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17β-Estradiol-Mediated Elevation of Peripheral White Blood Cell Count During Estramustine Phosphate Therapy for Prostate Cancer

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ABSTRACT

Background: In 1983, Daponte *et al.* first reported an increase in the peripheral white blood cell (WBCs) counts of prostate cancer patients treated with estramustine phosphate (EMP) therapy.
Objectives: In order to confirm Daponte's observation, we reviewed the clinical data of prostate cancer patients treated with EMP. We also examined the association between WBC counts and 17β-estradiol levels throughout the duration of the EMP therapy.
Patients and Methods: The study population comprised of 66 prostate cancer patients who were being treated with EMP. The complete blood count with a differential WBC count and the levels of serum 17β-estradiol and C-reactive protein measured during the therapy were compared with the baseline levels. The correlation between serum 17β-estradiol level and WBC count was calculated using the Pearson correlation test.
Results: We observed that the total WBC and the neutrophil counts were significantly elevated during the therapy. The serum 17β-estradiol level significantly correlated with the WBC count ($r^2 = 0.031$, $P = 0.002$). The granulocyte colony-stimulating factor levels measured during therapy were approximately 2-fold higher than the upper limit and decreased after cessation of the treatment ($P = 0.037$).
Conclusions: We successfully confirmed Daponte's observation. The increase in WBC counts was possibly attributable to elevated serum 17β-estradiol levels.

▸ *Implication for health policy/practice/research/medical education:*
Nonphysiological high-dose exposure of 17β-estradiol for prostate cancer patients resulted in significant elevation of neutrophil counts.

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1. Background

Estramustine phosphate (EMP) is a chemoendocrine agent used for the treatment of castration-resistant prostate cancer. EMP consists of 17β-estradiol (E2) bound to a nor-nitrogen mustard moiety. Initially, in the mid-1960s, EMP was synthesized for the treatment of breast cancer (1, 2). Later, in the 1970s, it was used for the treatment of prostate cancer and became commercially available in the Japanese market since 1984. EMP causes a variety of adverse effects, such as gastrointestinal toxicity, hepatic dysfunction, peripheral edema, and coagulation disorders. We had previously reported that inter-individual differences in adverse events and prognosis could be explained by single-nucleotide polymorphisms located on certain genes related to E2 metabolism (3-5). Most cytotoxic agents cause myelosuppression and neutropenia. However, EMP, also a cytotoxic agent, increases the white blood

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cell (WBC) count rather than causing leukocytopenia.

In a phase 2 clinical trial conducted by Alexander *et al.* on 44 postmenopausal women with breast cancer, no bone marrow suppression was observed in any Participants, and WBC counts rose in 38 of the 44 patients (86.4%) treated with EMP 420 mg daily (6). A clinical trial (EORTC 30762) conducted by Daponte *et al.* in 1983 was the first to report that EMP, but not diethylstilbestrol, increased the WBC count of patients with prostate cancer (7). Twenty-one patients with advanced prostate cancer were treated with EMP 240 mg *bid* for 2 months and 140 mg *bid* thereafter; the WBC counts increased in 17 patients (81.0%), while neutrophilic leukocytosis was observed in 4 patients (19.0%). We speculated as to why the WBC count increased during EMP therapy, since most cytotoxic agents cause leukocytopenia but not leukocytosis. Till date, Daponte's observation had not been confirmed by any large-scale studies.

In 2003, Molloy *et al.* demonstrated that E2 reduced the rate of apoptosis of neutrophils in a dose-dependent manner (8). Their study suggested a possible mechanism underlying the increase in the systemic neutrophil count observed in women, especially in the pregnancy phase. Subsequently, we hypothesized that elevation of E2 levels might affect the elevation of the WBC count during EMP therapy.

2. Objectives

In this study, to confirm Daponte's observation in a larger sample, we investigated changes in the WBC counts and its fraction in prostate cancer patients administered EMP therapy (duration, 2 years; dosages, 140 mg/day or 280 mg/day). We also measured the serum E2 levels to determine the correlation with WBC counts.

3. Patients and Methods

3.1. Patients

In this study, we retrospectively investigated the hematological and biochemical data of patients with advanced or recurrent prostate cancer who were on oral EMP therapy. A total of 71 patients received oral EMP therapy between June 1999 and November 2007. Our treatment concept and regimens have been described in detail elsewhere (9, 10). We excluded 5 patients from this analysis because of insufficient observation period (less than 3 months) or lack of hematological data. The clinical characteristics of the remaining 66 patients are shown in *Table 1*. The tumor status of the patients was evaluated by performing a digital rectal examination, transrectal ultrasonography, pelvic computed tomography, and bone scintigraphy, according to the 2002 TNM staging system for cancer (11). Eighteen patients had undergone radical prostatectomy, and their T-stage and Gleason score were determined on the basis of the pathological findings as well. The complete blood count with a differential WBC count and the levels of serum E2, and C-reactive protein (CRP) were measured before commencement of therapy and every 1–3 months thereafter. All patients were in-

Table 1. Clinical Characteristics of Patients

	EMP 140 mg/day + LHRHa [b] Analog (n = 25)	EMP [b] 280 mg/day (n = 41)	Total (n = 66)
Age at diagnosis, y			
Mean ± SD [b]	68.4 ± 8.4	68.4 ± 8.4	68.8 ± 8.1
Range	55 – 81	48 – 89	48 – 89
Pretreatment PSA level (ng/mL)			
Mean ± SD [b]	636.1 ± 1463.6	353.0 ± 1265.2	460 ± 1339.8
Range	0.2 – 5972.1	0.03 – 7265.0	0.03 – 7265.0
T-stage			
≤ T2	6	8 (1) [a]	14 (1) [a]
T3	13 (3) [a]	30 (12) [a]	43 (15) [a]
T4	6 (1) [a]	3 (1) [a]	9 (2) [a]
Gleason score			
≤ 6	5	4 (2)	9 (2) [a]
7	6	11 (4)	17 (4) [a]
8-10	13 (4) [a]	26 (8)	39 (12) [a]
Missing	1	0	1
Duration of treatment (days)			
Mean ± SD [b]	1207 ± 832.2	998.7 ± 682.1	1077.8 ± 743.3
Range	106 – 2533	108 – 3060	106 – 3060
Observation (days)			
Mean ± SD [b]	1621.0 ± 693.9	1435.4 ± 892.3	1505.8 ± 822.2
Range	106 – 2533	231 – 3531	106– - 3531

[a] Numbers written in the parenthesis mean number of patients diagnosed by specimens obtained from radical prostatectomy.
[b] Abbreviations: EMP, Estramustine phosphate; LHRHa, Luteinizing hormone-releasing hormone analog; SD, Standard deviation

formed of the investigational nature of this study, and they provided written informed consent.

3.2. Granulocyte Colony-Stimulating Factor Measurement

Stored serum samples extracted from only 3 patients who received EMP 280 mg/day were available; we measured the serum levels of granulocyte colony-stimulating factor (G-CSF) and granulocyte-monocyte colony-stimulating factor (GM-CSF) by using enzyme-linked immunosorbent assay (SRL Inc., Hachioji, Japan). These serum samples were stored at -80° C until the analyses.

3.3. Statistical Analyses

We compared the age of the patients using an unpaired t-test. The chi-square test was performed to compare the distribution of tumor status, clinical stage, Gleason sum, and castration status. The complete blood count and levels of E2, G-CSF, and GM-CSF were expressed as mean ± standard deviation (SD). The differences from the baseline values and the post-treatment data were analyzed for statistical significance by using the paired Student's t-test or the Dunnett-Hsu procedure. Intergroup differences were assessed using the analysis of covariance (ANCOVA) model, with the baseline value as a covariate. The correlation between E2 and WBC count was evaluated using the Pearson correlation test. Because the distribution of serum E2 levels was skewed, we logarithmically transformed the values to estimate the geometric mean. The data were analyzed using the Statistical Analysis System (SAS) program (JMP, Ver. 8.2.0, SAS Institute Inc., Cary, NC, USA). *P*-values of less than 0.05 were considered statistically significant.

4. Results

The mean changes in the WBC and neutrophil counts during and after cessation of EMP therapy are shown in *Figure 1*. The WBC counts increased rapidly and reached a plateau at 3 months. This increase was significant and dose-dependent. *Figure 2* shows a waterfall plot of changes in the WBC count at 3 months from the baseline. The WBC counts were higher than the baseline values in 84.0% (21/25) of the subjects who were on EMP 140 mg/day and in 90.2% (37/41) of subjects who were on EMP 280 mg/day. Throughout the treatment period, 5 patients (20.0%) who were on EMP 140 mg/day and 12 patients (29.3%) on EMP 280 mg/day developed leukocytosis (WBC count > 11,000/μL); none of the patients showed leukocytopenia. Twenty-five patients received luteinizing hormone-releasing hormone analog (LHRHa); 5 of these

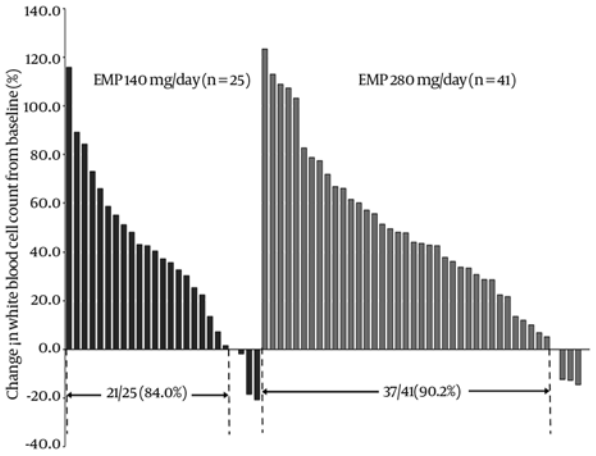

Figure 2. Waterfall Plot; Each Bar Indicates Individual Changes in the WBC Count at 3 Months From the Baseline

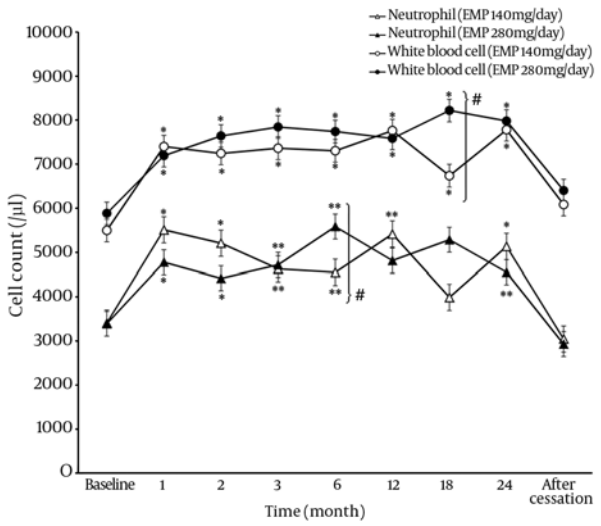

Figure 1. Mean Changes in WBC and Neutrophil Count During EMP Therapy and After Cessation of Therapy

*: $P < 0.001$, **: $P < 0.05$, #: $P < 0.005$

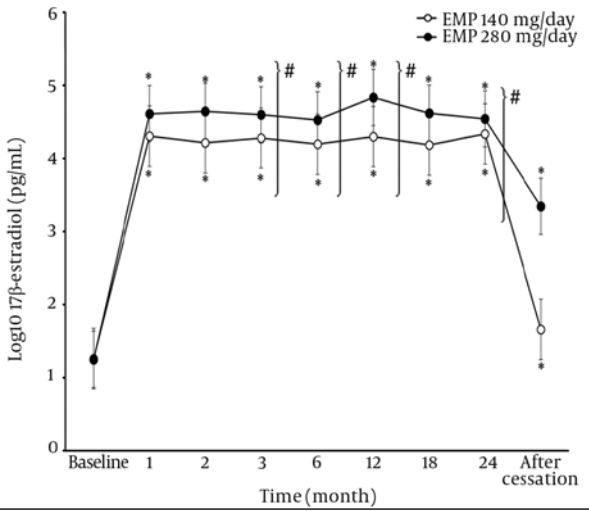

Figure 3. Mean Changes in E2 levels During EMP Therapy and After Cessation of Therapy

*: $P < 0.001$, **: $P < 0.05$, #: $P < 0.005$

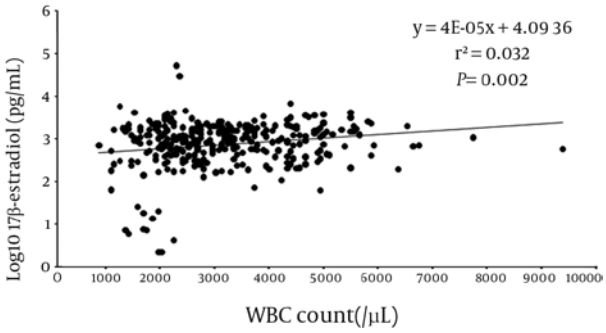

Figure 4. A Scatter Plot Showing the Relationship Between Geometric Mean of Serum E2 Levels and WBC Counts

patients (20.0%), who received EMP with LHRHa, and 12 (29.3%), who received EMP alone, developed leukocytosis (odds ratio [OR], 0.604; 95% confidence interval [CI], 0.170–1.909; $P = 0.398$). The median (inter-quartile range [IQR]) of serum E2 level was 17,360 (10,249–25,030) pg/mL in men on EMP 140 mg/day and 38,430 (25,530–49, 904) pg/mL on EMP 280 mg/day ($P < 0.0001$). The neutrophil counts and E2 levels significantly increased (*Figures 1 and 3*); however, other WBC fractions (eosinophils, basophils, monocytes, and lymphocytes) or CRP levels were not significantly different from the baseline values (data not shown). The Pearson correlation coefficient (r^2) between the geometric mean of serum E2 level and WBC count was 0.031 ($P = 0.002$; *Figure 4*). After cessation of treatment, WBC and neutrophil counts decreased, and the difference between these and the baseline values was no longer significant.

We could assess the serum GM-CSF and G-CSF levels in only 3 patients who received EMP 280 mg/day. The serum levels of GM-CSF were undetectable in all 3 samples. The mean ± SD of the G-CSF levels were 97.8 ± 41.5 pg/mL during the treatment period and 80.0 ± 51.9 pg/mL at 1 month after cessation of treatment ($P = 0.037$).

5. Discussion

We confirmed Daponte's findings in a 3-fold larger patient population and observed that serum E2 levels and WBC counts were maintained throughout the treatment period and decreased only after cessation of the EMP therapy. To our knowledge, this is the first report that shows a significant correlation between serum E2 levels and WBC counts during EMP therapy for prostate cancer.

E2, a major metabolite of EMP, inhibits the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), thus leading to decreased production of testosterone (9). In this study, the serum E2 level measured during EMP therapy was approximately 17,000 pg/mL in patients who were on EMP 140 mg/day and 38,000 pg/mL in patients who were on EMP 280 mg/day. O'Leary *et al.* reported that in the case of normal pregnancy, the serum E2 level increased gradually and reached 53.44 nmol/L (equal to 14, 557.34 pg/mL; molecular weight of E2, 272.39 kDa) at week 40 (12). Lurie *et al.* observed that WBC counts, particularly the neutrophil count, were elevated through the course of normal pregnancy (13). Bergstrøm *et al.* reported that hormone replacement therapy consisting of E2 caused leukocytosis (14). Interestingly, both the WBC counts and G-CSF levels increase significantly under exogenous gonadotropin stimulation (15). Salmassi *et al.* reported that gonadotropins influenced G-CSF production (16); however, Kitamura *et al.* showed that the serum levels of FSH and LH were very low during EMP therapy (9, 10). The nonphysiological level of E2 possibly caused the elevation in the WBC count, and E2 alone or its metabolites, but not FSH, LH, or LHRHa, may influence G-CSF levels and WBC counts. Our hypothesis is supported by a previous study that has provided evidence that neutrophil leukocytosis is caused by a high dose of ethynylestradiol (5.0 mg/kg) in female beagle dogs (17). Considering that the correlation coefficient between E2 and WBC was very low, further studies are required to investigate the association between WBC counts and other EMP metabolites.

EMP is usually administered along with other cytotoxic agents (i.e., docetaxel, paclitaxel). Docetaxel and paclitaxel can cause neutropenia. In a meta-analysis of individual patient data from randomized trials that assessed chemotherapy with or without EMP treatment in patients with castration-resistant prostate cancer, grade 3 or 4 neutropenia was noted in 41 of 275 (14.9%) patients who received chemotherapy without EMP and in 16 of 271 (5.9%) patients who received chemotherapy plus EMP (overall risk ratio [RR], 0.41; 95% CI, 0.23–0.71; $P = 0.002$) (18). EMP may have a protective effect against myelosuppression. The present study is the first to report a significant correlation between E2 levels and WBC counts during EMP therapy for prostate cancer. Further studies are warranted to understand the mechanism of leukocytosis caused by EMP therapy for prostate cancer.

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