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Research Article

Correlation of Lymphovascular Space Invasion and Invasive Circulating Tumor Cells in Patients with Epithelial Ovarian Cancer

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Abstract

Goals: Detection of circulating (CTCs) and invasive circulating tumor cells (iCTCs) in epithelial ovarian cancer (EOC) is feasible and prognostic. The deleterious impact of lymphovascular space invasion (LVSI) is well established in various malignancies but not yet extensively evaluated in EOC. The goals of this study were to evaluate the correlation between CTCs, iCTCs and LVSI and to assess the impact of LVSI on overall survival in women with EOC.

Methods: Peripheral blood samples from 85 women with EOC were assessed for the presence of CTCs and iCTCs using our functional cell adhesion matrix enrichment method. The histopathology slides were reviewed for histology, grade, LVSI presence or absence, extent (focal or multifocal) and organ site.

Results: Our data did not demonstrate any correlation between CTCs, iCTCs and LVSI. LVSI was significantly associated with decreased overall survival (median: 1194 vs. 2034 days) but not with stage, grade, debulking status, platinum sensitivity, or age. On univariate analysis, overall survival was significantly associated with LVSI, stage, debulking status, and platinum sensitivity. On multivariate analysis, only platinum sensitivity remained significantly associated with overall survival.

Conclusion: The absence of any correlation between LVSI and CTCs or iCTCs in EOC suggests tumor cell spread through these two pathways (lymphatic and vascular) may occur independently. The absence of an association between LVSI and overall survival when controlled for stage may be due to the overshadowing impact of overtly evident metastatic disease.

INTRODUCTION

Epithelial ovarian cancer (EOC), is the leading cause of death among gynecologic malignancies with approximately 22,440 cases (2.5% of all new female cancer cases) and 14,070 deaths (5.0% of all female cancer deaths) in the United States in 2018 [1]. According to data from SEER, "rates for new ovarian cancer cases have been falling on average 1.9% each year over the last 10 years. Death rates have been falling on average 2.2% each year over 2005-2014". Despite this modest improvement in outcome, the 5-year survival for women with epithelial ovarian cancer is only 46.5%. Survival is correlated with stage at diagnosis; the 5-year relative survival for women with distant disease is 28.9%, compared to 92.5% for those with localized disease [2].

The majority of women with epithelial ovarian cancer, approximately 60%, present with distant disease. Although the preponderance of women with EOC who undergo contemporary management with aggressive cytoreduction surgery and multi-agent chemotherapy achieve complete clinical remission, most are destined to recur within three years and die from the consequences of widespread, unresponsive metastatic disease.

Traditionally, it was believed that epithelial ovarian cancer spread by exfoliation and direct spread throughout the peritoneal cavity. Indeed, the bulk of EOC tumor mass is initially found within the peritoneal cavity and the majority of deaths are due to progressive intraperitoneal disease. However, extraperitoneal metastasis does occur, implying hematogenous or lymphatic dissemination of EOC cells. With changes in contemporary systemic therapy (e.g., intraperitoneal chemotherapy, bevacizumab), the frequency of extraperitoneal metastasis has increased [3].

The detection of circulating tumor cells (CTCs) in the peripheral blood of women with epithelial ovarian cancer has been proven to be feasible and prognostic [4-9]. As is true for a number of malignancies, including colorectal, prostate and breast cancer, CTCs are associated with adverse outcomes in women with epithelial ovarian cancer [9-12]. A significant

decrease in disease-free survival was observed in EOC patients with detectable CTCs. CTC-positive patients demonstrated a median disease-free survival time of 15 months vs. 35 months for CTC-negative patients. [4] A similar decrease was noted for patients with high CTC counts (defined as > 31.5 CTCs/ml): 15.0 months median survival versus 22.0 months median survival for CTC-low patients [4].

Lymphovascular space invasion (LVSI) is defined as endothelial-lined (capillary-like) spaces containing tumor cells that are contiguous with the stroma [13]. The deleterious impact of LVSI has been well-established in other gynecologic malignancies (e.g., vulvar, cervical, endometrial) [14-16]. In contrast, the significance of LVSI in ovarian cancer has not been extensively evaluated [17-21]. A review of two established ovarian cancer databases with examination of the associated histopathology slides concluded "the presence of LVSI is an independent predictive indicator of nodal metastasis and is associated with worse clinical outcome of patients with epithelial ovarian cancer" [22].

The goal of this study was to evaluate the correlation between circulating tumor cells, invasive circulating tumor cells and lymphovascular space invasion, and to assess the impact of lymphovascular space invasion on overall survival in women with epithelial ovarian cancer.

METHODS

Patients, Blood collection and Preparation

This study was approved by the Institutional Review Board overseeing human research at Stony Brook University. The study group consisted of 85 patients with epithelial ovarian cancer who underwent management by the Division of Gynecologic Oncology at Stony Brook University Hospital and participated in our ongoing circulating tumor cell research program. Clinical data were abstracted from the medical record and the Tumor Registry.

Blood collection and transport were previously described [4]. Two to twenty milliliters (mL) of blood were collected from patients using Vacutainer® tubes (Becton Dickinson; green top, lithium or sodium heparin as anticoagulant) and processed within 48 hours from collection. Blood was stored at 2-8ºC when storage longer than 4 hours was necessary.

Assessment of Lymphovascular Space Invasion

The histopathology (H&E) slides from each patient were reviewed by two gynecologic oncology pathologists. CT is a nationally recognized expert, including serving on the Gynecologic Oncology Group Pathology Committee for many years. The histologic type, grade, presence or absence of LVI, extent of the LVI (focal or multifocal) and location (organ site) was assessed. In accordance with published data demonstrating the lack of impact of LVSI quantity on survival, LVSI was analyzed in a dichotomous manner (i.e., presence *vs.* absence)[23].

Enumeration of Circulating Tumor Cells

Enrichment and identification of CTCs and iCTCs have been previously described [5,6]. Briefly, red blood cells in specimens were lysed and nuclear cells in serum-containing medium were seeded in Vita-Assay™ plates (Vitatex Inc., Stony Brook, NY) and incubated in 37°C CO_2 incubator for 2h. After removing floating cells by a one-time wash with PBS, adherent cells were collected, fixed and stained for flow cytometry and microscopy detection. Changes in CTCs and iCTCs were measured using an automated flow cytometer (FACSCalibur, BD Biosciences). Specifically, the antibodies and reagents used for flow cytometry were: phycoerythrin (PE)-conjugated anti-tumor progenitor (TP), antibodies (anti-CD44 and anti-seprase, Vitatex) or anti-epithelia (EPI) antibodies (ESA clone B29.1, Biomeda; EPCAM clone Ber-Ep4, DakoCytomation; CA125, DakoCytomation), allophycocyanin (APC)-conjugated anti-HL antibody cocktail (anti-CD45 clone 5B1, Miltenyi Biotech) and 7-aminoactinomycin D (7AAD). CTCs had nuclei that were stained with a nucleic acid (NA) dye, i.e., 7AAD, after cell fixation. Events were analyzed using FlowJo software (version 10 for Windows). To examine whether the cells identified as CTCs and iCTCs by flow cytometry could be imaged, parallel aliquots of blood samples from patients were subjected to microscopic imaging. For microscopic detection, anti-HL antibody against CD45 was used, followed by blue color alkaline-phosphatase–anti-alkaline- phosphatase (APAAP) secondary antibodies for CAM-avid immune cells, and staining with FITC- or TRITC conjugated EPI or TP antibodies and a NA dye, i.e., Hoechst 33342 (Invitrogen, Carlsbad, CA, USA) or DAPI (Life Technologies, Carlsbad, CA, USA). Stained cells in suspension were mounted using a Cytospin device. Microscopic analyses were performed on a Nikon E-400 inverted fluorescence microscope equipped with Microfire digital camera system and Image Pro Plus software.

In general, CTCs and iCTCs tended to be heterogeneous in size, had nuclei stained with a nucleic acid (NA) dye, and negatively stained with CD45. iCTCs exhibited CAM+ or TP+ Epi + NA + HL −, and CTCs were CAM ± or TP ± Epi + NA + HL −. The number of CTCs and iCTCs are absolute cell numbers that were captured from 1.0 mL of each patient's blood and counted by flow cytometry.

Statistical Analysis

Continuous variables were summarized by median and range; categorical variables were listed as frequencies. The continuous data were initially assessed for normality; as they were not normally distributed, univariate analysis was performed using the Mann–Whitney U test for non-parametric data. Fisher's exact tests were used to compare categorical data. A scatter plot was created and Pearson's correlation coefficient was used to assess the correlation between CTC and iCTC counts. Multivariate analysis was performed using the Cox proportional hazard regression test. Kaplan–Meier curves for overall survival were estimated and compared using the Log-rank test. The analyses were performed using SPSS version 9.4. The tests were two-tailed and p-values less than 0.05 were considered statistically significant.

RESULTS

Demographic and Clinical Data

The cases consisted of 69 serous carcinomas, six clear cell carcinomas, five carcinosarcomas, three undifferentiated carcinomas, and one each mixed (serous, mucinous and

endometrioid), and primary mucinous carcinomas. Three serous carcinomas were low-grade; the rest of carcinomas were high grade (82/85, 96.5%). The demographic and clinical data are described in Table 1. As expected, the majority (70/85, 82.4%), were advanced stage (III-IV), underwent optimal debulking (73/85, 85.9%), and had platinum sensitive disease (45/74, 60.8%). The median survival was 1302 days (range 6-5900 days). Only 16 patients (18.8%) are alive without disease; 60 patients (70.6%), had died of their disease, five (5.9%), were alive with disease and four (4.7%), had died of intercurrent disease unrelated to their ovarian cancer.

Assessment of Lymphovascular Space Invasion

LVSI was identified in 35 cases (41.2%); all high grade serous carcinomas except for one each clear cell carcinoma, carcinosarcoma and undifferentiated carcinoma. Nine cases (26.5% of cases with LVSI), had focal LVSI (found only on one slide). Overall, LVSI was located in the ovary (Figure 1A), or fallopian tube (Figure 1B), in 20 cases (58.8% of cases with LVSI); other sites included the peri-adnexal soft tissue (Figure 1C), omentum, colon, myometrium and appendix.

Table 1: Demographic and Clinical Data.

Age (median, range)	**63 years, 30-88 years**
Histology	
Serous	69
Clear cell	6
Carcinosarcoma	5
Undifferentiated	3
Mixed (serous, mucinous, endometrioid)	1
Mucinous (primary ovarian)	1
Stage	
IA	4
IC	6
IIA	1
IIB	1
IIC	3
IIIB	6
IIIC	46
IV	18
Debulking	
Optimal	73
Suboptimal	11
Biopsy only	1
Platinum Sensitive	
Yes	45
No	29
Unknown	11
Status	
NED	16
AWD	5
DOD	60
DICD	4
Overall survival (median, range)	1302 days (6-5900 days)

NED: No evidence of disease; AWD: Alive with disease; DOD: Dead of disease; DICD: Dead of intercurrent disease.

Circulating Tumor Cell and Lymphovascular Space Invasion Data

The circulating tumor cell and lymphovascular space invasion data are summarized in Table 2. Using the thresholds established in our previous studies [4,5]], 61 patients (71.8%), had high levels of circulating tumor cells (CTC ≥ 31.5 cells/ml) and 66 patients (77.6%) had high levels of invasive circulating tumor cells (iCTC ≥ 5 cells/ml).

High levels of CTCs were significantly associated with advanced stage (III/IV), high levels of iCTCs, and disease status, but not with grade, debulking status, platinum sensitivity, lymphovascular space invasion, age, or overall survival. High

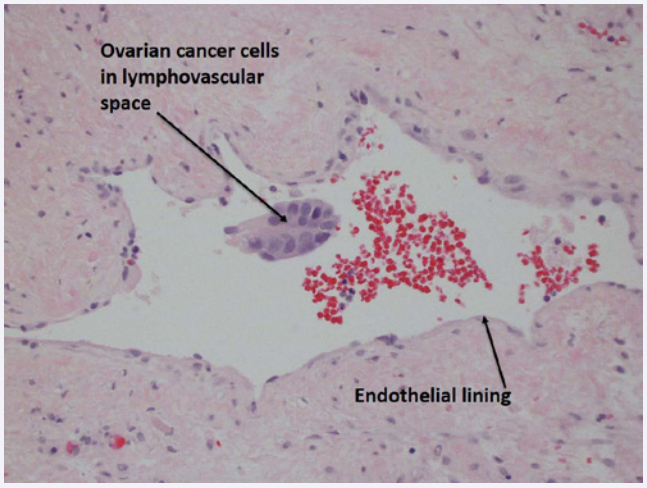

Figure 1a Lymphovascular space invasion in ovary.

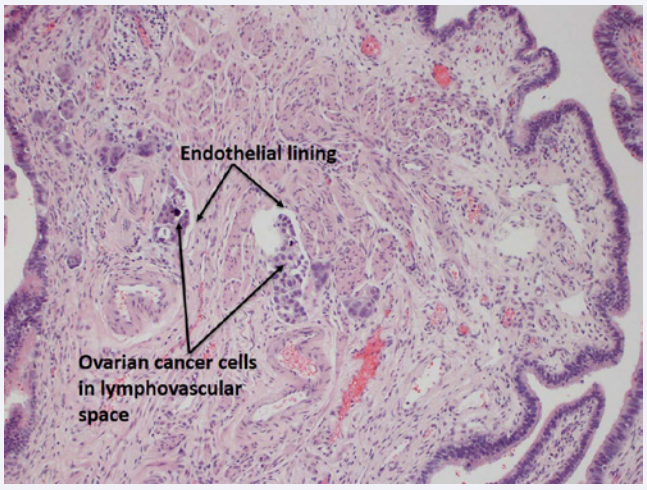

Figure 1b Lymphovascular space invasion in fallopian tube.

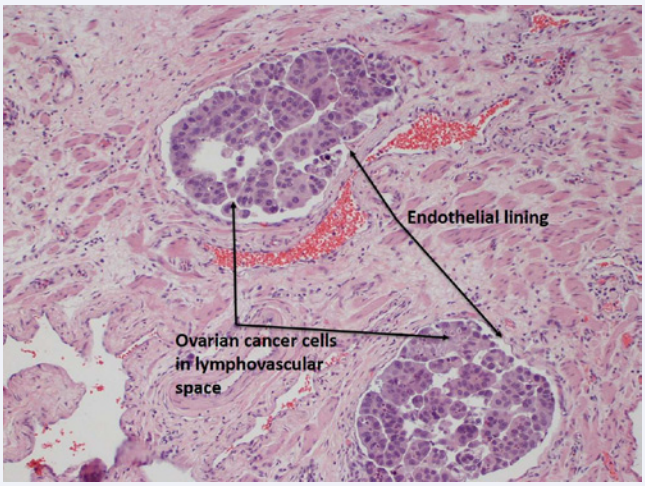

Figure 1c Lymphovascular space invasion in peri-adnexal soft tissues.

levels of iCTCs were significantly associated with advanced stage and high levels of CTCs, but not with grade, debulking status, platinum sensitivity, lymphovascular space invasion, age, or overall survival. Lymphovascular space invasion was significantly associated with decreased overall survival (median: 1194 vs. 2034 days, p =0.02), but not with stage, grade, debulking status, platinum sensitivity, median or high levels of CTCs or iCTS, or age.

On univariate analysis (Table 3), overall survival was significantly associated with stage, debulking status, platinum sensitivity and lymphovascular space invasion, but not with grade, high levels of CTCs or iCTCs, or age. On multivariate analysis (Table 4), only platinum sensitivity remained significantly associated with overall survival after controlling for the other significant factors identified on univariate analysis.

Kaplan-Meier curves comparing overall survival of patients with or without high levels of CTCs or iCTCs, and with or without

Table 2: Clinical Associations.

	LVSI			**CTC**			**iCTC**		
	Present	**Absent**	***p***	**< 31.5**	**≥ 31.5**	***p***	**< 5**	**≥ 5**	***p***
Stage			*0.26*			***0.0007***			***0.04***
I/II	4	11		10	5		7	8	
III/IV	31	39		14	56		12	58	
Grade			*0.14*			*0.32*			*1.00*
1	0	4		2	2		1	3	
3	35	46		22	59		18	63	
Debulking			*0.51*			*0.72*			*1.00*
Opt.	32	42		22	52		17	57	
Subopt.	3	8		2	9		2	9	
Plat. Sens.*			*0.06*			*0.80*			*1.00*
Yes	16	30		8	21		7	22	
No	17	12		14	31		10	35	
CTC			*1.00*						
< 31.5	9	14							
≥ 31.5	26	36							
iCTC			*0.79*			***0.0001***			
< 5	7	12		13	6				
≥ 5	28	38		11	55				
Status			*0.21*			***0.0479***			*0.55*
Alive	6	15		10	11		6	15	
Dead	29	35		14	50		13	51	
	Median		*p*	Median		*p*	Median		*p*
Age	59.6	62.3	*0.25*	60.2	61.56	*0.63*	63.0	60.7	*0.44*
CTC	95.0	63.6	*0.21*				14.5	94.4	***0.0001***
iCTC	39.7	34.2	*0.76*	7.0	48.1	***0.0001***			
OS	1194	2034	***0.02***	1797	1645	*0.66*	1955	1612	*0.36*

Abbreviations: CTC: circulating tumor cells; iCTC: invasive circulating tumor cells; Opt: optimal; Subopt: suboptimal; Plat. Sens: platinum sensitive; OS: overall survival.
Units: CTC and iCTC: cells/ml; age and OS: years
*Platinum sensitivity groups do not equal 85 as data were lacking for 11 patients

Table 3: Overall Survival (Univariate Analysis).

Factor	HR	95% CI	P
Stage: I/II *vs.* III/IV*	0.243	0.097-0.608	0.0025
Grade: 1 *vs.* 3	0.408	0.099-1.672	0.2128
Debulking: opt *vs.* subopt*	0.478	0.233-0.981	0.0443
Plat. Sens: no *vs.* yes*	3.463	1.997-6.004	< 0.0001
CTC: < 31.5 *vs.* ≥ 31.5	0.656	0.359-1.200	0.1710
iCTC: < 5 *vs.* ≥ 5	0.708	0.375-1.337	0.2866
Age^	1.002	0.982-1.023	0.8151
LVSI: neg *vs.* pos*	0.524	0.314-0.874	0.0134

Abbreviations: HR: hazard ratio; CI: confidence interval; CTC: circulating tumor cells; iCTC: invasive circulating tumor cells; Opt: optimal; Subopt: suboptimal; Plat. Sens: platinum sensitive; OS: overall survival; LVSI: lymphovascular space invasion
Units: CTC and iCTC: cells/ml; age and OS: years
* Significant
^ With each year of age, the hazard ratio increased by 0.25%; however, this was not significant

Table 4: Overall Survival (Multivariate Analysis).

Factor	HR	95% CI	P
Stage: I/II *vs.* III/IV	0.383	0.146-1.009	0.0521
Debulking: opt *vs.* subopt	0.865	0.391-1.911	0.7199
Plat. Sens: no *vs.* yes*	2.707	1.477-4.692	0.0013
LVSI: neg *vs.* pos	0.747	0.418-1.336	0.3254

Abbreviations: HR: hazard ratio; CI: confidence interval; Opt: optimal; Subopt: suboptimal; Plat. Sens: platinum sensitive; OS: overall survival; LVSI: lymphovascular space invasion
* Significant

detectable lymphovascular space invasion are shown in Figures 2A-C. Overall survival did not differ significantly for patients with or without high levels of CTCs or iCTCs but was significantly decreased for patients with lymphovascular space invasion (p=0.013).

A scatter plot with the regression line demonstrating the moderate correlation between the levels of CTCs and iCTCs is shown in Figure 3. The Pearson correlation coefficient (r) was 0.63.

DISCUSSION

The goal of this study was to evaluate the correlation between lymphovascular space invasion (LVSI) and circulating tumor cells in patients with epithelial ovarian cancer. To our knowledge, this is the first such study; despite an extensive search, we were unable to identify any comparable published reports in the literature.

Using the thresholds for circulating tumor cells (CTCs ≥ 31.5 cells/ml) and invasive circulating tumor cells (iCTCs ≥ 5 cells/ml) established in our prior studies [4,5], our data did not demonstrate any correlation between LVSI and CTCs or iCTCs. As a surrogate, given the absence of any published directly comparable reports, we identified two studies and one meta-analysis assessing the relationship between CTCs and lymph node metastasis [24-26]. Similar to our results, the results of these studies demonstrated that CTCs and lymph node metastases were not significantly correlated. In Cui's meta-analysis (including data from Sang and Obermayr's studies [24,25]), the pooled OR was 1.14 (95% CI: 0.67-1.93, Z = 0.481, P=0.630 fixed effect) [26]. These findings suggest that tumor cell spread through these two pathways (lymphatic and vascular) may occur independently.

Intravasation of the lymphatic or vascular vessels by cells within the primary tumor is an initial step leading to metastasis. The presence of circulating tumor cells is a direct consequence of intravasation into the vascular system [27]. Circulating tumor cells may lead to distant metastases, while lymph node metastases occur following tumor cell intravasation of the lymphatic system [28, 29]. According to Zavyalova, intravasation into the lymphatic vessels is easier compared to intravasation into the vascular vessels due to the absence of dense intraendothelial junctions, less stable walls and slower circulation in the lymphatic vessels [27].

It is difficult, and not general clinical practice, to

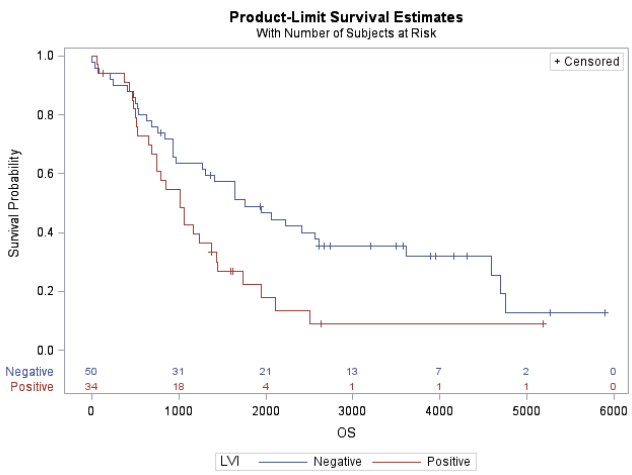

Figure 2a Kaplan-Meier Survival Curve (LVSI negative vs. positive). p=0.013.

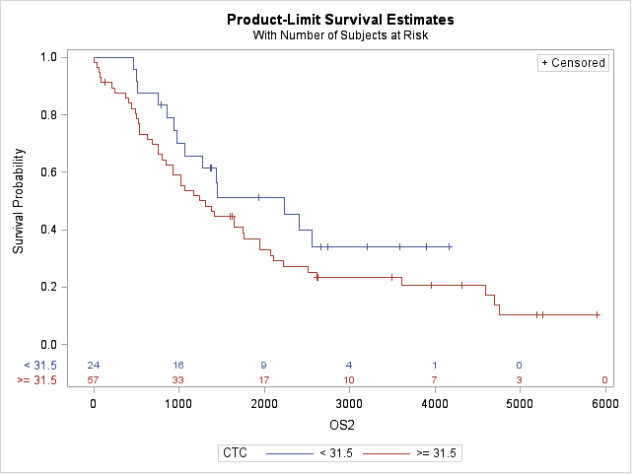

Figure 2b Kaplan-Meier Survival Curve (CTC < 31.5 vs. ≥ 31.5). p= 0.17.

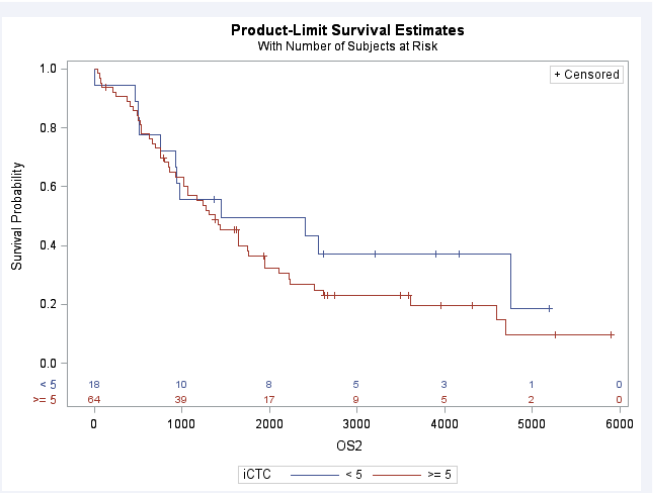

Figure 2c Kaplan-Meier Survival Curve (iCTC < 5 vs. ≥ 5). p= 0.28.

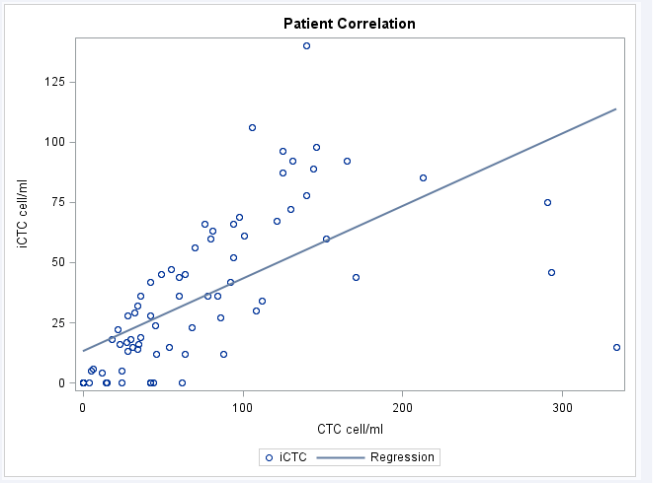

Figure 3 Correlation between CTC and Ictc. Pearson correlation coefficient r=0.63, r^2=0.40, p <0.0001.

discriminate between lymphatic and vascular spaces [29]. With immunohistochemistry, it is possible to distinguish between lymphatic and vascular vessels by comparing the results of staining with D2-40 (stains lymphatic vessels only) and CD34 or CD31 (stains both blood vessels and lymphatic vessels) [30]. Typically, though, identification of endothelial-lined (capillary-like) spaces containing tumor cells that are contiguous with the stroma [13], is generically reported as "lymphovascular space invasion". Further research is needed to improve the discrimination between lymphatic and vascular invasion in order to define their respective clinical impact [28].

The mechanism driving lymphovascular space invasion in epithelial ovarian cancer has not been clearly elucidated. Matsuo reported that estrogen receptor status was positively correlated with LVSI in high grade serous ovarian cancer, suggesting a potential role for the estrogen pathway [23]. Alternatively, tumoral vascular endothelial growth factor (VEGF), expression may contribute to development of LVSI [31,32].

The incidence of lymphovascular space invasion (LVSI) [13], was 41.2% in our study population, well within the reported incidence range for LVSI in epithelial ovarian cancer (36.4%-79.5%). [18, 19,22,33] The wide variation in the reported incidence of LVSI in ovarian cancer is multifactorial, including the absence of defined criteria and a reliable technique for identifying LVSI. Ultrastaging of sentinel lymph nodes increases the metastasis detection rate [34]. However, adopting such a process to assess LVSI in multiple tissues removed during cytoreduction of epithelial ovarian cancer is impractical.

Although LVSI has been well-established as a deleterious prognostic factor in numerous malignancies, gynecologic [14-16], and non-gynecologic [35,36], its significance in epithelial ovarian cancer remains poorly defined. The presence of LVSI in our study population was associated with a significant decrease in overall survival duration (median: 1194 *vs.* 2034 days, p = 0.02) and 5-year overall survival rate (17.7% vs. 30.0%, HR: 0.524, 95% CI: 0.314-0.874, P = 0.0134) on univariate analysis. However, on multivariate analysis controlling for stage, debulking status and platinum sensitivity, the presence of LVSI was no longer associated with the 5-year overall survival rate (HR: 0.747, 95% CI: 0.418-1.336, P = 0.3254).

Consistent with our findings, Mvunta reported the presence of LVSI was associated with a significant decrease in overall survival duration (78.2 versus 156 months, P= 0.009), on univariate analysis but not on multivariate analysis (HR: 1.962, 95% CI: 0.73–4.920, P = 0.153) [37]. Similarly, Faleiro-Rodrigues evaluated 104 primary epithelial ovarian cancers, none of which had received neoadjuvant chemotherapy, and reported the presence of LVSI was associated with a significant decrease in 5-year overall survival (39 vs. 75 months, P = 0.008), on univariate analysis but not on multivariate analysis (HR: 0.98, 95% CI: 0.45-2.19, P = 0.98) [38]. Matsuo used data from established ovarian cancer databases and review of histopathology slides to correlate the presence or absence of LVSI with survival outcome. Comparable to our findings, the presence of LVSI in their training and validation sets was associated with a significant decrease in the 5-year overall survival rate (OS) (Training Set: 26.5 *vs.* 67.7%, HR: 3.29, 95% CI: 1.32–8.24, P = 0.007. Validation Set: 89.6% *vs.* 85.0%, HR: 2.54, 95% CI: 1.64–3.92, P = 0.006) on univariate analysis. On multivariate analysis, controlling for stage and high-grade serous carcinoma, the presence of LVSI was no longer associated with a significant decrease in the 5-year overall survival rate (Training Set: HR: 2.16, 95% CI: 0.85–5.45, P = 0.10. Validation Set: HR: 1.87, 95% CI: 0.74–4.85, P = 0.18)[22]. Chen retrospectively reviewed clinical data and re-examination of histopathology slides of ovarian cancer patients to correlate the presence or absence of LVSI with overall survival. For the whole cohort, the presence of LVSI was associated with a significant decrease in 5-year overall survival rate (31% vs. 58%, HR and 95% CI not provided, P < 0.001) [32]. Li's meta-analysis suggested the presence of LVSI was associated with a significant decrease in OS (HR: 1.71, 95% CI: 1.42–2.07, P_{HR} < 0.001) [39]. In contrast to these studies, Masoumi-Moghaddam reported the presence of LVSI was not associated with a significant decrease in 5-year overall survival rate (HR: 0.625, 95% CI: 0.317–1.230, P = 0.173) [40].

Matsuo combined their training and validation sets to conduct a *post hoc* analysis of patients with apparent stage I disease. The presence of LVSI in this sub-group was not associated with a significant decrease in 5-year overall survival rate on univariate analysis (72.6% *vs.* 94.0%, HR: 3.77, 95% CI: 0.69–20.6, P = 0.10)[22]. In contradistinction to Matsuo's results, when Chen stratified for stage, the presence of LVSI was associated with a significant decrease in 5-year overall survival rate on univariate analysis of patients with early stage disease (44% *vs*. 70%, HR and 95% CI not provided, P < 0.001) but not those with advanced stage disease. [32] The presence of LVSI remained associated with a significant decrease in 5-year overall survival rate (HR: 2.20, 95% CI: 1.59–3.44, P < 0.001) for patients with early stage disease on multivariate analysis [33]. In our study, we had insufficient patients with stage I EOC to determine the impact of LVSI on overall survival on this sub-group.

According to Chen, one possible explanation for the discrepant impact of LVSI on overall survival in early compared to advanced stage disease relates to the presence of distant disease. In early stage disease, LVSI within the primary tumor indicates access to the lymphatic and/or vascular systems has occurred, portending a higher risk of recurrence compared to those patients without identifiable LVSI. In contrast, in advanced stage disease, the impact of LVSI is overshadowed by the presence of overtly evident metastatic disease [33].

In summary, our data did not demonstrate any correlation between lymphovascular space invasion and circulating tumor cells (CTCs or iCTCs), in epithelial ovarian cancer, suggesting tumor cell spread through these two pathways (lymphatic and vascular) may occur independently.

In accordance with published reports, the presence of LVSI in our study population was associated with a significant decrease in overall survival duration on univariate analysis. However, on multivariate analysis, the presence of LVSI was no longer associated with overall survival, possibly due to the greater impact of overtly evident metastatic disease.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST STATEMENT

According to the policy, three authors (M.L.P., C.L., and C.T.), do not have any relevant financial relationship with a commercial interest. The reported study was performed at Stony Brook Medicine as an NCI-funded, SBIR collaborative project between Vitatex Inc. and Stony Brook Medicine. W.C. has significant equity holdings or similar interests in the licensee Vitatex Inc. from SUNY Stony Brook for technology described in this presentation. W.C. is the inventor of patents for the Cell Adhesion Matrix (CAM), technology used in this study.

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