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Surgical staging and differential protein expression in endometrial cancers

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Summary

Purpose of Investigation: The benefit of comprehensive surgical staging in type I versus type II tumors is investigated and translationally associated with differentially expressed proteins. *Materials and Methods:* A single institutional database was queried for all uterine cancer patients and sample data was abstracted. Proteins identified from microarray data were further investigated at the immunohistochemical (IHC) level and correlated with sample data. *Results:* Lymph node dissection did not result in a survival benefit for either type I or type II uterine cancer. L1CAM is differentially expressed between tumor types. GRB7 was found to correlate with stage in type II cancers. Thrombomodulin was expressed at higher levels in all lymph node (LN) positive cancers. *Conclusions:* LN dissection did not provide a therapeutic benefit in endometrial cancers, especially type II cancers. Protein correlations with stage and histology demonstrate specific biologic profiles. Novel diagnostic and therapeutic targets for differentially expressed tumor proteins are identified.

Key words: Immunohistochemistry; Lymph node; Uterine cancer.

Introduction

Uterine adenocarcinoma is the most common female gynecologic cancer. An estimated 63,230 new cases of uterine cancer are predicted in the United States, with 11,350 deaths attributed to it, in the year 2018 [1]. Epidemiological and clinical studies suggest that endometrial cancers be separated into two groups by histologic appearance and behavior: type I tumors are the more common endometrioid adenocarcinomas, tend to be hormonally responsive, and have all an 83% stage five-year survival (5YS); type II tumors are more biologically aggressive and have a 53% all stage 5YS [2]. The main risk factor in type I carcinomas is hyperestrogenism. These cancers typically have a favorable prognosis with appropriate therapy. Type II cancers are poorly differentiated tumors, and are histologically represented by the serous and clear cell histologies. Type II tumors account for 3-5% of uterine carcinomas, but represent 50% of all relapses [3]. These type II tumors are classified as high risk, high grade, and are unresponsive to hormonal therapy.

Surgical staging, specifically lymph node dissection (LND), for uterine cancer has been questioned in two recent randomized controlled trials. Results from these trials conclude that, although a survival benefit cannot be demonstrated, LND maintains its importance in determining a patient's prognosis and tailoring adjuvant therapies [4, 5] Adjuvant treatment is usually recommended based on stage and pathologic risk factors (PRF). The national practice consensus for positive lymph node (LN) or type II patients allows for treatment of all stages to consist of chemotherapy, radiation therapy, or a combination of these therapies. The question of the necessity of LND in a type II population was then identified because practice patterns were changing and adjuvant chemotherapy was considered for most patients with type II cancer [6, 7]. The author specifically wanted to know whether LND conferred any diagnostic or therapeutic benefit to those patients with type II tumors, as early and late surgical morbidities can be significant.

A translational component was incorporated into this study. Clinically relevant genes, expressed at various levels in type II endometrial tumors, have been identified using high-throughput technologies such as cDNA microarrays [8]/ Santin *et al.* identified a number of differentially expressed genes using gene microarray analysis, comparing normal endometrial cells (NEC) to type II uterine tumors [9]. RT-PCR assays were used to validate the microarray data. Eleven genes were identified between the type II cancers and NEC and noted as upregulated: CDKN2A/ p14ARF, L1CAM, claudin-3, claudin-4, GRB-7, c-erbB2, kallikrein-6, kallikrein-10, IL-6, IL-18, and plasminogen

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activator receptor (PLAUR). Seven of these gene products (claudin-3, claudin-4, c-erB2, kallikrein-10, IL-6, IL-18, and PLAUR) had been studied to date of study initiation using immunohistochemistry (IHC) and results were available in published papers [10-12].

The goal of the translational portion of this study was to extend the characterization of those four remaining upregulated genes between normal endometrial cells/tissue, type II tumors, and to include type I tumors via IHC. These proteins were then correlated with sample data investigating clinical outcome parameters. The author then investigated seven additional proteins of interest.

Materials and Methods

Patient data was abstracted from the institutional database captured by ICD-9 diagnosis codes between 1989 and 2007 and entered into Excel after IRB approval. All patients underwent staging with hysterectomy, bilateral salpingo-oophorectomy (BSO), omental biopsy, and LND. Age, tumor stage (FIGO 1988), grade, lymphovascular space involvement (LVSI), lower uterine segment involvement (LUS), tumor size, depth of invasion (DOI), number of lymph nodes resected, site of lymph node dissection, and number of positive LNs were recorded. Patient medical comorbidities were listed. Progression free survival (PFS) and overall survival (OS) were queried from the institutional cancer registry and confirmed with the social security death index. Differences between patient characteristics, tumor characteristics, and IHC data were analyzed and compared to PFS and OS. There were 202 patients with type I tumors and 47 patients with type II tumors identified.

Paraffin embedded uterine cancer specimens were obtained from the pathology repository representing only patients from the above database. The type II tumors were matched by stage with the type I tumors. Fourteen normal endometrial controls were obtained from hysterectomy specimens performed for benign indications. The pathological nature of the type I, the type II, and the normal endometrial samples was verified by a gynecologic pathologist and with IHC to WT-1 and p53 as indicated. The most representative haematoxylin and eosin-stained block sections were used for each specimen. Protein expression was evaluated by IHC staining on the formalin-fixed tissue. Eleven different markers, including the four uninvestigated proteins identified via microarray by Santin *et al.*, (CDKN2A/p14ARF, L1CAM, GRB-7, kallikrein-6; CK5/6, calretinin, thrombomodulin, Ber-EP4, MMP1, MMP-3, and MMP9) were evaluated by IHC in 36 Type II, 42 type I cancers, and 14 normal endometrial tissue samples. The additional proteins were included for analysis based on literature review and expert pathologic recommendation.

Tissue sections were deparaffinized in xylene, rehydrated in graded alcohol, and transferred to PBS. The slides were rinsed with PBS and 3% hydrogen peroxide was used to block endogenous peroxidase. Slides were washed with PBS and incubated for 20 minutes with protein blocking solution consisting of PBS, 5% normal horse serum, and 1% normal goat serum. After excess blocking solution was removed, the slides were incubated with an anti-CDK2A/p14ARF antibody, anti-LCAM-1 antibody, anti-GRB-7 antibody, anti-kallikrein-6 antibody, anti-CK5/6 antibody, anti-calretinin antibody, anti-thrombomodulin antibody, anti-Ber-EP4 antibody, anti-MMP 1 antibody, anti-MMP 3 antibody, and anti-MMP 9 antibody. After 18 hours of incubation, the slides were washed and incubated with the appropriate dilution peroxi-

Table 1. — Patient characteristics.

Sample characteristics	n	Mean/%	SD	Min	Max
Type II cancer	249	18%	-	0	1
Age at diagnosis	249	58.24	10.30	27	84
Grade	249	1.91	0.81	1	3
Stage	249	1.69	0.99	1	4
DOI greater than half	247	38%	-	0	1
Positive LN	248	16%	-	0	1
Total number LN positive	248	0.40	1.37	0	15
Total LN	248	8.36	7.48	0	51
Total pelvic LN positive	248	0.36	1.30	0	15
Total Pelvic LN	242	7.48	7.01	0	51
Total para-aortic LN positive	248	0.04	0.24	0	2
Total para-aortic LN	242	0.89	2.04	0	13
Tumor size	248	4.58	3.15	0	22
LVSI yes	248	25%	-	0	1
LUS Involvement	248	44%	-	0	1
Chemotherapy yes	249	22%	-	0	1
Radiation yes	249	31%	-	0	1
Hypertension	249	48%	-	0	1
Diabetes	248	27%	-	0	1
Obesity	249	22%	-	0	1
Asthma	249	6%	-	0	1
Other Cancers	249	2%	-	0	1

LN = lymph nodes; LVSI = lymphovascular space invasion; LUS = lower uterine segment.

Table 2. — *Prognostic factors for overall survival.*

	Adjusted for cancer type*		Adjusted for type cancer and age category**	
Univariate variables	Hazard Ratio	*p*-value	Hazard Ratio	*p*-value
Grade	1.57	0.040	1.55	0.059
Stage (number)	1.42	0.021	1.45	0.016
DOI Greater than half	3.33	<.001	3.78	<.001
Positive LN	1.85	0.072	1.95	0.056
Total number LN positive	1.47	<.001	1.44	<.001
Total LN	1.01	0.77	1.01	0.57
Total pelvic LN positive	1.44	<.001	1.40	<.001
Total pelvic LN	1.02	0.46	1.02	0.34
Total para-aortic LN positive	3.96	<.001	3.42	<.001
Total para-aortic LN	0.91	0.28	0.92	0.33
Tumor max size	1.11	0.013	1.13	0.006
LVSI yes	2.52	0.002	2.72	0.001
LUS involvement	0.82	0.48	0.86	0.60
Chemotherapy yes	2.14	0.017	2.13	0.022
Radiation yes	1.14	0.65	1.27	0.43
Hypertension	1.26	0.40	1.23	0.45
Diabetes	0.93	0.81	0.91	0.77
Obesity	0.96	0.91	0.99	0.97
Asthma	3.88	<.001	4.40	<.001
Other cancers	1.94	0.36	2.00	0.35
Heart disease	3.33	<.001	3.57	<.001
Thyroid disease	0.95	0.91	0.96	0.93

Univariate Cox Model: outcome = time until death; DOI = depth of invasion LN = lymph nodes; LVSI = lymphovascular space invasion.
** Cancer types: type I, type II ** Age categories (at diagnosis): less than 50 years, 50-60 years, and greater than 60 years.*

dase conjugated to anti-rabbit/mouse universal IgG, anti-goat IgG, at the above dilutions. Cases with less than 30% staining in tumor cells were considered negative for expression, while positive cases were classified to have more than 30% staining.

SPSS 16.0 was used for analysis. Cox regression analysis was used to assess associations of patient variables with patient PFS and OS. For the translational portion of the data analysis Fisher's exact test was used for each pair-wise comparison. For the protein correlations, the Spearman rank correlation was used. Correlations were done within the cancer types, and then the two cancer types were combined to assess protein presence and stage.

Results

The author identified 249 patients with uterine cancer who had comprehensive surgical staging including LND. The average age of the type I tumor patients was 57 years and for the type II patients, 62 years. Forty-eight percent of patients had hypertension and 27% had diabetes. Differences between tumor type cohorts were not significant for age or comorbidities. Eighteen percent were type II cancer patients. The postoperative management of these patients varied. Table 1 demonstrates patient characteristics.

The author found a statistically significant difference in OS between those with type II tumors and type I tumors. The median survival in years for the type I cancer group was 15.4 versus 3.8 for the type II cancer group.

The author then looked, via a univariate Cox model, at the hazard ratio (HR) for decreased OS for each tumor type. Statistically significant independent risk factors for poor prognosis and death included grade, stage, DOI greater than half, LVSI, and total number of positive LN (Table 2). All the results significantly associated with survival in the univariate models were then entered into a multivariate model. Positive LN, DOI greater than half, and type II histology were statistically significant for decreasing OS (Table 3).

Because a variable of interest was "site" (pelvic v. para-aortic) and "number of LN removed" compared to OS, other models were run to see if adjusting for the number of corresponding LN removed and their location would have any effect on the associations with survival (Table 4). The total number of LN removed and OS yielded a HR of 1.0. The author also looked at total number of LN removed, adjusting for the number of positive LN, and the HR continued to be 1.0. It does not appear that identifying or removing a greater number of LN is associated with a better survival (debulking) in either tumor type.

In each model it appears that as more positive LN were identified/removed, there was a higher risk of death. Even after adjusting for grade, stage, and other indicators of poor prognosis, we see via multivariate analysis that those with three or more positive LNs removed had a six-fold greater risk of death (95% CI 1.79, 21.91) than those with zero positive LNs (Table 5).

The author then investigated the absence or presence of protein by cancer type compared to normal endometrium controls (Table 6). L1CAM was upregulated in type II tumors compared to type I tumors, as well as in type II tumors compared to control (type II different than type I $p < 0.001$; type II different than control $p < 0.01$). Calretinin was upregulated in type II tumors compared to the control ($p < 0.01$). Kallikrein-6 was also upregulated in type II tumors compared to the control ($p < 0.001$) and in type I tumors compared to the control ($p < 0.01$). CDK2a/p14ARF was also upregulated in both tumor types compared to the control ($p < 0.001$). MMP3 was upregulated only in type II tumors compared to control ($p < 0.01$).

The absence or presence of each protein was then correlated with stage (Table 7). GRB7 showed a 17% higher correlation in type II cancers with progressively increasing stage. Calretinin showed a 10% higher correlation in type II cancers, again with progressively increasing stage. Figure 1 represents this data in bar graph form. The author then wanted to investigate if there was any relationship of an in-

Table 3. — *Multivariate prognostic factors for overall survival.*

		95% Confidence interval		
Univariately significant covariates	Hazard ratio	Lower	Upper	*p*-value
Stage (number)	0.91	0.61	1.36	0.64
DOI greater than half	2.85	1.47	5.53	0.002
Total pelvic LN positive	1.27	1.03	1.55	0.024
Total para-aortic LN positive	2.43	1.13	5.20	0.022
Tumor size	1.04	0.93	1.17	0.52
LVSI yes	1.48	0.71	3.05	0.29
Chemotherapy yes	1.04	0.46	2.39	0.92
Asthma	3.39	1.33	8.68	0.011
Heart disease	2.94	1.41	6.13	0.004
Type II Cancer	2.62	1.14	6.01	0.023

Multivariate Cox Model: outcome = time until death; LN = lymph nodes; DOI = depth of invasion; LVSI = lymphovascular space invasion.

Table 4. — *Prognostic value of LND for overall survival.*

Site and number of LN removed	Hazard ratio	*p*-value
Total LN	1.00	0.99
Total pelvic-LN	1.01	0.66
Total para-aortic LN	0.84	0.16

Univariate Cox Model: outcome = time until death; LN = lymph nodes.
** Adjusted for cancer type, age category, and positive LNs of same type*

Table 5. — *Number of positive LNs removed and hazard ratio for death.*

Categorized total positive	Hazard ratio	95% confidence interval		
		Lower	Upper	*p*-value
Positive LNs				
Zero positive LNs (ref)	1			-
1-2 positive LNs	2.01	0.94	4.30	0.072
3-4 positive LNs	6.56	1.69	25.53	0.007
5 or more positive LNs	30.04	5.24	172.23	0.000
Type II	2.22	1.20	4.11	0.011

Multivariate Cox Model; LN = lymph node.

Table 6. — *Protein absence or presence by cancer type.*

Protein		Group					
		Type I		Type II		Control	
		Count	(%)	Count	(%)	Count	(%)
BerEp4	Absent	2	(5%)	3	(9%)	4	(29%)
	Present	39	(95%)	32	(91%)	10	(71%)
Calretinin	Absent	29	(73%)	20	(57%)	14	(100%)
	Present	11	(28%)	15	(43%)		(0%)
CK56	Absent	17	(43%)	8	(23%)	3	(21%)
	Present	23	(58%)	27	(77%)	11	(79%)
GRB7	Absent	37	(90%)	27	(77%)	12	(86%)
	Present	4	(10%)	8	(23%)	2	(14%)
Kallikrein6	Absent	24	(59%)	13	(37%)	14	(100%)
	Present	17	(41%)	22	(63%)		(0%)
L1CAM	Absent	39	(95%)	19	(54%)	14	(100%)
	Present	2	(5%)	16	(46%)		(0%)
MMP9	Absent	12	(29%)	19	(54%)	2	(14%)
	Present	29	(71%)	16	(46%)	12	(86%)
MMP1	Absent	1	(2%)	2	(6%)	2	(14%)
	Present	40	(98%)	33	(94%)	12	(86%)
MMP3	Absent	6	(15%)	4	(11%)	7	(50%)
	Present	35	(85%)	31	(89%)	7	(50%)
p14ARF	Absent	3	(7%)	2	(6%)	14	(100%)
	Present	38	(93%)	33	(94%)		(0%)
Thrombomodulin	Absent	35	(85%)	29	(83%)	13	(93%)
	Present	6	(15%)	6	(17%)	1	(7%)

Serous diff than control $p < 0.01$
Endo diff than control $p < 0.01$
Serous diff than control $p < 0.001$
Endo diff than serous $p < 0.001$
Serous diff than control $p < 0.01$
Serous diff than control $p < 0.01$
Endo and serous both diff than control $p < 0.001$

* *Fisher's exact test used for p-values; Diff = different; endo = endometroid.*

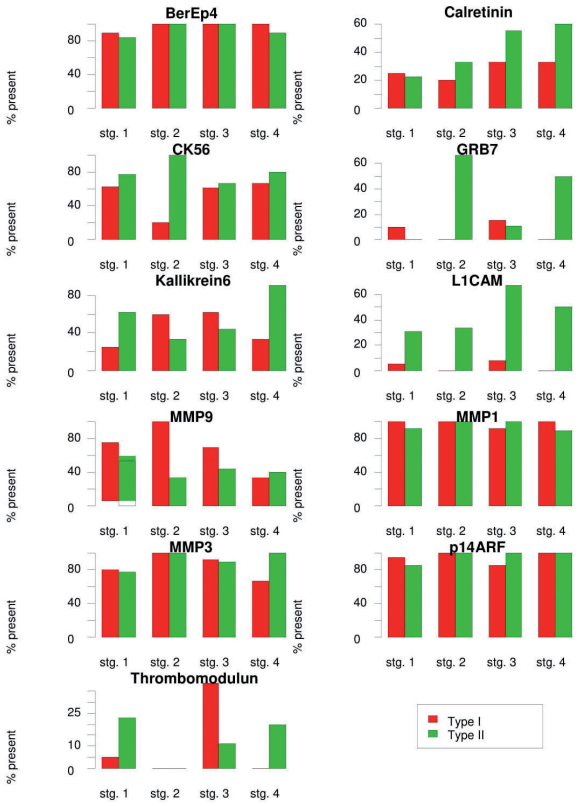

Figure 1. — Expression levels of protein by cancer type and stage.

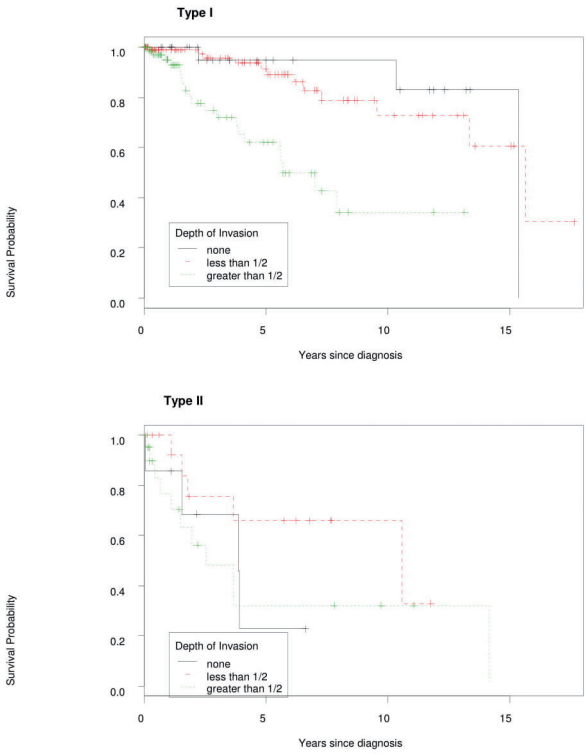

Figure 2. — Two-dimensional gel demonstrating protein expression in cervical overall survival by depth of invasion.

Table 7. — *Protein absence or presence correlated with stage.*

		Cancer Type		
		Type I	Type II	Combined
BerEp4	Correlation Coefficient	0.22	0.10	0.14
	p-value	0.171	0.565	0.228
	n	41	35	76
Calretinin	Correlation Coefficient	0.08	0.32	0.24
	p-value	0.628	0.058	0.039
	n	40	35	75
CK56	Correlation Coefficient	-0.02	-0.01	0.03
	p-value	0.919	0.968	0.814
	n	40	35	75
GRB7	Correlation Coefficient	0.01	0.42	0.27
	p-value	0.963	0.012	0.018
	n	41	35	76
Kallikrein6	Correlation Coefficient	0.27	0.21	0.25
	p-value	0.082	0.236	0.027
	n	41	35	76
L1CAM	Correlation Coefficient	0.01	0.20	0.22
	p-value	0.974	0.250	0.056
	n	41	35	76
MMP9	Correlation Coefficient	-0.09	-0.11	-0.15
	p-value	0.562	0.540	0.187
	n	41	35	76
MMP1	Correlation Coefficient	-0.16	-0.02	-0.08
	p-value	0.319	0.913	0.501
	n	41	35	76
MMP3	Correlation Coefficient	0.08	0.28	0.17
	p-value	0.637	0.103	0.139
	n	41	35	76
p14ARF	Correlation Coefficient	-0.10	0.28	0.09
	p-value	0.539	0.102	0.421
	n	41 35 76		
Thrombomodulin	Correlation Coefficient	0.28	-0.04	0.11
	p-value	0.075	0.822	0.360
	n	41	35	76

Spearman's Correlation used for p-values.

dividual protein with LN involvement, i.e. Stage IIIc. Thrombomodulin showed a statistically significant difference (p=0.017) specific to Stage IIIc uterine cancer (Table 8). This trend continued when separated between tumor types.

Five-year OS and 5Y PFS were evaluated by histology. Type II cancers were shown to have a significant decrease in OS and PFS. The 5YS for all stages of type I cancers was 82%, and 45% for type II cancers. The 5YS was then stratified by age and tumor type. It appears that age over 60 significantly decreased the 5YS and was tumor type dependent. The 5YS for type I tumors was 97% for age less than 50 and 80% for ages above 51. The 5Y PFS for type I tumors was 97% for age less than 50, 80% for ages 51-60, and 70% for ages older than 60. The 5YS and 5Y PFS for type II tumors was 100% for age less than 50, 50% for ages 51-60, and 30% for ages older than 60.

Table 8. — *Protein expression correlated with Stage 3c uterine cancer.*

Protein		Stage 3c				
		No positive LNs n=30		At least one positive LN n=11		*p*-value
BerEp4	Absent	2	(7%)	0	(0%)	0.38
	Present	28	(93%)	11	(100%)	
Calretinin	Absent	22	(73%)	7	(70%)	0.84
	Present	8	(27%)	3	(30%)	
CK56	Absent	13	(45%)	4	(36%)	0.63
	Present	16	(55%)	7	(64%)	
GRB7	Absent	27	(90%)	10	(91%)	0.93
	Present	3	(10%)	1	(9%)	
Kallikrein6	Absent	19	(63%)	5	(45%)	0.30
	Present	11	(37%)	6	(55%)	
L1CAM	Absent	29	(97%)	10	(91%)	0.45
	Present	1	(3%)	1	(9%)	
MMP9	Absent	9	(30%)	3	(27%)	0.86
	Present	21	(70%)	8	(73%)	
MMP1	Absent	0	(0%)	1	(9%)	0.095
	Present	30	(100%)	10	(91%)	
MMP3	Absent	5	(17%)	1	(9%)	0.54
	Present	25	(83%)	10	(91%)	
p14ARF	Absent	2	(7%)	1	(9%)	0.79
	Present	28	(93%)	10	(91%)	
Thrombomodulin	Absent	28	(93%)	7	(64%)	0.017
	present	2	(7%)	4	(36%)	

Spearman's correlation used for p-values.

Survival and PFS were analyzed for the three depth of invasion categories (Figures 2 and 3). Because the survival profile seemed similar between "none" and "less than half" categories, these groups were merged in further analysis. This correlates with the latest FIGO 2009 restaging. The 5YS for type I tumors was 97% for DOI less than half and 62% for greater than half. The PFS was 97% for less than half and 58% for greater than half. The 5YS for type II tumors was 45% for less than half and 30% for greater than half. The PFS was 45% for less than half and 35% for greater than half.

The 5YS and 5Y PFS by the number of positive LNs removed were distinguished between type I and II tumors (Figures 4 and 5). As more positive LNs were removed, the OS decreased for type I tumors. The 5YS in type I tumors with no positive LNs was 89%, one to two positive LNs was 68%, and greater than two positive LNs was 40%. However, it appears that the 5YS is not related to the number of positive LNs removed in type II tumors as the 5YS for no positive LNs was 45%, one to two positive LNs was

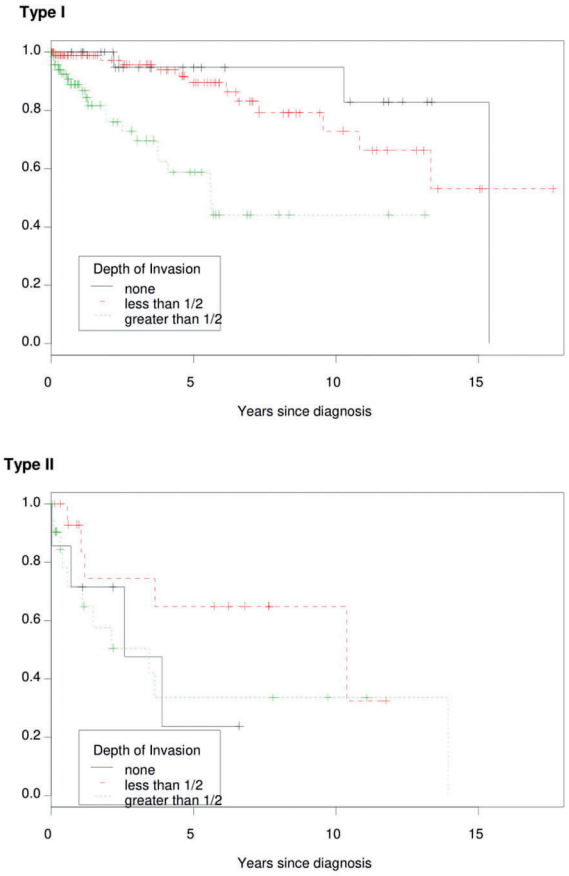

Figure 3. — Progression free survival by depth of invasion.

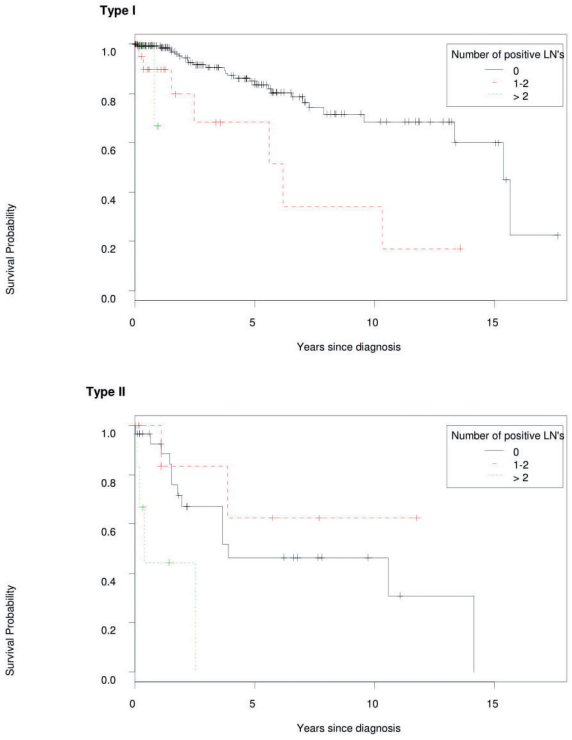

Figure 4. — Overall survival by number of positive LNs removed.

64%, and greater than two positive LNs was 0. The 5Y PFS for type I tumors was 85% for no positive LNs, 74% for one to two positive LNs, and 0 for greater than two positive LNs removed. For type II tumors, the 5Y PFS was 47% for no positive LNs, 59% for one to two positive LN's, and 0 for greater than two positive LNs.

Discussion

The goal of this study was to assess the value of LND between uterine cancer histological subtypes. A secondary goal was to extend the characterization of upregulated genes identified by microarray between normal endometrial tissue, type I, and type II uterine cancers via IHC. LN dissection was not found to provide any survival benefit in endometrial cancers, especially type II cancers. Protein correlations with stage and histology demonstrate specific biologic profiles. Novel diagnostic and therapeutic targets for differentially expressed tumor proteins were identified.

This study confirmed, on many levels, the known adverse risk factors of tumor type, age, grade, stage, LVSI, and LN positivity with respect to PFS and OS. There is still much discussion regarding a benefit to LN dissection, either for diagnostic or therapeutic purposes. This retrospective study was initiated in an attempt to decrease potential surgical morbidity, because adjuvant chemotherapy with or without radiation therapy is often given to patients who have any stage of type II cancer. This study found that LN dissection did not provide any therapeutic benefit, especially for type II cancers. This is supported by the Benedetti-Panici *et al.* study [5] but contrary to other notable studies [13-15]. The author also assessed the total number of LNs removed adjusting for the number of positive LNs and there was not sufficient evidence that removing more LNs helps prognosis. Studies supporting the data presented here exist. Goldberg *et al.* found that complete surgical staging including omentectomy and LND in addition to hysterectomy and BSO with maximal cytoreduction, compared to hysterectomy and BSO with optional pelvic LN sampling, did not contribute to a better 5Y PFS or OS [16].

The author obtained the published RNA microarray data and further assessed the results on the IHC level in an attempt to correlate protein differences between tumor types, as well as in combination with adverse PRF. Protein correlations with stage and histology demonstrated specific biologic profiles.

L1CAM was identified as the most differentially expressed protein in this IHC analysis, as it was significantly upregulated in type II tumors. L1CAM is a multifunctional transmembrane cell adhesion protein. L1CAM has been identified in-vitro as a target gene of β-catenin-TCF sig-

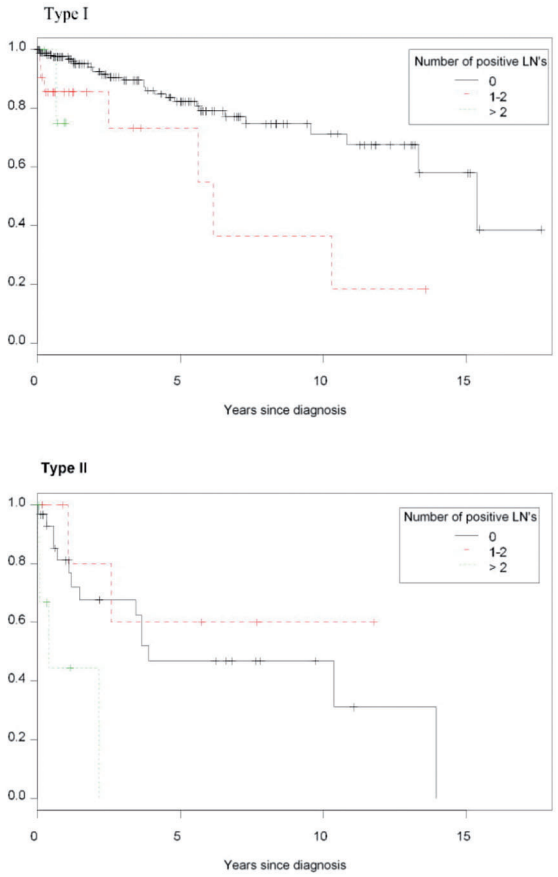

Figure 5. — Progression free survival by number of positive LNs removed.

naling in colorectal cancer cells: L1CAM was localized at the invasive front of tumor tissue that expressed a loss of β-catenin and E-cadherin on the cell surface [17]. The β-catenin-TCF transcriptional complex was found to activate the L1 gene, which conferred increased cell motility, transformation, and tumorigenesis [18].

L1CAM has also been identified as a cofactor for intercellular signals involved in cell migration and adhesion [19]. L1CAM was identified in secretory membrane vesicles in serous ovarian cancer cells. The enhanced migration of L1-expressing cells was found to be dependent on metalloproteinase activity [20]. An exocytosis-released L1 ectodomain was found to travel via ascites and stimulate cancer cell migration by binding to αvβ5 integrins [21].

The other proteins found to be differentially upregulated then understandably play a role. MMP3 was upregulated in type II cancers compared to controls. This molecule has been shown to cleave L1CAM [22], possibly assisting in the dispersion of metastatic cells via disruption of adhesion molecules specific to type II tumors. Calretinin was upregulated in type II tumors as well. This protein may enhance migration through calcium signaling pathways [23]. Thrombomodulin was found at a higher level in both types of node positive cancers, possibly mediating a lymphatic transport mechanism versus an ascitic-peritoneal transport. Kallikrein-6 and CDK2a/p14ARF were both upregulated in the cancer types compared to control, supporting the RNA microarray data for a role in carcinogenesis.

The strengths in this study are found in the comprehensive staging that all patients received and that treatment was provided at a single institution. The limitations with this study lie in the study design. The retrospective nature of this study depended on the availability and accuracy of the medical records and cannot compare to a randomized study. The 1988 FIGO staging system for uterine cancer staging was used for baseline stage, as the study was conceived before the 2009 staging modification. However for data analysis in this paper, due to no difference found, Staged IB and IC cancers were merged, analogous to the 2009 classification, and supporting the validity of the 2009 staging. Therefore the data presented here is in alignment with current outcome parameters. For the translational component, RT-PCR would have confirmed the IHC analysis for both tumor types and in the control tissues.

Comprehensive surgical staging, risk stratified by known uterine cancer normograms [24, 25], is the current standard of care. Adjuvant chemotherapy is recommended for consideration in type II tumors. Positive LN basins are variably included in the adjuvant radiation fields and some clinicians value the anatomical information obtained from LND, in addition to the prognostic knowledge of stage. The benefit of chemotherapy alone or in combination with radiation continues to leave treatment disposition open to interpretation. This retrospective review may support the exclusion of LN dissection, especially in type II tumors, if adjuvant chemotherapy and/or radiation is practiced and tumor subtype is conclusively known preoperatively.

IHC has advanced our knowledge base by identifying gene products (proteins) which are differentially expressed between tumors. The larger presence of L1CAM in type II uterine cancers may explain their biological aggressiveness and higher stage at presentation due to altered tumor signaling, cell migration, and adherence throughout the abdomino-pelvic cavity. Due to the differential distribution of L1CAM in type II cancers, this protein may represent a worthy diagnostic and therapeutic marker for this category of endometrial tumors. The other documented proteins may also play a role in future targeted therapies based on their biological properties.

Conclusion

A survival benefit of lymph node dissection between endometrial cancer types was explored, and protein correlations with stage and histology demonstrated specific biologic profiles.

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