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Chemotherapy-induced neutropenia as a biomarker of survival in advanced ovarian carcinoma: An exploratory study of the Gynecologic Oncology Group ☆

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HIGHLIGHTS

- Chemotherapy-induced neutropenia has emerged as an important biomarker in several solid tumors.
- Chemotherapy-induced neutropenia may be a biomarker for survival in untreated advanced ovarian cancer.
- Absence of chemotherapy-induced neutropenia may reflect under-dosing and ultimately attenuated antineoplastic effect.

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ABSTRACT

Objective. To determine whether chemotherapy-induced neutropenia (C-iN) is associated with improved survival in a population of primary advanced ovarian cancer and peritoneal carcinoma patients treated with a carboplatin plus paclitaxel chemotherapy backbone.

Methods. A post-hoc exploratory analysis of Gynecologic Oncology Group (GOG) protocol 182 was performed. Landmark analysis was conducted on all patients with progression-free survival >18 weeks from the time of study entry. Neutropenia was defined as the absolute neutrophil count <1000 mm^3. The occurrence of C-iN was analyzed according to demographic, clinicopathologic, and therapeutic intent, including age, body surface area, and treatment arm. Progression-free survival (PFS) and overall survival (OS) were estimated using the Kaplan–Meier method. The Cox proportional hazards model was used to evaluate independent prognostic factors and to estimate their effects on PFS and OS.

Results. Neutropenic data was available for 3447 patients. Neutropenic (n = 3196) and non-neutropenic groups (n = 251) were similar in demographic and clinicopathologic characteristics. Neutropenic patients experienced significantly improved survival compared to non-neutropenic patients with the adjusted hazard ratio (HR) for death being 0.86 (95% confidence interval 0.74–0.99; p = 0.041). There was no survival benefit associated with any of the treatment arms among patients with C-iN.

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Conclusion. These data suggest that C-iN may represent a clinical biomarker associated with a survival advantage for patients with untreated advanced ovarian cancer. The absence of C-iN may indicate under-dosing and ultimately attenuated anti-neoplastic effect in vulnerable populations.

Introduction

Current chemotherapy regimens are associated with numerous potential toxicities, with the incidence of hematologic toxicities, such as neutropenia, varying greatly with the particular regimen. In 2006, the American Society of Clinical Oncology (ASCO) and more recently, in 2011, the European Organization for Research and Treatment of Cancer (EORTC) released updated guidelines on the use of hematopoietic colony-stimulating factors. In each publication, a comprehensive review of chemotherapy regimens used in current practice with the corresponding risk of neutropenia was reviewed [1,2]. Carboplatin plus paclitaxel, the standard adjuvant regimen for the treatment of ovarian carcinoma, carries approximately a 30–90% risk of neutropenia.

Gynecologic Oncology Group protocol 182 (GOG 182) was an international phase 3 randomized trial for advanced ovarian and peritoneal carcinoma conducted during the current platinum–taxane era from February 2001 to September 2004 [3]. The objectives of the study were to determine whether the incorporation of an additional cytotoxic agent improves overall survival (OS) and progression-free survival (PFS). The carboplatin plus paclitaxel doublet was included in each of the five study arms which were designed to administer 8 cycles of chemotherapy each. The four experimental arms tested gemcitabine, pegylated liposomal doxorubicin, or topotecan as a chemotherapy triplet and/or sequential doublet. An interim analysis was triggered when the pre-planned 272 events occurred on the reference arm, and the study was closed with 4312 patients enrolled. There were no improvements in either OS or PFS associated with any experimental regimen [3]. The carboplatin–paclitaxel–gemcitabine triplet was associated with increased grade 4–5 neutropenia.

The relationship between toxicity and response is not well understood. Because body surface area (BSA) dosing does not account for the complex processes of cytotoxic drug elimination, an unpredictable variation in effect may occur leading to easily recognized over-dosing as well as under-dosing, the latter resulting in significantly reduced anti-cancer effect. For this reason, some investigators advocate a "toxicity adjusted dose" in which drug-specific toxicity may be used as a biomarker for accurate dosing [4]. Because GOG-182 contains the largest number of primary advanced ovarian/peritoneal carcinoma that have been prospectively treated with the current standard chemotherapy regimen of carboplatin plus paclitaxel, we chose to use this study to test the hypothesis that chemotherapy-induced neutropenia may serve as a clinical biomarker for survival in this population.

Methods

Background on GOG protocol 182 methodology & study parameters

A post-trial exploratory analysis was performed on data from patients enrolled and treated through GOG, the primary group participating in the study. Eligibility criteria included a histologic diagnosis of epithelial ovarian cancer or primary peritoneal carcinoma, International Federation of Gynecologists and Obstetricians (FIGO) stage III or IV, with either optimal (≤1 cm residual disease) or suboptimal residual disease following initial surgery [3]. Patients with ovarian tumors of low malignant potential as well as those who had received prior chemotherapy for any abdominal or pelvic tumor were excluded. Patients must have had adequate bone marrow function with an absolute neutrophil count (ANC) greater than or equal to 1500/μL (equivalent to Common Toxicity Criteria v.3 grade 1).

Carboplatin AUC 6 and paclitaxel 175 mg/m^2 every 21 days for 8 cycles constituted the control arm. This dosing for the carboplatin–paclitaxel backbone was also used in the investigational arms, with minor modifications to equilibrate anticipated toxicity and provide adequate exposure to experimental regimens. Sequential doublets utilized 4 cycles of the experimental regimen (gemcitabine 1000 mg/m^2 d1, 8 plus carboplatin AUC 6, d8 or topotecan 1.25 mg/m^2 d1–3 plus carboplatin AUC 5, d3) followed by 4 cycles of carboplatin–paclitaxel with dosing equivalent to the control arm [3]. For the remaining two investigational arms, carboplatin AUC 5 plus paclitaxel 175 mg/m^2 was incorporated into a triplet containing either gemcitabine 800 mg/m^2 d1, 8 or pegylated liposomal doxorubicin 30 mg/m^2 d1 every other cycle. For patients with an abnormally low serum creatinine, the creatinine clearance for carboplatin dosing was estimated using a minimal value of 0.6 mg/dl. The maximum body surface area (BSA) used for dose calculations was 2.0 m^2 for all treatment arms [3]. When feasible, the dose, schedule, and sequence of drug administration was adjusted for each treatment arm to approach uniform anticipated hematologic toxicity across the entire study population, based on available data from phase I trials.

Table 1
Eligible patient demographics and clinical characteristics.

	N	
Age years	3447	51.2[a] 58.8[b] 66.5[c]
Race/ethnicity	3447	
White		90.5% (3119)
Black		4.2% (146)
Other		5.3% (182)
Performance status	3447	
Normal, asymptomatic		48.6% (1674)
Symptomatic, ambulatory		45.1% (1553)
Symptomatic, in bed		6.4% (220)
Top-level FIGO stage	3447	
III		86.2% (2972)
IV		13.8% (475)
Histology	3447	
Serous		83.3% (2871)
Clear cell/mucinous		3.5% (121)
Other		13.2% (455)
CA-125 μg/mL	3348	85[a] 205[b] 531[c]
Ascites	3361	
No		26.1% (877)
Yes		73.9% (2484)
Tumor residual	3447	
Microscopic		24.6% (847)
Optimal (0.1–1 cm)		48.1% (1659)
Suboptimal (>1 cm)		27.3% (941)
Interval cytoreduction	3447	
No		96.6% (3330)
Yes		3.4% (117)
Treatment	3447	
CP		20.2% (698)
CPG		20.0% (689)
CPD		19.8% (683)
CT → CP		19.9% (685)
CG → CP		20.1% (692)
Myelosuppression	3447	
Non-neutropenic		7.3% (251)
Neutropenic		92.7% (3196)

a, *b* and *c* represent the lower quartile *a*, the median *b*, and the upper quartile *c* for continuous variables.
N is the number of non-missing values.
Numbers after percents are frequencies.

Patients were not permitted to receive prophylactic hematopoietic cytokines such as filgrastim (G-CSF), PEG-filgrastim (Neulasta), or sargramostim (GM-CSF) unless they experienced treatment delays or recurrent neutropenic complications after treatment modifications. Importantly, hematopoietic growth factors were not permitted to avoid initial chemotherapy dose modifications as stipulated in the protocol.

Dose-limiting neutropenia (DLT-ANC) was defined by the occurrence of febrile neutropenia or prolonged grade 4 neutropenia persisting ≥7 days. Febrile neutropenia was defined as fever of unknown origin without clinically or microbiologically documented infection with ANC <1000 cells/mm^3 and fever ≥38.5 °C [3]. The first occurrence of DLT-ANC in the absence of dose-limiting thrombocytopenia (DLT-PLT) was to be managed by one-level reduction in drug dosages as outlined in the protocol, with G-CSF administered with the second occurrence. When DLT-ANC was accompanied by DLT-PLT, the first occurrence was managed by one-level reduction in dosages and the second occurrence with the addition of G-CSF and decreasing carboplatin AUC by one unit.

Ancillary data analysis

In this exploratory analysis, demographic and clinicopathologic data on GOG-enrolled subjects on each of the five treatment arms were collected. The single exclusion criterion was missing data on minimum ANC. Neutropenia was defined as a nadir value of ANC <1000/mm^3 at a chemotherapy cycle (of any duration). Landmark analysis was performed on patients with progression-free survival >18 weeks from the time of study entry. Landmark analysis is a type of survival analysis that classifies patients according to some intermediate, non-outcome event that is nevertheless a response to treatment. In a landmark analysis, the starting point for measuring survival is moved from a patient's study entry to some later time when the event of interest has been observed in most patients. The landmark point of 18 weeks was selected because 18 weeks was the 99th quantile of time to a neutropenic event on the carboplatin–paclitaxel control arm of another GOG phase III trial in this population (protocol 218).

Categorical variables were compared between the myelosuppression groups by the Pearson chi-square test, and continuous variables by the Wilcoxon–Mann–Whitney test [5,6]. Progression-free survival (PFS) and overall survival (OS) were estimated using the Kaplan–Meier method [7]. The stratified Cox proportional hazards model (stratified by treatment arm of protocol 182) was used to evaluate independent prognostic factors and to estimate their covariate-adjusted effects on PFS and OS [8]. Because approximately 5% of the patients had at least one prognostic factor missing, missing values were generated by simple imputation before modeling, under the assumption of data missing at random (MAR). The nonlinearity of the effect of continuous variables was assessed using restricted cubic splines [9]. All statistical tests were two-tailed with the significance level set at $\alpha = 0.05$. Statistical analyses were performed using the R programming language and environment [10].

Results

A total of 3447 patients (93.5%) met the inclusion criteria and had PFS >18 weeks. The median age was 58.8 years, 90.5% were white, and approximately 94% had a GOG performance status of 0 or 1. Only 27% had suboptimal residual disease following primary cytoreductive surgery. Protocol-directed chemotherapy was distributed evenly across

Table 2
Patient characteristics by myelosuppression.

	N	Non-neutropenic	Neutropenic	Test statistic
		$N = 251$	$N = 3196$	
Age years	3447	50.5[a] 57.9[b] 65.6[c]	51.3[a] 58.9[b] 66.7[c]	p = 0.313[1]
Race/ethnicity	3447			p = 0.488[2]
White		90.8% (228)	90.5% (2891)	
Black		5.2% (13)	4.2% (133)	
Other		4.0% (10)	5.4% (172)	
Performance status	3447			p = 0.083[2]
Normal, asymptomatic		41.8% (105)	49.1% (1569)	
Symptomatic, ambulatory		50.6% (127)	44.6% (1426)	
Symptomatic, in bed		7.6% (19)	6.3% (201)	
Top-level FIGO stage	3447			p = 0.073[2]
III		82.5% (207)	86.5% (2765)	
IV		17.5% (44)	13.5% (431)	
Histology	3447			p = 0.116[2]
Serous		78.9% (198)	83.6% (2673)	
Clear cell/mucinous		5.2% (13)	3.4% (108)	
Other		15.9% (40)	13.0% (415)	
CA-125 μg/mL	3348	86.7[a] 214.8[b] 554.4[c]	85.0[a] 204.8[b] 531.0[c]	p = 0.954[1]
Ascites	3361			p = 0.177[2]
No		22.4% (55)	26.4% (822)	
Yes		77.6% (190)	73.6% (2294)	
Tumor residual	3447			p = 0.409[2]
Microscopic		21.1% (53)	24.8% (794)	
Optimal (0.1–1 cm)		49.8% (125)	48.0% (1534)	
Suboptimal (>1 cm)		29.1% (73)	27.2% (868)	
Interval cytoreduction	3447			p = 0.208[2]
No		95.2% (239)	96.7% (3091)	
Yes		4.8% (12)	3.3% (105)	
Treatment	3447			p = 0.001[2]
CP		29.5% (74)	19.5% (624)	
CPG		13.1% (33)	20.5% (656)	
CPD		17.9% (45)	20.0% (638)	
CT → CP		18.7% (47)	20.0% (638)	
CG → CP		20.7% (52)	20.0% (640)	

a, *b* and *c* represent the lower quartile *a*, the median *b*, and the upper quartile *c* for continuous variables.
N is the number of non-missing values.
Numbers after percents are frequencies.
Tests used: [1]Wilcoxon test; [2]Pearson test.

Table 3
Multivariate progression-free survival analysis.

	aHR	95% CI	p
Myelosuppression			
Non-neutropenic	1.00	*Referent*	–
Neutropenic	0.90	0.78–1.03	0.129
Age (years)[a]	1.00	1.00–1.01	0.015
BSA (m^2)[b]	1.02	1.01–1.04	0.013
Race/ethnicity			
White	1.00	*Referent*	–
Black	1.06	0.89–1.26	0.517
Other	0.81	0.69–0.96	0.013
Performance status			
Normal, asymptomatic	1.00	*Referent*	–
Symptomatic, ambulatory	1.03	0.95–1.11	0.446
Symptomatic, in bed	1.14	0.98–1.33	0.079
Stage			
III	1.00	*Referent*	–
IV	1.33	1.20–1.47	<0.001
Histology			
Serous	1.00	*Referent*	–
Clear cell/mucinous	1.60	1.31–1.96	<0.001
Other	0.87	0.77–0.97	0.011
CA-125 (μg/mL)[c]	1.01	1.01–1.02	<0.001
Ascites			
No	1.00	*Referent*	–
Yes	1.24	1.13–1.36	<0.001
Tumor residual			
Microscopic	1.00	*Referent*	–
Optimal (0.1–1 cm)	1.57	1.42–1.74	<0.001
Suboptimal (>1 cm)	1.75	1.56–1.97	<0.001
Interval cytoreduction			
No	1.00	*Referent*	–
Yes	0.86	0.71–1.05	0.151

[a] adjusted Hazard Ratio denotes the change in risk of progression or death associated with an increase of 1 year in age.
[b] adjusted Hazard Ratio denotes the change in risk of progression or death associated with an increase of 0.1 m^2 in BSA.
[c] adjusted Hazard Ratio denotes the change in risk of progression or death associated with a 10% increase in CA-125 (μg/mL).

the five treatment arms, and 92.7% (n = 3196) of the study group experienced at least one neutropenic event during treatment. These data appear in Table 1.

Neutropenic and non-neutropenic groups were similar in demographic and clinicopathologic characteristics, including age, race/ethnicity, performance status, FIGO stage, histology, baseline CA-125, and surgical outcome (Table 2). In terms of treatment allocation, the percentages of neutropenic and non-neutropenic patients among the carboplatin–paclitaxel–pegylated liposomal doxorubicin triplet and the two sequential chemotherapy doublet arms were similar. However, as expected, less neutropenia was observed on the control arm comprised of a single chemotherapy doublet, while a larger percentage of patients on the carboplatin–paclitaxel–gemcitabine triplet experienced neutropenia (p = 0.001) (Table 2).

On multivariate analysis, factors which significantly impacted PFS included age, BSA, non-White/non-Black race, stage IV, clear cell/mucinous histology, CA-125, presence of ascites, and tumor residual (Table 3). When non-neutropenic patients were used as the reference, the adjusted hazard ratio (HR) for disease progression in neutropenic patients was 0.90 (95% CI 0.78–1.03, p = 0.129) (Table 3). The median PFS for the non-neutropenic vs neutropenic groups is 14.1 months (95% CI, 12.9–17.2 months) and 16.7 months (95% CI, 16.1–17.2 months), respectively (Fig. 1A).

Significant prognostic factors for OS included all of the above (except race) and performance statuses 2–3, plus the occurrence of neutropenia (Table 4). The adjusted HR for death in neutropenic patients was 0.86 (95% CI 0.74–0.909, p = 0.041) (Table 4). The median OS for the non-neutropenic vs neutropenic groups is 38.2 months (95% CI, 35.2–44.9 months) and 47.0 months (95% CI, 45.0–48.9 months), respectively (Fig. 1B).

The functional forms of several variables in the OS model are not only significantly but also nonlinear. The partial effect of age on the OS model appears in Fig. 2a. The change in risk before age 50 years is not significant given the wide confidence intervals, but after 50 risk appears to increase sharply. In Table 4, the change in risk of death associated with an increase of one year in age is given over the two intervals defined by the changepoint (<50 years, ≥50 years). Fig. 2b depicts the partial effect of BSA on the OS model, which has a changepoint at 1.87 m^2 (95% CI, 1.52–1.95 m^2) where the previously flat risk begins to increase. The change in risk over the two intervals defined by the changepoint is also presented in Table 4.

To test whether a survival advantage among neutropenic patients was conferred by a specific regimen, a survival model was generated which included an interaction term between indicators for neutropenia and the treatment regimen. In this analysis the interaction term was not statistically significant, suggesting that neither myelosuppression subgroup

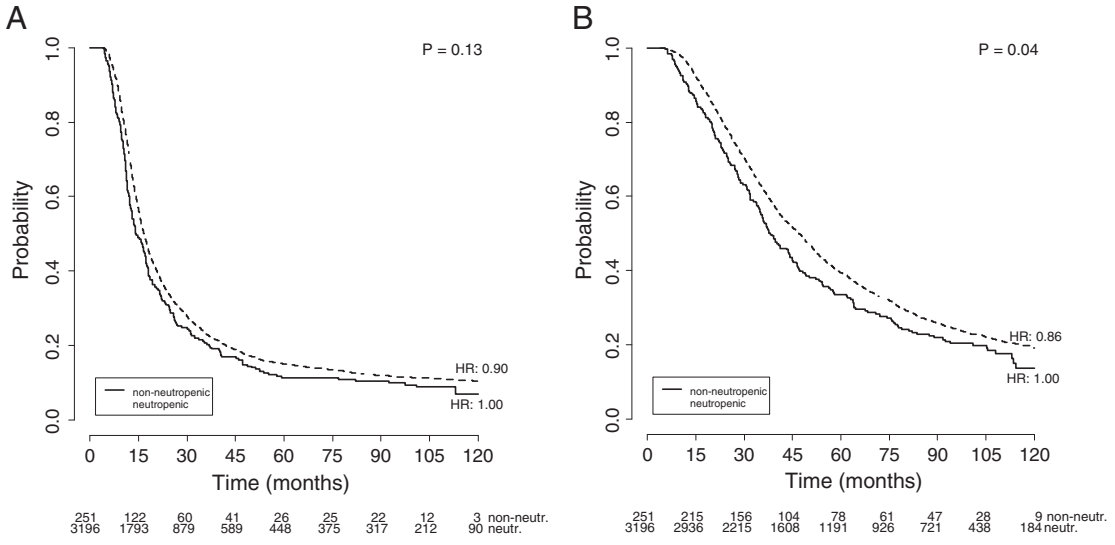

Fig. 1. Panel A (left). Kaplan–Meier curves of progression-free survival for all patients, stratified by myelosuppression. Figures below the time (months) axis indicate the numbers of patients at risk. The p-value is from the Wald test to compare hazard ratios between the myelosuppression subgroups in the multivariate model. Panel B (right). Kaplan–Meier curves of overall survival for all patients, stratified by myelosuppression. Figures below the time (months) axis indicate the numbers of patients at risk. The p-value is from the Wald test to compare hazard ratios between the myelosuppression subgroups in the multivariate model.

Table 4
Multivariate overall survival analysis.

	aHR	95% CI	p
Myelosuppression			
Non-neutropenic	1.00	*Referent*	–
Neutropenic	0.86	0.74–0.99	0.041
Age (years)[a]			<0.001[b]
<50	1.00	0.99–1.01	
≥50	1.01	1.01–1.02	
BSA (m^2)[c]			0.002[b]
<1.9	1.00	0.96–1.03	
≥1.9	1.06	1.03–1.11	
Race/ethnicity			
White	1.00	*Referent*	–
Black	1.11	0.92–1.34	0.279
Other	0.85	0.71–1.02	0.073
Performance status			
Normal, asymptomatic	1.00	*Referent*	–
Symptomatic, ambulatory	1.06	0.98–1.15	0.162
Symptomatic, in bed	1.22	1.04–1.42	0.015
Stage			
III	1.00	*Referent*	–
IV	1.41	1.26–1.57	<0.001
Histology			
Serous	1.00	*Referent*	–
Clear cell/mucinous	1.80	1.46–2.23	<0.001
Other	0.88	0.78–1.00	0.047
CA-125 (μg/mL)[d]			<0.001[b]
<680	1.02	1.01–1.03	
≥680	1.01	1.00–1.01	
Ascites			
No	1.00	*Referent*	–
Yes	1.29	1.17–1.42	<0.001
Tumor residual			
Microscopic	1.00	*Referent*	–
Optimal (0.1–1 cm)	1.56	1.40–1.74	<0.001
Suboptimal (>1 cm)	1.80	1.58–2.03	<0.001
Interval cytoreduction			
No	1.00	*Referent*	–
Yes	0.75	0.60–0.93	0.008

[a] adjusted Hazards Ratios denote the change in risk of death associated with an increase of 1 year in age over the given intervals.
[b] p-Values for continuous nonlinear predictors are from the overall test of their significance in the model.
[c] adjusted Hazards Ratios denote the change in risk of death associated with an increase of 0.1 m^2 in BSA over the given intervals.
[d] adjusted Hazards Ratios denote the change in risk of death associated with a 10% increase in CA-125 (μg/mL) over the given intervals.

(i.e. neutropenic vs non-neutropenic) had a preferential benefit from also being in a particular treatment arm. Similarly, interactions between neutropenia and age, and neutropenia and BSA, were not significant.

Discussion

In this exploratory analysis, chemotherapy-induced neutropenia (C-iN) was associated with improved OS in a large untreated population with advanced ovarian and peritoneal carcinoma. Patients developing neutropenia during the first 6 cycles of chemotherapy had a 14% reduction in the risk of death compared to patients without neutropenia. Because an association between C-iN and improved PFS was not observed, post-progression therapy may have a greater, if as yet poorly understood, impact among neutropenic patients, but this is just conjecture. Nevertheless, these results are consistent with the observations reported by others who have noted that C-iN is positively correlated with patient outcomes in a variety of solid tumors including non-small cell lung, colorectal, gastric, breast, cervical, and ovarian cancer [11–21]. Shitara et al. conducted a meta-analysis comprised of 9528 patients from 13 prospective and retrospective studies that evaluated neutropenia or leucopenia as a prognostic factor for survival [21]. There was a 31% reduction in risk of death for patients with high-grade neutropenia or leucopenia compared to patients with lower grade or lack of cytopenia (HR 0.69; 95% CI 0.65–0.75) [21].

Rocconi et al. first reported the association of C-iN and survival in 255 patients with primary advanced ovarian carcinoma treated with 6 cycles of platinum–taxane therapy [16]. Demographic and clinicopathologic factors were similar between patients who had experienced neutropenia during treatment (n = 203) and those who never had neutropenia (n = 52). Neutropenic patients demonstrated improvements in PFS (14 vs 6 months; p = 0.01), OS (45 vs 29 months; p = 0.03), and platinum sensitivity rates (69% vs 44%; p = 0.001) [16]. Further improvements in PFS and platinum sensitivity correlated with increasing number of neutropenic episodes.

More recently, the results of a large Gynecologic Cancer Intergroup trial (SCOTROC-4) were reported by Banerjee et al. [20]. The investigators evaluated the efficacy and tolerability of intrapatient dose escalation of single agent carboplatin in a randomized trial of untreated stage IC-IV ovarian cancer. Nearly 1000 patients were randomized to flat dosing versus dose escalation and although the dose escalation as per protocol was feasible in the majority of patients, a futility analysis led to study closure at a median follow-up time of 26 months. There were no statistically significant differences in median PFS or median OS. In univariate analysis, C-iN was associated with improved PFS [20]. High baseline neutrophils (and other hematological parameters including the difference between baseline white blood cell count and neutrophils) were associated with reduced PFS. The impact of C-iN on survival disappeared in multivariable analysis, leaving the authors to speculate that the baseline counts override the absolute nadir count [20]. It is

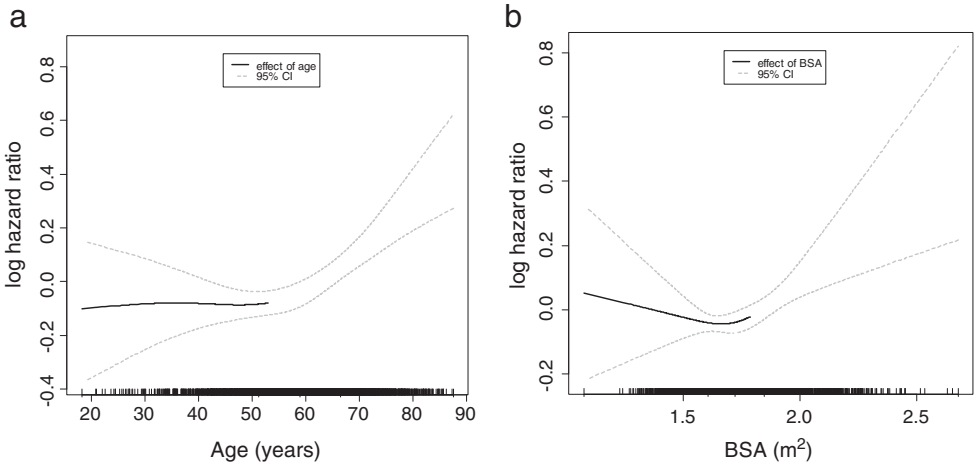

Fig. 2. Panel a (left). Three-knot restricted cubic spline plot of the partial effect of age on the log hazard ratio of the overall survival model. Panel b (right). Three-knot restricted cubic spline plot of the partial effect of body surface area (BSA) on the log hazard ratio of the overall survival model.

difficult to extrapolate the SCOTROC-4 study population to the United States as the majority of patients in the trial were suboptimally debulked and none received combination platinum–taxane-based therapy.

If C-iN can be validated as a prognostic biomarker in ovarian cancer, issues concerning appropriate dosing, schedule and route of delivery of chemotherapy become implicit. Current strategies for dose intensification have been designed to exploit the dose–response curve using heated intraoperative intraperitoneal chemotherapy, postoperative intraperitoneal/intravenous chemotherapy, and weekly, dose-dense schedules. Because of significant inter-patient variation of drug clearance, in vitro assays that accurately reflect pharmacokinetic phenomenon are not easily reproducible among patients. Intra-person C-iN may be used as an in vivo bioassay to directly track dose intensification and biologic effect, and indirectly, oncologic outcome.

Although dosing is based on a patient's estimated body surface area (BSA), there are little data supporting such a strategy. Nearly two decades ago, Gurney described the limitations of BSA dosing which does not account for the complex process of cytotoxic drug elimination [22]. This can lead to a variation in effect with unrecognized underdosing occurring in up to 30% of patients. Such patients are at risk of reduced anti-tumor effect and ultimately, poor oncologic outcome. There exists compelling clinical evidence that reductions in standard dose intensity may compromise PFS and OS in the curative setting.

Two groups at particularly high risk of under-dosing include obese patients and the elderly. Historically, these groups have performed poorly when compared to non-obese patients and younger women. Overweight and obese patients have historically been capped at a BSA of 2.0 m^2, and the elderly are often treated at reduced dosages empirically. Concerns about overdosing obese cancer patients using actual body weight are largely unfounded, and with modern supportive care, most elderly patients with cancer can tolerate standard dosages of chemotherapy [23–25]. The occurrence of C-iN in these two groups may correlate with outcome. Although we were unable to demonstrate significant interactions between neutropenia and BSA or neutropenia and age, our survival models clearly indicate an increase in risk of death at thresholds of age (Fig. 2a) and BSA (Fig. 2b).

Significant strengths of this study include that the analyses were performed on prospectively collected data from a large population of women with newly diagnosed ovarian cancer treated using standardized dosing. Quality control was overseen by the National Cancer Institute and its Data Safety Monitoring Board.

Limitations of this study include that the investigational treatments were expected to produce a high frequency of neutropenia. This resulted in a very large group with C-iN and a much smaller group without C-iN, ultimately limiting the power and generalizability of our findings. Additionally, this analysis did not include BRCA1/2 typing. Loss of BRCA1/2 may be present in 20% of the treated population and would be expected to interfere with DNA repair and increase nadirs and survival. Finally, we have not established cause and effect in this analysis. It is entirely possible that C-iN is a biomarker for something completely different.

If the cancer stem cell hypothesis is invoked, biologic plausibility to support the relationship between C-iN and survival becomes discernible. Approximately 150 years ago, the origin of cancer from "stem cell" populations was introduced [26,27]. Because certain subpopulations of cancer cells have inherited normal stem cell properties including capacity for self-renewal, ability to differentiate, activate anti-apoptotic pathways, and metastasize, cancer stem cell response correlates directly with survival [28–31]. Taken further, the relative amount of C-iN (i.e., differentiated cell response) would be expected to correlate with survival (i.e. cancer stem cell response). It has been postulated that with increasing severity of neutropenia, a greater fractional kill of cancer stem cells occurs, potentially improving survival. Further work is required to determine whether the severity (rather than just the occurrence) of neutropenia correlates with clinical outcome.

Mechanistically, single nucleotide polymorphisms (SNPs) in DNA repair genes may result in neutropenia and prevent removal of platinum-DNA adducts, augmenting the response to anti-neoplastic therapy [32]. Neutrophils have also been implicated in the modification of the microenvironement in pre-metastatic tissues, facilitating colonization by cancer cells. Finally, neutrophil elastase has been shown to directly stimulate tumor cell proliferation in human lung adenocarcinomas [33]. A direct pro-tumoral role has also been described for neutrophil-associated matrix metalloprotease type 9 (MMP-9) on angiogenesis and early carcinogenesis [34].

Accumulating evidence supports a relationship between increased neutrophils and resistance to anti-vascular endothelial growth factor (VEGF) therapy. Shojaei et al. have demonstrated that G-CSF can induce angiogenesis and render tumors refractory to anti-VEGF therapy [35,36]. Conceivably, the management of C-iN with exogenous G-CSF could be pro-angiogenic and increase risk of progression. Recently these discussions have become increasingly relevant with four phase 3 randomized trials in primary advanced ovarian cancer and in populations with platinum-sensitive and platinum-resistant recurrences demonstrating improved PFS with the integration of anti-VEGF therapy using the monoclonal antibody, bevacizumab [37–40]. It is unclear whether resistance to bevacizumab is due to the host endothelium having a limited impact on tumor growth for a limited period of time when VEGF is "blocked" or due to the emergence of resistant clones. Clones can become resistant to intracellular tyrosine kinase inhibitors and may have a greater impact on intracellular signal transduction than external levels of VEGF. These phenomena are likely to propel further investigation to unravel the molecular cascade which governs the relationship between the occurrence of and degree of severity of C-iN and survival in ovarian carcinoma.

Conflict of interest

Dr. Michael Bookman served on ad-hoc advisory boards for Eli Lilly, Glaxo-SmithKline, and Genentech-Roche Oncology to facilitate the development of clinical trials in ovarian cancer. He also served as study chair for GOG-182 (the study source for this manuscript). Currently he serves as Chair of the GOG Ovarian Committee, which manages clinical trials in ovarian cancer.

All other co-authors have no conflicts of interest to declare.

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