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# Use of hematologic biomarkers during chemotherapy predicts survival in ovarian cancer patients

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**Summary**

*Objective:* The optimal strategy for combining chemotherapy with immunotherapy in ovarian cancer patients is currently under investigation. Increasing evidence indicates that the lymphopenia induced by chemotherapy may promote homeostatic proliferation and thereby enhance antitumor immunity. Furthermore, there has been much discussion and even discord over the effects of anemia and blood transfusion in the perichemotherapy period. The goals of this retrospective study were to determine the timing of chemotherapy induced lymphopenia and to observe perichemotherapy hemoglobin levels, and the impact of the timing and depth of lymphopenia and anemia on clinical outcomes of ovarian cancer patients. *Materials and Methods:* A chart review was performed on 115 patients identified in the electronic medical record from May 2005 until May 2011. Identified patients were only those who received at least six cycles of carboplatin and paclitaxel under the present authors' care for primary peritoneal, ovarian, or fallopian tube carcinoma. Specifically, the authors focused on lymphocyte and hemoglobin nadir and the reconstitution kinetics for this population. For each patient's lymphocyte count, nadir values were abstracted from weekly complete blood counts. They then split the population into two groups based on whether the nadir occurred at or after the nine-week mark (third cycle) for the lymphopenia data; this point was chosen because it was good for prognosis and it corresponds to patients whose trajectories bottom out. The intrachemotherapy hemoglobin levels were observed and an exploratory analysis was performed to attempt to identify a range that significantly effected patient outcomes. *Results: Lymphocytes:* The nadir of absolute lymphocyte concentrations is associated with platinum status and clinical response (Figure 1A). 94/115 patients had a lymphocyte count nadir after the third cycle of chemotherapy. 71/94 (75.5%) were platinum sensitive, 21/94 (22.3%) were resistant, and 2/94 (2.1%) were refractory. Of those that experienced a nadir before three cycles, ten (47.6%) were sensitive, ten (47.6%) were resistant, and one (4.7%) was refractory ($p = 0.04$). Considering nadir values continuously, both overall survival (OS, $p = 0{:}0068$) and progression free survival (PFS, $p = 0{:}0321$) were strongly associated with late nadir points. Twenty-one of the 115 patients had a nadir value earlier than the third draw and this was associated with progressive disease, platinum resistance, poor overall survival, and poor progression free survival. The effect sizes were great [median OS 33 *vs*. 66 months median PFS, 14 *vs*. 38 months, early *vs*. late nadir respectively (Figure 1B)]. *Hemoglobin*: A mean Hb less than 12.5 is associated with both overall survival (OS) (HR = 2.11, 95% CI: 1.03-4.33; $p = 0{:}042$) and progression free survival (PFS) (HR = 1.91, 95% CI: 1.02−3.56; $p = 0{:}041$), as were low Hb level at outset of chemotherapy and a decreasing Hb trend over the course of treatment. Furthermore, for each cycle of chemotherapy in which the hemoglobin was recorded at a value less than 11, hazard increased, with OS (HR = 3.51, 95% CI: 1.63−7.54, $p = 0{:}0013$), and PFS (HR = 2.20, 95% CI:1.12−4.33; $p = 0{:}0223$). Deeper analysis revealed that outcomes were significantly affected when a patient had three or more cycles with Hb less than 11 with both OS (HR = 2.34, 95% CI: 1.37−4.01; Wald-Test $p = 0{:}0020$, Log Rank $p = 0.00145$) and PFS (HR = 1.88, 95% CI: 1.17−3.02; Wald-Test $p = 0{:}009$, Log Rank $p = 0.00743$). *Conclusion:* The nadir of absolute lymphocyte concentrations is an independent predictor of overall survival and progression free survival. This is an easily measurable biomarker which can be utilized for identifying patients that will be likely to respond to immunomodulation. Furthermore, this evidence showing significant improvement in OS and PFS with two or less cycles with hemoglobin < 11 sheds new light on the need for further studies on growth stimulating factors and blood transfusion during this treatment period.

*Key words:* Hematologic biomarkers; Chemotherapy induced lymphopenia; Homeostatic proliferation;

## Introduction

The optimal strategy for combining chemotherapy with immunotherapy in ovarian cancer patients is currently unknown. As clinicians, we have long been monitoring the hematologic compartment for indication of potential danger when administering chemotherapy. While we know that neutropenia presents a particularly susceptible time for potential morbidity, mortality, and survival, there is little evidence to support or refute the use of parameters such as hemoglobin levels or absolute lymphocyte counts in predicting outcomes for our patients. There are many conflicting opinions on how anemia effects outcome in patients receiving chemotherapy. In epithelial ovarian cancer (EOC), several studies report a prognostic association between pretreatment serum hemoglobin and eventual survival outcomes [1, 2]. Little attention has been given to the trajectory of this candidate biomarker during the course of chemotherapy. Further dissension is seen when discussing benefits and harms of perioperative and perichemotherapy blood transfusion [3]. The present authors hypothesized that the number of cycles during which a patient is anemic reflects the dynamics of their disease and treatment and will therefore

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**A**

| | | ≥ 9 weeks | < 9 weeks | *p*-value |
|---|---|---|---|---|
| n | | 94 | 21 | |
| Site | Fallopian | 3 | 1 | 0.638 |
| | Ovary | 72 | 14 | |
| | Primary peritomeal | 19 | 6 | |
| Age | | 58.8 | 61.6 | 0.364 |
| BMI | | 29.5 | 27.1 | 0.103 |
| Baseline CA 125 | | 1147.1 | 1093.0 | 0.895 |
| Stage | I/II | 19 | 2 | 0.484 |
| | III/IV | 64 | 15 | |
| Grade | 1 | 9 | 2 | 0.396 |
| | 2 | 22 | 2 | |
| | 3 | 54 | 14 | |
| Histology | Endometrioid | 11 | 1 | 0.581 |
| | Mixed | 9 | 1 | |
| | Other | 12 | 2 | |
| | Serous | 62 | 17 | |
| Debulking | OPT | 71 | 16 | 1.000 |
| | SUB | 22 | 5 | |
| Clinical response | CR | 74 | 12 | 0.060 |
| | PD | 18 | 9 | |
| | PR | 2 | 0 | |
| Platinum sensitivy | REF | 2 | 1 | 0.040 |
| | RES | 21 | 10 | |
| | SENS | 71 | 10 | |

**B**

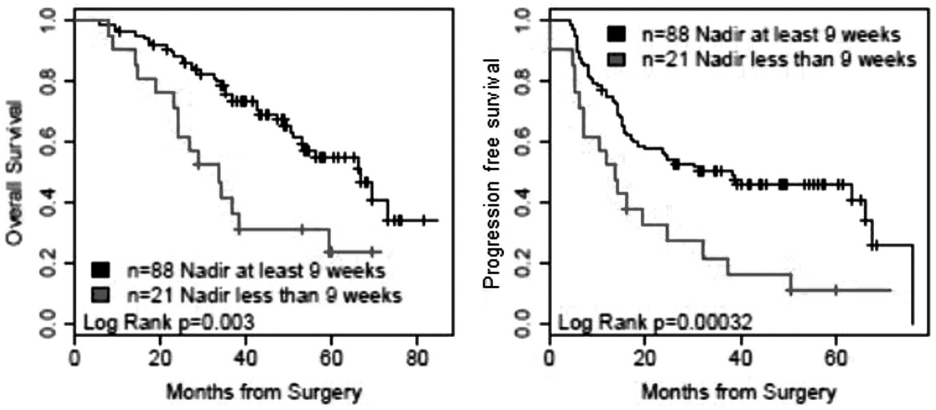


Figure 1. — A) Patient demographics regarding the lymphocyte nadir before and after the third cycle of chemotherapy. B) Overall survival and progression free survival in patients that had a lymphocyte count nadir before and after the third cycle.

**A** **B**

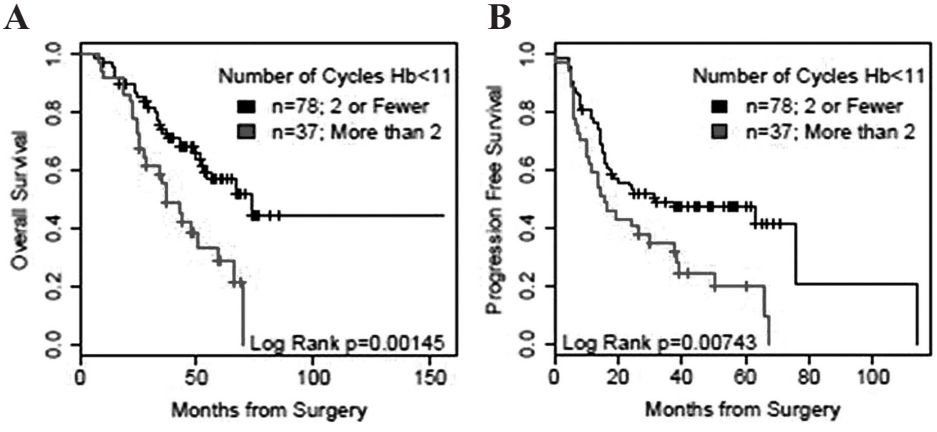


Figure 2. — Patients with three or more cycles of chemotherapy in which their Hb was less than 11 had significantly worse A) OS and B) PFS. They were also more likely to have progressive disease after treatment.

be associated with eventual outcome. However, when discussing another hematologic counterpart, a relative paucity of data seems evident when discussing absolute lymphocyte counts in relation to chemotherapy. Increasing evidence indicate that the lymphopenia induced by chemotherapy may promote homeostatic proliferation and thereby enhance antitumor immunity [4]. The goals of this portion of the study were to determine the frequency of chemotherapy induced lymphopenia, the impact of the timing of lymphopenia on clinical outcomes of ovarian cancer patients, and to ascertain if there is an optimal level at which hemoglobin was observed to have the most favorable outcomes for our patients.

## Materials and Methods

After IRB approval was obtained, a chart review was performed on 115 patients identified in the electronic medical record from May 2005 until May 2011. Identified patients were only those who received at least six cycles of carboplatin and paclitaxel under the authors' care for primary peritoneal, ovarian, or fallopian tube carcinoma. Specifically, they focused on hemoglobin levels, lymphocyte nadir, and the reconstitution kinetics for this population.

For each patient's lymphocyte counts, nadir values were abstracted from weekly complete blood counts. The authors then performed an exploratory analysis. When analyzing for the effect of lymphopenia, they then split the population into two groups based on whether the nadir occurred at or after the nine-week mark (third cycle); this point was chosen because it was good for prognosis and it corresponds to patients whose trajectories bottom out.

Hemoglobin levels from each cycle of therapy were recorded for patients receiving combination carboplatin and paclitaxel chemotherapy. Patients transitioned to another treatment regimen due to adverse reaction, toxicity, or intolerance were excluded. An exploratory analysis was then performed to find any beneficial or detrimental hemoglobin level. The survival analysis was based on a Cox regression analysis.

## Results

The nadir of absolute lymphocyte concentrations was associated with platinum status and clinical response (Figure 1A). 94/115 patients had a lymphocyte count nadir after the third cycle of chemotherapy. 71/94 (75.5%) were platinum sensitive, 21/94 (22.3%) were resistant, and 2/94 (2.1%) were refractory. Of those that experienced a nadir before three cycles, ten (47.6%) were sensitive, ten (47.6%) were resistant, and one (4.7%) was refractory ($p = 0.04$). Considering nadir values continuously, both overall survival (OS, $p = 0.0068$) and progression free survival (PFS, $p = 0.0321$) were strongly associated with late nadir points. Twenty-one of the 115 patients had a nadir value earlier than the third draw and this was associated with progressive disease, platinum resistance, poor OS, and poor PFS. The effect sizes are great [median OS: 33 *vs*. 66 months ($p = 0.003$), median PFS: 14 *vs*. 38 months ($p = 0.0032$), early *vs.* late nadir, respectively (Figure 1B)].

The hypothesis was validated: for every anemic (hemoglobin <11 g/dL) chemotherapy cycle, hazard of progression and death increases (OS, HR = 3.51, 95% CI: 1.63−7.54, $p = 0.0013$; PFS, HR = 2.20, 95% CI: 1.12−4.33; $p = 0.0223$). Further, patients with three or more anemic cycles experience greater morbidity and mortality (OS, HR = 2.34, 95% CI: 1.37−4.01; log-rank $p = 0.00145$) and have decreased PFS (HR = 1.88, 95% CI: 1.17−3.02; log-rank $p = 0.00743$, Figure 2). For patients with less than three anemic cycles *vs*. those with three or more, the median OS was 73.3 *vs*. 36.8 months, respectively ($p = 0.00145$). The median PFS was also significantly different at 32.4 *vs*. 15.9 months, respectively ($p = 0.00743$). In addition, mean hemoglobin less than 12.5 is associated with prognosis (OS, HR = 2.11, 95% CI: 1.03−4.33; $p = 0.042$; PFS, HR = 1.91, 95% CI: 1.02−3.56; $p = 0.041$).

## Discussion

Chemotherapy regimens that cause direct tumor cell death also produce several off target effects including lymphopenia and its associated immune suppression. Recent evidence demonstrates that tumor growth is a result of immune modulation by activating immune regulatory pathways, such as modulation of tumor antigen expression, altered expression of T cell activation and inhibitory molecules, and antigen processing and presentation [4]. Recent studies have shown that chemotherapy induced lymphopenia and subsequent hematological reconstitution resets the immunological thermostat in tumor-bearing hosts and possibly reinitiates an effective immune deterrence against minimal residual disease [4]. Some tumors have been found to contain lymphocytic infiltrate exhibiting oligoclonal expansion that recognize tumor antigens and display tumor-specific cytolytic activity when tested directly ex vivo [5]. In addition, there are significant differences in PFS and OS in patients with versus without tumor infiltrating lymphocytes (TILs) in EOC (38% five years *vs.* 4.5%) [6]. Lymphopenia and neutropenia caused by chemotherapy has been historically associated with poor clinical outcomes. However, it has also been observed that chemotherapy induced lymphopenia causes a "reboot" of the immune system which is associated with supranormal levels of circulating homeostatic cytokines like IL-7 and IL-15, that can enhance T-cell activation and proliferation, termed as homeostatic proliferation (HP), leading to augmented immune protection against tumor [7].

The incidence of anemia in patients with solid tumor malignancies is as high at 59% [8]. Anemia is associated with poor performance status, decreased functional capacity, and poorer quality of life [9-12]. While it has been established that low pretreatment hemoglobin level has ill effects on OS[3], it is not clear how intra-chemotherapy anemia effects outcome. This evidence showing significant improvement in OS and PFS with fewer than three cycles of hemoglobin < 11 g/dL sheds new light on the need for further studies on growth stimulating factors and blood transfusion during this

treatment period. We must further ascertain what the intrinsic and extrinsic differences are between these patients with less than three cycles of anemia and those with three or more.

## Conclusion

In conclusion, more studies must be done to further delineate the effects of growth factors and blood transfusion in the perichemotherapy period. However, the present authors have shown that the nadir of absolute lymphocyte concentrations is an independent predictor of OS and PFS. This is an easily measurable biomarker which can be utilized for identifying patients that will be likely to respond to immunomodulation.

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