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Evaluation of venous thrombosis and tissue factor in epithelial ovarian cancer

Joshua G. Cohen [a], Emily Prendergast [b], Julia E. Geddings [c], Ann E. Walts [d], Hasmik Agadjanian [b], Yohei Hisada [c,e], Beth Y. Karlan [b], Nigel Mackman [c,e], Christine S. Walsh [b,*]

[a] Division of Gynecologic Oncology, University of California, Los Angeles, 200 Medical Plaza, Suite 220, Los Angeles, CA 90095, USA
[b] Division of Gynecologic Oncology, Cedars-Sinai Medical Center, 8635 West 3rd Street, Suite 280W, Los Angeles, CA 90048, USA
[c] Division of Hematology/Oncology, University of North Carolina, Chapel Hill, 2312 MBRB, 111 Mason Farm Rd, CB#7126, Chapel Hill, NC 27599, USA
[d] Department of Pathology and Laboratory Medicine, Cedars-Sinai Medical Center, 8700 Beverly Boulevard, Los Angeles, CA 90048, USA
[e] K.G. Jebsen TREC, The Faculty of Health Sciences, UiT- The Arctic University of Tromsø, 9037 Tromsø, Norway

HIGHLIGHTS

- Epithelial ovarian cancer patients with VTE have worse overall survival.
- Microvesicle TF activity and plasma TF are not predictive of VTE in EOC patients.
- Ovarian clear cell tumors have greater expression of TF than high grade serous tumors.

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ABSTRACT

Objective. Ovarian clear cell carcinoma (OCCC) and high grade serous ovarian cancer (HGSOC) are associated with the highest risk of VTE among patients with epithelial ovarian cancer (EOC). Tissue factor (TF) is a transmembrane glycoprotein which can trigger thrombosis. We sought to evaluate if there is an association between VTE and tumor expression of tissue factor (TF), plasma TF, and microvesicle TF (MV TF) activity in this high-risk population.

Methods. We performed a case-control study of OCCC and HGSOC patients with and without VTE. 105 patients who underwent surgery at a tertiary care center between January 1995 and October 2013 were included. Plasma TF was measured with an enzyme-linked immunosorbent assay. A TF-dependent Factor Xa generation assay was used to measure MV TF activity. Immunohistochemical (IHC) analysis was performed to evaluate tumor expression of TF.

Results. 35 women with OCCC or HGSOC diagnosed with VTE within 9 months of surgery were included in the case group. Those with VTE had a worse OS, $p < 0.0001$, with a greater than three-fold increase in risk of death, HR 3.33 (CI 1.75–6.35). There was no significant difference in median plasma TF level or MV TF activity level between patients with and without VTE. OCCC patients had greater expression of TF in their tumors than patients with HGSOC, $p < 0.0001$.

Conclusions. TFMV activity and plasma TF level were not predictive of VTE in this patient population. Given the extensive expression of TF in OCCC tumors, it is unlikely IHC expression will be useful in risk stratification for VTE in this population.

1. Introduction

Ovarian cancer is the deadliest gynecologic cancer in the United States and one of the malignancies with the highest risk for venous thromboembolism (VTE). There will be 21, 980 new cases with 14,240 deaths due to ovarian cancer this year in the United States alone [1]. Among gynecologic oncology patients, mortality rates are six-fold higher in those diagnosed with pulmonary embolism (PE) [2]. In patients with ovarian cancer the estimated incidence of VTE is 5.6% to 9.7%, and VTE is associated with decreased overall survival (OS) [3,4].

High grade serous ovarian carcinoma (HGSOC) and ovarian clear cell carcinoma (OCCC) worldwide account for 45% and 5% of epithelial

* Corresponding author at: Cedars-Sinai Medical Center, 8635 West 3rd Street, Suite 280W, Los Angeles, CA 90048, USA.
E-mail address: Christine.Walsh@cshs.org (C.S. Walsh).

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ovarian cancers (EOC) respectively [5]. HGSOC and OCCC are the two subtypes of EOC associated with the highest risk of VTE (16%) contributing to a lower survival than observed in other epithelial subtypes [6–8]. The 11 to 42% prevalence of VTE reported in OCCC is almost twice the rate in other subtypes of EOC [9–11]. VTE diagnosed during primary treatment of OCCC is an independent prognostic factor for death when patients are controlled for stage, (HR 3.6, p = 0.005) [12].

Based on an analysis of 30,531 operations, the Veteran Affairs Surgical Quality Improvement Program concluded that adherence to hospital based guidelines designed to reduce the incidence of perioperative VTE had no impact on the development of VTE in patients undergoing surgery [13]. Improved risk stratification is needed to identify which women with EOC would benefit from alternative prophylactic measures. This study was designed to investigate the association between tissue factor (TF) and thrombosis in EOC patients at high risk of VTE.

TF is a 47 kDa transmembrane glycoprotein that is essential for hemostasis [14]. TF will form a binary complex with Factor VIIa and initiate coagulation activation in vivo [14]. It is expressed in many cancer types with expression increasing based on histologic grade [15]. TF has been implicated in cancer-associated thrombosis through multiple mechanisms [15,16]. In patients with ovarian cancer, preoperative plasma levels of TF are significantly elevated and have been identified as an independent prognostic factor for cancer-related death [17]. TF circulates on microvesicles (MVs), which are small (0.1–1 μm) membrane vesicles that are released from activated or apoptotic cells [18]. Yokota et al. has shown that MVs containing active TF-Factor VIIA complexes are secreted by ovarian cancer cells and that enhanced secretion occurs under hypoxic conditions [19]. Levels of TF-positive MVs and MV TF activity have been linked to thrombosis in various cancers but the only reproducible association is between MV TF activity and VTE in pancreatic cancer [20].

Various strategies have been developed to predict the risk of VTE in cancer patients [21]. There is a paucity of data to guide further risk stratification for ovarian cancer patients. Combining D-dimer with other biomarkers including platelets, leukocytes, hemoglobin and clinical parameters (i.e. tumor type) represent the best available risk-stratification strategies for VTE in cancer patients [22]. MV TF activity levels in blood and TF expression in tumors may help predict risk of VTE in various cancers [23]. As patients with HGSOC and OCCC are at higher risk of VTE than patients with other EOC, evaluation of TF in this group of women could help assess the potential role of TF in risk stratification for VTE. In this case-controlled study, we hypothesize tumor expression of TF, plasma TF, and TFMV activity are associated with development of VTE in women with OCCC and HGSOC.

2. Methods

2.1. Study population

In this IRB-approved study we identified women with OCCC or HGSOC diagnosed with or without VTE who underwent surgery at a single tertiary care center between January 1995 and October 2013. Clinical data were collected until September 2016. Patients with documented deep venous thromboembolism (DVT) or pulmonary embolism (PE) within 9 months of cytoreductive surgery were included in this study. 9 months was chosen as all patients had completed chemotherapy by this time point. A control cohort of Cedars-Sinai Medical Center patients without thrombosis was matched for age at initial diagnosis and stage during the same time period. A 1:2 case to control matching was attempted based on age at diagnosis and stage.

Women with primary or recurrent ovarian, fallopian tube, or primary peritoneal carcinoma were included in the study. Clinical data including race, age, smoking history, preoperative body mass index (BMI) within 3 weeks of surgery, past medical history, menstrual history, family history, and treatment history were collected through retrospective chart review. Optimal resection was defined intraoperatively as <1 cm residual disease. Venous thromboembolic events were classified in relation to date of surgery. Analysis was based on individual patients and not on number of thrombotic events per patient.

When available, patient plasma samples were collected within 3 months of surgery. Patient samples were initially collected in ethylenediaminetetraacetic acid (EDTA) containing tubes and processed by centrifuging at 3000 × *g* for 10 min at room temperature to produce platelet-poor plasma (PPP) with platelets <10,000/μL. The supernatant was collected and stored at −80 °C. Specimens were thawed no more than twice. A negative value indicates the plasma draw took place prior to event of interest (i.e. −1 indicates the plasma sample was drawn one day before surgery). The median time from plasma draw to VTE diagnosis for OCCC and HGSOC patients was −1 day (range −30 to 8) and −2 days (range −159 to 124) respectively. The median times from plasma draw to surgery for OCCC cases, OCCC controls, HGSOC cases, and HGSOC controls were as follows: 0 days (range −6 to 0), 0 days (range −21 to 0), 0 days (−69 to 0), and 0 days (−12 to 0). The median time from VTE diagnosis to surgery for OCCC and HGSOC patients was 3 days (range −128 to 275) and 1.5 days (range −124 to 159) respectively.

TF antigen and MV TF activity levels were determined for the subset of patients who had plasma samples collected perioperatively, processed as PPP, and stored at −80 °C. Available samples by patient group were as follows: 5 (26%) OCCC patients with VTE, 11 (29%) OCCC patients without VTE, 14 (88%) HGSOC patients with VTE, and 29 (90%) HGSOC patients without VTE.

2.2. TF immunohistochemical staining

Immunohistochemical (IHC) assessment was performed for all patients with or without VTE as previously described [17]. Nonspecific tissue binding was blocked with a 20 min incubation using normal horse serum (Vector Laboratories, Burlingame, CA) diluted in phosphate buffer solution (PBS). The mouse antihuman TF monoclonal antibody (4509, Sekisui Diagnostics, Stamford, CT) was diluted in 1× PBS with 1% bovine serum albumin (BSA) (A-7030, Sigma-Aldrich, St. Louis, MO), and 1% Tween 20 (Sigma-Aldrich, St. Louis, MO) at a 1:50 dilution and then applied to the tissue overnight at 4 °C. Sections were further incubated with a biotinylated horse anti-mouse immunoglobulin (IgG) (SK 4002, Vector ABC Kit Mouse IgG, Vector Labs, Burlingame, CA) followed by an avidin-biotin-peroxidase (ABC) complex (SK 4002, Vector ABC Kit Mouse IgG, Vector Labs, Burlingame, CA). TF antigen was visualized after treatment with the 3,3′-diaminobenzidine horse radish peroxidase substrate (SK 4105, ImmPact, Vector Labs, Burlingame, CA) and counterstained with hematoxylin.

Based on its known expression of TF, pancreatic cancer was included as positive control in each staining run [24]. Pancreatic cancer with normal mouse IgG (Vector Labs, Burlingame, CA) substituted for the TF primary antibody served as the negative control in each staining run. A negative control slide with pancreatic cancer was also included with each group of tumor specimens. Three observers (JGC, AEW, CW) blinded to the clinical data and TFMV results, jointly evaluated the TF immunostained slides using a multihead microscope and a modified H-score to determine TF expression by each tumor [25]. For each tumor, the percentage of tumor cells stained at each of three intensities [0 = no staining to 3 = strong staining] was recorded. In each case, only areas of well-preserved tumor were used for IHC evaluation. When present, discordant scoring was resolved by re-review of slides and discussion until a consensus was reached. An overall score was then assigned to each tumor based on the following formula: 3*(% of tumor cells showing 3+ intensity) + 2*(% of tumor cells showing 2+ intensity) + 0*(% of tumor cells showing 0/1+ intensity).

2.3. TF enzyme-linked immunosorbent assay

Plasma TF levels were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (EK0928, Boster

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