Expression of Tissue Factor in Epithelial Ovarian Carcinoma Is Involved in the Development of Venous Thromboembolism

OPEN

Expression of Tissue Factor in Epithelial Ovarian Carcinoma Is Involved in the Development of Venous Thromboembolism

Manabu Sakurai, MD, Koji Matsumoto, MD, PhD,* Masahiko Gosho, PhD,† Akiko Sakata, MD,‡ Yoshihiko Hosokawa, MD,* Yuri Tenjimbayashi, MD,* Takashi Katoh, MD,* Ayumi Shikama, MD,* Haruna Komiya, MD,* Hiroo Michikami, MD,* Nobutaka Tasaka, MD,* Azusa Akiyama-Abe, MD,* Sari Nakao, MD, PhD,* Hiroyuki Ochi, MD, PhD,* Mamiko Onuki, MD, PhD,* Takeo Minaguchi, MD, PhD,* Hiroyuki Yoshikawa, MD, PhD,* and Toyomi Satoh, MD, PhD**

Objectives: Our 2007 study of 32 patients with ovarian cancer reported the possible involvement of tissue factor (TF) in the development of venous thromboembolism (VTE) before treatment, especially in clear cell carcinoma (CCC). This follow-up study further investigated this possibility in a larger cohort.

Methods: We investigated the intensity of TF expression (ITFE) and other variables for associations with VTE using univariate and multivariate analyses in 128 patients with epithelial ovarian cancer initially treated between November 2004 and December 2010, none of whom had received neoadjuvant chemotherapy. Before starting treatment, all patients were ultrasonographically screened for VTE. The ITFE was graded based on immunostaining of surgical specimens.

Results: Histological types were serous carcinoma (n = 42), CCC (n = 12), endometrioid carcinoma (n = 15), mucinous carcinoma (n = 53), and undifferentiated carcinoma (n = 6). The prevalence of VTE was significantly higher in CCC (34%) than in non-CCC (17%, $P = 0.03$). As ITFE increased, the frequencies of CCC and VTE increased significantly ($P < 0.001$ and $P = 0.014$, respectively). Multivariate analysis identified TF expression and pretreatment dimerized plasmin fragment D level as significant independent risk factors for VTE development. These factors showed particularly strong impacts on advanced-stage disease ($P = 0.021$).

Conclusions: The 2007 cohort was small, preventing multivariate analysis. This study of a larger cohort yielded stronger evidence that the development of VTE in epithelial ovarian cancer may involve TF expression in cancer tissues.

Key Words: Tissue factor, Venous thromboembolism, Epithelial ovarian cancer

Received May 24, 2016, and in revised form August 18, 2016.
Accepted for publication August 24, 2016.

(*Int J Gynecol Cancer* 2017;27: 37–43)

Faculty of Medicine, Departments of *Obstetrics and Gynecology and †Clinical Trial and Clinical Epidemiology, and ‡Department of Pathology, Tsukuba Human Tissue Diagnostic Center, University of Tsukuba, Tsukuba, Japan.

ISSN: 1048-891X
DOI: 10.1097/IGC.0000000000000848

Address correspondence and reprint requests to Manabu Sakurai, MD, Faculty of Medicine, Department of Obstetrics and Gynecology, University of Tsukuba, 1-1-1 Tennoudai, Tsukuba, Ibaraki 305-8575, Japan. E-mail: m-sakurai@md.tsukuba.ac.jp.

Supported in part by Grants-in-Aid for Scientific Research (nos. 26861310 and 26462513) from the Ministry of Education, Science, and Culture, Japan. The funding sources played no role in the study design, data collection and analysis, writing of the manuscript, or decision to publish.

The authors declare no conflicts of interest.

Venous thromboembolism (VTE) is known to occur as a perioperative complication, particularly after intrapelvic and intra-abdominal surgeries.[1] Venous thromboembolism also occurs readily after surgery for ovarian cancer.[2] In 1865, Trousseau[3] reported that, for some patients with cancer in a hypercoagulable state, the cancer can actually cause VTE. We reported that VTE (mainly subclinical) develops before the start of treatment in 26.7% of patients with ovarian cancer, 9.9% of patients with endometrial cancer, and 4.8% of patients with cervical cancer.[4–6] In terms of histological type, the frequency of VTE occurring before the start of treatment of ovarian cancers is significantly higher for clear cell carcinoma (CCC) (50.0%) than for non-CCC (19.0%; $P = 0.02$), whereas among endometrial cancers, the pretreatment frequency of VTE is significantly higher in nonendometrioid adenocarcinomas including CCC (38.1%) than in endometrioid adenocarcinomas (6.0%; $P = 0.0002$).[4,5]

Tissue factor (TF), or blood coagulation factor III, initiates extrinsic blood coagulation at the time of tissue damage. Recent research has elucidated that TF is involved in the hypercoagulable state seen in patients with malignant tumors.[7] In 2007, we performed immunohistochemical studies, investigated the expression of TF in 32 patients with ovarian cancer in our hospital, and reported the possibility that TF is involved in the development of VTE in patients with ovarian cancer before starting treatment.[8] However, that study included surgical specimens obtained from 15 patients after they had undergone neoadjuvant chemotherapy (NAC), whereas another patient had a yolk sac tumor in addition to epithelial ovarian cancer (EOC). The validity of the results was thus potentially limited by the inability to perform multivariate analyses due to the small sample size. The cohort in this study was increased in size and restricted to patients with EOC who had not received NAC to clarify the relationship between TF and the development of VTE using multivariate analysis.

MATERIALS AND METHODS

Study Population

The study protocol was approved by the ethics committee at the study hospital. All protocols were carried out in accordance with the principles of the Declaration of Helsinki. Participants were composed of 128 patients who underwent initial therapy at the study hospital between November 2004 and December 2010 and had been pathologically given a diagnosis of EOC. Informed consent was obtained from all patients for the use of surgical specimens for research purposes. The clinical stage of each specimen was decided in accordance with the International Federation of Gynecology and Obstetrics 2014 classifications. Although 181 patients were treated during the study period, 53 patients who underwent NAC were excluded considering the impact of anticancer drug exposure on immunostaining in tumor cells. The clinical stages of these 53 patients were stage II in 2 patients, stage III in 33 patients, and stage IV in 18 patients. The histological types were serous carcinoma in 45 patients, CCC in 5 patients, mucinous carcinoma in 2 patients, and undifferentiated carcinoma in 1 patient. Twelve patients in whom NAC was not expected to lead to the total elimination of cancer in the initial surgery, 23 patients in whom surgery needed to be cut short because of complications including VTE or a poor general condition, and 18 patients in the NAC group who took part in a phase III trial of upfront debulking surgery versus NAC for stage III/IV ovarian, tubal, and peritoneal cancers[9] were ultimately chosen for treatment, bringing the total number of patients included in this study to 128.

Immunostaining was performed on surgical specimens obtained from 126 patients who had undergone radical surgery for EOC and biopsy tissues obtained from 2 patients who had undergone exploratory laparotomy.

Immunohistochemistry

We performed immunostaining on tissue specimens from all patients in this study, using the same technique reported in our 2007 investigation.[8] In brief, we prepared 3-mm-thick sections from 3 sites of paraffin block specimens from each patient. The anti-TF antibody (Cedarlane Laboratories, Burlington, NC) used as the primary antibody was diluted 50-fold and biotinylated by the avidin-biotin-peroxidase complex method (Vector ABC Elite kit; Vector Laboratories, Burlingame, CA), followed by color development with diaminobenzidine tetrahydrochloride. As positive controls, we used sections of the umbilical cord, which is known to stain brightly for TF,[10] whereas negative controls were sections that had been incubated in normal mouse serum.

The intensity of TF expression (ITFE) was graded into the following 4 levels based on the proportion of all cell populations that stained positively for TF: negative, no apparent positive tumor cells; weakly positive, less than 50% positive tumor cells; moderately positive, greater than or equal to 50% positive tumor cells with weak intensity; and strongly positive, greater than or equal to 50% positive tumor cells with strong intensity (Fig. 1).[11]

All evaluations of the immunohistological results were performed by 2 independent observers blinded to the results of hematological examinations and histological diagnoses. Mean values were used as the final values.

Detection of Deep Vein Thrombosis

Examination for VTE was performed using the same method we have reported elsewhere.[4,8] Leg vein ultrasonography was performed on all patients using an ATL HDI5000 system (Philips Medical Systems, Bothell, WA) with a 3- to 7.5-MHz transducer, to detect deep vein thrombosis (DVT). Output, pulse repetition frequency, and wall thump filter settings were adjusted for venous vascular studies. Bilateral iliac, femoral, great saphenous, popliteal, peroneal, posterior tibial, and soleal veins were assessed for the presence/absence of DVT. The iliac and femoral veins were examined with the patient supine, whereas all other veins were assessed in the upright position. All vessels were imaged in both short-axis cross-section (transverse image) and long-axis cross-section (longitudinal image). Manual compression using a transducer and color Doppler imaging were performed to observe the lumina of veins and search for blood clots. Pelvic veins were also examined using the Valsalva maneuver. However, the absence of a response to the Valsalva maneuver would raise the suspicion of a blood flow disorder in the proximal vein, so

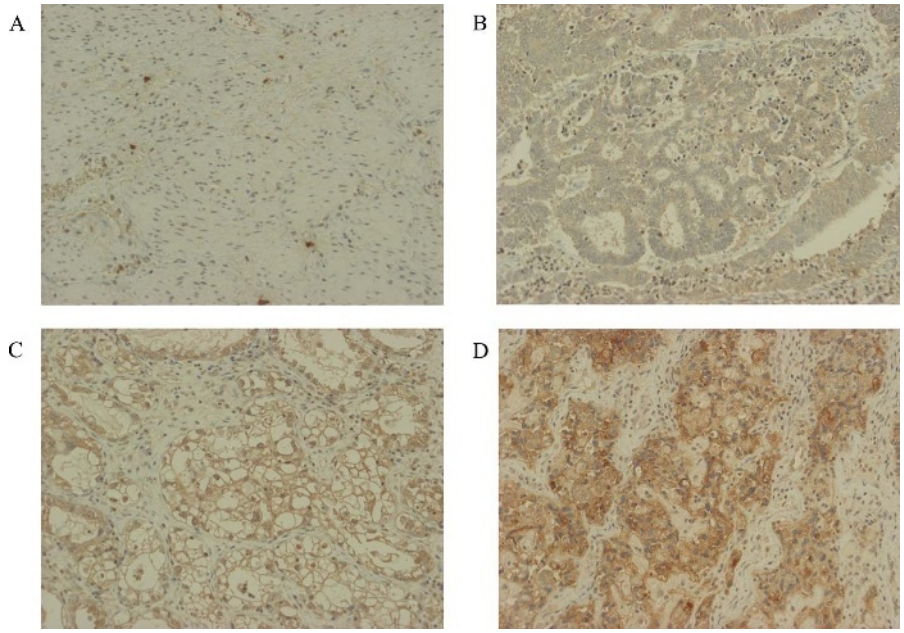

FIGURE 1. Immunohistochemical staining for TF in EOC tissues. A, Negative control. B, Weakly positive (<50% positive tumor cells). C, Moderately positive (≥50% positive tumor cells with weak intensity). D, Strongly positive (≥50% positive tumor cells with strong intensity). Percentages are based on the proportion of the entire tumor cell population positive for TF. All pictures were taken at original magnification ×200.

thrombi in the pelvic veins were diagnosed based on the results of contrast-enhanced computed tomography (CT). All patients underwent extensive imaging using CT and magnetic resonance imaging to detect the spread of pelvic tumors and blood clots in the iliac veins and inferior vena cava.

Detection of Pulmonary Thromboembolism

All patients in whom DVT was detected on leg vein ultrasonography or pelvic contrast-enhanced CT were further examined for the presence or absence of pulmonary thromboembolism on contrast-enhanced CT of the chest or pulmonary blood flow scintigraphy using Tc-99.

Statistical Analysis

Categorical data are summarized as frequency and percentage, whereas continuous data are summarized as mean and standard deviation. Categorical data were subjected to Fisher exact test. Continuous data were tested using Student *t* test for differences between 2 groups and by an analysis of variance for comparisons of 3 or more groups. The development of VTE was investigated using univariate and multivariate analyses with a logistic model. Age, body mass index (BMI), stage, pretreatment dimerized plasmin fragment D (D-dimer or DD) level, histological type, and ITFE were used as variables in the univariate analysis to identify potential risk factors for VTE development. Variables showing significance were then subjected to multivariate analysis. Variables showing strong confounding suggestive of multicollinearity as well as a low *P* value were selected and included in the multivariate analysis model. To evaluate the contribution of ITFE and histology in each stage, we used additional multivariate models that included ITFE, stage, and DD level; the interaction between ITFE and stage; the interaction among histological type, stage, and DD level; and the interaction between histological type and stage. All analyses were performed using SAS version 9.4 software (SAS Institute, Cary, NC), and values of $P < 0.05$ were considered statistically significant.

RESULTS

Subject Characteristics

At the time of initial treatment, mean age was 56.6 years (range, 31–88 years), and mean BMI was 22.4 kg/m^2 (range, 13.3–35.4 kg/m^2). Mean pretreatment DD level was 4.2 μg/mL (range, 0.1–20.0 μg/mL). For the 128 patients, stage was I in 55 patients, II in 22 patients, III in 37 patients, and IV in 14 patients, whereas the histological type was serous in 42 patients, mucinous in 12 patients, endometrioid in 15 patients, CCC in 53 patients, and undifferentiated in 6 patients (Table 1).

TF Expression in Cancer Tissues

Tissue factor was expressed in 71 patients (weakly positive, n = 23; moderately positive, n = 32; strongly positive, n = 16), representing 55.5% of the 128 patients.

The ITFE did not correlate with patient age, BMI, or pretreatment DD level. According to stage, ITFE was 81.8% for stage I and 45.4% for stage II but was significantly lower for stages III and IV, at 29.7% and 35.7%, respectively ($P < 0.001$). Incidences according to histological type were 94.3% (50/53) for CCC, 23.8% (10/42) for serous carcinoma, 26.7% (4/15) for endometrioid carcinoma, 58.3% (7/12) for mucinous carcinoma, and 0% (0/6) for undifferentiated carcinoma. The ITFE was significantly higher in CCC than in non-CCC ($P < 0.001$) and was also stronger in CCC (Table 2). As ITFE increased from

TABLE 1. Patient characteristics (N = 128)

Age, mean (SD), y	56.6 (11.3)
BMI, mean (SD), kg/m^2	22.4 (3.4)
DD, mean (SD), μg/mL	4.2 (5.6)
FIGO stage, n (%)	
I	55 (43)
II	22 (17)
III	37 (29)
IV	14 (11)
Histology, n (%)	
Serous carcinoma	42 (33)
CCC	53 (41)
Endometrioid carcinoma	15 (12)
Mucinous carcinoma	12 (9)
Undifferentiated carcinoma	6 (5)

FIGO, International Federation of Gynecology and Obstetrics; SD, standard deviation.

negative to mild, moderate, and strong, the incidence of VTE increased significantly (P_{trend} = 0.014) to 16%, 17%, 38%, and 38%, respectively (Table 3).

Risk Factors for the Development of VTE Before Starting Treatment

Venous thromboembolism developed before starting treatment in 31 patients (24.2%) (DVT alone, n = 20; DVT with PE, n = 8; PE alone, n = 3), and the rate of subclinical disease was 96.8%. Screening was performed according to a DD level with a negative predictive value of 96.1%.[12] Nonetheless, patients with silent PE alone were likely overlooked among the patients who did not undergo CT because no DVT was identified—we consider that the rate is extremely low.

TABLE 3. Incidence of VTE for each ITFE

	Incidence of VTE, n (%)	*P*
ITFE		0.014
Negative	9/57 (15.8)	
Weakly positive	4/23 (17.4)	
Moderately positive	12/32 (37.5)	
Strongly positive	6/16 (37.5)	

P_{trend} = 0.014.

Mean (SD) DD level was 7.4 (6.4) μg/mL in patients with VTE, which is significantly higher than in patients without VTE (3.2 [5.0] μg/mL; $P < 0.001$). Venous thromboembolism developed in 34.0% (18/53) of the patients with CCC, which is significantly higher than the incidence of 17.3% (13/75) among patients with non-CCC ($P = 0.03$). Tissue factor–positive patients developed VTE at an incidence of 31.0% (22/71), which is significantly higher than the incidence of 15.8% (9/57) in TF-negative patients ($P = 0.0496$). Comparison of the negative/weakly positive patient group with the moderately/strongly positive patient group showed a significant difference in VTE incidences, at 16.3% (13/80) and 37.5% (18/48; $P = 0.01$), respectively. The ITFE was therefore entered into the multivariate analysis using those 2 groups (negative/weakly positive vs moderately/strongly positive). A strong correlation was found between CCC and ITFE, and because multicollinearity was seen (Spearman correlation coefficient, 0.66), simultaneous incorporation of CCC and ITFE in the multivariate analysis model was considered statistically inappropriate. Accordingly, when ITFE was selected

TABLE 2. Patient characteristics according to ITFE in cancer tissues

	ITFE				
	Negative (N = 57)	Weakly Positive (N = 23)	Moderately Positive (N = 32)	Strongly Positive (N = 16)	*P**
Age, mean (SD), y	56.4 (11.8)	55.6 (13.6)	58.3 (9.9)	55.5 (9.6)	0.797
BMI, mean (SD), kg/m^2	22.8 (3.6)	22.7 (3.9)	21.9 (2.8)	21.4 (2.6)	0.376
DD, mean (SD), μg/mL	4.3 (5.6)	3.1 (4.6)	4.8 (6.1)	4.5 (6.6)	0.723
FIGO stage, n (%)					
I	10 (18.2)	16 (29.1)	18 (32.7)	11 (20.0)	<0.001
II	12 (54.5)	2 (9.1)	5 (22.7)	3 (13.6)	
III	26 (70.3)	5 (13.5)	5 (13.5)	1 (2.7)	
IV	9 (64.3)	0 (0)	4 (28.6)	1 (7.1)	
Histology, n (%)					
CCC	3 (5.7)	10 (18.9)	25 (47.2)	15 (28.3)	<0.001
Non-CCC	54 (72.0)	13 (17.3)	7 (9.3)	1 (1.3)	

*Analyzed using Student *t* test for continuous variables and Fisher exact test for categorical variables.
FIGO, International Federation of Gynecology and Obstetrics; SD, standard deviation.

as the factor with the lower *P* value and multivariate analysis was performed, the results showed that pretreatment DD level and ITFE represented significant independent risk factors for the development of VTE before the start of treatment (Table 4). We also examined the impact of histology and TF on VTE development before the start of treatment by early- and advanced-stage diseases. Although no significant difference was seen between CCC and non-CCC in either early- or advanced-stage diseases, a tendency was seen for VTE development to increase in CCC. When TF was moderately/strongly positive, no significant difference was seen in early-stage diseases, but a tendency toward an increased risk of VTE development was seen. In advanced-stage diseases, the risk of VTE development was significantly increased ($P = 0.021$; Table 5).

DISCUSSION

This study was able to confirm that TF is involved in the pretreatment development of VTE in EOC, particularly in CCC.

Uno et al[8] studied 32 patients with ovarian cancer and revealed a relationship between TF expression in tumor tissues and the development of VTE before the start of treatment. However, that result was only shown in univariate analysis because of the small size of the study cohort. This study focused on EOC as the histological type and enrolled 4 times as many patients, enabling multivariate analysis but generating the same results and confirming the earlier findings. We were also able to verify that the incidence of VTE development before the start of treatment increased significantly as ITFE increased. Tissue factor binds to blood coagulation factor VII, which is released on damage to the vascular endothelial cells and others and promotes extrinsic coagulation.[13] We surmised that the mechanism involves TF present in tumors being released into the blood vessels and leading to an increased incidence of VTE development.

Uno et al[8] reported pretreatment VTE development in 45.5% (5/11) of patients with CCC, which is significantly higher than the frequency in patients with non-CCC. Some subsequent studies found development rates of 15% to 42%,[14–16] which

TABLE 4. Logistic regression analysis for VTE

		Univariate		Multivariate	
Characteristics	**Incidence, %**	**OR (95% CI)**	***P***	**Adj OR (95% CI)**	***P***
Age, y					
<60	17/81 (21.0)	Reference	0.26		
≥60	14/47 (29.8)	1.60 (0.70–3.64)			
BMI, kg/m^2					
<25	28/108 (25.9)	Reference	0.30		
≥25	3/20 (15.0)	0.50 (0.14–1.85)			
Expression of TF					
Negative	9/57 (15.8)	Reference	0.014		
Weakly positive	4/23 (17.4)	1.60 (1.10–2.32)			
Moderately positive	12/32 (37.5)	-			
Strongly positive	6/16 (37.5)	-			
Negative	9/57 (15.8)	Reference	0.049		
Positive	22/71 (31.0)	2.39 (1.00–5.73)			
Negative/weakly positive	13/80 (16.3)	Reference	0.008	Reference	0.007
Moderately/strongly positive	18/48 (37.5)	3.09 (1.34–7.11)		3.59 (1.43–8.99)	
Stage (FIGO)					
I/II	19/77 (24.7)	Reference	0.88		
III/IV	12/51 (23.5)	1.00 (0.96–1.04)			
Histology					
Non-CCC	13/75 (17.3)	Reference	0.03		
CCC	18/53 (34.0)	2.45 (1.07–5.60)			
Serous carcinoma	7/42 (16.7)	Reference	0.17		
Nonserous carcinoma	24/86 (27.9)	1.94 (0.76–4.95)			
Pretreatment plasma DD level, μg/mL					
<2.0	8/74 (10.8)	Reference	<0.0001	Reference	<0.0001
≥2.0	23/54 (42.6)	6.25 (2.44–14.3)		3.59 (1.43–8.99)	

Adj, adjusted; CI, confidence interval; FIGO, International Federation of Gynecology and Obstetrics; OR, odds ratio.

TABLE 5. Effect of TF and histological type on the risk of VTE in early- and advanced-stage diseases

Factor	Stage	Adj OR (95% CI)	*P*
TF (moderately/strongly positive vs negative/weakly positive)	Early (I or II)	2.21 (0.69–7.13)	0.184
	Advanced (III or IV)	6.44 (1.32–31.36)	0.021
Histological type (CCC vs non-CCC)	Early (I or II)	2.48 (0.74–8.37)	0.143
	Advanced (III or IV)	3.69 (0.77–17.81)	0.104

Adj, adjusted; CI, confidence interval; OR, odds ratio.

are higher than those in patients with non-CCC. Likewise, this study found pretreatment VTE development in 33.9% (18/53) of patients with CCC and confirmed that the rate was significantly elevated. The possibility was suggested that the histological type of ovarian cancer represents a confounding factor that skews TF expression and VTE development. For that reason, this study applied multivariate analysis using 2 factors: ITFE with a lower *P* value and pretreatment DD level. That analysis confirmed both factors as significant risk factors (TF, $P = 0.007$; DD level, $P < 0.0001$). Moreover, when multivariate analysis was performed for CCC and DD level, both represented significant risk factors (CCC, $P = 0.014$; DD level, $P < 0.0001$). We saw no significant difference in histology in our examination of the respective impacts of histology and TF on VTE development before the start of treatment of early- and advanced-stage diseases. In advanced-stage diseases, TF expression significantly increased the risk of VTE development ($P = 0.021$), suggesting that the impact of TF expression on VTE development is more evident in advanced-stage diseases.

Tissue factor is involved in the early stages of thrombus formation via the extrinsic coagulation process and can be regarded as the cause of VTE. D-dimer is a degradation product remaining after fibrin formation[14] and can be regarded as merely a result of thrombus formation. Accordingly, the production of TF by EOC can be considered an essential risk factor for the pretreatment development of VTE.

We were able to confirm that TF is particularly markedly expressed in CCC compared with other histological types of EOC. In the report by Uno et al,[8] the 15 NAC-treated patients accounted for nearly half of the total 32 patients, and the possibility that the effects of anticancer agents in the analyzed tissue specimens had biased the results of TF immunostaining was thus unable to be excluded. No pretreatment biopsies were taken from patients with EOC undergoing NAC at our hospital if cancer of the ovaries, fallopian tubes, or peritoneum was suspected on image diagnosis; if cells consistent with a malignant surface epithelial-stromal tumor were seen in an aspiration cytology of the tumor, pleural effusion, or ascites; or if biopsy and advanced-stage diagnosis by microscopy during NAC could be skipped because the patient met the criteria of CA125 of greater than 200 U/mL and carcinoembryonic antigen of less than 20 ng/mL.[9,17,18]

This study ruled out such potential bias by excluding patients who had received NAC and studying only specimens obtained before the administration of anticancer agents. We thus consider that the present report has greater credibility with regard to the evaluation of TF expression in patients with EOC.

In summary, this study confirmed that (1) TF expression in tumors represents an independent, significant risk factor for the pretreatment development of VTE, with a stronger influence in advanced stages; (2) increases in ITFE are followed by an increased incidence of VTE development; (3) both the incidence and ITFE are higher in CCC than in non-CCC; and (4) CCC shows a higher incidence of pretreatment VTE development compared with non-CCC. These findings strongly suggest that the high ability of CCC to produce TF is involved in the pretreatment development of VTE in CCC.

REFERENCES

1. Turpie AGG, Chin BSP, Lip GYH. Venous thromboembolism: pathophysiology, clinical features, and prevention. *BMJ*. 2002;325:887–890.
2. von Tempelhoff GF, Heilmann L, Hommel G, et al. Hyperviscosity syndrome in patients with ovarian carcinoma. In: *Cancer*. vol. 82. 1998:1104–1111.
3. Trousseau A. *Phlegmasia Alba Dolens. Clinique Medicale de l'Hotel-Dieu de Paris*. 3rd ed. Paris, France: JB Balliere et Fils; 1865:654–712.
4. Satoh T, Oki A, Uno K, et al. High incidence of silent venous thromboembolism before treatment in ovarian cancer. *Br J Cancer*. 2007;97:1053–1057.
5. Satoh T, Matsumoto K, Uno K, et al. Silent venous thromboembolism before treatment in endometrial cancer and the risk factors. *Br J Cancer*. 2008;99:1034–1039.
6. Satoh T, Matsumoto K, Tanaka YO, et al. Impact of changes in haematocrit level and platelet count on thromboelastometry parameters. *Thromb Res*. 2013;131:127–132.
7. Kasthuri RS, Taubman MB, Mackman N. Role of tissue factor in cancer. *J Clin Oncol*. 2009;27:4834–4838.
8. Uno K, Homma S, Satoh T, et al. Tissue factor expression as a possible determinant of thromboembolism in ovarian cancer. *Br J Cancer*. 2007;96:290–295.
9. Onda T, Matsumoto K, Shibata T, et al. Phase III trial of upfront debulking surgery versus neoadjuvant chemotherapy for stage III/IV ovarian, tubal and peritoneal cancers: Japan Clinical Oncology Group Study JCOG0602. *Jpn J Clin Oncol*. 2008;38:74–77.
10. Callander NS, Varki N, Rao LV. Immunohistochemical identification of tissue factor in solid tumors. *Cancer*. 1992;70:1194–1201.
11. Hamada K, Kuratsu J, Saitoh Y, et al. Expression of tissue factor correlates with grade of malignancy in human glioma. *Cancer*. 1966;77:1877–1883.
12. Wells PS, Anderson DR, Rodger M, et al. Evaluation of D-dimer in the diagnosis of suspected deep-vein thrombosis. *N Engl J Med*. 2003;349:1227–1235.
13. Furie B, Furie BC. The molecular basis of blood coagulation. *Cell*. 1988;53:505–518.

14. Diaz ES, Walts AE, Karlan BY, et al. Venous thromboembolism during primary treatment of ovarian clear cell carcinoma is associated with decreased survival. *Gynecol Oncol*. 2013;131:541–545.
15. Duska LR, Garrett L, Henretta M, et al. When 'never-events' occur despite adherence to clinical guidelines: the case of venous thromboembolism in clear cell cancer of the ovary compared with other epithelial histologic subtypes. *Gynecol Oncol*. 2010;116:374–377.
16. Matsuura Y, Robertson G, Marsden DE, et al. Thromboembolic complications in patients with clear cell carcinoma of the ovary. *Gynecol Oncol*. 2007;104:406–410.
17. Onda T, Kobayashi H, Nakanishi T, et al. Feasibility study of neoadjuvant chemotherapy followed by interval debulking surgery for stage III/IV ovarian, tubal, and peritoneal cancers: Japan Clinical Oncology Group Study JCOG0206. *Gynecol Oncol*. 2009;113:57–62.
18. Onda T, Satoh T, Saito T, et al. Comparison of treatment invasiveness between upfront debulking surgery versus interval debulking surgery following neoadjuvant chemotherapy for stage III/IV ovarian, tubal, and peritoneal cancers in a phase III randomised trial: Japan Clinical Oncology Group Study JCOG0602. *Eur J Cancer*. 2016;64:22–31.

