CORRELATION OF LYMPHOVASCULAR SPACE INVASION AND INVASIVE CIRCULATING TUMOR CELLS IN PATIENTS WITH EPITHELIAL OVARIAN CANCER

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INTRODUCTION

Epithelial ovarian cancer (EOC) is the leading cause of death from gynecologic malignancies in the United States with 22,440 cases and 14,070 deaths (2.5%/5.0% of all new female cancer cases/deaths, respectively) in 2018¹. Traditionally, it was believed that epithelial ovarian cancer spreads by exfoliation and direct spread throughout the peritoneal cavity. 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 occurs, implying hematogenous or lymphatic dissemination. The deleterious impact of lymphovascular space invasion (LVSI) is well-established but has not been extensively evaluated in EOC. A review 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”².

The detection of circulating (CTCs) and invasive circulating tumor cells (iCTCs) in the peripheral blood of women EOC is feasible and prognostic³⁻⁸. CTCs are associated with adverse outcomes in women with EOC³.

The goal of this study was to evaluate the correlation between lymphovascular space invasion and invasive circulating tumor cells in patients with epithelial ovarian cancer.

METHODS

The Institutional Review Board at Stony Brook University approved this study. The study group consisted of 85 patients with EOC who were managed by the Division of Gynecologic Oncology and participated in our CTC research program. Clinical data were abstracted from the medical record and the Tumor Registry.

Assessment of LVSI: The histopathology (H&E) slides from each patient were reviewed by two gynecologic oncology pathologists. The histologic type, grade, presence or absence of LVSI, extent of the LVSI (focal or multifocal) and location (organ site) was assessed.

Enumeration of CTC/iCTC: Enrichment and identification of CTCs and iCTCs have been previously described⁴⁻⁵.

Statistical Analysis: Continuous variables were summarized by median and range; categorical variables were listed as frequencies. The continuous data 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. Box and whisker plots were created to compare LVSI and iCTC/CTC count. A scatter plot was created and Pearson's correlation coefficient was used to assess the correlation between LVSI 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 < 0.05 were considered statistically significant.

RESULTS

- LVSI present in 41.2% of cases
- High levels of CTCs^{3,4} (CTC ≥ 31.5 cells/ml) present in 71.8% of cases
- High levels of iCTCs^{3,4} (iCTC ≥ 5 cells/ml) present in 77.6% of cases
- High levels of CTCs significantly associated with advanced stage, high levels of iCTCs, and disease status, but not with grade, debulking status, platinum sensitivity, lymphovascular space invasion, age, or overall survival.
- High levels of iCTCs significantly associated with advanced stage and high levels of CTCs, but not with grade, debulking status, platinum sensitivity, disease status, lymphovascular space invasion, age, or overall survival.
- LVSI significantly associated with decreased overall survival but not with stage, grade, debulking status, platinum sensitivity, disease status, median or high levels of CTCs or iCTS, or age.
- LVSI not significantly associated with overall survival on multivariate analysis.

Overall Survival Analysis

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
Cytoreduction: 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
Multivariate Analysis			
Stage: I/II vs. III/IV	0.383	0.146-1.009	0.0521
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

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%; N.S.

LVSI vs. iCTC Count

LVSI vs. CTC Count

LVSI in Peri-Adnexal Soft Tissue

LVSI Overall Survival: Univariate

CONCLUSIONS

- LVSI and CTC/iCTC counts were not correlated.
- LVSI was not associated with decreased overall survival on multivariate analysis.
- High levels of CTCs and iCTCs were not associated with decreased overall survival on multivariate analysis.
- Possible reasons include:
 - Absence of a reliable technique for identifying LVSI. Ultrastaging to assess LVSI in multiple tissues removed during cytoreduction of epithelial ovarian cancer is impractical;
 - CTCs may access the circulation individually or in small numbers without creating visible embolic tumor masses;
 - Lymphatic vs. vascular spaces;
 - Post-progression therapy;
 - Insufficient number of samples (Type II error)
- Additional study is warranted

REFERENCES

1. <https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2018.html>
2. Matsuo K, Sheridan TB, Yoshino K, Miyake T, Hew KE, Im DD, Rosenshein NB, Mabuchi S, Enomoto T, Kimura T, Sood AK, Roman LD. Significance of lymphovascular space invasion in epithelial ovarian cancer. *Cancer Medicine* 1:156-164, 2012
3. Fan TM, Zhao Q, Chen JJ, Chen WT, Pearl ML. Clinical significance of circulating tumor cells detected by an invasion assay in peripheral blood of patients with ovarian cancer. *Gynecol Oncol* 112:185-191, 2009
4. Pearl ML, Zhao Q, Yang J, Dong H, Zhang Q, Golightly M, Zucker S, Chen W-T. Prognostic analysis of invasive circulating tumor cells (iCTCs) in epithelial ovarian cancer. *Gynecol Oncol* 134:581-590, 2014
5. Pearl ML, Dong H, Tulley S, Zhang Q, Golightly M, Zucker S, Chen W-T. Treatment monitoring of patients with epithelial ovarian cancer using invasive circulating tumor cells (iCTCs). *Gynecol Oncol* 137:229-38, 2015
6. Tulley S, Zhao Q, Dong H, Pearl ML, Chen WT. Vita-Assay method of enrichment and identification of circulating cancer/circulating tumor cells (CTCs). *Methods Mol Biol* 1406:107-119, 2016
7. Pearl ML, Dong H, Zhao Q, Tulley S, Dombroff M, Chen WT. iCTC drug resistance (CDR) testing ex vivo for evaluation of available therapies to treat patients with epithelial ovarian cancer. *Gynecol Oncol* 147:426-432, 2017
8. Romero-Laorden N, Olmos D, Fehm T, Garcia-Donas J, Diaz-Padilla I. Circulating and disseminated tumor cells in ovarian cancer: A systematic review. *Gynecol Oncol* 133:632-639, 2014

ACKNOWLEDGEMENT

This study was supported by Small Business Innovative Research (SBIR) grant R44CA140047 from the NCI awarded to Vitatex Inc. that holds a subcontract with Stony Brook Medicine.

W-T.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 Cell Adhesion Matrix (CAM) technology and several antibody reagents used in this study.