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Original Article

Prognostic value of preoperative serum bilirubin levels in ovarian cancer

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Abstract: Bilirubin is a promising prognostic factor for non-liver disease-related deaths in various cancers. We investigated the association between preoperative serum bilirubin levels and oncological outcomes in patients with ovarian cancer. We retrospectively analyzed the clinical data of 282 patients with epithelial ovarian carcinoma (EOC), and grouped them according to optimal threshold values of total bilirubin (TBIL), direct bilirubin (DBIL), and indirect bilirubin (IBL) measured by receiver operating characteristic curve analysis. Univariate and multivariate Cox proportional hazards regression analyses were used to evaluate various parameters that might affect overall survival (OS) and progression-free survival (PFS) in patients with EOC. The optimal cutoff values for TBIL, DBIL, and IBIL levels were 9.65 μmol/L, 2.95 μmol/L, and 6.75 μmol/L, respectively. Increased TBIL, DBIL, and IBIL levels correlated with the serum carbohydrate antigen (CA)-125 levels, International Federation of Gynecology and Obstetrics stage, and pathological differentiation (all P<0.05). Univariate analysis revealed longer OS and PFS in patients with high TBIL (≥9.65 μmol/L) and IBIL (≥6.75 μmol/L) levels (P<0.05). Multivariate analysis showed that patients with high IBIL levels (≥6.75 μmol/L) had significantly longer OS and PFS than those with low IBIL levels (<6.75 μmol/L) [hazard ratio (HR) = 0.333, 95% confidence interval (CI): 0.123~0.904, P<0.05; HR = 1.814, 95% CI: 1.169~2.816, P<0.05]. Therefore, IBIL is a potential independent prognostic factor for OS and PFS in patients with EOC. The higher the IBL level, the better the prognosis of patients with EOC.

Keywords: Bilirubin, epithelial ovarian carcinoma, overall survival, progression-free survival, prognosis

Introduction

Ovarian cancer is the leading cause of death in patients with gynecologic malignancies [1]. Because of the insidious onset of ovarian cancer, early diagnosis is difficult, and most patients are diagnosed at the late stage. Metastases [International Federation of Gynecology and Obstetrics (FIGO) stage III-IV] occur in 70% of newly diagnosed patients with ovarian cancer. Advanced ovarian cancer has a high degree of malignancy, fast metastasis and invasion, easy recurrence of chemotherapy resistance, and a poor prognosis [2]. The 5-year survival rate of patients is only 25% [3]. Therefore, to guide surgery and evaluate follow-up treatment, it is necessary to explore the indexes that can predict the degree of ovarian cancer malignancy before surgery and analyze the prognosis. Factors influencing the prognosis of ovarian cancer include tumor intrinsic factors and host-related factors [4]. Identification of these factors may contribute to the assessment of ovarian cancer prognosis and development of individualized treatment to improve ovarian cancer survival. Compared with traditional prognostic indicators such as tumor size, tumor stage, and degree of differentiation, blood biochemical indicators are increasingly popular because they are easily obtained, non-invasive, and show high predictive efficacy [5].

Bilirubin, comprising direct bilirubin (20%) and indirect bilirubin (80%), is the end product of hemoglobin metabolism. It has long been used as a marker of lesions in the liver, gallbladder, and blood systems [6]. Interestingly, many experimental and clinical studies in recent years have demonstrated that bilirubin plays an important protective role in anti-inflammation,

anti-oxidation, and anti-tumorigenesis. In different tumor models, such as those of the colon and adenocarcinoma, bilirubin can induce apoptosis and inhibit proliferation *in vitro* [7]. Serum bilirubin, as an endogenous antioxidant, increases moderately, improving the ability to scavenge oxidative free radicals in cancer patients [8]. Serum bilirubin as a prognostic marker in various malignant tumors has been explored, such as its relationship with the prognosis of non-small cell lung cancer [9], breast cancer [10], gastric cancer [11], and colorectal cancer [12]. However, the relationship between serum bilirubin levels and the prognosis of ovarian cancer patients has not yet been reported. Therefore, we intend to fill this gap by retrospectively analyzing the clinical data of 282 ovarian cancer patients.

Materials and methods

Study subjects

The clinical data of 282 patients with epithelial ovarian carcinoma (EOC) diagnosed by pathology, who had undergone prior surgical treatment at Suzhou Hospital affiliated to Nanjing Medical University from January 2007 to December 2018, were retrospectively collected. The inclusion criteria were as follows: (1) the patients with EOC had undergone initial treatment; (2) the postoperative histopathological diagnosis of the patient was clear; (3) the tumor site, diameter, pathological type, and depth of infiltration were all obtained by biopsy pathological examination; and (4) the histopathological report was read by two pathologists. The exclusion criteria were as follows: (1) the patient died during the perioperative period; (2) the patient had received radiation, chemotherapy, and other anti-tumor treatment preoperatively; (3) the patient had other tumors; (4) the patient had serious liver and kidney damage, autoimmune diseases, thrombosis, and bleeding diseases; (5) the patient had an infectious disease lasting almost 2 weeks; and (6) no liver function examination results were available at one week prior to surgery.

Case information

The following case information was collected: (1) general information such as the age of the patient at the time of disease onset, body mass index (BMI), menopause status, pregnancy history, delivery mode, number of abortions, cesarean section history, tubal ligation history, and preliminary symptoms (e.g., abdominal distension, abdominal pain, and irregular vaginal bleeding); (2) preoperative auxiliary examination of patients [CA125, human epididymis protein 4 (HE4), testing of total bilirubin (TBIL), direct bilirubin (DBIL), and indirect bilirubin (IBIL) levels, ultrasound, computed tomography (CT) or magnetic resonance imaging (MRI)]; (3) surgery and postoperative information, such as tumor size, location, ascites status, pelvic adhesion, postoperative residual lesion size, lymph node metastasis, FIGO staging (2015), tumor differentiation degree, endometrial lesion status, uterine fibroid status, chemotherapy, chemotherapy drug resistance, disease recurrence, and survival. Postoperative follow-up lasted for at least 5 years. Follow-up was conducted every 1-3 months for 2 years and then every 3-6 months for the subsequent 3 years. The diagnosis of recurrence was based on imaging examination (e.g., B ultrasound, CT, MRI, positron emission tomography-CT) to detect disease recurrence or metastatic lesions. The elevation of the levels of tumor markers (including serum carbohydrate antigen (CA) 125 and HE4) alone was not considered as a criterion for disease recurrence. Overall survival (OS) and progression-free survival (PFS) were included in the survival analysis. OS is the time from initial surgery to death or the last follow-up, and PFS is the time from initial surgery to tumor progression or recurrence.

Statistical analysis

SPSS 23.0 statistical software was used for data analysis. Enumeration data were expressed as rates (%), normal distribution measurement data were expressed as means ± standard deviation, and non-normal distribution measurement data were expressed as medians (interquartile interval). Chi-square test or Fisher's exact test was used for enumeration data comparison, and the Mann-Whitney U test was used for measurement data comparison. The optimal cut-off points of age, tumor size, TBIL, DBIL, IBL, and CA125 were determined by receiver operating characteristic curve analysis. The Kaplan-Meier method was used for

Table 1. Clinical and morphological characteristics of EOC patients

Characteristics	No of patients (%)
Age (year) (median [IQR])	54 (47.75, 65.00)
BMI (kg/m^2)	21.56±2.46
Menopause	
Pre-	130 (46.10)
Post-	152 (53.90)
Gravid	
0	24 (8.51)
≥8	258 (91.49)
Parity	
0	26 (9.22)
≥9	256 (90.78)
Induced abortion	
0	144 (51.06)
≥5	138 (48.94)
Cesarean section history	
Yes	28 (9.93)
No	254 (90.07)
Tube ligation history	
Yes	16 (5.67)
No	266 (94.33)
Breast cancer history	
Yes	12 (4.26)
No	270 (95.74)
Hypertension history	
Yes	74 (26.24)
No	208 (73.76)
Diabetic mellitus history	
Yes	22 (7.80)
No	260 (92.20)
Symptoms	
Bloating	
Yes	130 (46.10)
No	152 (53.90)
Abdominal paid	
Yes	90 (31.91)
No	192 (68.09)
Abnormal vaginal bleeding	
Yes	22 (7.80)
No	260 (92.20)
Cachexia	
Yes	28 (9.93)
No	254 (90.07)
Pre-surgery CA125 (U/mL) (median [IQR])	111.80 (49.53, 579.55)
Pre-surgery HE4 (pmol/L) (median [IQR])	67.00 (44.75, 324.50)
Pre-surgery TBIL (μmol/L) (median [IQR])	9.90 (7.80, 12.93)
Pre-surgery DBIL (μmol/L) (median [IQR])	2.60 (2.08, 3.50)
Pre-surgery IBL (μmol/L) (median [IQR])	7.40 (5.60, 9.20)

survival analysis. A *P*-value <0.05 indicated statistical significance. The survival curves were constructed using GraphPad Prism5 software.

Results

Basic information and clinicopathological characteristics of patients

The general information of onset age, body mass index (BMI), menopause, pregnancy, labor, abortion, cesarean section, tubal ligation, and the first symptoms such as abdominal distention, abdominal pain, and irregular vaginal bleeding are shown in **Table 1**. The median values of TBIL, DBIL and IBL were 9.90, 2.60 and 7.40 μmol/L, respectively (**Table 1**). The histopathological type of ovarian cancer: serous cancer was 169 cases, accounting for 59.93% of the total. 41.84% of them were early stage ovarian cancer (FIGO I-II), 58.16% were advanced stage ovarian cancer (FIGO III-IV) (**Table 2**).

Determination of the optimal interception point

According to the comparison of patients' prognosis with the absolute value of TBIL, DBIL and IBIL, the Receiver Operating Characteristic (ROC) curve was drawn, respectively. When the TBIL was 9.65 μmol/L, the DBIL was 2.95 μmol/L and the IBIL was 6.75 μmol/L, reaching the maximum value of Youden's index (0.233, 0.142 and 0.237). Therefore, the optimal cut-off points of TBIL, DBIL and IBIL were selected, and the area under ROC curve (AUC) was 0.572 (95% CI: 0.501~0.643, *P*-value <0.05), 0.453 (95% CI: 0.383~0.523, *P*-value = 186), 0.599 (95% CI: 0.530~0.667, *P*-value <0.05), respectively, with sensitivity of 0.618, 0.449 and 0.640. The specificity was 0.615, 0.692 and 0.596, respectively (**Figure 1**).

Table 2. The surgico-pathological characters and treatment-related variables of EOC patients

Characteristics	No of patients (%)
Tumor size (mm) (median [IQR])	85 (50, 126)
Side of ovarian tumor	
Unilateral	218 (77.30)
Bilateral	64 (22.70)
Ascites	
Yes	200 (70.92)
No	82 (29.08)
Pelvic adhesion	
Yes	40 (14.18)
No	242 (85.82)
Residual disease	
No or ≤o cm	244 (86.52)
>1 cm	38 (13.48)
Metastasis of lymph node	
Yes	194 (68.79)
No	88 (31.21)
Histotype	
Serous	169 (59.93)
Others	113 (40.07)
FIGO Stage	
I	108 (38.30)
II	10 (3.55)
III	120 (42.55)
IV	44 (15.60)
Early or late Stage	
I+II	118 (41.84)
III+IV	164 (58.16)
Differentiation	
High	24 (8.51)
Medium	56 (19.86)
Low	202 (71.63)
Endometrial disorders	
Endometrial polyp	22 (7.80)
Endometrial cancer	2 (0.71)
Myoma of uterus	64 (22.70)
Cervical intraepithelial neoplasia	4 (1.42)
Chemotherapy	
Yes	180 (63.83)
No	102 (36.17)
Chemo-resistance	
Yes	106 (37.59)
No	176 (62.41)

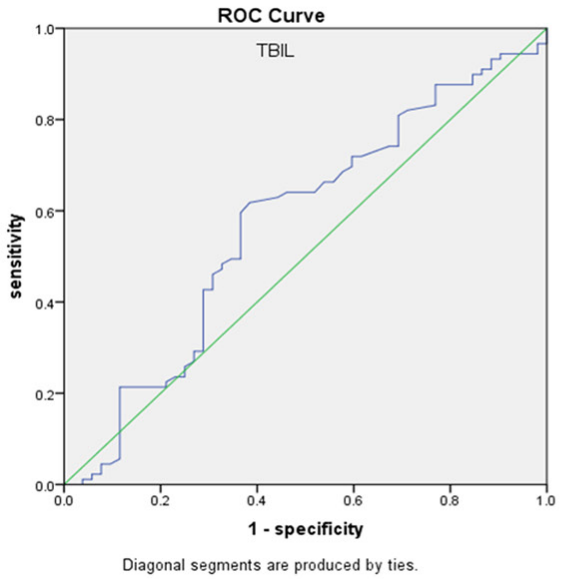
Age, preoperative CA125 level and tumor size were selected according to the ROC curve to obtain the optimal interception point (65 years old, 124 U/mL and 9 cm) for the analysis of influencing factors of OS (**Figure 1**).

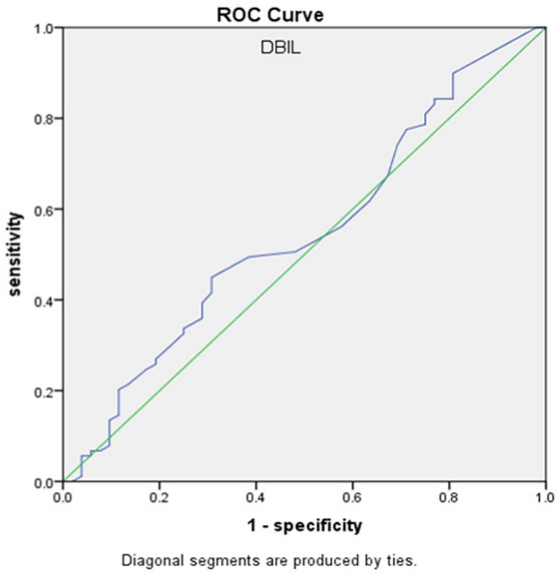
Correlation between preoperative TBIL, DBIL and IBIL in patients with ovarian epithelial carcinoma and clinicopathological features

There were statistically significant differences in CA125 level, ascites, FIGO stage and pathological differentiation between the low TBIL level group (≤h.65 μmol/L) and the high TBIL level group (>9.65 μmol/L) (*P*-value <0.05) (**Table 3**). There were statistically significant differences in CA125 level, ascites, FIGO stage and pathological differentiation between the low DBIL level group (≤2.95 μmol/L) and the high DBIL level group (>2.95 μmol/L) (*P*-value <0.05) (**Table 4**). There were statistically significant differences in CA125 level, FIGO stage and pathological differentiation between patients with low IBIL level (cally μmol/L) and patients with high IBIL level (>6.75 μmol/L) (*P*-value <0.05) (**Table 5**).

Univariate and multivariate analysis of OS and PFS in patients with ovarian epithelial carcinoma

Univariate COX regression analysis of OS in patients with ovarian epithelial carcinoma showed that TBIL level (<9.65 vs s 5 vs μmol/L) and IBIL level (<6.75 vs s 5 vs μmol/L) were the influencing factors of OS (*P*-value <0.05). The difference in survival curve was statistically significant (*P*-value = 0.001, chi-square = 12.020; *P*-value = 0.002, chi-squared = 9.384) (**Figure 2**). In addition, menopause, tubal ligation, CA125 level (<124 vs ≥124 U/L), tumor size (<9 vs ≥9 cm), lymph node metastasis, FIGO stage, early and late stage of tumor, pathological differentiation, chemotherapy and chemotherapy resistance were all influencing factors of OS (*P*-value <0.05). Multivariate COX regression analysis showed that oviduct ligation, FIGO stage, chemotherapy, chemotherapy resistance and IBIL level (<6.75 vs s 5 vs μmol/L) were the influencing factors for OS in EOC patients, and the difference was statistically significant (HR = 2.685, 95% CI: 1.111~6.491, *P*-value <0.05; HR = 13.307, 95% CI: 5.890~30.062, *P*-value = 0.000; HR = 3.216, 95% CI: 1.089~9.498, *P*-value <0.05; HR = 4.801, 95% CI: 2.357~9.781, *P*-value = 0.000;


ROC Curve
TBIL
sensitivity
1 - specificity
0.0
0.2
0.4
0.6
0.8
1.0
Diagonal segments are produced by ties.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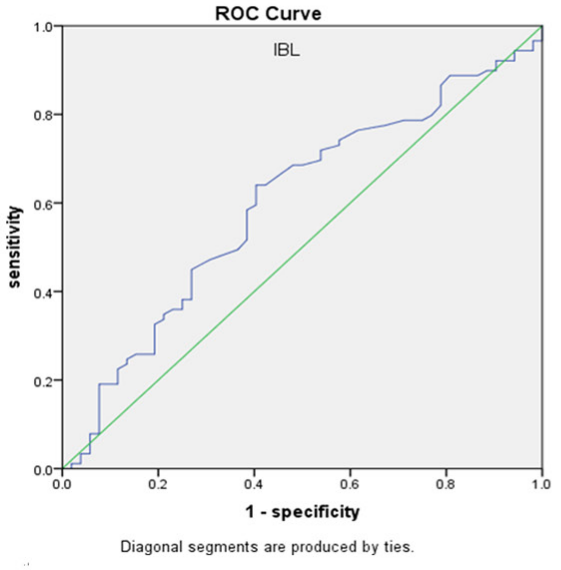
ROC Curve
IBL
sensitivity
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0.6
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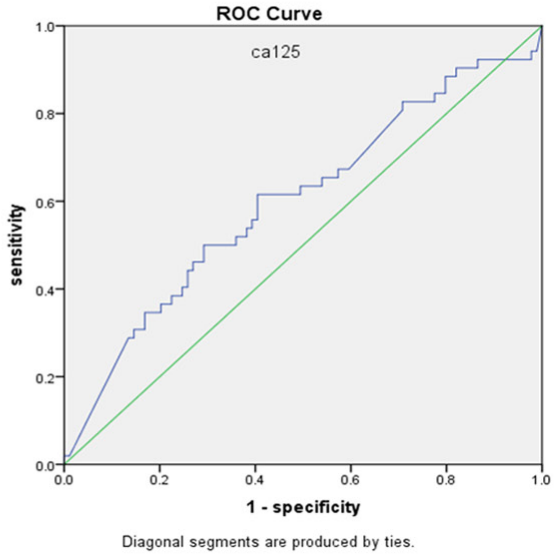
Diagonal segments are produced by ties.


ROC Curve
ca125
sensitivity
1 - specificity
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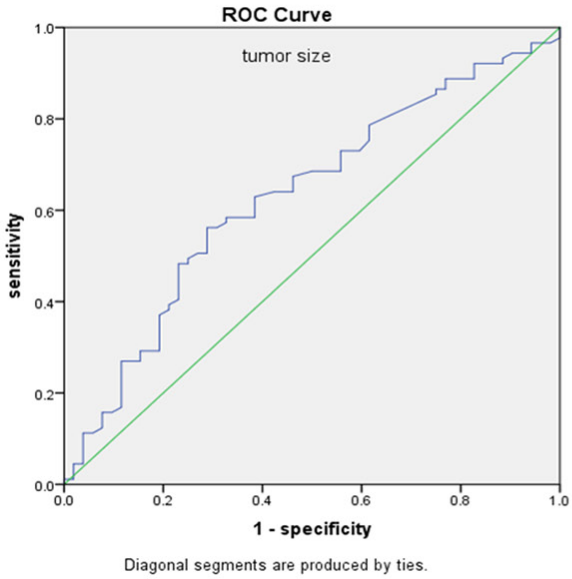
0.2
0.4
0.6
0.8
1.0
Diagonal segments are produced by ties.


ROC Curve
tumor size
sensitivity
1 - specificity
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0.2
0.4
0.6
0.8
1.0
Diagonal segments are produced by ties.

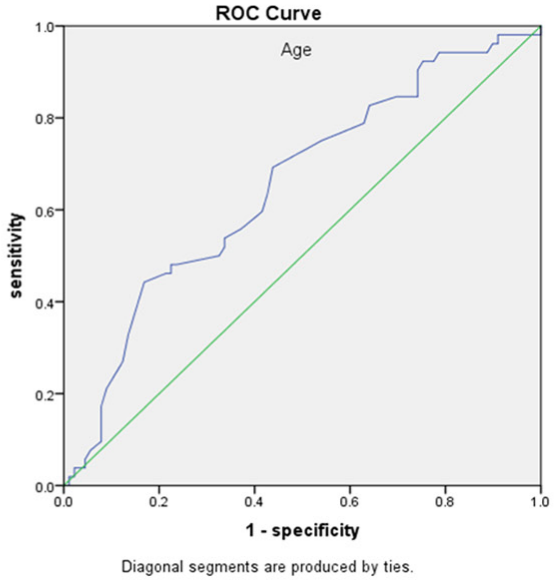
ROC Curve
Age
sensitivity
1 - specificity
0.0
0.2
0.4
0.6
0.8
1.0
Diagonal segments are produced by ties.

Figure 1. ROC curve to build a predictive model for risk of ovarian cancer. TBIL, IBL, DBIL, preoperative CA125 level, tumor size and age were selected according to the ROC curve to obtain the optimal interception point (9.65 μmol/L, 6.75 μmol/L, 2.95 μmol/L, 124 U/mL, 9 cm and 65 years old) for the analysis of influencing factors of OS.

Table 3. Correlation between preoperative TBIL in patients with ovarian epithelial carcinoma and clinicopathological features

Characteristics	TBIL (μmol/L)		*P*-value
	≤valu (N = 132)	>9.65 (N = 150)	
Age (year) (median [IQR])	54.0 (20.0)	52.0 (17.0)	0.398
BMI (kg/m^2)	21.56±2.23	21.55±2.66	0.996
Menopause			0.839
Pre-	60	70	
Post-	72	80	
Tube ligation history			0.442
Yes	6	10	
No	126	140	
Presurgery CA125 (U/mL) (median [IQR])	210.4 (553.7)	78.0 (497.4)	0.044
Presurgery HE4 (pmol/L) (median [IQR])	67.4 (286.5)	67.0 (333.0)	0.817
Tumor size (mm) (median [IQR])	77 (73)	92 (79)	0.527
Ascites			0.000
Yes	108	92	
No	24	58	
Metastasis of lymph node			0.411
Yes	94	100	
No	38	50	
Hisotype			0.317
Serous	75	94	
Others	57	56	
FIGO Stage			0.000
I	30	78	
II	4	6	
III	68	52	
IV	30	14	
Early or late Stage			0.000
I+II	34	84	
III+IV	98	66	
Differentiation			0.000
High	6	18	
Medium	12	44	
Low	114	88	
Myoma of uterus			0.090
Yes	24	40	
No	108	110	
Chemotherapy			0.000
Yes	100	80	
No	32	70	
Chemo-resistance			0.119
Yes	64	42	
No	36	38	

Table 4. Correlation between preoperative DBIL in patients with ovarian epithelial carcinoma and clinicopathological features

Characteristics	DBIL (μmol/L)		*P*-value
	≤valu (N = 170)	>2.95 (N = 112)	
Age (year) (median [IQR])	53.0 (18.5)	55 (18.0)	0.221
BMI (kg/m^2)	21.66±2.32	21.40±2.78	0.404
Menopause			0.286
Pre-	74	56	
Post-	96	56	
Tube ligation history			0.055
Yes	6	10	
No	164	102	
Presurgery CA125 (U/mL) (median [IQR])	213.5 (649.8)	61.7 (311.3)	0.004
Presurgery HE4 (pmol/L) (median [IQR])	67.0 (252.2)	75.7 (363.4)	0.895
Tumor size (mm) (median [IQR])	77 (74)	97 (78)	0.889
Ascites			0.000
Yes	136	64	
No	34	48	
Metastasis of lymph node			0.299
Yes	113	81	
No	57	31	
Hisotype			0.077
Serous	109	60	
Others	61	52	
FIGO Stage			0.006
I	55	53	
II	4	6	
III	86	34	
IV	25	19	
Early or late Stage			0.003
I+II	59	59	
III+IV	111	53	
Differentiation			0.000
High	5	19	
Medium	24	32	
Low	141	61	
Myoma of uterus			
Yes	30	34	
No	140	78	
Chemotherapy			0.058
Yes	116	64	
No	54	48	
Chemo-resistance			0.103
Yes	76	34	
No	40	30	

HR = 0.333, 95% CI: 0.123~0.904, *P*-value <0.05) (**Table 6**).

Univariate COX regression analysis of PFS in patients with ovarian epithelial carcinoma sh-

Table 5. Correlation between preoperative IBIL in patients with ovarian epithelial carcinoma and clinicopathological features

Characteristics	IBL (μmol/L)		*P*-value
	≤valu (N = 126)	>6.75 (N = 156)	
Age (year) (median [IQR])	54.0 (21.0)	52.0 (17.0)	0.676
BMI (kg/m^2)	21.73±2.25	21.42±2.62	0.288
Menopause			0.057
Pre-	66	64	
Post-	60	92	
Tube ligation history			0.170
Yes	4	12	
No	122	144	
Presurgery CA125 (U/mL) (median [IQR])	207.2 (646.6)	78.0 (483.5)	0.043
Presurgery HE4 (pmol/L) (median [IQR])	73.9 (281.0)	61.0 (191.8)	0.603
Tumor size (mm) (median [IQR])	72 (76)	92 (77)	0.767
Ascites			0.866
Yes	90	110	
No	36	46	
Metastasis of lymph node			0.733
Yes	88	106	
No	38	50	
Hisotype			0.180
Serous	81	88	
Others	45	68	
FIGO Stage			0.000
I	31	77	
II	5	5	
III	64	56	
IV	26	18	
Early or late Stage			0.000
I+II	36	82	
III+IV	90	74	
Differentiation			0.000
High	7	17	
Medium	7	49	
Low	112	90	
Myoma of uterus			0.059
Yes	22	42	
No	104	114	
Chemotherapy			0.001
Yes	94	86	
No	32	70	
Chemo-resistance			0.423
Yes	58	48	
No	36	38	

owed that all the other factors were influencing factors of PFS except age and DBIL level (<2.95 vs s 5 vs μmol/L) (*P*-value <0.05). TBIL and IBL survival curves were statistically significant (*P*-value = 0.000, chi-squared = 15.990; *P*-value = 0.001, chi-squared = 11.356) (**Figure**

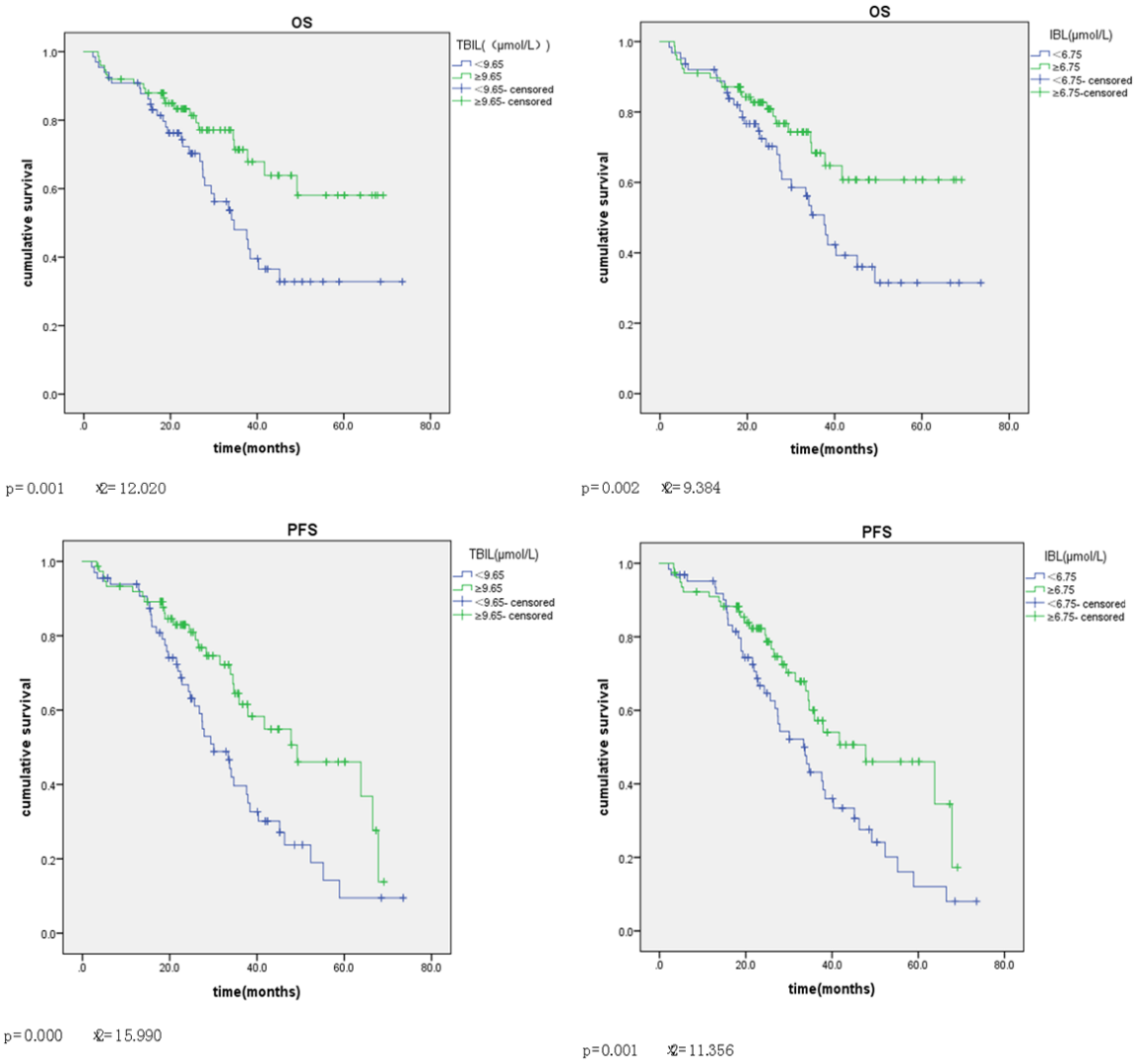

Figure 2. Univariate analysis of overall survival in EOC patients. Kaplan-Meier survival curves showing the effects of TBIL and IBL for OS and PFS. TBIL and IBL survival curves were statistically significant for OS (*P*-value = 0.001, chi-square = 12.020; *P*-value = 0.002, chi-squared = 9.384) and for PFS (*P*-value = 0.000, chi-squared = 15.990). *P*-value = 0.001, chi-squared = 11.356).

2). Higher levels of TBIL and IBL had longer OS and PFS (**Figure 2**). Multivariate COX regression analysis showed that tubal ligation, FIGO stage, early and late stage of tumor, chemotherapy, chemotherapy resistance and IBIL level (<6.75 vs s 5 vs μmol/L) were the influencing factors of PFS in EOC patients, and the difference was statistically significant (HR = 2.940, 95% CI: 1.326~6.522, *P*-value <0.05; HR = 6.171, 95% CI: 3.339~11.406, *P*-value = 0.000; HR = 0.250, 95% CI: 0.083~0.755, *P*-value <0.05; HR = 3.296, 95% CI: 1.015~10.703, *P*-value <0.05; HR = 5.702, 95% CI: 3.169~10.259, *P*-value = 0.000; HR = 1.814, 95% CI: 1.169~2.816, *P*-value <0.05) (**Table 7**).

Discussion

Numerous studies have shown that oxidative stress may be involved in affecting many tumor behaviors, including survival, proliferation, chemotherapy resistance, radiation resistance, angiogenesis, and distant metastasis [13]. Oxidative stress can activate many proteins, such as Ras, P13K/Akt, and ERK1/2 [14]. After activation, these proteins usually upregulate the expression and activity of matrix metalloproteinase (MMP) [15], further promoting tumor cell invasion and metastasis [16]. *In vitro* experiments have shown that bilirubin can inhibit the activation of ERK1/2 and expression of

Table 6. Univariate and multivariate analysis of potential prognostic factors for overall survival

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Age (years) (<65 vs ≥65)	0.864	0.556~1.342	0.515			
Menopause (yes vs no)	2.417	1.600~3.650	0.000	1.235	0.699~2.182	0.467
Tube ligation history (yes vs no)	2.476	1.288~4.759	0.007	2.685	1.111~6.491	0.028
Hypertension history (yes vs no)	1.486	0.979~2.255	0.063			
Presurgery CA125 (U/mL) (<124 vs ≥124)	1.839	1.238~2.732	0.003	0.568	0.299~1.080	0.084
Tumor size (cm) (<9 vs ≥9)	0.460	0.303~0.698	0.000	0.674	0.395~1.149	0.147
Ascites (yes vs no)	0.644	0.408~1.017	0.059			
Metastasis of lymph node (yes vs no)	3.217	1.886~5.487	0.000	0.843	0.393~1.808	0.660
FIGO Stage (I vs II vs III vs IV)	3.702	2.769~4.949	0.000	13.307	5.890~30.062	0.000
Early or late stage (I+I vs III+IV)	10.783	5.585~20.820	0.000	0.280	0.055~1.423	0.125
Differentiation (high vs medium vs low)	5.978	3.059~11.682	0.000	0.367	0.128~1.055	0.063
Chemotherapy (yes vs no)	1.683	1.077~2.629	0.022	3.216	1.089~9.498	0.034
Chemo-resistance (yes vs no)	8.636	5.007~14.896	0.000	4.801	2.357~9.781	0.000
TBIL (μmol/L) (<9.65 vs ≥9.65)	0.548	0.370~0.811	0.003	0.577	0.305~1.090	0.090
DBIL (μmol/L) (<2.95 vs ≥2.95)	0.663	0.437~1.006	0.053			
IBL (μmol/L) (<6.75 vs ≥6.75)	0.505	0.340~0.750	0.001	0.333	0.123~0.904	0.031

Table 7. Univariate and multivariate analysis of potential prognostic factors for progress free survival

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Age (years) (<65 vs ≥65)	1.012	0.704~1.453	0.950			
Menopause (yes vs no)	1.725	1.230~2.419	0.002	0.867	0.540~1.393	0.555
Tube ligation history (yes vs no)	2.341	1.291~4.244	0.005	2.940	1.326~6.522	0.008
Hypertension history (yes vs no)	1.623	1.139~2.313	0.007	1.220	0.774~1.923	0.391
Presurgery CA125 (U/mL) (<124 vs ≥124)	1.767	1.255~2.487	0.001	0.586	0.338~1.014	0.056
Tumor size (mm) (<9 vs ≥9)	0.659	0.466~0.932	0.018	0.925	0.604~1.417	0.720
Ascites (yes vs no)	0.671	0.456~0.988	0.043	1.054	0.643~1.728	0.834
Metastasis of lymph node (yes vs no)	2.738	1.793~4.182	0.000	0.872	0.479~1.588	0.654
FIGO Stage (I vs II vs III vs IV)	3.375	2.652~4.295	0.000	6.171	3.339~11.406	0.000
Early or late stage (I+II vs III+IV)	8.513	5.099~14.210	0.000	0.250	0.083~0.755	0.014
Differentiation (high vs medium vs low)	5.897	3.527~9.861	0.000	1.336	0.603~2.958	0.475
Chemotherapy (yes vs no)	2.925	1.893~4.519	0.000	3.296	1.015~10.703	0.047
Chemo-resistance (yes vs no)	7.295	4.788~11.113	0.000	5.702	3.169~10.259	0.000
TBIL (μmol/L) (<9.65 vs ≥9.65)	0.509	0.363~0.715	0.000	1.081	0.439~2.657	0.866
DBIL (μmol/L) (<2.95 vs ≥2.95)	0.702	0.493~1.001	0.050	1.199	0.676~2.128	0.535
IBL (μmol/L) (<6.75 vs ≥6.75)	0.567	0.405~0.795	0.001	1.814	1.169~2.816	0.008

MMP-2, as well as damage the invasion ability of nasopharyngeal carcinoma cells [17]. *In vivo* studies in mice have shown that bilirubin inhibits the lung metastasis of nasopharyngeal carcinoma cells [18]. Bilirubin is not only associated with the occurrence and development of tumors, but also with the prognosis of patients with cancers such as lung cancer [19], breast cancer [20], colorectal cancer [21], and oral squamous cell carcinoma [22]. A case-control study assessed the correlation between antioxidant levels in the body and the risk of breast cancer and found that moderate increases in serum bilirubin levels were positively correlated with a reduced risk of breast cancer [20]. Data from the third National Health and

Nutrition Examination Survey of the United States population showed that the serum bilirubin levels were negatively correlated with colon cancer risk [23]. The correlation between bilirubin levels and ovarian cancer prognosis is still lacking. In this study, patients with higher preoperative serum TBIL and IBL levels showed prolonged OS and PFS compared with those with lower preoperative TBIL and IBL levels.

The main source of bilirubin is hemoglobin released by aging or apoptotic red blood cells [24]. Heme first produces biliverdin, which produces IBL in the liver under the action of biliverdin reductase (BLVRA and BLVRB) [25]. Under the action of glucuronyltransferase, IBL combines with glucuronic acid to form DBIL, which is then excreted into the small intestine with bile [26]. Bilirubin can be transformed into biliverdin again by the oxidation of glutathione [27]. Studies have shown that biliverdin has strong antioxidant properties at the cellular level [28]. Even in the presence of highly oxidizing molecules such as nitrite, superoxide, and hydroxyl radicals, biliverdin can protect cellular macromolecular compounds such as lipids and proteins from oxidative stress [29]. The bilirubin-biliverdin redox cycle further proves that bilirubin has potential antioxidant activity [30]. In addition, bilirubin exists mainly in the body as a fat-soluble diacid, which can freely enter and leave the phospholipid membrane of body cells and function in antioxidation. Thus, bilirubin may play an important physiological role as an intracellular antioxidant [31].

Bilirubin and biliverdin play regulatory roles in many biological processes and are effective endogenous activators of aromatic hydrocarbon receptors [aryl hydrocarbon receptor (AhR)] [32]. AhR is a ligand-activated transcription factor that acts on various genes, including heme oxygenase (HMOX)-1, cytochrome P450 mono-oxygenase1A1/2 (CYP1A1/2), cytochrome P450 mono-oxygenase2A6 (CYP2A6), and uridine diphosphoglucuronosyl transferase1A1 (UGT1A1), and participates in the biotransformation and transport of bilirubin [33]. The AhR signaling pathway seems to have a broader impact because it is part of known complex networks such as cell cycle regulation, mitogen-activated protein kinase (MAPK) cascade activation, and nuclear factor-erythroid-2-like signals [encoded by NFE2L2 (also known as Nrf2)] [34]. These pathways induce a series of genes related to AhR/Nrf2 signaling [35]. Target genes include genes related to apoptosis, the T helper cell-mediated immune response, and cell proliferation and differentiation (vascular endothelial cells, smooth muscle cells, and macrophages) [36].

In addition to bilirubin production, BLVRA has several other important biological functions, including the unique multi-specific (serine/threonine/tyrosine) kinase activity, which contributes to cell signal transduction [25]. BLVRA and HMOX1 can translocate from the cytoplasm to the nucleus and activate various signaling pathways by oxidative stress, including those involved in survival, the stress response, Jak-Stat, transforming growth factor-β, activated B nuclear factor kappa light chain enhancer, and p38MAPK signaling, as well as regulation of the expression of HMOX itself and p38MAPK [37]. Biliverdin and BLVRA have also been shown to regulate protein kinase C, a serine/threonine kinase associated with carcinogenesis [38]. This complex network suggests that intracellular bilirubin should be considered part of an antioxidant cell system through which cells can regulate their function [39]. Therefore, it is reasonable to assume that each cell and/or tissue may have a different intracellular bilirubin threshold level, which may determine the level of protection against oxidation.

This study found that the increased level of serum bilirubin correlates with better prognosis in patients with EOC, but the effective bilirubin threshold level still needs to be determined using a multicenter, large-sample study. Thus, bilirubin is not only a metabolite but also plays an important physiological role as an intracellular antioxidant that can resist the immunosuppression of proto-presenting cells and T cells and inhibit adhesion molecule expression and immune cell migration. In addition, bilirubin has an extensive inhibitory effect on protein phosphorylation, resulting in the regulation of intracellular signal pathways, with significance in vascular and autoimmune pathology as well as cancer. Moreover, bilirubin has been shown to inhibit the proliferation of the neointima and vascular smooth muscle cells *in vivo* and *in vitro*. Furthermore, bilirubin can inhibit the growth of tumor cells and may induce apoptosis. These concepts shed new light on bilirubin

metabolism and suggest the possibility of using bilirubin as a new tool for improving autoimmunity and treating cancer. As our understanding of bilirubin and related metabolic enzymes continues to increase, some scholars have suggested the use of the term "bilirubinomics" to describe this research field [40].

This study has important clinical application value. Preoperative liver function examination is a routine blood examination with low cost and little trauma. If applied in clinical practice, it can effectively minimize medical costs. Due to the limitation of objective conditions, this study has some drawbacks. First, this is a single-center study with a small number of cases included; thus, it is necessary to further expand the sample size in a follow-up study or carry out a multi-center study. Furthermore, no information on the history of alcohol consumption, coffee intake [41], and cigarette smoking [42] was collected in this study. Those factors may cause liver damage, which may affect serum bilirubin levels.

This study included 282 patients who had undergone prior ovarian cancer surgery and analyzed the clinical data on the age at the time of disease onset, BMI, menopause, pregnancy, labor, number of abortions, history of cesarean section, history of tubal ligation, and general information of initial symptoms (such as abdominal distension, abdominal pain, and irregular vaginal bleeding), surgical pathology, and long-term prognosis. Preoperative TBIL, DBIL, and IBL levels showed significant correlation with CA125 levels, the FIGO stage, and pathological differentiation among patients in the preoperative TBIL level group (>9.65 μmol/L vs ≤9.65 μmol/L), DBIL level group (>2.95 μmol/L vs ≤2.95 μmol/L), and IBIL level group (>6.75 μmol/L vs ≤s vs μmol/L). Survival curve analysis showed that patients with higher levels of TBIL and IBIL showed prolonged OS and PFS compared with those with lower levels. The preoperative TBIL and IBIL levels correlated with the prognosis of EOC. The preoperative IBIL levels were found to be independent prognostic factors for OS and PFS in patients with EOS.

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Disclosure of conflict of interest

None.

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References

[1] White KM, Seale H and Harrison R. Enhancing ovarian cancer care: a systematic review of guideline adherence and clinical variation. BMC Public Health 2019; 19: 296.

[2] Kumar S, Long J, Kehoe S, Sundar S and Cummins C. Quality of life outcomes following surgery for advanced ovarian cancer: a systematic review and meta-analysis. Int J Gynecol Cancer 2019; 29: 1285-1291.

[3] Canaz E, Grabowski JP, Richter R, Braicu EI, Chekerov R and Sehouli J. Survival and prognostic factors in patients with recurrent low-grade epithelial ovarian cancer: an analysis of five prospective phase II/III trials of NOGGO metadata base. Gynecol Oncol 2019; 154: 539-546.

[4] Christophersen MK, Hogdall C and Hogdall E. The prospect of discovering new biomarkers for ovarian cancer based on current knowledge of susceptibility loci and genetic variation (Review). Int J Mol Med 2019; 44: 1599-1608.

[5] Kawakami E, Tabata J, Yanaihara N, Ishikawa T, Koseki K, Iida Y, Saito M, Komazaki H, Shapiro JS, Goto C, Akiyama Y, Saito R, Saito M, Takano H, Yamada K and Okamoto A. Application of artificial intelligence for preoperative diagnostic and prognostic prediction in epithelial ovarian cancer based on blood biomarkers. Clin Cancer Res 2019; 25: 3006-3015.

[6] Feng L, Gu S, Wang P, Chen H, Chen Z, Meng Z, and Liu L. Pretreatment values of bilirubin and albumin are not prognostic predictors in patients with advanced pancreatic cancer. Cancer Med 2018; 7: 5943-5951.

[7] Rao P, Suzuki R, Mizobuchi S, Yamaguchi T and Sasaguri S. Bilirubin exhibits a novel anti-can-

cer effect on human adenocarcinoma. Biochem Biophys Res Commun 2006; 342: 1279-1283.
[8] Vitek L. Role of bilirubin in the prevention of cardiovascular diseases and cancer. Cas Lek Cesk 2016; 155: 10-14.
[9] Jiang D, Shi J, Yuan M, Duan X, Li L and Li Q. Levels of serum bilirubin in small cell lung cancer and non-small cell lung cancer patients. Cell Mol Biol (Noisy-le-grand) 2018; 64: 71-76.
[10] Liu X, Meng QH, Ye Y, Hildebrandt MA, Gu J and Wu X. Prognostic significance of pretreatment serum levels of albumin, LDH and total bilirubin in patients with non-metastatic breast cancer. Carcinogenesis 2015; 36: 243-248.
[11] Sun H, He B, Nie Z, Pan Y, Lin K, Peng H, Xu T, Chen X, Hu X, Wu Z, Wu D and Wang S. A nomogram based on serum bilirubin and albumin levels predicts survival in gastric cancer patients. Oncotarget 2017; 8: 41305-41318.
[12] Gao C, Fang L, Li JT and Zhao HC. Significance and prognostic value of increased serum direct bilirubin level for lymph node metastasis in Chinese rectal cancer patients. World J Gastroenterol 2016; 22: 2576-2584.
[13] Radestad E, Klynning C, Stikvoort A, Mogensen O, Nava S, Magalhaes I and Uhlin M. Immune profiling and identification of prognostic immune-related risk factors in human ovarian cancer. Oncoimmunology 2019; 8: e1535730.
[14] Tell G and Gustincich S. Redox state, oxidative stress, and molecular mechanisms of protective and toxic effects of bilirubin on cells. Curr Pharm Des 2009; 15: 2908-2914.
[15] Zeng L, Qian J, Zhu F, Wu F, Zhao H and Zhu H. The prognostic values of matrix metalloproteinases in ovarian cancer. J Int Med Res 2019: 48: 300060519825983.
[16] Gencer S, Cebeci A and Irmak-Yazicioglu MB. Matrix metalloproteinase gene expressions might be oxidative stress targets in gastric cancer cell lines. Chin J Cancer Res 2013; 25: 322-333.
[17] Yao JJ, Kou J, Peng QH, Dong J, Zhang WJ, Lawrence WR, Zhang F, Zhou GQ, Wang SY and Sun Y. Prognostic value of serum bilirubin in southern Chinese patients with advanced nasopharyngeal carcinoma. Clin Chim Acta 2018; 484: 314-319.
[18] Deng CC, Xu M, Li J, Luo XL, Zhu YJ, Jiang R, Zhang MX, Lei JJ, Lian YF, Zou X, You R, Chen LZ, Feng QS, Bei JX, Chen MY and Zeng YX. Unconjugated bilirubin is a novel prognostic biomarker for nasopharyngeal carcinoma and inhibits its metastasis via antioxidation activity. Cancer Prev Res (Phila) 2016; 9: 180-188.
[19] Song YJ, Gao XH, Hong YQ and Wang LX. Direct bilirubin levels are prognostic in non-small cell lung cancer. Oncotarget 2018; 9: 892-900.
[20] Xu P, Ling ZL, Zhang J, Li Y, Shu N, Zhong ZY, Chen Y, Di XY, Wang ZJ, Liu L and Liu XD. Unconjugated bilirubin elevation impairs the function and expression of breast cancer resistance protein (BCRP) at the blood-brain barrier in bile duct-ligated rats. Acta Pharmacol Sin 2016; 37: 1129-1140.
[21] Yang L, Ge LY, Yu T, Liang Y, Yin Y and Chen H. The prognostic impact of serum bilirubin in stage IV colorectal cancer patients. J Clin Lab Anal 2018; 32: e22272.
[22] Zhang H, Li G, Zhu Z, Zheng Y, Wu Y, Zhang W, Gu N, Wang X and Song X. Serum bilirubin level predicts postoperative overall survival in oral squamous cell carcinoma. J Oral Pathol Med 2018; 47: 382-387.
[23] Zucker SD, Horn PS and Sherman KE. Serum bilirubin levels in the U.S. population: gender effect and inverse correlation with colorectal cancer. Hepatology 2004; 40: 827-835.
[24] Adeosun SO, Moore KH, Lang DM, Nwaneri AC, Hinds TD Jr and Stec DE. A novel fluorescence-based assay for the measurement of biliverdin reductase activity. React Oxyg Species (Apex) 2018; 5: 35-45.
[25] Weaver L, Hamoud AR, Stec DE and Hinds TD Jr. Biliverdin reductase and bilirubin in hepatic disease. Am J Physiol Gastrointest Liver Physiol 2018; 314: G668-G676.
[26] Chiddarwar AS, D'Silva SZ, Colah RB, Ghosh K and Mukherjee MB. Genetic variations in bilirubin metabolism genes and their association with unconjugated hyperbilirubinemia in adults. Ann Hum Genet 2017; 81: 11-19.
[27] Grant DJ and Bell DA. Bilirubin UDP-glucuronosyltransferase 1A1 gene polymorphisms: susceptibility to oxidative damage and cancer? Mol Carcinog 2000; 29: 198-204.
[28] Gordon DM, Adeosun SO, Ngwudike SI, Anderson CD, Hall JE, Hinds TD Jr and Stec DE. CRISPR Cas9-mediated deletion of biliverdin reductase A (BVRA) in mouse liver cells induces oxidative stress and lipid accumulation. Arch Biochem Biophys 2019; 672: 108072.
[29] Zhang Y, Ding Y, Lu T, Zhang Y, Xu N, McBride DW, Tang J and Zhang JH. Biliverdin reductase-A attenuated GMH-induced inflammatory response in the spleen by inhibiting toll-like receptor-4 through eNOS/NO pathway. J Neuroinflammation 2018; 15: 118.
[30] Konickova R, Vankova K, Vanikova J, Vanova K, Muchova L, Subhanova I, Zadinova M, Zelenka J, Dvorak A, Kolar M, Strnad H, Rimpelova S, Ruml T, J Wong R and Vitek L. Anti-cancer effects of blue-green alga Spirulina platensis, a natural source of bilirubin-like tetrapyrrolic compounds. Ann Hepatol 2014; 13: 273-283.
[31] Lee S, Lee Y, Kim H, Lee DY and Jon S. Bilirubin nanoparticle-assisted delivery of a small mole-

cule-drug conjugate for targeted cancer therapy. Biomacromolecules 2018; 19: 2270-2277.
[32] Biagioli M, Carino A, Fiorucci C, Annunziato G, Marchiano S, Bordoni M, Roselli R, Giorgio CD, Castiglione F, Ricci P, Bruno A, Faccini A, Distrutti E, Baldoni M, Costantino G and Fiorucci S. The Aryl Hydrocarbon Receptor (AhR) mediates the counter-regulatory effects of pelargonidins in models of inflammation and metabolic dysfunctions. Nutrients 2019; 11: 1820.
[33] Bock KW. Aryl hydrocarbon receptor (AHR): from selected human target genes and crosstalk with transcription factors to multiple AHR functions. Biochem Pharmacol 2019; 168: 65-70.
[34] Neavin DR, Lee JH, Liu D, Ye Z, Li H, Wang L, Ordog T and Weinshilboum RM. Single Nucleotide polymorphisms at a distance from Aryl Hydrocarbon Receptor (AHR) binding sites influence AHR ligand-dependent gene expression. Drug Metab Dispos 2019; 47: 983-994.
[35] Molzer C, Wallner M, Kern C, Tosevska A, Zadnikar R, Doberer D, Marculescu R and Wagner KH. Characteristics of the heme catabolic pathway in mild unconjugated hyperbilirubinemia and their associations with inflammation and disease prevention. Sci Rep 2017; 7: 755.
[36] Safe S, Han H, Goldsby J, Mohankumar K and Chapkin RS. Aryl Hydrocarbon Receptor (AhR) ligands as selective AhR modulators: genomic studies. Curr Opin Toxicol 2018; 11-12: 10-20.
[37] Gao Y, Cao Z, Yang X, Abdelmegeed MA, Sun J, Chen S, Beger RD, Davis K, Salminen WF, Song BJ, Mendrick DL and Yu LR. Proteomic analysis of acetaminophen-induced hepatotoxicity and identification of heme oxygenase 1 as a potential plasma biomarker of liver injury. Proteomics Clin Appl 2017; 11.
[38] Sugishima M, Wada K, Unno M and Fukuyama K. Bilin-metabolizing enzymes: site-specific reductions catalyzed by two different type of enzymes. Curr Opin Struct Biol 2019; 59: 73-80.
[39] Gonzalez-Sanchez E, Perez MJ, Nytofte NS, Briz O, Monte MJ, Lozano E, Serrano MA and Marin JJG. Protective role of biliverdin against bile acid-induced oxidative stress in liver cells. Free Radic Biol Med 2016; 97: 466-477.
[40] Mathew LG, Beattie NR, Pritchett C and Lanzilotta WN. New insight into the mechanism of anaerobic heme degradation. Biochemistry 2019; 58: 4641-4654.
[41] Shafiei F, Salari-Moghaddam A, Milajerdi A, Larijani B and Esmaillzadeh A. Coffee and caffeine intake and risk of ovarian cancer: a systematic review and meta-analysis. Int J Gynecol Cancer 2019; 29: 579-584.
[42] Zhou A, Minlikeeva AN, Khan S and Moysich KB. Association between cigarette smoking and histotype-specific epithelial ovarian cancer: a review of epidemiologic studies. Cancer Epidemiol Biomarkers Prev 2019; 28: 1103-1116.