FIGO 2009) system. Retrospective chart review collected clinical data from patients' records including relevant clinical demographics, surgicopathologic variables, follow-up data, adjuvant treatment, and recurrence.

### *Slide preparation*

Lymph node paraffin blocks were re-evaluated by two pathologists to ensure negative metastasis by H&E. Available pathologic specimens were freshly sliced from formalin-fixed paraffin blocks at 5  $\mu$ m and mounted on 1-2 slides per block for staining and interpretation. Sections were cut 1-2 days prior to immunostaining to avoid potential problems in antigen recognition due to storage degradation of cut tissue sections on glass slides. Immunostaining was performed using pancytokeratin monoclonal antibody [AE1/AE3- ab27988] (Abcam Inc., Cambridge, MA) per previous techniques and protocol (Gonzales Bosquet et al., 2003; abcam website). Stained slides were evaluated by both pathologists for presence or absence of micrometastases. Micrometastasis was defined as positive staining of cells <2 mm in greatest dimension. Patients were grouped into micrometastases positive or negative groups and statistics were run on all of the collected data.

### *Statistics*

Categorical data was compared utilizing Chi-squared test or Fisher's exact test and continuous variables were compared using Student's t-test. All statistical

## Endometrial cancer micrometastases

calculations were performed using SPSS 17.0 Software for Macintosh (Chicago, IL).

### Results

#### Patient characteristics

51 patients met eligibility requirements and were included in our study. The cohort had a mean age of  $61.5 \pm 12.1$  with a mean BMI of  $30.4 \pm 7.8$  (Table 1). Most patients had Stage IA (84%) tumors with grade 2/3 histology (51%). 20 of 51 (39%) patients received paraaortic lymphadenectomy which incorporated all grade 3 and most of grade 2 histologies. Eleven patients (22%) received adjuvant therapy.

#### Lymph node evaluation

A total of 151 lymph node paraffin blocks were obtained, with all being negative for metastasis on re-evaluation using H&E. Mean number of lymph nodes per patient was  $12.3 \pm 5.3$ . Of all paraffin blocks evaluated for MM, only 2 blocks were positive with pancytokeratin (1.3%). This accounted for 2 patients (3.8%) MM rate.

#### Survival data

Both patients that had documented MM by

**Table 1.** Demographics.

Demographics	N=51
Mean Age	61.5±12.1
Mean BMI	30.4±7.8
Stage	
IA	43 (84%)
IB	8 (16%)
Grade	
1	25 (49%)
2	18 (35%)
3	8 (16%)
Mean Lymph Nodes	12.3±5.3
Adjuvant Radiation Therapy	11 (22%)
Median Follow-up (mos)	23.7

**Table 2.** Studies evaluating the incidence of positive lymph nodes by immunohistochemistry for micrometastasis in endometrial cancer patients.

Study	Year	No. of Patients	FIGO Stage	Positive MM Lymph nodes (%)
Pelosi [16]	2003	16	I	0 (0%)
Bosquet [14]	2003	16	I/II	2 (12.5)
Niikura [17]	2006	20	I/II/III	0 (0%)
McCoy	2010	51	I	2 (3.9%)
Total		103		4 (3.9%)

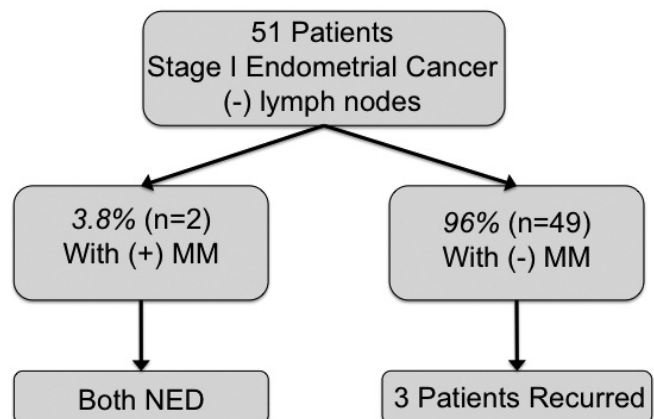
pancytokeratin were FIGO stage IB with grade 2/3 histology. Each received adjuvant whole pelvic radiation therapy for uterine risk factors and remain without evidence of disease at 23 and 24 months respectively. In the 49 patients (96%) that were negative for MM, 6.1% (n=3) recurred. All recurrences were documented in the pelvis (2 vaginal recurrences and 1 pelvic side-wall) and were treated with whole pelvic radiation therapy ± vaginal brachytherapy. These recurrences occurred within 15 months median follow-up of surgical management and all are without evidence of disease. (Fig. 1)

Of note, original uterine characteristics for recurrences were as follows: 1) vaginal recurrence: <50% myometrial invasion with no lymphovascular space invasion; 2) vaginal recurrence: >50% myometrial invasion with no lymphovascular space invasion; 3) pelvic side-wall recurrence: <50% myometrial invasion with no lymphovascular space invasion.

### Discussion

Being able to properly identify early stage cancer patients that would benefit from adjuvant therapy continues to be of utmost importance. Irrespective of disease type, lymph node metastasis remains one of the most important risk factors for recurrence and death. Despite the rationale for ultrastaging of lymph node metastasis in endometrial cancer patients, techniques have thus far been unsuccessful in improving survival.

In our study, the overall incidence of positive micrometastasis in H&E lymph node negative patients was less than 4%. This low number is consistent in the literature among surgical stage I patients (Table 2) and in our opinion does not warrant routine evaluation of IHC for pancytokeratin in H&E negative lymph nodes. Collectively, 4 studies that evaluated H&E plus IHC



**Fig. 1.** Schematic of patients evaluated in our study. The overwhelming majority of patients with negative lymph nodes by H&E staining were also negative for micrometastasis with immunohistochemistry. Both patients with positive micrometastasis remain without any evidence of disease. MM: micrometastasis; NED: no evidence of disease.

revealed that 4% of patients with negative H&E demonstrated positive MM by IHC (Gonzalez Bosquet et al., 2003; Pelosi et al., 2003; Niikura et al., 2007) (Table 2). However in regards to detecting MM, other modalities might lead to higher rates of discovery. For example, combining serial sectioning to H&E and IHC has led to positive MM rates up to 15% (Bezu et al., 2010).

Despite higher detection rates of MM, the true clinical utility of identifying MM in endometrial cancer has yet to be defined. To date, no data has demonstrated that the identification and treatment of endometrial cancer patients with MM improves survival. Both patients that had positive MM received adjuvant radiation therapy for other factors and are currently without evidence of disease at 15 and 16 months respectively. This data mirrors the breast cancer literature that most patients with detected MM received adjuvant radiation therapy anyway.

Even if a survival advantage exists, it would be increasingly difficult to detect. This is likely due to multiple factors including: 1) the low incidence of MM in H&E negative lymph nodes 2) paradigm shift towards treating high risk and intermediate high risk early staged endometrial cancer patients with adjuvant therapy 3) most patients with early stage endometrial cancer have favorable prognosis currently 4) the benefit of improved survival with lymphadenectomy in lymph node negative patients (Kilgore et al., 1995). Considering these factors, the number of patients needed to detect a true survival difference secondary to MM alone would be very difficult and require an extremely large cohort.

Although the presence of MM has yet to confer a greater risk of recurrence and/or death in this patient population, further study is needed. The ultimate goal of properly identifying patients that would benefit from adjuvant treatment is still closely approximated with detection rate of lymph node positivity. Other methods that could improve detection of lymph node positive disease, include other IHC stains, serial sectioning, sentinel lymph nodes, polymerase chain reaction (PCR) techniques, as well as nanoparticle imaging techniques.

Our data compliments other literature with regard to detecting micrometastasis in endometrial cancer patients with negative lymph nodes. Although various techniques might demonstrate increased micrometastasis in endometrial cancer, demonstrating that detection of micrometastasis and treatment improves survival is paramount to this debate. Based on our findings and the literature to date, we cannot recommend incorporating the routine use of IHC detection of micrometastasis in surgical stage I endometrial cancer.

---

*Acknowledgements.* The authors have no financial conflicts to disease. This manuscript was presented at the Society of Gynecologic Oncologists 008 Annual Meeting in Tampa, FL

---

## References

- Bezu C., Coutant C., Ballester M., Feron J.G., Rouzier R., Uzan S. and Daraï E. (2010). Ultrastaging of lymph node in uterine cancers. *J. Exp. Clin. Cancer Res.* 29, 5.
- Boronow R.C., Morrow C.P., Creasman W.T., Disaia P.J., Silverberg S.G., Miller A. and Blessing J.A. (1984). Surgical staging in endometrial cancer: clinical-pathologic findings of a prospective study. *Obstet. Gynecol.* 63, 825-832.
- Breast Cancer Study Group (1990). Prognostic importance of occult axillary lymph node micrometastases from breast cancers. *International (Ludwig) Breast Cancer Study Group. Lancet* 335, 1565-1568.
- Cote R.J., Hawes D., Chaiwun B. and Beattie E.J. Jr (1998). Detection of occult metastases in lung carcinomas: progress and implications for lung cancer staging. *J. Surg. Oncol.* 69, 265-274.
- Creasman W.T., Morrow C.P., Bundy B.N., Homesley H.D., Graham J.E. and Heller P.B. (1987) Surgical pathologic spread patterns of endometrial cancer A Gynecologic Oncology Group Study. *Cancer* 60, 2035-2041.
- de Mascarel I., Bonichon F., Coindre J.M. and Trojani M. (1992) Prognostic significance of breast cancer axillary lymph node micrometastases assessed by two special techniques: reevaluation with longer follow-up. *Br. J. Cancer.* 66, 523-527.
- Delpech Y., Coutant C., Daraï E. and Barranger E. (2008). Sentinel lymph node evaluation in endometrial cancer and the importance of micrometastases. *Surg. Oncol.* 17, 237-245.
- Gonzalez Bosquet J., Keeney G.L., Mariani A., Webb M.J. and Cliby W.A. (2003). Cytokeratin staining of resected lymph nodes may improve the sensitivity of surgical staging for endometrial cancer. *Gynecol. Oncol.* 91, 518-525.
- Greenson J.K., Isenhardt C.E., Rice R., Mojzisek C., Houchens D. and Martin E.W. Jr (1994). Identification of occult micrometastases in pericolic lymph nodes of Duke's B colorectal cancer patients using monoclonal antibodies against cytokeratin and CC49. Correlation with long-term survival. *Cancer* 73, 563-569.
- Izbicki J.R., Hosch S.B., Pichlmeier U., Rehders A., Busch C., Niendorf A., Passlick B., Broelsch C.E. and Pantel K. (1997). Prognostic value of immunohistochemically identifiable tumor cells in lymph nodes of patients with completely resected esophageal cancer. *N. Engl. J. Med.* 337, 1188-1194.
- Jemal A., Siegel R., Ward E., Hao Y., Xu J. and Thun M.J. (2009). *Cancer statistics, 2009. CA Cancer J. Clin.* 59, 225-249.
- Kilgore L.C., Partridge E.E., Alvarez R.D., Austin J.M., Shingleton H.M., Noojin F. 3rd and Conner W. (1995). Adenocarcinoma of the endometrium: survival comparisons of patients with and without pelvic node sampling. *Gynecol. Oncol.* 56, 29-33.
- Maehara Y., Oshiro T., Endo K., Baba H., Oda S., Ichiyoshi Y., Kohnoe S. and Sugimachi K. (1996) Clinical significance of occult micrometastasis lymph nodes from patients with early gastric cancer who died of recurrence. *Surgery* 119, 397-402.
- Morrow C.P., Bundy B.N., Kurman R.J., Creasman W.T., Heller P., Homesley H.D. and Graham J.E. (1991). Relationship between surgical-pathological risk factors and outcome in clinical stage I and II carcinoma of the endometrium: a Gynecologic Oncology Group study. *Gynecol. Oncol.* 40, 55-65.
- Niikura H., Okamoto S., Yoshinaga K., Nagase S., Takano T., Ito K. and Yaegashi N. (2007). Detection of micrometastases in the sentinel

*Endometrial cancer micrometastases*

- lymph nodes of patients with endometrial cancer. *Gynecol. Oncol.* 105, 683-686.
- Pelosi E., Arena V., Baudino B., Bellò M., Giusti M., Gargiulo T., Palladin D. and Bisi G. (2003). Pre-operative lymphatic mapping and intra-operative sentinel lymph node detection in early stage endometrial cancer. *Nucl. Med. Commun.* 24, 971-975.
- Reich O., Winter R., Pickel H., Tamussino K., Haas J. and Petru E. (1996). Does the size of pelvic lymph nodes predict metastatic involvement in patients with endometrial cancer? *Int. J. Gynecol. Cancer* 6, 445-447.

Accepted August 18, 2011