IL17a and IL21 combined with surgical status predict the outcome of ovarian cancer patients

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Abstract

Aside from tumor cells, ovarian cancer-related ascites contains the immune components. The aim of this study was to evaluate whether a combination of clinical and immunological parameters can predict survival in patients with ovarian cancer. Ascites specimens and medical records from 144 ovarian cancer patients at our hospital were used as the derivation group to select target clinical and immunological factors to generate a risk-scoring system to predict patient survival. Eighty-two cases from another hospital were used as the validation group to evaluate this system. The surgical status and expression levels of interleukin 17a (IL17a) and IL21 in ascites were selected for the risk-scoring system in the derivation group. The areas under the receiver operating characteristic (AUROC) curves of the overall score for disease-free survival (DFS) of the ovarian cancer patients were 0.84 in the derivation group, 0.85 in the validation group, and 0.84 for all the patients. The AUROC curves of the overall score for overall survival (OS) of cases were 0.78 in the derivation group, 0.76 in the validation group, and 0.76 for all the studied patients. Good correlations between overall risk score and survival of the ovarian cancer patients were demonstrated by sub-grouping all participants into four groups (P for trend <0.001 for DFS and OS). Therefore, a combination of clinical and immunological parameters can provide a practical scoring system to predict the survival of patients with ovarian carcinoma. IL17a and IL21 can potentially be used as prognostic and therapeutic biomarkers.

Key Words

- ▶ ascites
- ▶ interleukin 17
- ▶ interleukin 21
- ▶ risk-scoring system
- ▶ tumor immunology
- ▶ ovarian carcinoma

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Introduction

Ovarian carcinoma is the most lethal gynecologic malignancy (Jemal *et al.* 2011). The standard treatment for this disease is debulking surgery followed by platinum-based chemotherapy; however, the overall survival (OS) rate is

around 35% (Cannistra 2004, Kim *et al.* 2012). The high mortality rate is due to vague symptoms, diagnosis at a late stage and a lack of accurate biomarkers (Suh *et al.* 2010, Wang *et al.* 2015). An effective diagnosis may be made via

a combination of robust biomarkers specific for ovarian cancer and current clinical methods. Therefore, further research to identify accurate ovarian cancer biomarkers is needed to allow for early detection and diagnosis and subsequently better care. Furthermore, these biomarkers would be prognostic factors to predict the disease course and the response to treatment (Bast *et al.* 2009, Suh *et al.* 2010).

Cancer arises in the tumor microenvironment that is both a cause and a consequence of tumorigenesis. Tumor and host cells co-evolve dynamically through indirect and direct cellular interactions (Casey *et al.* 2015). Many of the hallmarks of cancer are related to the microenvironment, including the ability to induce proliferation and angiogenesis, and avoid apoptosis, hypoxia, and immune detection (Hanahan & Weinberg 2011). Ovarian cancer is characterized to spread primarily by tumor cell implantation in the peritoneal cavity. In addition to solid tumors, ascites is also frequently noted in patients with ovarian cancer. In these fluids, several kinds of tumor-associated cells including immunocytes and also regulatory elements such as cytokines and chemokines are found in ascites. Therefore, ascites can be considered to be part of the tumor microenvironment and ideal to adequately reflect the relationship between host immunity and tumor cells in the cancer microenvironment (Yigit *et al.* 2011, Fridman *et al.* 2012).

Ovarian carcinoma has been demonstrated to be immunogenic, however the underlying interaction between host immunity and tumor-associated cells is not clear (Zhang *et al.* 2003, Curiel *et al.* 2004, Tomsova *et al.* 2008). The presence of tumor infiltrating lymphocytes has been positively correlated with survival in ovarian cancer patients (Zhang *et al.* 2003, Raspollini *et al.* 2005). However, ovarian cancer can create an immunosuppressive microenvironment to escape immune elimination (Latha *et al.* 2014). One of the escape mechanisms is a shift in the immune response from helper T1 (Th1) toward Th2, a process mediated by cytokines (Yigit *et al.* 2011). A lower level of the pro-inflammatory Th17-related cytokine, interleukin 17 (IL17), has also been reported in more advanced ovarian cancer-associated ascites (Hirahara *et al.* 2001, Kryczek *et al.* 2009). In addition to modulating the status of host immunity, alterations of Th1-, Th2-, and Th17-related cytokines in ascites can be considered to be immune biomarkers to evaluate disease severity and prognosis (Kryczek *et al.* 2009, Yigit *et al.* 2011, Cândido *et al.* 2013, Chen *et al.* 2013).

Few studies have reported using a combination of clinical and immunological parameters to predict survival in ovarian cancer patients. The aim of this study was to

evaluate whether a combination of clinical parameters and cytokine profiles in ascites can predict survival in patients with ovarian cancer. Therefore, Th1 (interferon gamma (IFN γ) and tumor necrosis factor alpha (TNF α)-, Th2 (IL4, IL6, and IL10)-, and Th17 (IL17a, IL17f, IL21, IL22, and IL23)-related cytokines were selected for analysis in this study (Dong 2008, Oreja-Guevara *et al.* 2012). Ascites specimens and medical records from 144 ovarian cancer patients in our hospital were used as the derivation group to select target clinical and immunological factors to generate a risk-scoring system to predict patient survival. Eighty-two cases from another hospital were used as the validation group to evaluate this risk-scoring system. The clinical application of this risk-scoring system was also discussed.

Materials and methods

Patients and specimens

From July 2003 to September 2012, 226 women diagnosed with ovarian carcinoma who received debulking or staging surgery were enrolled. One hundred and forty-four of the 226 patients were from National Taiwan University Hospital (NTUH) in northern Taiwan, and the remaining 82 cases were from National Cheng Kung University Hospital (NCKUH) in southern Taiwan. All of the patients who received neoadjuvant chemotherapy before surgery were excluded. The Institutional Review Boards of both hospitals approved the study protocol. Ascites specimens were collected during surgery and centrifuged at 760 *g* for 5 min into cellular elements and supernatant. The supernatant was kept at -20°C until analysis (Chen *et al.* 2012a,b).

The medical records of the patients were prospectively reviewed until December 2013 to obtain clinical parameters including age, operative findings, pathological findings, disease relapse, and prognosis. The characteristics of disease were defined according to the system of International Federation of Gynecology and Obstetrics (FIGO) (1971). Stage I and II diseases were considered to be early stage, and stages III and IV as advanced stage. Except for the women with stage IA and grade I tumors, all patients received three to six courses of adjuvant platinum-based chemotherapy. The maximum diameters of postoperative residual tumors were also recorded. When the maximum diameter of a residual tumor was ≤ 1 cm, the surgical status was defined as being optimal debulking surgery. Otherwise, the surgical status was defined as sub-optimal.

After completion of the primary treatment, history taking, pelvic/rectal examination, and regional lymph nodal palpation would be arranged every 3 months for 3 years, and every 6 months thereafter. Serum levels of carcinoembryonic antigen and carcinoma antigen 125 were measured at each visit, and smears of the vaginal cuff were performed annually. Magnetic resonance imaging or computerized tomography was arranged for suspected disease relapse. Recurrence was considered when tumor marker levels were greater than or equal to twofold the upper limit of normal in two consecutive tests with 2-week intervals, findings of imaging studies and aspiration cytology were abnormal, or when there was histological confirmation from a tissue biopsy. The period of time from completion of the primary treatment until the diagnosis of disease recurrence was defined as disease-free survival (DFS). The time from the diagnosis of disease until the date of death or last visit was defined as OS (Chen et al. 2012a,b).

Cytokine measurements (cytokine bead array)

In this study, ten cytokines including IFN γ , TNF α , IL4, IL6, IL10, IL17a, IL17f, IL21, IL22, and IL23 were evaluated. Levels of these cytokines were measured using a customized Procarta cytokine profiling kit (Affimatrix, Santa Clara, CA, USA) as in our previous study (Chen et al. 2013). In the assays, the threshold of detection for the various cytokines ranged from 0.10 to 0.92 pg/ml respectively. Intra- and inter-assay coefficients of variation for these cytokines were <10.0%. The details were shown in Supplementary Table 1, see section on supplementary data given at the end of this article.

Development of the risk-scoring system

The studied population was divided into two groups, participants from NTUH as the NTUH set (derivation group) and those from NCKUH as the NCKUH set (validation group). There were two steps for the development of the risk-scoring system that was modified from the methodology of Chan et al. (2014).

First, a univariate Cox proportional hazard model was used to select the significant risk predictors for the OS of the derivation group. The expression levels of all cytokines were divided into high and low groups by median levels in order to facilitate statistical analysis. The median levels for IFN γ , TNF α , IL4, IL6, IL10, IL17a, IL17f, IL21, IL22, and IL23 were 4.5, 2.9, 2.5, 942.5, 5.8, 16.9, 21.3, 135.2, 18.0, and 16.0 pg/ml respectively.

Tests for proportional hazards assumption of the Cox regression model were performed. All of the risk predictors were time-independent (Supplementary Table 2, see section on supplementary data given at the end of this article). Moreover, the effects of product terms (Supplementary Table 3) and the correlation coefficients (Supplementary Table 4) between clinical parameters (FIGO stage, histology, tumor grade, and optimal surgery) and dichotomized immunological factors were evaluated.

Second, multivariate Cox regression analysis was performed including the significant risk predictors selected from the univariate analysis. The regression coefficients of significant risk factors in the multivariate analysis were divided by the smallest regression coefficient (for IL21 in this study) before being rounded to an integer value to generate the risk scores.

The sensitivity and specificity of the predictive scores for disease relapse and disease-related death of the women with ovarian cancer were estimated using receiver operating characteristic (ROC) curves. We calculated the areas under the ROC curves (AUROC) of the derivation group, validation group, and all patients. To evaluate the validity of the scoring system in the prediction of clinical outcomes of the ovarian cancer patients, all of the cases were categorized into four groups based on the predictive scores. The survival functions of different groups were compared by Cox proportional hazard regression.

Statistical analysis

All statistical analyses were performed using SPSS for Windows version 15.0 (SPSS, Inc.). The clinicopathological characteristics between two sets were compared using the χ^2 test for dichotomized variables and the Mann–Whitney U test for continuous variables. The expression levels of the cytokines between two sets or among various histological subtypes were analyzed by the Mann–Whitney U test or Kruskal–Wallis test. Statistical significance was considered as $P < 0.05$.

Results

Characteristics of the studied population

In total, 226 women with ovarian cancer were enrolled as the study population, of whom 144 were in the NTUH set and 82 in the NCKUH set. The clinicopathological characteristics of these cases are shown in Table 1. The mean follow-up duration was 29.3 months in the NTUH

Table 1 Clinicopathological characteristics of the studied ovarian carcinoma patients

Characteristic	NTUH set (n=144)	NCKUH set (n=82)	Studied population (n=226)
Follow-up period ^{*,a} (mean $\pm$ s.d., months)	29.3 $\pm$ 22.0	33.7 $\pm$ 23.0	30.9 $\pm$ 22.4
Patients			
Age ^{*,a} (mean $\pm$ s.d., years)	54.5 $\pm$ 11.6	55.2 $\pm$ 12.5	54.8 $\pm$ 12.0
Disease status (cases (%))			
FIGO stage ^{*,b}			
Early	45 (31.2%)	24 (29.3%)	69 (30.5%)
Advanced	99 (68.8%)	58 (70.7%)	157 (69.5%)
Histology ^{*,b}			
Serous	65 (45.1%)	42 (51.2%)	107 (47.3%)
Mucinous	5 (3.5%)	8 (9.8%)	13 (5.8%)
Clear cell	35 (24.3%)	15 (18.3%)	50 (22.1%)
Endometrioid	21 (14.6%)	12 (14.6%)	33 (14.6%)
Undifferentiated	18 (12.5%)	5 (6.1%)	23 (10.2%)
Tumor grade ^{*,b,c}			
1	20 (14.2%)	4 (5.3%)	24 (11.1%)
2	13 (9.2%)	8 (10.7%)	21 (9.7%)
3	108 (76.6%)	63 (84%)	171 (79.2%)
Debulking surgery ^{†,b}			
Optimal	86 (59.7%)	63 (76.8%)	149 (65.9%)
Suboptimal	58 (40.3%)	19 (23.2%)	77 (34.1%)
Outcome (cases (%))			
Disease recurrence ^{*,b}			
Yes	72 (50%)	46 (56.1%)	118 (50%)
No	72 (50%)	36 (43.9%)	108 (50%)
Disease-related death ^{*,b}			
Yes	38 (26.4%)	29 (35.4%)	67 (29.6%)
No	106 (73.6%)	53 (64.6%)	159 (70.4%)

* $P > 0.05$ and $^{\dagger}P = 0.01$. FIGO, International Federation of Gynecology and Obstetrics; NTUH, National Taiwan University Hospital; NCKUH, National Cheng Kung University Hospital.

^aBy Mann–Whitney U test.

^bBy χ^2 test.

^cUnavailable data for three patients in NTUH set and seven patients in NCKUH.

set and 33.7 months in the NCKUH set. The mean age at diagnosis of ovarian cancer was 54.5 years in the NTUH set and 55.2 years in the NCKUH set. There were no significant differences in the distribution of disease status, including FIGO stage, histology, and tumor grade or the percentages of disease recurrence and disease-related death, between the two sets. More patients (76.8%) in the NCKUH set received optimal surgery than in the NTUH set (59.7%; $P = 0.01$).

The median levels of the ten cytokines from the 226 samples of ovarian carcinoma-related ascites are presented in Table 2. The expression levels of all cytokines in ascites were different between the two sets except for IL6. However, the higher or lower levels of cytokine expression were not particularly confined to one medical institute. As shown in Table 3, the IFN γ , IL10, IL17a, and IL23 levels of patients with various histological subtypes were different. The patients with serous ovarian carcinoma tended to have higher expression levels of IFN γ and IL10 and lower expression levels of IL17a in their ascites.

Development of the risk-scoring system with a combination of surgical status and IL17a and IL21 levels

The results of univariate and multivariate Cox regression analyses to evaluate the risk predictors for the OS of cases in the NTUH set (derivation group) are shown in Tables 4 and 5. In univariate analysis, advanced disease, grade 3 tumors, no optimal (sub-optimal) surgery and dichotomized immunologic parameters of IFN γ , IL17a, and IL21 expressed in the cancer-associated ascites were significantly associated with the OS of the 144 women (Table 4). In multivariate analysis, sub-optimal cytoreductive surgery (hazard ratio (HR): 2.3 (95% CI 1.01–5.11), $P = 0.048$) had a significant impact on the OS of the patients. The other two significant predictors for OS were lower expression levels of IL17a (HR: 3.0 (95% CI 1.10–8.03), $P = 0.032$) and higher expression levels of IL21 (HR: 2.2 (95% CI 1.02–4.59), $P = 0.045$). Therefore, surgical status and the expression levels of IL17a and IL21 in ascites were selected to develop the risk-scoring system in the derivation group (Table 5).

Table 2 Expression levels of various cytokines in ascites of the ovarian cancer patients

Cytokines (median (range), pg/ml)	NTUH set (n = 144)	NCKUH set (n = 82)	P value ^a	Studied population (n = 226)
IFN γ level	4.5 (0.6–339.0)	26.0 (0.2–403.2)	<0.001	10.8 (0.2–403.2)
TNF α level	2.9 (0.6–77.1)	5.1 (1.8–1600.0)	<0.001	4.0 (0.6–1600.0)
IL4 level	2.5 (0.3–73.3)	8.6 (5.9–720.0)	<0.001	6.9 (0.3–720.0)
IL6 level	942.5 (0.5–15 600.0)	595.0 (22.8–15 800.0)	0.81	916.6 (0.5–15 800.0)
IL10 level	5.8 (0.6–83.3)	5.0 (3.5–129.0)	0.049	5.4 (0.6–129.0)
IL17a level	16.9 (1.0–43.2)	9.7 (2.2–37.2)	0.04	15.3 (1.0–43.2)
IL17f level	21.3 (13.0–1710.0)	18.8 (16.4–120.0)	<0.001	20 (13.0–1710.0)
IL21 level	135.2 (22.0–1206.0)	96.8 (11.4–963.0)	0.03	121.5 (11.4–1206.0)
IL22 level	18.0 (1.2–130.0)	8.9 (3.8–29.4)	<0.001	13.1 (1.2–130.0)
IL23 level	16.0 (8.0–770.0)	18.0 (10.0–56.0)	0.01	17.0 (8.0–770.0)

NTUH, National Taiwan University Hospital; NCKUH, National Cheng Kung University Hospital; IFN γ , interferon gamma; TNF α , tumor necrosis factor alpha; IL, interleukin.

^aBy Mann–Whitney *U* test.

Risk scores were calculated as the β coefficient of optimal surgery (or IL17a level) divided by the β coefficient of IL21 level, and then rounded to an integer value (Table 5). Therefore, sub-optimal surgical status was scored as 1 and optimal status as 0. An IL17a level <17 pg/ml in the ascites was scored as 1 and an IL17a level $\geq$ 17 pg/ml as 0. An IL21 level $\geq$ 135 pg/ml in the ascites was scored as 1, and an IL21 level <135 pg/ml as 0. The overall score for each ovarian cancer patient ranged from 0 to 3.

Validation of the risk-scoring system

The ROC curves of the overall score for disease recurrence in the derivation group (NTUH set; AUROC, 0.84; 95% CI 0.78–0.91), validation group (NCKUH set; AUROC, 0.85; 95% CI 0.76–0.94), and all patients (AUROC, 0.84; 95% CI

0.79–0.89) are shown in Fig. 1A. The ROC curves of the overall score for disease-related death in the derivation group (NTUH set; AUROC, 0.78; 95% CI 0.69–0.86), validation group (NCKUH set; AUROC, 0.76; 95% CI 0.66–0.86), and all studied patients (AUROC, 0.76; 95% CI 0.69–0.82) are shown in Fig. 1B.

Clinical application of the risk-scoring system

According to the overall scores of the women with ovarian cancer, the 226 patients were divided into four groups. As demonstrated in Fig. 2A, the median DFS was 32 months for the women with an overall score of 0, 19 months for an overall score of 1, 9 months for an overall score of 2 and 4 months for an overall score of 3 (HR: 3.1 (95% CI 2.50–3.93), *P* for trend (*P*_{trend}) <0.001). In addition, the median OS was 32 months for the

Table 3 Expression levels of various cytokines in ascites of the ovarian cancer patients with various histological subtypes in all studied population

Cytokines (median (range), pg/ml)	Histological subtype					P value ^a
	Serous (n = 107)	Mucinous (n = 13)	Clear cell (n = 50)	Endometrioid (n = 33)	Undifferentiated (n = 23)	
IFN γ level	18.2 (0.6–403.2)	1.4 (0.6–225.1)	3.1 (0.2–268.3)	1.4 (0.3–288.3)	12.7 (0.6–96.0)	0.001
TNF α level	4.0 (0.6–1600.0)	4.0 (0.6–242.0)	4.0 (0.6–632.0)	6.2 (0.6–137.0)	4.0 (0.6–13.1)	0.41
IL6 level	1440.0 (1.1–15 600.0)	147.0 (22.9–1870.0)	1710.0 (1.0–15 800.0)	425.0 (0.5–13 700.0)	113.0 (0.5–12 000.0)	0.10
IL4 level	6.93 (0.45–720.0)	6.9 (0.8–11.9)	5.9 (0.5–16.6)	7.8 (0.3–18.3)	6.9 (0.5–14.2)	0.30
IL10 level	6.9 (0.6–129.0)	3.9 (0.6–8.1)	5.1 (0.6–105.0)	5.3 (0.6–83.3)	4.2 (0.6–29.9)	0.03
IL17a level	9.5 (1.8–42.1)	27.0 (3.2–36.0)	20.8 (1.1–42.0)	27.0 (1.8–43.2)	20.1 (1.0–39.0)	<0.001
IL17f level	19.8 (13.0–1710.0)	19.1 (16.0–39.5)	21.0 (16.0–530.0)	20.9 (16.0–172.0)	21.0 (16.4–51.0)	0.56
IL21 level	135.0 (19.9–963.0)	65.0 (25.2–679.1)	125.8 (11.4–1206.0)	100.8 (13.0–1154.0)	112.2 (32.0–736.0)	0.10
IL22 level	12.4 (3.8–130.0)	16.0 (4.5–24.0)	14.8 (6.1–52.8)	10.3 (4.3–44.0)	11.1 (1.2–43.0)	0.52
IL23 level	16.0 (8.0–770.0)	18.0 (11.2–23.0)	18.0 (10.8–67.0)	18.0 (10.0–76.0)	16.0 (10.0–28.3)	0.004

IFN γ , interferon gamma; TNF α , tumor necrosis factor alpha; IL, interleukin.

^aBy Kruskal–Wallis test.

Table 4 Univariate Cox proportional hazards model for overall survival of 144 patients with ovarian carcinoma in the NTUH set

NTUH set (n = 144)	HR (95% CI) ^a	P value
FIGO stage (advanced/early)	7.4 (2.28–24.30)	0.001
Histology (serous/non-serous ^b)	1.4 (0.72–2.57)	0.35
Tumor grade (3/1 and 2)	3.0 (1.06–8.59)	0.04
Optimal surgery (no/yes)	4.5 (2.24–8.89)	<0.001
IFN γ level ^c (high/low)	1.9 (1.02–3.71)	0.045
TNF α level ^c (high/low)	0.8 (0.44–1.61)	0.60
IL4 level ^c (high/low)	0.6 (0.30–1.31)	0.22
IL6 level ^c (high/low)	1.9 (0.96–3.82)	0.06
IL10 level ^c (high/low)	1.3 (0.60–2.50)	0.41
IL17a level ^c (high/low)	0.2 (0.09–0.47)	<0.001
IL17f level ^c (high/low)	1.1 (0.57–2.24)	0.74
IL21 level ^c (high/low)	2.8 (1.40–5.71)	0.004
IL22 level ^c (high/low)	0.9 (0.49–1.82)	0.86
IL23 level ^c (high/low)	0.7 (0.37–1.42)	0.34

NTUH, National Taiwan University Hospital; HR, hazard ratio; FIGO, International Federation of Gynecology and Obstetrics; IFN γ , interferon gamma; TNF α , tumor necrosis factor alpha; IL, interleukin.

^aCox regression model.

^bNon-serous includes mucinous, clear cell, endometrioid, and undifferentiated types.

^cCytokine expression levels were dichotomized into high and low groups by median values.

women with an overall score of 0, 27 months for an overall score of 1, 22 months for an overall score of 2, and 20 months for an overall score of 3 (HR: 2.6 (95% CI 1.97–3.44), $P_{\text{trend}} < 0.001$) (Fig. 2B). Two out of 49 patients (4.1%) with an overall score of 0, 37 out of 81 (45.7%) with an overall score of 1, 54 out of 71 (76.1%) with an overall score of 2, and all 25 cases (100.0%) with an overall score of 3 had disease recurrence.

Discussion

Ovarian carcinoma is a general concept for a series of distinct diseases molecularly and etiologically that share an anatomical location (Vaughan *et al.* 2011). A dualistic model proposed by Kurman & Shih (2010) classified several different types of epithelial ovarian cancers into type I and II sub-groups. In type I tumors, the disease usually presents at a low stage with a clinically indolent course, however, type II tumors are highly aggressive disease and usually present at an advanced disease. Even with the same histology (high-grade serous carcinoma), intratumoral genetic heterogeneity related to treatment resistant clone selection has been reported (Cooke *et al.* 2010).

The prediction of survival for ovarian cancer patients is difficult using a single factor; however, it has been reported to be possible with a combination of clinicopathological characteristics and traditional laboratory blood analysis (Chi *et al.* 2008, Chan *et al.* 2012, Previs *et al.* 2014). The main purpose of predictive models is to provide an insight into who will benefit the most from a particular treatment modality while avoiding excessive health care costs and potentially catastrophic toxicity that would ultimately lead to discontinuation of therapy. Ascites, a common manifestation of ovarian carcinoma, is an ideal material to evaluate interactions between the host immunity and tumor cells in the disease micro-environment. In previous studies, the cytokine profiles expressed in ovarian cancer-associated ascites have been reported to serve as biomarkers to predict the clinical

Table 5 Multivariate Cox proportional hazards model for overall survival of 144 patients with ovarian carcinoma in the NTUH set

NTUH set (n = 144)	HR (95% CI) ^a	β coefficient	P value	Risk score ^b
FIGO stage (advanced/early)	4.0 (0.80–19.98)	1.387	0.09	–
Tumor grade (3/1 and 2)	0.8 (0.25–2.81)	–0.172	0.78	–
IFN γ level ^c (high/low)	1.0 (0.48–2.08)	–0.007	0.98	–
Optimal surgery				
Yes	1			0
No	2.3 (1.01–5.11)	0.818	0.048	1
IL17a level ^c (pg/ml)				
≥ 17	1			0
< 17	3.0 (1.10–8.03)	1.088	0.032	1
IL21 level ^c (pg/ml)				
< 135	1			0
≥ 135	2.2 (1.02–4.59)	0.771	0.045	1

NTUH, National Taiwan University Hospital; HR, hazard ratio; FIGO, International Federation of Gynecology and Obstetrics; IFN γ , interferon gamma; TNF α , tumor necrosis factor alpha; IL, interleukin.

^aCox regression model.

^bRisk scores: i) β coefficient of optimal surgery/ β coefficient of IL21 level, then rounded to integer value and ii) β coefficients of IL17a level/ β coefficients of IL21 level, then rounded to integer value.

^cCytokine expression levels were dichotomized into high and low groups by median values.

Figure 1

Receiver operating characteristic (ROC) curves of the overall score for disease relapse (A) and disease-related death (B) of ovarian cancer patients in the derivation group (NTUH set), validation group (NCKUH set), and all patients. NTUH, National Taiwan University Hospital; NCKUH, National Cheng Kung University Hospital; AUROC, area under the ROC curves.

course of cancer patients (Yigit *et al.* 2011, Cândido *et al.* 2013, Chen *et al.* 2013). Theoretically, a combination of clinical parameters and cytokine profiles in ascites could provide a predictive system to evaluate the outcomes of ovarian cancer patients. The present study demonstrated that a combination of surgical status, IL17a and IL21 expression levels in ascites could be used in a practical risk-scoring system to predict the outcome of patients (Table 5), and this system was validated by the derivation group (NTUH set), the validation group (NCKUH set) and all of the studied patients (Fig. 1). Furthermore, good correlations between the overall predictive risk scores and survival of the patients with ovarian cancer were

noted by sub-grouping all of the patients into four groups (Fig. 2).

CD4⁺ T cells can differentiate into different lineages of Th cells with distinct developmental regulation and biological functions. Th17 cells have been identified as a new lineage of effector Th cells, and shown to be important in immune responses to various immune diseases, including malignancies. The Th17-related cytokines include IL17a, IL17f, IL21, IL22, and IL23 (Dong 2008). Fialová *et al.* suggested that the development of antitumor immune responses in ovarian cancer patients is highly dynamic. The recruitment of Th17 cells with higher IL17 and IL21 expression levels in ovarian cancer tissues has been reported in the early stages of disease (Fialová *et al.* 2013), and a lower level of IL17 has been reported in more advanced ovarian cancer-associated ascites (Kryczek *et al.* 2009). The expression levels of IL17a and IL21 in

Figure 2

(A) Survival curves for disease-free survival of all 226 ovarian cancer patients based on the overall scores. (B) Overall survival of all 226 ovarian cancer patients based on the overall scores.

ascites were important predictors for the OS of ovarian cancer patients in this study (Table 5). Th17-related cytokines may therefore play a crucial role in the outcomes of ovarian cancer patients.

Presently, clinical trials focusing on Th17 and its related cytokines for cancer treatment are ongoing, especially for ovarian carcinoma. IL17 has been reported to potentially be a favorable prognostic marker of antitumor immune response and an indicator of the chemosensitivity of ovarian carcinoma (Kryczek *et al.* 2009, Droeser *et al.* 2013). Thus, targeting Th17 and its related cytokines may be a potential strategy for the treatment of ovarian cancer. After being validated by a prospective clinical trial with more participants, measuring the expression levels of Th17-related cytokines in ascites and then applying the risk-scoring system developed in this study may be helpful before providing current chemotherapeutic agents or cancer immunotherapy for ovarian cancer patients. In addition, alternative therapeutic methods would be considered and developed for cases with unfavorable expression profiles of Th17-related cytokines in their ascites.

Alterations of immunocytes and cytokines in the tumor microenvironment have been reported to be related to the prognosis of ovarian cancer patients in several previous studies (Zhang *et al.* 2003, Yigit *et al.* 2011, Cândido *et al.* 2013, Chen *et al.* 2013, Webb *et al.* 2014). To the best of our knowledge, no current risk-scoring system includes immune-related factors. Therefore, the first significant strength of this study is that we developed and validated a practical risk-scoring system with IL17a, IL21 and surgical status to predict the survival of ovarian cancer patients. Second, our study showed that the Th17-related cytokines, IL17a and IL21, may have the potential to be prognostic and therapeutic biomarkers.

There are several limitations to this study. Differences in the levels of Th17-related cytokines expressed in serum and ascites were not explored. In addition, the relationship between Th17-related cytokine levels and variations in immune effector and suppressor cells in ascites were not investigated either. Therefore, further studies are needed to evaluate the underlying immunological regulation in the ovarian cancer microenvironment.

We previously reported that IFN γ levels in ascites could independently predict the OS of ovarian cancer patients (Chen *et al.* 2013). To explore more potential immunological predictors, Th1-, Th2-, and Th17-related cytokines were included in the current survey. In addition to IL17a and IL21, the expression level of IFN γ in ascites still significantly influenced the OS of the 144 patients in univariate

analysis (Table 4). However, only IL17a and IL21 showed significant impacts on the OS in multivariate analysis (Table 5). Therefore, IL17a and IL21 may play more important roles in carcinogenesis; however, further studies are needed to elucidate the associated mechanisms.

In conclusion, we used a combination of clinical and immunologic parameters to develop a practical scoring system to predict the outcome of patients with ovarian carcinoma. Good correlations between the overall predictive risk score and survival of the ovarian cancer patients were demonstrated. In addition, IL17a and IL21 may have the potential to be therapeutic biomarkers.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/ERC-15-0145>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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