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Original Article

Possible surrogate marker for an effective dose-dense chemotherapy in treating ovarian cancer

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ABSTRACT

Objective: To dissect the correlated hematologic markers that reflect the clinical outcome or treatment response in patients receiving dose-dense chemotherapy with a combination of platinum (cisplatin or carboplatin) and paclitaxel.

Materials and Methods: From 2009 to 2014, we enrolled 55 ovarian cancer patients (total 67 courses) including first-line, persistent, platinum-sensitive, or platinum-resistant disease in MacKay Memorial Hospital, Taipei, Taiwan. Weekly pretreatment complete blood counts and calculated ratios [platelet/neutrophil ratio, neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), neutrophil/monocyte ratio, platelet/monocyte ratio, lymphocyte/monocyte ratio] during dose-dense chemotherapy were collected. By grouping these hematologic biomarkers into three different response subgroups (responsive, stable, and nonresponsive) according to CA125 trend, the data were analyzed using one-way analysis of variance, and using *post hoc*-Tukey test for comparing each other. A p value < 0.05 was considered to be statistically significant.

Results: Absolute counts of lymphocytes and platelets, PLR, platelet/neutrophil ratio, platelet/monocyte ratio (all $p < 0.001$), and NLR ($p = 0.013$) had statistically significant differences. Moreover, using box-and-whisker plot, absolute count of lymphocyte, PLR, and NLR showed most remarkable discrepancy in responsive, stable, and nonresponsive patients. Subgroup analysis for primary, platinum-sensitive, and platinum-resistant patients further revealed that PLR and NLR were significantly correlated to the outcome of dose-dense chemotherapy.

Conclusion: Lower PLR or lower NLR had better treatment response for dose-dense chemotherapy and are possible markers for representing treatment response in dose-dense chemotherapy. For a clinician, this is useful for timing when to switch to another chemotherapy regimen.

Introduction

Epithelial ovarian cancer is the fifth leading cause of cancer-related death among women. Evidence has established tumor debulking surgery followed by a platinum-based chemotherapy as the first-line therapy for advanced ovarian cancer, yielding response rates of > 80%. However, for platinum-resistant ovarian cancer, second-line cytotoxic agents only achieve a 15–20% response rate. Nowadays studies are still seeking another possible mode, schedule, or regimen of chemotherapeutic strategies. Chemotherapy refinement and optimization might be the other focus to overcome the development of drug resistance and improve the survival of the patients. The standard regimen of chemotherapy for advanced ovarian cancer is combination of platinum (cisplatin or carboplatin) and paclitaxel and response rates have been reported in 73% of those receiving cisplatin/paclitaxel and in 60% of those receiving cisplatin/cyclophosphamide combinations [1].

The concept of dose-dense therapy was defined by the Norton–Simon regression hypothesis, which was proposed in the 1970s and suggests that the rate of tumor regrowth between

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Table 1
Patient demographics.

Patient number	55
Course number	67 (1–3 courses)
First	11 (16%)
Persistent disease	4 (6%)
Platinum-sensitive	26 (39%)
Platinum-resistant	26 (39%)
Age (y)	54.8 (33–81)
Treatment duration	194.6 (45–1122)

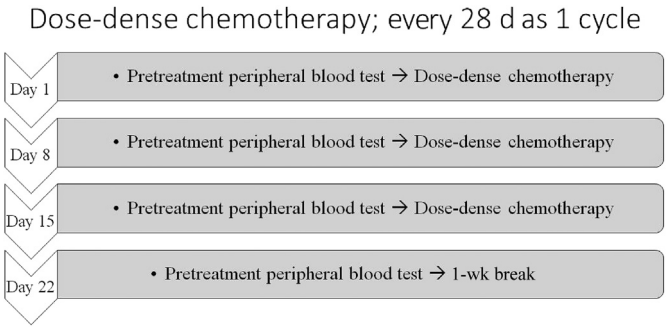

Figure 1. Schedule for dose-dense chemotherapy. The patients had pretreatment peripheral blood tests on the mornings of Day 1, Day 8, and Day 15. If the result of blood test was acceptable, dose-dense chemotherapy with paclitaxel (60–80 mg/m^2) plus platinum [carboplatin (area under the curve = 1.2–2) or cisplatin (25–30 mg/m^2)] was performed. If not, chemotherapy is delayed until bone marrow recovery. One cycle is dose-dense chemotherapy continuing for 3 weeks followed by a 1-week break.

treatments will be proportional to the rate of tumor growth. It means that tumors given less time to regrow between treatments are more likely to be destroyed [2]. Despite the uncertain mechanism, clinically, we have found therapeutic efficacy of dose-dense chemotherapy in treating ovarian epithelial cancer including first-line or salvaged settings. Moreover, it is noteworthy that dose-dense chemotherapy is effectively applied even for platinum-resistant disease. Many researchers have reported that the patients receiving dose-dense chemotherapy have better tolerance and a higher response rate (about 43–60%) [3,4].

Recently, many studies have investigated the mechanism of dose-dense chemotherapy, either the cytotoxic or immunogenic effect. Previously we have documented a correlation between the induction of serum interferon-γ and interleukin-2 and the efficacy of chemotherapy by weekly low-dose carboplatin and paclitaxel in the patients having platinum-resistant ovarian cancer, indicating a role of antitumor immunity in dose-dense chemotherapy [5].

Cancer patients who receive multiple chemotherapies are usually immunocompromised. They are relatively difficult to be induced strong antitumor immunity. Clinically there is a need to evaluate which patient would benefit from this mode of dose-dense treatment. We conducted this study in order to determine if there were hematological markers [neutrophil count, lymphocyte count, platelet count, monocyte count, and ratios, such as platelet/neutrophil ratio (PNR), neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), neutrophil/monocyte ratio (NMR), platelet/monocyte ratio (PMR), and lymphocyte/monocyte ratio (LMR)] in patients with ovarian cancer receiving dose-dense chemotherapy.

Materials and methods

Patient selection

From 2009 to 2014 (Table 1), primary or recurrent epithelial ovarian cancer patients who had received dose-dense chemotherapy as adjuvant or salvage chemotherapy in MacKay Memorial Hospital, Taipei, Taiwan were enrolled in our retrospective study.

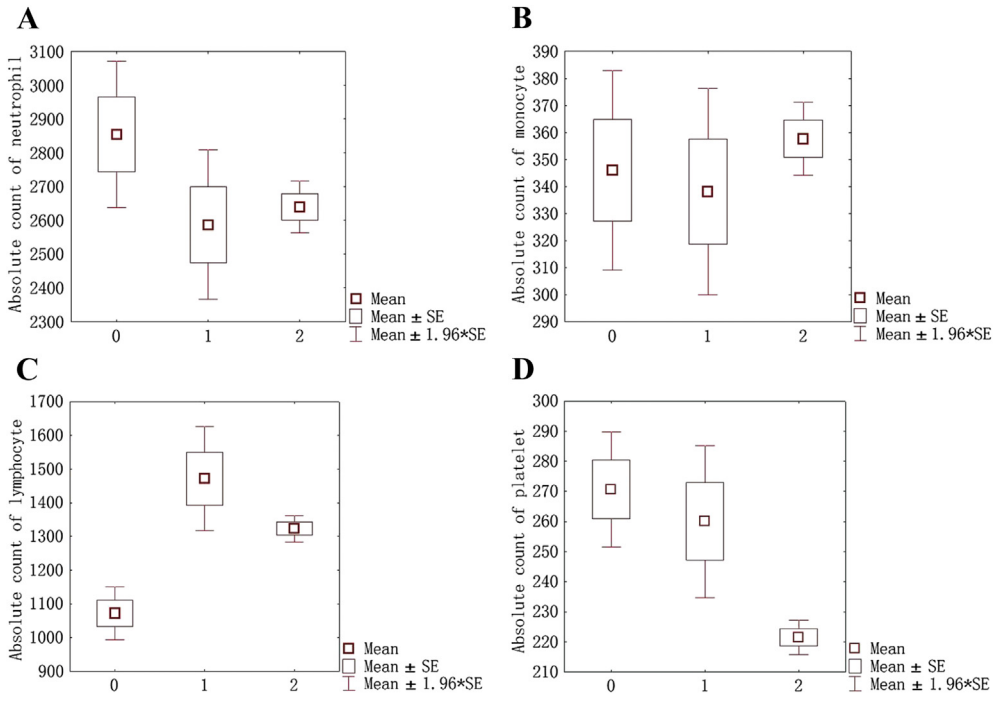

Figure 2. The distribution of blood cell count [(A) neutrophils; (B) monocytes; (C) lymphocytes; (D) platelets] in nonresponsive, stable, and responsive groups: using box-and-whisker plot. Absolute count of lymphocytes showed remarkable difference in the three subgroups. 0 = nonresponsive; 1 = stable; 2 = responsive; Mean ± 1.96 SE = 95% confidence interval; SE = standard error.

Data collection

We started to retrieve the data after getting the approval of the institutional review board. From the medical records we first excluded the patients of tumor marker CA125 noninformative subgroup (pretreatment CA125 was < 35 U/mL). We documented the hematologic data of every weekly pretreatment complete blood count and also calculated the six ratios by division of any two basic blood cell counts: PLR, PNR, PMR, NLR, NMR, and LMR during the dose-dense chemotherapy treatment period. Monthly tumor marker CA125 was collected due to an index of the definition of dose-dense chemotherapy efficacy.

Schedule for dose-dense chemotherapy in MacKay Memorial Hospital

The schedule for dose-dense chemotherapy in MacKay Memorial Hospital (Figure 1) was paclitaxel (60–80 mg/m^2) and platinum [carboplatin (area under the curve = 1.2–2) or cisplatin (25–30 mg/m^2)] on Day 1, Day 8, and Day 15, every 28 days as one cycle. Our patients received routine blood tests including complete blood count and biochemistry profile (fasting sugar, liver enzyme, renal function, and electrolyte: Na^+, K^+ and Cl^-) on the morning of the treatment day.

The eligibility criteria for blood tests were: adequate bone marrow (absolute neutrophil count > 1500/μL, platelets > 100 K/μL); liver enzyme (pretreatment alanine aminotransferase and/or aspartate aminotransferase ≤ 2 upper limit of normal); and renal function (creatinine ≤ 1.2 mg/dL). The exclusion criteria for blood tests were thrombocytopenia (platelets < 100×10^9/L), or neutropenia (absolute neutrophil count < 1500×10^6/L), or infection episode [signs or symptoms: fever (≥ 38°C), leukocytosis (white blood cells ≥ 10×10^9/L), or positive result of blood or urine culture]. In these cases, chemotherapy should be delayed and restarted after condition resolution.

The definition of dose-dense chemotherapy efficacy

We defined the efficacy of dose-dense chemotherapy by monthly CA125 levels [6,7]. We evaluated the trend of CA125 of each patient and grouped the treatment course into three possible different responses. There are three kinds of treatment response: responsive, stable, and nonresponsive periods (some courses may have only one kind of treatment response).

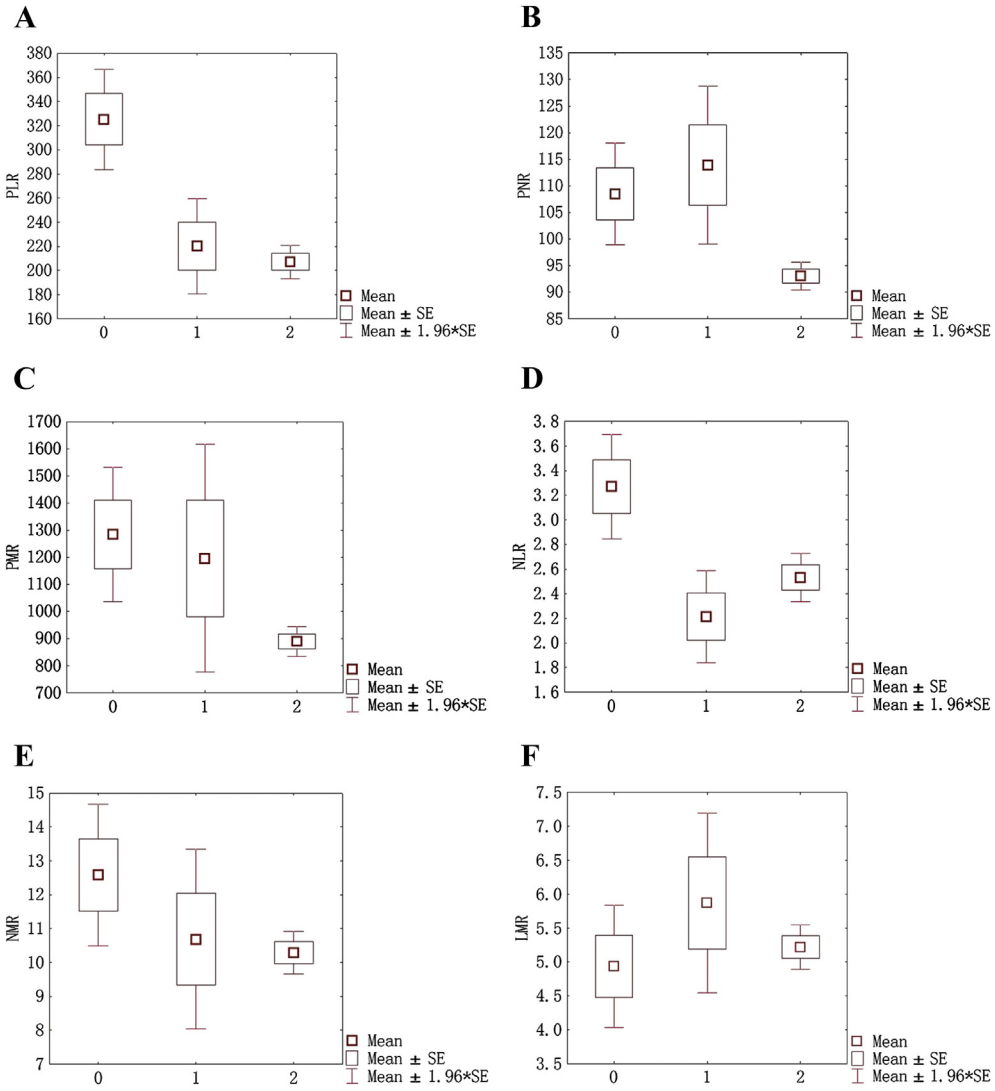

Figure 3. The distribution of ratios [(A) platelet/lymphocyte ratio (PLR); (B) platelet/neutrophil ratio (PNR); (C) platelet/monocyte ratio (PMR); (D) neutrophil/lymphocyte ratio (NLR); (E) neutrophil/monocyte ratio (NMR); (F) lymphocyte/monocyte ratio (LMR)] in nonresponsive, stable, and responsive groups: using box-and-whisker plot. PLR and NLR showed remarkable difference in the three subgroups. 0 = nonresponsive; 1 = stable; 2 = responsive; Mean ± 1.96 SE = 95% confidence interval; SE = standard error.

Responsive period: the trend of CA125 levels declined from the pretreatment point to the nadir point of CA125 $\geq$ 50%, or dropped below the upper limit of normal (35 U/mL).

Stable period: ineligible periods of responsive or nonresponsive criteria, or stationary CA125 levels but greater than upper limit of normal (35 U/mL).

Nonresponsive period: the trend of CA125 was elevated, doubling of CA-125 levels from either the upper limit of normal (35 U/mL) or the nadir CA-125 level.

Statistical analysis and comparison

We grouped 55 cases (totally 67 courses of dose-dense chemotherapies) into three different treatment periods (responsive, stable, and nonresponsive) according to CA125 trend. The three different periods of blood cell count or ratio were analyzed using one-way analysis of variance and using *post hoc*-Tukey test. A p value < 0.05 was considered to be statistically significant. Statistical analysis was performed using STATISTICA software (Dell Software in Tulsa, Oklahoma, USA).

Results

From 2009 to 2014, there were 55 patients with ovarian cancer who received dose-dense chemotherapy recruited into this study. Some patients had two or three treatment courses intermittently, therefore, the total of treatment courses was 67. The median age of the patients was 54.8 years with a range of 33–81 years. The median treatment duration was 194.6 days (range, 45–1122 days). Of the 67 courses, 30 (45%) were prescribed for platinum-resistant or persistent diseases.

Initially, we found that absolute count of lymphocytes, absolute count of platelets, PLR, PNR, PMR (all $p < 0.001$), and NLR ($p = 0.013$) had statistically significant differences. Using box-and-whisker plot, we further determined the distribution of blood cell count or ratio in nonresponsive, stable, and responsive subgroups (Figures 2 and 3). If the three boxes showed remarkable discrepancy, this was significant not only statistically but also clinically. We found absolute count of lymphocytes, PLR, and NLR had most remarkable discrepancy. In *post hoc*-Tukey test, we analyzed the relationship between each two subgroups comparison (Table 2; nonresponsive vs. stable, nonresponsive vs. responsive, stable vs. responsive). If we combinated the responsive and stable subgroups as identical effective treatment subgroup in opposition to nonresponsive subgroup, we also found the results of subdivision of p values (statistically significant in absolute count of lymphocytes, PLR, and NLR) in post hoc-Tukey test were same as the results of box-and whisker plot distribution.

In the clinical situation, we regard *stable* and *responsive* response as effective treatment and usually keep the chemotherapy regimen constant. Depending on *post hoc*-Tukey test, absolute count of lymphocytes, PLR, and NLR showed statistical significance in the nonresponsive versus stable and nonresponsive versus responsive subgroups. However, subgroup analysis for primary, platinum-sensitive, and platinum-resistant patients demonstrated that PLR and NLR correlated most to the outcome of dose-dense chemotherapy ($p < 0.001$, $p = 0.006$, and $p = 0.011$ in primary, platinum-sensitive, and platinum-resistant groups for PLR; $p = 0.006$, $p = 0.5$, and $p = 0.044$ in primary, platinum-sensitive, and platinum-resistant groups for NLR).

We concluded that PLR and NLR are possible markers for dose-dense chemotherapy in treating ovarian cancer. These hematologic markers are more available markers than CA125 and immunogenic markers. Thus we can usually obtain relevant information from the weekly pretreatment hematologic data and decide the timing to switch to another salvage chemotherapy regimen.

Discussion

Ovarian cancer is known to be associated with inflammation [8]. There is often a complex host–tumor relationship with most tumors having inflammatory cells and mediators present in their microenvironment [2]. This results in the production of chemokines, cytokines, and prostaglandins, which not only recruit inflammatory cells such as neutrophils, mast cells, and macrophages, but also stimulate angiogenesis and cell proliferation [8]. Many studies have reported that this process of lymphopenia has prognostic significance and is associated with poor outcome in a variety of tumors including renal, pancreatic, and ovarian cancers [9,10].

Previous studies have demonstrated the association of both NLR and PLR with poor prognosis in various tumor types. Recent research reported that PLR is a novel independent prognostic marker in patients with ovarian cancer [11–13]. However, most studies explored the correlation between pretreatment or preoperative blood cell count or ratio and prognosis. There is no study that showed these potential biomarker fluctuation and treatment response relationships during chemotherapy period. This is the first study to prove that PLR and NLR have dynamic correlation with treatment response. From the distribution of hematologic markers in nonresponsive, stable, and responsive subgroups, we can see that the patients with lower PLR or lower NLR had better treatment response for dose-dense chemotherapy.

Previously, we have documented a correlation between the induction of serum interferon-γ and interleukin-2 and the efficacy of chemotherapy by weekly low-dose carboplatin and paclitaxel in the patients having platinum-resistant ovarian cancer, indicating a role of antitumor immunity in the dose-dense chemotherapy [5]. We believed there should be a mechanism (other than cytotoxicity) responsible for the tumor killing, which has been jeopardized in

Table 2
Statistical analysis (1-way ANOVA *post hoc*-Tukey honest significant difference test) for absolute count of lymphocytes, absolute count of platelets, platelet/lymphocyte ratio (PLR), platelet/neutrophil ratio (PNR), platelet/monocyte ratio (PMR), neutrophil/lymphocyte ratio (NLR), comparison of nonresponsive versus stable, nonresponsive versus responsive, stable versus responsive subgroups; marked differences are significant at $p < 0.05$.

	Nonresponsive	Stable	Responsive
Absolute count of lymphocytes			
Nonresponsive	—	< 0.001	< 0.001
Stable	< 0.001	—	0.103
Responsive	< 0.001	0.103	—
Absolute count of platelets			
Nonresponsive	—	0.719	< 0.001
Stable	0.714	—	0.002
Responsive	< 0.001	0.002	—
PLR			
Nonresponsive	—	0.002	< 0.001
Stable	0.002	—	0.874
Responsive	< 0.001	0.874	—
PNR			
Nonresponsive	—	0.682	< 0.001
Stable	0.682	—	< 0.001
Responsive	< 0.001	< 0.001	—
PMR			
Nonresponsive	—	0.831	< 0.001
Stable	0.831	—	0.042
Responsive	< 0.001	0.042089	—
NLR			
Nonresponsive	—	0.037	0.017
Stable	0.037	—	0.652
Responsive	0.017	0.652	—

ANOVA = analysis of variance.

traditional maximum tolerated dose chemotherapy. Recently, we have noted that dose-dense chemotherapy acts through an inflammasome-related pathway where NK and also γδ T cells were triggered (data not shown). The results of the recent studies suggest that there may be a key of induced tumor-specific immune response. In this regard, great interest has been generated in elucidating the role of cancer inflammation with tumor burden and prognosis. It is well known that inflammation is associated with different stages of tumor and development including initiation, promotion, malignant conversion, invasion, and metastasis [2].

The role of dose-dense chemotherapy for ovarian cancer is getting more important. In this study, we have not only established the relation of hematologic counts and chemotherapy response but also provided a possible surrogate marker to determine whether we should continue the dose-dense chemotherapy in patients receiving this mode of treatment. Further research elucidating the underlying mechanism of this relation is mandated.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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