THE CORRELATION BETWEEN INCREASED APOPTOSIS AND DECREASED PERIPHERAL BLOOD WBC IN PATIENTS RECEIVING CHEMOTHERAPY FOR OVARIAN CANCER

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ABSTRACT

Objective: The purpose of this study was to determine whether the decrease of WBC is correlated with the increase of apoptosis induced by cytotoxic drugs in patients who received neoadjuvant polychemotherapy for ovarian cancer and whether the reduction of peripheral blood WBC can be predicted by the detection of apoptosis. Methods: The study included 25 patients who received neoadjuvant polychemotherapy for ovarian cancer after operation. Total 2 ml of venous blood was collected from these subjects within 24 hours before chemotherapy and at the fifth day after the beginning of chemotherapy. Peripheral blood WBC count was performed and its apoptosis was analyzed using flow cytometry (FCM) and DNA electrophoresis. Results: 68% (17/25) of the patients had a decrease in WBC after chemotherapy. The average counts of WBC were 5.19±1.36×10⁹/L and 4.36±1.56×10⁹/L, the distributions were 4.10~8.60×10⁹/L and 2.00~7.90×10⁹/L before and after chemotherapy respectively. At the same time, 64%(16/25) of the patients had an increase in apoptotic cells. The proportions of apoptosis were 4.01±2.59% and 5.66±1.36%, the distributions were 1.05~11.02% and 0.8~14.08% before and after chemotherapy respectively. Both the decrease of WBC and the increase of apoptosis were statistical significant (P<0.05). The coefficient between the decrease of WBC and the increase of apoptosis is 0.646(P<0.05). The sensitivity of the quantitative analysis of apoptosis using FCM for clinical early diagnosis of the decrease of WBC is 82%, the speciality is 75% and the accuracy is 80%. Conclusion: The increased apoptosis induced by cytotoxic drugs contributed to the chemotherapy-associated reduction of WBC at some extend, there were somewhat correlation between them. The detection of peripheral apoptosis could be of some help to assess the decrease and scientific bases for the administration of G-CSF, GM-CSF to obtain the optimal cost-effectiveness of clinical chemotherapy.

Key words: Ovarian cancer; Chemotherapy; Apoptosis; WBC; Correlation

As it is known, chemotherapy is an important adjuvant therapy for patients with ovarian cancer after operation, for ovarian cancers are still difficult to be discovered and diagnosed at early stage. Furthermore, ovarian cancer cells are sensitivity to the platinum-based chemotherapy regimens, a positive effect can also be achieved even for the patients at late stage and the survival duration of the patients can be prolonged, therefore, chemotherapy has become a crucial treatment for patients with ovarian cancer to obtain long time survival. However, the cytotoxic drugs can not selectively kill the cancer cells without any side effects. The decrease of peripheral blood WBC due to myelosuppression caused by cytotoxic drugs contributes to the limitation of regular chemotherapy and it has been a life-threaten factor for the patients. Consequently, to forecast the decrease of peripheral blood WBC and perform protective therapies as early as possible have become a hot area of clinical researches. Evidences were provided that two kinds of drug that regularly used for ovarian cancer: cisplatin and cyclophosphamide caused apoptosis in several tumors. For the reasons above, in our study, we detected the apoptosis of total peripheral blood WBC in patients with ovarian cancer based on the specific evidences of morphology and molecular biology.
before and after chemotherapy. Then, we analyzed the correlation between the increase of apoptosis and the decrease of peripheral blood WBC to explore the means to forecast the reduction of WBC in patients receiving chemotherapy for ovarian cancer.

**PATIENTS AND METHODS**

**Patients and Drug Administration**

The study included 25 women who received neoadjuvant polychemotherapy after operation for ovarian cancer. Systematic examination was performed to the patients to insure that the patients had no immunological diseases, infections and others that affect the counts of WBC before the beginning of chemotherapy. Chemotherapy was begun once the parameters were affirmed in normality. Neoadjuvant polychemotherapy regimen consisted of cisplatin (60-70 mg/m²) and cyclophosphamide (600-700 mg/m²). Patients who received drugs for rapidly declined WBC were rejected from the study.

**Preparation of Purified WBC and Count of Total Peripheral Blood WBC**

Total 2 ml of venous blood was collected from patients within 24 h before chemotherapy and at the fifth day after the beginning of chemotherapy using heparin (100 U/ml) as anticoagulant. After incubation at 37°C for 2 h in 10-folds volume of PBS (pH 7.4), erythrocytes were lysed by addition of hypotonic lysis buffer (NH₄Cl solution 8.29 g/L containing EDTA0.037g/L and KHCO₃ 1g/L) and WBC were centrifuged and resuspended in PBS (pH7.4). The preparation contained greater than 97% polymorphonuclear leukocytes of which 95% were neutrophils cells. Cell viability was greater than 98% as determined by trypan blue exclusion.

The amount of peripheral blood WBC inclined to be affected by physical factors, therefore the samples must be taken on fasted condition in the morning. Total white blood cell (WBC) counts were performed by certified hematology laboratory.

**Apoptosis Analysis**

Apoptosis of WBC was assessed by two distinct methods, analysis of apoptotic nuclei (hypodiploid) by FCM and detection of DNA double-stranded breaks by DNA electrophoresis.

**The Quantitative Analysis of Apoptosis Using FCM**

1 - 5 × 10⁶ purified WBC were fixed with 70% ethanol for 30 min at 4°C. After washed with PBS twice, the cells were gently resuspended in 0.5 ml hypotonic fluorochrome solution (100 mg/L propidium iodide, 100 mg/L RNase in PBS pH7.4, they were both purchased from Sigma Company) for 30 min, then immediately analyzed using flow cytometry. Cell debris and adhesive cells were excluded by gating on physical parameters. Data were analyzed using cell FIT cell-cycle analysis versin 2.0 software (Becton Dickinson). For each sample, 1 × 10⁵ cells were counted. The percentage of hypodiploid nuclei reported in DNA fluorescence histograms reflected the relative proportion of apoptotic cells.

**The Qualitative Analysis of Apoptosis Using DNA Electrophoresis**

2% agarose electrophoresis was performed to assess DNA fragmentation. The purified WBC were pelleted by centrifugation, lysed and incubated in TE overnight at 37°C by the addition of 10% SDS and 2mg/L proteinase. DNA was extracted twice with phenol/chloroform/ isopropanol (25:24:1), and precipitated with 1 / 10 volume of NaAC(3M/L) and 1 volume of ethanol (100%) at -20°C for 10 min. Electrophoresis was conducted in 0.5 × TBE buffer at 150V for about 25 min. After staining with ethridium bromide, DNA was visualized by UV examination for photography (Marker was purchased from a Branch of Takara Co., Da Lian, China).

**Statistical Analysis**

Data were expressed as $\bar{x} \pm s$. The values were compared using the student's two-tailed $t$ test. Differences were regards as significant if $P<0.05$. The relationships between the increased apoptosis and the reduction of WBC were analyzed using line regression method, the sensitivity of apoptosis detection as a means of early diagnosis of WBC reduction was accessed using clinical epidemiology.

**RESULT**

**Total WBC Counts before and after Chemotherapy**

Of 25 patients, 17(68%) had a decrease in WBC after chemotherapy. Among them, ten patients were lower than 4.0 × 10⁹/L, the counts of WBC were 5.19±1.36 × 10⁹/L and 4.36±1.56 × 10⁹/L, the distributions were 4.10~8.60 × 10⁹/L and 2.00~7.90 × 10⁹/L before and after chemotherapy respectively. The decrease of WBC had statistical significance ($P<0.05$) (paired student’s two-side test) (Figure 1).