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Association between Serum Levels of Soluble Tumor Necrosis Factor Receptors/CA 125 and Disease Progression in Patients with Epithelial Ovarian Malignancy

A Gynecologic Oncology Group Study

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BACKGROUND. A prospective study was undertaken within the Gynecologic Oncology Group to determine whether serum levels of soluble tumor necrosis factor receptors I (sTNFR-I) and II (sTNFR-II), alone or in combination with CA 125, were associated with clinicopathologic characteristics or outcome in patients with epithelial ovarian malignancies.

METHODS. Quantitative immunoassays were performed on valid pretreatment serum specimens obtained from patients with epithelial ovarian malignancies to assess levels of sTNFR-I, sTNFR-II, and CA 125. The authors then analyzed the results of these immunoassays for potential correlations with clinicopathologic characteristics and outcome.

RESULTS. The median age of the 139 women evaluated was 59 years. Seventy-eight percent had Stage III or IV disease, and 58% had serous carcinomas. sTNFR-II was

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associated with age ($P = 0.013$), and CA 125 was associated with histologic subtype ($P = 0.0009$). In addition, sTNFR-I ($P = 0.037$) and CA 125 ($P < 0.0001$) were associated with extent of disease. After adjusting for patient age, histologic subtype, and extent of disease, all three biomarkers were predictive of progression-free survival, but not overall survival, when the combination was included in the model. The authors observed a 51% reduction (hazard ratio [HR], 0.49; 95% confidence interval [CI], 0.24–0.99), a 2.9-fold increase (HR, 2.87; 95% CI, 1.15–7.20), and a 22% increase (HR, 1.22; 95% CI, 0.99–1.51) in the risk of progression for each unit increase in the log-transformed levels of sTNFR-I, sTNFR-II, and CA 125, respectively.

CONCLUSIONS. The observations made in the current study—that among patients with low or high CA 125 levels, those with high sTNFR-I levels and low sTNFR-II levels had the lowest risk, that patients with low-low or high-high sTNFR-I and sTNFR-II levels, respectively, had an intermediate risk, and that patients with low sTNFR-I levels and high sTNFR-II levels had the highest risk of progression—suggested the potential value of simultaneous assessment of all three biomarkers in patients with epithelial ovarian malignancies. ***Cancer* 2004;101:106–15.**

KEYWORDS: soluble tumor necrosis factor receptor I, soluble tumor necrosis factor receptor II, CA 125, biomarkers, ovarian carcinoma.

In 2003, the American Cancer Society estimated that 25,400 women in the United States would develop epithelial ovarian malignancies and that 14,300 patients would die of such malignancies.[1] Although the 5-year survival rates for patients diagnosed and treated for localized or regional disease were 95% and 81%, respectively,[1] the relative 5-year survival rate for patients with distant disease decreased to 31%.[1,2] The prognosis for patients with epithelial ovarian malignancy is dependent on well established clinicopathologic factors such as age, performance status, International Federation of Gynecology and Obstetrics (FIGO) disease stage, tumor grade, extent of residual disease at the completion of initial cytoreductive surgery, use of platinum-containing drugs for chemotherapy, and, in some studies, histologic cell type.[3] Yet even when accounting for the above factors, outcomes tend to be relatively unpredictable. It is likely that biologic heterogeneity contributes to the unpredictable nature of the development and progression of ovarian carcinoma.

CA 125, the most extensively studied and clinically utilized serum tumor marker for epithelial ovarian malignancy, is detectable in 80% of patients.[4] Levels of this epithelial cell surface glycoprotein antigen can be elevated in the serum before clinical development of primary and recurrent ovarian carcinoma. Despite shortcomings of suboptimal specificity and sensitivity, serum concentration of CA 125 has been used in the clinical setting to evaluate ovarian masses and to monitor disease status and response to treatment in patients with ovarian carcinoma. Data from several studies have demonstrated a relationship between prechemotherapy serum CA 125 levels and prognosis in women with epithelial ovarian malignancies.[5–8] At least two of these analyses,[5,6] however, failed to demonstrate predictive value when adjustments were made for traditional clinicopathologic factors. Furthermore, the biologic role that CA 125 plays in disease progression remains elusive.

Tumor necrosis factor (TNF) and related pleiotropic cytokines have been implicated as fundamental mediators in processes such as apoptosis, immunity, tumorigenesis, metastasis, and angiogenesis.[9] The multiple biologic effects of TNF are mediated by two cell surface TNF receptors (TNFRs)—namely, TNFR-I (or 'p55 receptor') and TNFR-II (or 'p75 receptor'), which have molecular weights of 55 kilodaltons (kD) and 75 kD, respectively.[10–12] Both TNFR-I and TNFR-II are characterized by the presence of several cysteine repeats in their extracellular domains, whereas their intracellular N-terminal domains contain no significant homology.[13] TNFR-I contains a cytoplasmic death domain and can induce apoptosis, whereas TNFR-II binds TNF receptor–associated factors, which are Zn RING/finger proteins implicated in gene regulation and antiapoptotic signaling.[9] These two receptors often are differentially expressed on the surfaces of many target cells, with TNRF-I expressed more ubiquitously and TNFR-II expressed predominantly on lymphoid cells.[14] Naylor et al.[15] examined 12 primary tumor specimens obtained from women with epithelial ovarian malignancies and showed that TNFR-I was selectively expressed in malignant epithe-

lial cells, but not in infiltrating immune cells, in all tumor specimens examined, whereas TNRF-II was expressed at the tumor-stromal interface and in the macrophages within the glandular lumina.

Shed-soluble TNF receptors I (sTNFR-I) and II (sTNFR-II), which have approximate molecular weights of 30 and 40 kD, respectively,[11,16,17] were identified in urine[18–22] as natural inhibitors of TNF that appear to function by competing for TNF and preventing cytokine-mediated activation of cell surface TNFR-I and TNFR-II.[18–21,23] Circulating sTNFR-I and sTNFR-II are generated from the cleavage of the 55 kD and 75 kD cell surface TNF receptors TNFR-I and TNFR-II, respectively. Like other soluble cytokine receptors, sTNFR are believed to result from limited proteolysis of membrane-bound precursors. Metalloproteases have been implicated in this process.[24–27] The extracellular domains of TNFR-I and TNFR-II are proteolytically cleaved to produce 30 kD (sTNFR-I) and 40 kD (sTNFR-II) soluble fragments, respectively. "Shedding" can be induced by a variety of cytokines,[28,29] including TNF itself,[30] as well as agents such as retinoids, thiols, and phorbol esters,[26,31] in vitro. A number of laboratories have identified elevated levels of sTNFR-I and sTNFR-II in the biologic fluids of patients with a variety of solid malignancies,[32–34] including epithelial ovarian tumors.[35–38] The principal objective of the current study was to prospectively investigate the relationship of pretreatment serum levels of sTNFR-I, sTNFR-II, and CA 125, individually or in combination with each other, with outcome in women with epithelial ovarian malignancies who were treated in Gynecologic Oncology Group (GOG) Phase III trials.

MATERIALS AND METHODS

Study Description

GOG Protocol 148 was designed as a double-blind study comprising 165 women with all FIGO stages of epithelial ovarian malignancy. Protocol eligibility required a satisfactory pretreatment serum specimen to have been obtained from patients enrolled in a randomized Phase III treatment trial conducted by the GOG between 1994 and 1998. Patients with autoimmune diseases and those receiving immunosuppressive therapy for a condition other than their malignancy or a condition predating the development of their malignancy were ineligible. Although the study initially recruited patients from two Phase III protocols, it was necessary to open enrollment to six such trials (GOG Protocols 114, 132, 152, 157, 158, or 162) to meet the cohort size requirement. Each of these randomized Phase III treatment protocols used similar clinical evaluation and management schemes, including comparable staging and surgical procedures, platinum-based chemotherapy, and clinical follow-up methods. Two patients enrolled in Protocol 114 (for "optimal" FIGO Stage III disease) who were found to have earlier-stage tumors on pathologic review and were excluded from the treatment protocol were included in the current biomarker study. Approval for both GOG 148 and the concomitant treatment protocol was obtained from the institutional review boards at all participating GOG institutions in accordance with assurances filed with and approved by the U.S. Department of Health and Human Services. In addition, signed informed consent was obtained from the women enrolled in GOG 148 to participate in the current serum biomarker protocol as well as the frontline treatment protocol.

Serum Collection Procedure

The protocol required that the pretreatment serum specimen be collected after initial exploratory laparotomy was performed for staging and/or surgical cytoreduction but before the initiation of frontline chemotherapy. For each patient, peripheral whole blood specimens were collected in serum separator tubes, and the serum sample was recovered and either immediately shipped with a cold pack to the laboratory at the University of California–Irvine Medical Center (Orange, CA) or stored at −20 °C until shipment. Upon receipt, the serum sample was thawed, split into aliquots, and stored at −80 °C. A satisfactory serum specimen was obtained from 139 (84%) of the 165 women who were enrolled in GOG 148. There were 26 (16%) patients for whom a satisfactory pretreatment serum specimen was not provided; i.e., the specimens were not obtained as specified by the protocol ($n = 23$), the specimens were contaminated with hemolyzed blood ($n = 2$), or the specimens were obtained 1 day after the initiation of primary chemotherapy ($n = 1$).

Quantification of the sTNFR-I and sTNFR-II Levels in Serum Specimens Using an Enzyme-Linked Immunosorbent Assay

Aliquots of the pretreatment serum specimens were analyzed to quantify the level of sTNFR-I or sTNFR-II using a previously described enzyme-linked immunosorbent assay (ELISA).[36] Sample absorbance in individual wells was analyzed at 405 nm using an EAR AT ELISA plate reader (SLT-Lab Instruments, Salzburg, Austria). Concentrations of sTNFR-I or sTNFR-II (in pg/mL) then were interpolated based on the results of the standard samples run on the same plates. The lower limit of detection for the sTNFR-I and sTNFR-II assays was 20 pg/mL. Samples and stan-

dards were evaluated in duplicate, and < 10% variability was observed between duplicate samples.

Quantification of CA 125 Levels in Serum Samples Using a Radioimmunoassay

An aliquot of each patient's pretreatment serum specimen was analyzed to quantify the level of CA 125 using a commercially available CA 125 II radioimmunoassay according to the manufacturer's instructions, including recommendations for quality assurance (Centocor). Radioactivity in individual wells was counted for 1 minute using a well-type gamma counter. Serum sample levels (in units [U] per milliliter) were calculated using a standard curve. Samples and standards were evaluated in duplicate, and assays of individual samples were repeated as above for cases in which the standard error was > 10%.

Statistical Methods

Biomarker and clinical data were analyzed using SPSS (Version 10.0; SPSS Inc., Chicago, IL) and SAS software (Version 8.0; SAS, Cary, NC). Levels of sTNFR-I (in pg/mL), sTNFR-II (in pg/mL), and CA 125 (in U/mL) were evaluated using a natural log (ln) transformation to better approximate a normal distribution for each marker. The associations between serum biomarker levels and clinical variables were evaluated using an analysis of variance,[39] and the Tukey procedure was used for multiple comparisons of means. Effects of the biomarkers on progression-free survival (PFS) and overall survival were evaluated using Cox proportional hazards regression models.[40] The likelihood ratio test was used to evaluate the goodness of fit of each of the overall models, and the Wald test was performed to assess the association between individual covariates and outcome. Hazard ratios were estimated based on the fitted Cox model, and the survival distribution for the Cox model was estimated by entering a specific value for each variable included in the fitted model, as described by Kalbfleisch and Prentice.[41] PFS was calculated as the time in months from enrollment in the frontline treatment protocol to disease progression or death in the case of noncensored events, or to the date of last contact in the case of censored events (i.e., for patients who were alive with no evidence of disease progression). Overall survival was calculated as the time from enrollment in the treatment protocol to death in the case of noncensored events, or to the date of last contact in the case of censored events (i.e., for patients who were alive, regardless of disease status).

Associations of the pretreatment serum levels of sTNFR-I, sTNFR-II, and CA 125 with each other were assessed using sample (Pearson) correlation coefficients. There was a strong positive correlation between sTNFR-I and sTNFR-II (correlation coefficient, 0.84), and there were weak but statistically significant correlations between CA 125 and sTNFR-I (correlation coefficient, 0.35), and between CA 125 and sTNFR-II (correlation coefficient, 0.22). Due to the observed correlations among the serum biomarkers, additional statistical analyses were performed in which the raw concentration of each biomarker was natural log transformed and standardized. These analyses demonstrated that the observed predictive value, which was observed when all three biomarkers were included in the model at the same time, did not result from an overinflated variance that was attributable to the multicollinearity among these biomarkers (data not shown).

A detailed exploratory analysis was performed to determine whether there was any potential clinical value associated with expressing pretreatment serum levels of sTNFR-I and sTNFR-II as categoric, rather than continuous, variables. Although this analysis confirmed the clinical value of categorizing pretreatment levels of CA 125 using a cutoff of 30–35 U/mL, there was no evidence to suggest that either sTNFR-I or sTNFR-II should be categorized using the median, the mean, or 2 times the upper limit of normal as a cutoff (data not shown). In the current analysis, all three biomarkers were evaluated as continuous variables.

RESULTS

Among the 139 eligible patients participating in the current serum biomarker study, the median age at protocol entry was 59 years. Eighty-seven percent of patients were Caucasian, 78% had FIGO Stage III or Stage IV disease, and 58% had serous carcinomas (Table 1). All patients were treated in randomized Phase III clinical trials for epithelial ovarian malignancy—31 (~22%) for FIGO Stage I or II disease, 75 (~54%) for optimal FIGO Stage III tumors, and 33 (~ 24%) for suboptimal Stage III or IV carcinoma. Table 2 shows the distribution of patients randomized to the different GOG frontline treatment trials. All patients received a combination of a platinum compound and paclitaxel as frontline adjuvant therapy, except for three patients (enrolled in GOG 132) who received either a platinum compound or paclitaxel alone.

The first hypothesis tested in the current study was that biomarker levels would be associated with specific clinicopathologic characteristics. Table 3 indicates that advanced age was associated with higher sTNFR-II levels ($P = 0.013$), but not with sTNFR-I or CA 125 levels. In contrast, none of the three markers was associated with initial performance status assessed using the Zubrod scoring system (Table 3) or

TABLE 1
Characteristics of Eligible Patients

Characteristic	No. of patients (%)
Age (yrs)	
< 50	42 (30.2)
50–59	40 (28.8)
60–69	38 (27.3)
> 70	19 (13.7)
Race/ethnicity	
White	121 (87.1)
Black	7 (5.0)
Hispanic	6 (4.3)
Other	5 (3.6)
FIGO stage and debulking status	
I	21 (15.1)
II	10 (7.2)
III, optimally debulked	75 (54.0)
III, suboptimally debulked	22 (15.8)
IV, suboptimally debulked	11 (7.9)
Tumor grade	
1	17 (12.2)
2	41 (29.5)
3	81 (58.3)
Histologic cell type	
Serous adenocarcinoma	81 (58.3)
Endometrioid adenocarcinoma	23 (16.5)
Clear cell carcinoma	11 (7.9)
Mucinous adenocarcinoma	8 (5.8)
Other[a]	16 (11.5)

FIGO: International Federation of Gynecology and Obstetrics.
[a] Includes nine patients with mixed epithelial carcinoma; five patients with adenocarcinoma, unspecified; and two patients with undifferentiated carcinoma.

with tumor grade (Table 4). Compared with other histologic subtypes, serous subtype was associated with higher serum levels of CA 125 ($P = 0.0009$), but not with levels of sTNFR-I or sTNFR-II. To assess the relationship between serum biomarker levels and tumor burden, FIGO stage and the amount of macroscopic residual disease after primary cytoreductive surgery were combined into one variable, which was called *FIGO stage–debulking status*. Patients were categorized as having early-stage disease (Stage I or II disease, in no case associated with macroscopic residual disease); optimally debulked advanced-stage disease (Stage III disease in which the maximum diameter of the largest macroscopic residual tumor implant after primary cytoreductive surgery was < 1 cm); or suboptimally debulked advanced-stage disease (Stage III disease in which the maximum diameter of the largest macroscopic residual tumor implant was > 1 cm, or Stage IV disease). As shown in Table 4, the progression from early-stage disease to optimally debulked advanced-stage disease to suboptimally debulked advanced-stage disease was associated with higher pretreatment serum levels of sTNFR-I ($P = 0.037$) and CA 125 ($P < 0.0001$), but not with sTNFR-II.

The primary hypothesis of the current study was that serum levels of sTNFR-I and sTNFR-II, alone or in combination with levels of CA 125, predict patient outcome. Unadjusted and adjusted Cox proportional hazard regression models were used to determine the predictive value of pretreatment levels of sTNFR-I, sTNFR-II, and/or CA 125 for PFS and overall survival. As shown in Table 5, the use of unadjusted models demonstrated that the pretreatment levels of sTNFR-I and sTNFR-II were not associated with PFS or overall survival. In contrast, pretreatment levels of CA 125 were significantly associated with worse PFS (hazard ratio [HR], 1.36; confidence interval [CI], 1.14–1.61), but not with worse overall survival. After adjusting for the prognostic clinicopathologic covariates, none of the serum biomarkers was significantly associated with PFS or overall survival when the serum biomarkers were individually included in the model. Simultaneous inclusion of all three biomarkers in both the unadjusted and adjusted Cox models revealed that sTNFR-I, sTNFR-II, and CA 125 were associated with risk of disease progression (Table 5). Most notably, after adjusting for age, extent of disease, and histologic subtype, we observed a 51% reduction (HR, 0.49; CI, 0.24–0.99), a 2.9-fold increase (HR, 2.87; CI, 1.15–7.20), and a 22% increase (HR, 1.22; CI, 0.99–1.51) in the relative risk of progression for each unit increase in the log-transformed levels of sTNFR-I, sTNFR-II, and CA 125, respectively. The value of this combination of serum biomarkers in predicting overall survival, however, was not observed (Table 5).

Due to the inherent challenge of visualizing the clinical significance of hazard ratios associated with continuous variables, simulation hazard plots were generated to illustrate the combined impact on PFS of the serum levels of sTNFR-I and sTNFR-II in patients with low or high serum CA 125 levels. The adjusted survival plots in Figure 1 illustrate that high sTNFR-I levels or low sTNFR-II levels were associated with significant prolongation of PFS; this effect was magnified when both conditions were met, even after adjustment for clinicopathologic variables. In contrast, low sTNFR-I levels or high sTNFR-II levels were associated with significant decreases in PFS; this effect also was magnified when both conditions were met. This relationship of sTNFR-I and sTNFR-II to PFS was robust, in that after stratifying by CA 125 levels, the same reciprocal relationship of sTNFR-I and sTNFR-II with PFS was maintained; an elevation in CA 125 simply shifted all PFS curves to the left. Progression-free survival was consistently better for patients with high

TABLE 2
Distribution of GOG 148 Patients According to Protocol and Type of Therapy

	FIGO stage–debulking status					
	High-risk early-stage	Optimally debulked stage III		Suboptimally debulked Stage III/IV		
GOG treatment protocol	157	114	158	132	152	162
Cisplatin				2		
Paclitaxel				1		
Cisplatin + paclitaxel (24-hour infusion)		8	30	2	19	4
Cisplatin + paclitaxel (96-hour infusion)						5
Carboplatin + paclitaxel (three cycles)	12					
Carboplatin + paclitaxel (six cycles)	17		25			
Carboplatin/cisplatin + paclitaxel		14				
Total	29	22	55	5	19	9

GOG: Gynecologic Oncology Group; FIGO: International Federation of Gynecology and Obstetrics.

TABLE 3
Association between Serum Biomarker Levels and Patient Characteristics

		ln (sTNFR-I)		ln (sTNFR-II)		ln (CA 125)	
Characteristic	No. of patients	Mean ± SD	*P* value	Mean ± SD	*P* value	Mean ± SD	*P* value
Age (yrs)			0.075		0.013		0.980
< 55	59	7.89 ± 0.53		7.76 ± 0.38		4.76 ± 1.18	
≥ 55	80	8.07 ± 0.59		7.94 ± 0.42		4.75 ± 1.14	
Zubrod performance status[a]			0.125		0.317		0.191
Asymptomatic (score 0)	61	7.91 ± 0.58		7.82 ± 0.43		4.61 ± 1.08	
Symptomatic (score 1 or 2)	78	8.06 ± 0.56		7.89 ± 0.41		4.87 ± 1.20	

sTNFRI/II: soluble tumor necrosis factor receptor I/II; SD: standard deviation.

[a] An initial Zubrod performance score of 0 indicates that the patient was asymptomatic, whereas a score of 1 or 2 indicates that the patient was symptomatic and either fully ambulatory (score 1) or in bed < 50% of the time (score 2).

sTNFR-I and low sTNFR-II, and worse for low sTNFR-I and high sTNFR-II, compared with patients who had either high sTNFR-I and high sTNFR-II or low sTNFR-I and low sTNFR-II. This pattern was observed in all patients regardless of age, stage-debulking status, and histologic subtype.

DISCUSSION

The results of the current prospective multicenter cooperative group study suggest that distinct relationships exist between baseline serum levels of sTNFR-I or sTNFR-II compared with CA 125 and historically important clinical and pathologic variables in women with all stages of epithelial ovarian malignancy. Although none of the three markers investigated was associated with initial performance status or tumor grade, sTNFR-II was associated with age, sTNFR-I and CA 125 were associated with extent of disease (an aggregate of FIGO stage and amount of macroscopic residual disease after primary cytoreductive surgery), and CA 125 was associated with histologic subtype. In contrast, Gadducci et al.[36] reported that serum levels of sTNFR-I and sTNFR-II were both associated with clinical evidence of disease but not with the amount of residual disease after primary surgery, whereas Onsurd et al.[37] demonstrated that sTNFR-I and sTNFR-II levels were correlated with extent of disease as measured by disease stage or by positive ascites. Gadducci et al.[36] also found no association between sTNFR-I or sTNFR-II levels and tumor grade.

The current serum biomarker study also provided compelling evidence for a more refined risk assessment model for ovarian cancer progression when the serum concentrations of all three biomarkers are simultaneously included in unadjusted or adjusted Cox regression analyses. It is generally accepted that patients with CA 125 levels in the lowest percentile (≤ 50 U/mL) tend to have a more favorable outcome than

TABLE 4
Association between Serum Biomarker Levels and Surgicopathologic Characteristics

Characteristic	No. of patients	ln (sTNFR-I)		ln (sTNFR-II)		ln (CA 125)	
		Mean ± SD	*P* value	Mean ± SD	*P* value	Mean ± SD	*P* value
Tumor grade			0.967		0.900		0.565
1	17	7.96 ± 0.52		7.86 ± 0.42		4.48 ± 1.14	
2	41	8.00 ± 0.57		7.89 ± 0.41		4.75 ± 1.15	
3	81	8.00 ± 0.59		7.85 ± 0.42		4.81 ± 1.16	
Histologic subtype			0.197		0.342		< 0.0009
Serous	81	8.03 ± 0.53		7.89 ± 0.40		5.00 ± 1.05[a]	
Clear cell or mucinous	19	7.77 ± 0.65		7.73 ± 0.47		3.94 ± 0.99[a]	
Other	39	8.02 ± 0.60		7.87 ± 0.41		4.64 ± 1.25	
FIGO stage–debulking status			0.037		0.173		< 0.0001
I/II	31	7.79 ± 0.60[a]		7.77 ± 0.42		3.92 ± 0.94[a]	
III, optimal debulking	75	8.01 ± 0.54	7.85 ± 0.40		4.74 ± 1.03[a]		
III/IV, suboptimal debulking	33	8.15 ± 0.57[a]		7.96 ± 0.43		5.58 ± 1.03[a]	

SD: standard deviation; sTNFR-I/II: soluble tumor necrosis factor receptor I/II; FIGO: International Federation of Gynecology and Obstetrics.
[a] Comparison of the means between indicated groups was significant at the $P < 0.05$ level.

TABLE 5
Association between Pretreatment Serum Biomarker Levels and Patient Outcome

Characteristic	Progression-free survival				Overall survival			
	Unadjusted for covariates		Adjusted for covariates[a]		Unadjusted for covariates		Adjusted for covariates[a]	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
sTNFR-I model								
ln (sTNFR-I)	1.21 (0.85–1.72)	0.304	1.06 (0.73–1.53)	0.770	1.23 (0.80–1.87)	0.343	1.03 (0.66–1.61)	0.904
sTNFR-II model								
ln (sTNFR-II)	1.49 (0.92–2.41)	0.108	1.37 (0.82–2.30)	0.228	1.44 (0.81–2.54)	0.216	1.26 (0.68–2.33)	0.460
CA 125 model								
ln (CA 125)	1.36 (1.14–1.61)	< 0.001	1.15 (0.94–1.40)	0.174	1.19 (0.97–1.47)	0.094	0.93 (0.74–1.18)	0.571
Combined model with sTNFR-I, sTNFR-II, and CA 125								
ln (sTNFR-I)	0.55 (0.29–1.04)	0.066	0.49 (0.24–0.99)	0.047	0.80 (038–1.69)	0.552	0.78 (0.36–1.68)	0.524
ln (sTNFR-II)	2.43 (1.08–5.48)	0.032	2.87 (1.15–7.20)	0.024	1.65 (0.63–4.31)	0.304	1.72 (0.62–4.78)	0.301
ln (CA 125)	1.42 (1.18–1.71)	< 0.001	1.22 (0.99–1.51)	0.065	1.19 (0.96–1.49)	0.120	0.94 (0.73–1.21)	0.623

CI: confidence interval; sTNFR-I/II: soluble tumor necrosis factor receptor I/II.
[a] Adjusted hazard ratios are controlled for patient age (< 55 years vs. ≥ 55 years), histologic subtype (serous adenocarcinoma vs. clear cell carcinoma or mucinous adenocarcinoma vs. other histologic subtypes), and an aggregate stage-debulking variable (Stage I or II vs. optimally debulked Stage III vs. suboptimally debulked Stage III or IV epithelial ovarian malignancy). All models with adjusted variables were significant at a level of $P < 0.001$.

do patients with CA 125 levels in the highest percentile (≥ 242 U/mL). The current study shows that among patients with low or high CA 125 levels, those with sTNFR-I levels in the highest quartile (≥ 4172 pg/mL) and sTNFR-II levels in the lowest quartile (≤ 2009 pg/mL) had the lowest risk of disease progression, whereas patients with sTNFR-I levels in the lowest quartile (≤ 2028 pg/mL) and sTNFR-II levels in the highest quartile (≥ 3387 pg/mL) had the highest risk. Patients with low-low or high-high sTNFR-I and sTNFR-II levels respectively had an intermediate risk. Specific levels were used to illustrate the predictive value of these markers for patients with a particular profile. However, these arbitrary cutoff values are not critical, because each of these serum biomarkers provides distinct prognostic information for each unit increase in (log-transformed) concentration.

To our knowledge, only a limited number of studies have been published that have evaluated the potential prognostic value of the sTNFRs in women with ovarian carcinoma.[35,36] Grosen et al.[35] demonstrated that sTNFRs were more sensitive than CA 125 in de-

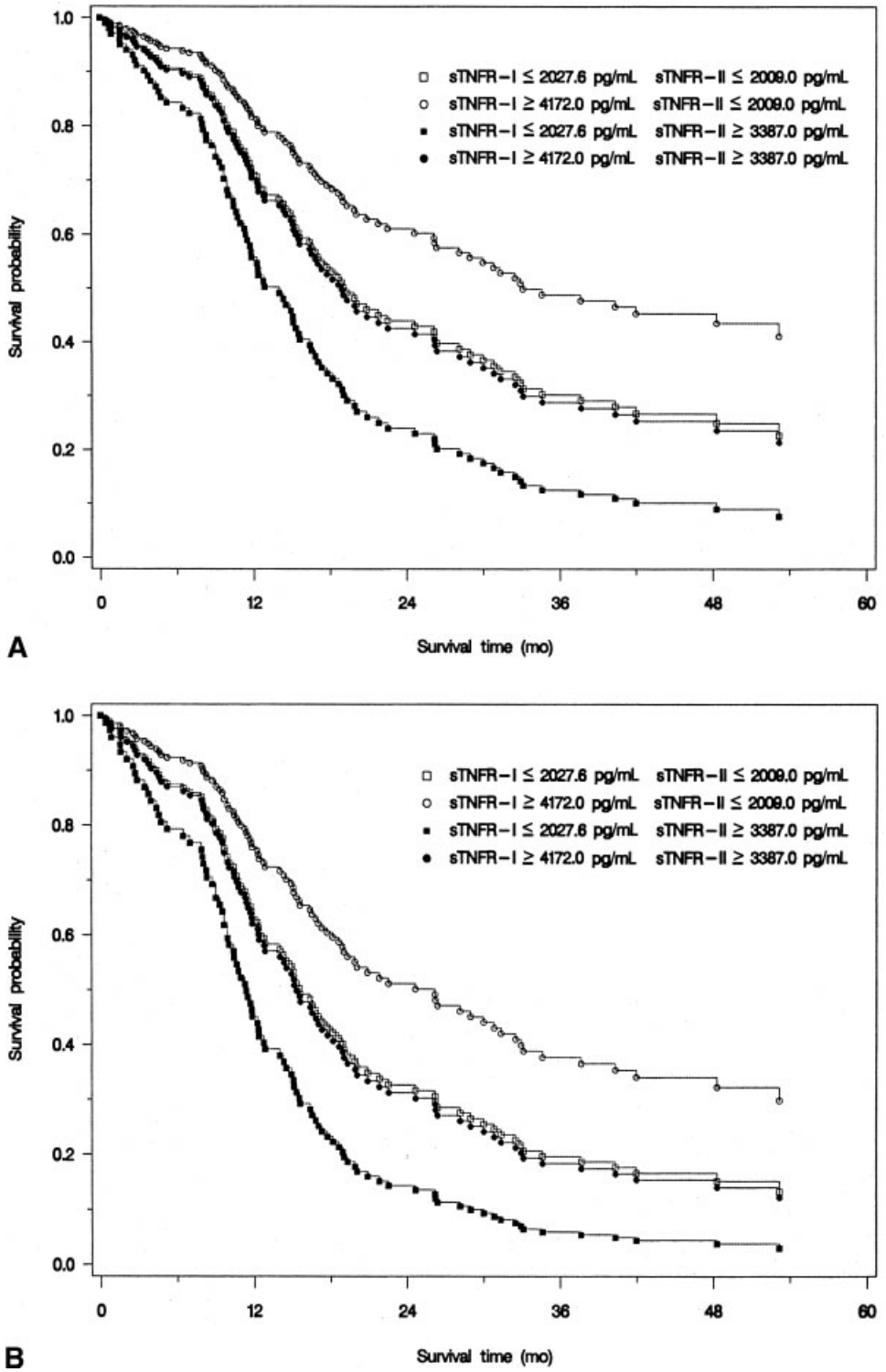

FIGURE 1. Estimated progression-free survival (PFS) curves for patients with specified serum levels of soluble tumor necrosis factor receptor I (sTNFR-I), soluble tumor necrosis factor receptor II (sTNFR-II), and CA 125 conditioned on patient age, stage–debulking status, and histologic subtype. Patients were age $\geq$ 55 years with optimally debulked Stage III serous papillary adenocarcinoma of the ovary and CA 125 levels of (A) $\leq$ 5.15 units (U) per milliliter (lowest quartile) or (B) $\geq$ 242.4 U/mL (highest quartile). The sTNFR-I cutoff level was set at 2027.6 pg/mL (25th percentile) or 4172 pg/mL (75th percentile). The sTNFR-II cutoff level was set at 2009 pg/mL (25th percentile) or 3387 pg/mL (75th percentile). Individual serum specimens exhibited $<$ 10% variability in the quantification of sTNFR-I and sTNFR-II.

tecting disease status and measuring response to therapy, but this component of a larger retrospective biomarker study was performed using banked serum specimens from 14 patients with epithelial ovarian tumors who had been clinically followed for 1–3 years. Gadducci et al.[36] showed that patients who died of ovarian carcinoma progression or who were alive with clinical evidence of disease 2 years after primary surgery and frontline treatment with a platinum-based regimen had higher serum levels of sTNFR-I or

sTNFR-II than did patients who were alive without evidence of disease. It is difficult to directly compare the results presented in the current study with those reported by Gadducci et al.,[36] due to the following differences in study design: type of study (prospective vs. retrospective), biomarker data (log-transformed concentrations vs. nontransformed concentrations), outcome data (detailed time-to-event PFS and overall survival data with $\leq$ 9 years of follow-up vs. PFS data at a single time point), cohort size ($n = 139$ vs. $n = 24$), and statistical analysis methods (unadjusted and adjusted Cox regression modeling vs. Student t test).

The current study was not designed to demonstrate a causal link between the levels of these three serum biomarkers (sTNFR-I, sTNFR-II, and CA 125) and PFS, but it was able to provide suggestive evidence that the serum levels of sTNFR-I and sTNFR-II predict risk of disease progression in patients with ovarian carcinoma who have low or high levels of CA 125. The biological mechanisms that are operating in these patients to cause the differential serum levels of these three biomarkers and alter the risk of progression may be a challenge to unravel. For example, Gatanaga et al.[28] observed that exposure of ovarian tumor cells to the cytokines interleukin-1β and interferon gamma, but not interleukin-4, caused modest increases in sTNFR-I levels over the levels associated with spontaneous release, as well as dramatic increases in sTNFR-II levels. This observation suggests that selective mechanisms exist for the generation of sTNFR-I compared with sTNFR-II in vivo. In addition, the biologic and/or clinical characteristics correlated with the levels of these soluble receptors in patients with epithelial ovarian malignancies are likely to be influenced by the cell type–selective distribution of TNFR-I and TNFR-II, whose distinct signaling pathways result in the induction of diverse cellular responses. These characteristics also are likely to be affected by levels of sTNFR-I and sTNFR-II and the affinity of these molecules for their ligands, including (but possibly not limited to) TNF and lymphotoxin. Perhaps high serum levels of sTNFR-I predict a subset of advanced lesions that are more apt to respond to cytotoxic therapy that is in keeping with a susceptibility to apoptosis hypothesis whereas high serum levels of sTNFR-II may indicate an impairment of cell-mediated anti-tumor immunologic mechanisms. The relation between serum sTNFR-I and sTNFR-II levels and disease outcome also could be mediated by other processes, such as tumorigenesis, metastasis, and/or angiogenesis, that are known to be regulated by TNF superfamily molecules and their receptors.[9,42]

Use of the three independent serum tumor markers investigated together with traditional predictive variables may enable refinement of the current staging system for ovarian carcinoma; this possibility merits further study. The identification of molecular markers that predict prognosis independently of known clinicopathologic factors may have clinical utility with respect to "molecular staging" of disease and may also provide clues regarding the identities of the critical biologic factors that drive the disease process. Such information will allow refinements in primary prevention, early detection, and therapeutic strategies, which could result in the use of tailored treatment not only to improve 5-year survival rates and quality of life but also to prevent suffering and death due to ovarian carcinoma. Therefore, additional research, including a validation study involving a larger cohort, is needed to establish the combined role of these three serum biomarkers in the management of patients with epithelial ovarian malignancies.

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