Leukocyte Cell Population Analysis From the Coulter Automatic Blood Cell Analyzer DxH800 to Monitor the Effect of G-CSF

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Background: Granulocyte colony-stimulating factor (G-CSF) induces the formation of toxic granulation neutrophils (TGNs), which are found in many inflammatory responses. Cell population data (CPD) may be able to clarify the effect of G-CSF, and potentially help doctors in discriminating the effect of G-CSF from other inflammatory situations. **Methods:** To achieve this, we performed analyses of leukocyte CPD from normal controls and healthy donors that had received G-CSF for peripheral blood stem cells (PBSCs) mobilization (G-CSF group). **Results:** Two hundred and seventy-one subjects were enrolled as normal controls, and 21 subjects were enrolled in the G-CSF group. Mean volume (MN-V)-neutrophils (NE), mean axial light loss (MN-AL2)-NE, and all standard deviation (SD) parameters increased significantly, whereas all light scattering parameters, mean median angle light scatter (MN-MALS)-NE, mean upper median angle light scatter (MN-UMALS)-NE, mean lower

median angle light scatter (MN-LMALS)-NE, and mean low angle light scatter (MN-LALS)-NE reduced significantly in the G-CSF group. MN-V-lymphocytes (LY) from the G-CSF group showed no significant difference ($P = 0.143$), whereas MN-V-monocytes (MO) were significantly decreased ($P < 0.001$). Receiver operating characteristic (ROC) curves for the discrimination of the G-CSF group from normal controls showed excellent sensitivity in SD-LALS-NE (at 30.85, sensitivity 95.2%, specificity 76.0%), MN-AL2-NE (at 134.5, sensitivity 90.5%, specificity 83.0%), and SD-AL2-NE (at 16.4, sensitivity 95.2%, specificity 95.2). Several CPD parameters of lymphocytes and monocytes, as well as neutrophils can be used as markers for determining the effect of G-CSF. **Conclusion:** Our data show that many CPD of leukocytes can be considered to be useful parameters of the effect of G-CSF. J. Clin. Lab. Anal. 26:194–199, 2012. © 2012 Wiley Periodicals, Inc.

Key words: granulocyte colony-stimulating factor; leukocyte cell population; toxic granulation neutrophils

INTRODUCTION

Granulocyte colony-stimulating factor (G-CSF) has sometimes been used after chemotherapy and allogeneic stem-cell transplantation (SCT) to shorten the neutropenic phase (1–3). It was reported that G-CSF induced the formation of toxic granulation neutrophils (TGNs), which have prominent azurophilic cytoplasmic granules in blood smears stained by the Wright or May-Grünwald-Giemsa technique (4, 5). However, these changes in neutrophils can also be detected in several inflammatory responses including infection, tissue damage, and other inflammatory diseases (6–8). In addition, the degree of toxic change and neutrophil size was variable; some neutrophils showed toxic changes whereas others did not, even within the same samples. Therefore, microscopic examination by

experts can be subjective, and not reproducible (9). In particular, specific features in samples after G-CSF are more difficult to quantify with the naked eye alone. Recently, the automatic blood cell analyzer DxH800 (Coulter Inc., Miami, FL) has been shown to be effective, revealing significant cell population data (CPD) for each subpopulation of white blood cells (WBCs).

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Received 30 December 2010; Accepted 22 December 2011
DOI 10.1002/jcla.21493

Published online in Wiley Online Library (wileyonlinelibrary.com).

In this study, we analyzed leukocyte CPD of normal controls and healthy donors that had received G-CSF for peripheral blood stem cells (PBSCs) mobilization to clarify the effect of G-CSF on the CPD. The purpose of our study was to determine the usefulness of CPD as a marker for determining the effect of G-CSF.

MATERIALS AND METHODS

Two hundred and seventy-one subjects without hematological disorders (157 males and 114 females from 14 to 80 years of age) were enrolled as normal controls. Twenty-one healthy donors that received G-CSF (G-CSF group) for PBSCs mobilization (10 males and 11 females from 28 to 71 years of age) were selected to investigate the effects of G-CSF on CPD. The donor work-up included medical history, physical examination, ECG, chest x-ray, blood count, blood chemistry, coagulation screening, and testing for infectious disease markers. Samples with infection were excluded based on medical history, physical examination, and lab findings, and normal controls were selected since an infection caused toxic changes in the neutrophils and the presence of band neutrophils in the blood, which were translated into a change of CPD. The blood samples were analyzed using DxH800, and complete blood count (CBC) and CPD of neutrophils, lymphocytes, and monocytes were obtained.

The CPD of the leukocytes are listed in Table 1. The mean (MN) and standard deviation (SD) values of volume, conductivity, and five-angle light scattering parameters, corresponding to a total of 14 parameters, were collected. The light scattering parameters were as follows: median angle light scatter (MALS), upper median angle light scatter (UMALS), lower median angle light scatter (LMALS), low angle light scatter (LALS), and axial light loss (AL2). The light scattering parameters reflected cellular complexity, granularity, and nuclear structure, but AL2 reflected cell size on the basis of absorbed light. The protocols of this study were approved by the Catholic Medical Center Institutional Review Board.

PBSCs Mobilization Procedure from Healthy Donor

The 21 healthy donors were all genetically related siblings of the patients who underwent allogeneic SCT for hematologic malignancies at the Catholic Blood and Marrow Transplantation Center between 2009 and 2010. All donors were mobilized with G-CSF (Filgrastim, Dong-A Pharmaceutical, Seoul) at doses of 10 µg/kg/day, subcutaneously once a day. G-CSF was administered for 4 consecutive days and leukapheresis procedures were commenced on Day +4 of the G-CSF administration. The analyses of CPD were done from a blood sample on Day +4, before leukapheresis.

TABLE 1. CPD of Neutrophils, Lymphocytes, and Monocytes and Abbreviations

Neutrophils		Lymphocytes		Monocytes	
Mean volume	MN-V-NE	Mean volume	MN-V-LY	Mean volume	MN-V-MO
SD volume	SD-V-NE	SD volume	SD-V-LY	SD volume	SD-V-MO
Mean conductivity	MN-C-NE	Mean conductivity	MN-C-LY	Mean conductivity	MN-C-MO
SD conductivity	SD-C-NE	SD conductivity	SD-C-LY	SD conductivity	SD-C-MO
Mean median angle light scatter	MN-MALS-NE	Mean median angle light scatter	MN-MALS-LY	Mean median angle light scatter	MN-MALS-MO
SD median angle light scatter	SD-MALS-NE	SD median angle light scatter	SD-MALS-LY	SD median angle light scatter	SD-MALS-MO
Mean upper median angle light scatter	MN-UMALS-NE	Mean upper median angle light scatter	MN-UMALS-LY	Mean upper median angle light scatter	MN-UMALS-MO
SD upper median angle light scatter	SD-UMALS-NE	SD upper median angle light scatter	SD-UMALS-LY	SD upper median angle light scatter	SD-UMALS-MO
Mean lower median angle light scatter	MN-LMALS-NE	Mean lower median angle light scatter	MN-LMALS-LY	Mean lower median angle light scatter	MN-LMALS-MO
SD lower median angle light scatter	SD-LMALS-NE	SD lower median angle light scatter	SD-LMALS-LY	SD lower median angle light scatter	SD-LMALS-MO
Mean low angle light scatter	MN-LALS-NE	Mean lower angle light scatter	MN-LALS-LY	Mean lower angle light scatter	MN-LALS-MO
SD low angle light scatter	SD-LALS-NE	SD lower angle light scatter	SD-LALS-LY	SD lower angle light scatter	SD-LALS-MO
Mean axial light loss	MN-AL2-NE	Mean axial light loss	MN-AL2-LY	Mean axial light loss	MN-AL2-MO
SD axial light loss	SD-AL2-NE	SD axial light loss	SD-AL2-LY	SD axial light loss	SD-AL2-MO

SD, standard deviation.

Statistical Analysis

The average values of CPD parameters are expressed as $MN \pm SD$. To determine differences in effects between G-CSF and normal controls, statistical analyses were conducted using Mann–Whitney's *U*-test. A *P*-value of ≤ 0.05 was regarded as statistically significant. We plotted receiver operating characteristic (ROC) curves to determine the area under the curve (AUC) with cutoff levels for the optimal combination of sensitivity and specificity. The sensitivity and specificity of each parameter for differences in effects between G-CSF and normal controls were analyzed. Statistical studies were performed using the Statistical Package for the Social Sciences, version 13.0 (SPSS, Inc, Chicago, IL).

RESULTS

Neutrophil CPD Analyses between Normal Control and the G-CSF Group

Table 2 shows the results of CPD analyses between normal controls and the G-CSF group. We noted significant differences in all neutrophil CPD. MN-V-NE and MN-AL2-NE significantly increased in the G-CSF group relative to the normal controls ($P < 0.001$). All other light scattering parameters, MN-MALS-NE, MN-UMALS-NE, MN-LMALS-NE, and MN-LALS-NE significantly reduced in the G-CSF group ($P < 0.001$). In addition, MN-C-NE was significantly decreased in the G-CSF group ($P < 0.001$). All SD parameters, including SD-C-NE increased significantly in the G-CSF group ($P < 0.001$). Figure 1 shows ROC of neutrophil CPD for discrimination of the G-CSF group from normal controls.

Lymphocyte CPD Analyses between Normal Controls and the G-CSF Group

For the analyses of lymphocyte, all lymphocyte CPD except MN-V-LY, MN-LALS-LY revealed significant differences between the G-CSF group and normal controls. There was no significant difference in MN-V-LY of the G-CSF group relative to the normal controls ($P = 0.143$). However, MN-AL2-LY significantly increased in the G-CSF group relative to the normal controls ($P < 0.001$). All light scattering parameters except MN-LALS-LY were significantly higher in the G-CSF group than in the normal control. MN-C-NE significantly increased in the G-CSF group ($P = 0.009$). All SD parameters including SD-C-NE increased significantly in the G-CSF group ($P < 0.001$). Figure 2 shows ROC of lymphocyte CPD for discrimination of the G-CSF group from normal control.

Monocyte CPD Analyses between Normal Controls and the G-CSF Group

For the analyses of monocytes, MN-V-MO decreased significantly in the G-CSF group relative to the normal controls ($P < 0.001$). However, MN-AL2-MO significantly increased in the G-CSF group relative to the normal controls ($P = 0.003$), whereas MN-LALS-MO reduced significantly in the G-CSF group ($P = 0.004$). However, other light scattering parameters, MN-MALS-MO, MN-UMALS-MO were significantly higher in the G-CSF group. Among SD parameters, SD-V-MO, SD-C-MO, SD-UMALS-MO, SD-LALS-MO, SD-AL2-MO significantly increased in the G-CSF group. Figure 3 shows the ROC of monocyte CPD for discrimination of the G-CSF group from normal controls.

DISCUSSION

G-CSF has been identified as a glycoprotein that stimulates the survival, proliferation, differentiation, and functional activation of neutrophilic granulocytes both in vivo and in vitro (10, 11). When the serum concentration of G-CSF is increased, it is known that TGNs increase in the blood. Takeshita et al. reported that by using a high concentration of G-CSF (10–100 $\mu\text{g/mL}$), G-CSF-induced TGN formation (4). Another study using physiological levels of G-CSF showed that neutrophils changed to TGN-like cells in response to 1000 pg/mL of G-CSF added in peripheral blood from a healthy subject (5).

However, since all previous studies were performed with microscopic examinations using the naked eye alone, it was difficult to accurately quantify changes. In particular, it would be impossible to detect changes in lymphocytes and monocytes. In this context, we performed analyses of leukocyte CPD to determine the usefulness of CPD as a marker for investigating the effect of G-CSF.

In this study, we noted significant differences between samples from healthy donors that had received G-CSF (G-CSF group) and normal controls with regards to MN-V-NE and SD-V-NE. This showed that the neutrophils of subjects in the G-CSF group were larger and their size variations were greater than in the normal controls. Meanwhile, the monocyte volume parameter (MN-V-MO) of the G-CSF group decreased and there was no difference in the lymphocyte volume parameter (MN-V-LY), even though large variations in size (SD-V-LY, SD-V-MO) were observed. These CPD of monocytes and lymphocytes make it possible to compare the change of morphology by administration of G-CSF, which is impossible by microscopic examinations. All lymphocyte SD parameters increased in the G-CSF group compared with normal controls. This showed that certain lymphocytes were altered as a result of G-CSF administration. In this study, we

TABLE 2. The leukocytes CPD of G-CSF group compared with normal control

Neutrophil													
Type	MN_V_ NE	SD_V_ NE	MN_C_ NE	SD_C_ NE	MN_ MALS_ NE	SD_ MALS_ NE	MN_ UMALS_ NE	SD_ UMALS_ NE	MN_ LMALS_ NE	SD_ LMALS_ NE	MN_ LALS_ NE	SD_ LALS_ NE	SD_ AL2_ NE
Normal (n = 271)	154.4 ± 8.1	19.9 ± 2.7	145.0 ± 10.6	6.5 ± 1.9	138.4 ± 4.8	10.3 ± 1.2	140.3 ± 9.1	11.8 ± 2.2	129.9 ± 10.4	13.6 ± 2.4	156.7 ± 12.1	29.5 ± 4.1	129.2 ± 5.6
G-CSF group (n = 21)	177.2 ± 9.1	32.0 ± 1.6	137.6 ± 5.6	8.2 ± 1.3	123.9 ± 6.7	13.9 ± 1.6	134.5 ± 6.7	18.0 ± 4.7	107.9 ± 9.6	18.5 ± 2.5	123.5 ± 26.8	42.5 ± 6.4	141.2 ± 5.8
P-value*	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Lymphocyte													
Type	MN_V_ LY	SD_V_ LY	MN_C_ LY	SD_C_ LY	MN_ MALS_ LY	SD_ MALS_ LY	MN_ UMALS_ LY	SD_ UMALS_ LY	MN_ LMALS_ LY	SD_ LMALS_ LY	MN_ LALS_ LY	SD_ LALS_ LY	SD_ AL2_ LY
Normal (n = 271)	89.3 ± 4.0	14.4 ± 1.7	117.9 ± 8.1	9.6 ± 2.4	75.3 ± 4.9	15.6 ± 1.4	78.4 ± 8.6	18.2 ± 2.0	64.2 ± 5.8	18.4 ± 1.9	30.6 ± 1.5	9.3 ± 1.1	55.4 ± 4.2
G-CSF group (n = 21)	90.2 ± 6.1	19.7 ± 3.7	114.5 ± 3.0	11.9 ± 1.6	86.5 ± 4.1	18.2 ± 2.7	91.1 ± 7.5	21.2 ± 3.4	74.1 ± 2.8	20.7 ± 2.9	30.2 ± 2.7	10.7 ± 2.0	61.5 ± 4.9
P-value*	0.143	<0.001	0.009	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.368	0.001	<0.001
Monocyte													
Type	MN_V_ MO	SD_V_ MO	MN_C_ MO	SD_C_ MO	MN_ MALS_ MO	SD_ MALS_ MO	MN_ UMALS_ MO	SD_ UMALS_ MO	MN_ LMALS_ MO	SD_ LMALS_ MO	MN_ LALS_ MO	SD_ LALS_ MO	SD_ AL2_ MO
Normal (n = 271)	171.4 ± 6.4	18.9 ± 2.0	125.2 ± 8.9	6.4 ± 2.3	88.5 ± 4.2	11.2 ± 1.4	99.7 ± 7.5	12.3 ± 1.9	72.4 ± 6.0	14.2 ± 1.8	77.3 ± 9.4	23.4 ± 4.1	104.8 ± 7.1
G-CSF group (n = 21)	169.3 ± 12.5	32.8 ± 6.6	115.6 ± 8.4	15.0 ± 9.7	89.1 ± 12.7	11.9 ± 2.9	100.7 ± 14.6	18.2 ± 6.3	71.3 ± 11.8	15.2 ± 2.9	66.6 ± 18.4	27.4 ± 4.8	110.2 ± 18.3
P-value*	<0.001	<0.001	<0.001	<0.001	0.040	0.297	0.025	<0.001	0.411	0.072	0.004	<0.001	0.003

G-CSF, granulocyte colony-stimulating factor.

*Statistical significances were tested by Mann-Whitney's U-test.

Fig. 1. ROC curves of neutrophil CPD for discrimination of the G-CSF group from normal controls. SD-LALS-NE at 30.85, sensitivity 95.2%, and specificity 76.0%; MN-AL2-NE at 134.5, sensitivity 90.5%, and specificity 83.0%; SD-AL2-NE at 16.4, sensitivity 95.2%, and specificity 95.2%.

Fig. 2. ROC curves of lymphocyte CPD for discrimination of the G-CSF group from normal controls. MN-MALS-LY at 78.5, sensitivity 95.2%, and specificity 76.4%; MN-UMALS-LY at 87.5, sensitivity 85.7%, and specificity 92.3%; MN-LMALS-LY at 69.5, sensitivity 95.2%, and specificity 77.1%.

used samples after applying extrinsic G-CSF with a dose of 10 $\mu\text{g/kg/day}$ for 4 consecutive days. However, the serum concentration of G-CSF can also increase during inflammation responses. Kawakami et al. examined serum G-CSF levels in patients with infections and reported, the serum G-CSF level in the acute stage of infection was significantly higher than after the recovery phase, which was the same as the level in normal controls (12). Although we excluded samples that had infection, the possibility

Fig. 3. ROC curves of monocyte CPD for discrimination of the G-CSF group from normal controls. SD-V-MO at 26.8, sensitivity 95.2%, and specificity 100%; SD-C-MO at 6.90, sensitivity 76.2%, and specificity 72.7%; SD-AL2-MO at 15.1, sensitivity 81.0%, and specificity 93.7%.

of including undetected infection remains. Thus, further analyses of leukocyte CPD according to serum G-CSF levels including physiological levels are needed.

Interestingly, in blood samples of G-CSF group, many neutrophil CPD were similar to the CPD of patients with acute bacterial infection. One possible hypothesis to explain this similarity is that TGNs also increase after an inflammatory response, which is the defense mechanism against infection and tissue damage. Chaves et al. showed a significant increase of neutrophil volume and significant decrease of neutrophil light scatter by the Coulter-automated hematology analyzer in bacteremic cases (13). Another study from Chaves et al. in 70 patients with positive blood cultures for bacteria reported that the neutrophil volume distribution width significantly increased in the bacteremic patients ($P < 0.001$) (14). Our previous analyses in 117 patients with bacterial sepsis and MN-V-NE and MN-AL2-NE were significantly increased in the sepsis patients relative to the normal controls ($P < 0.05$) (data not shown). However, several lymphocyte and monocyte CPD of the G-CSF group were different from those of previously analyzed sepsis samples (unpublished data). Particularly with sepsis samples, MN-V-LY and MN-V-MO were significantly increased, whereas MN-V-LY of the G-CSF group was not significantly different compared with normal controls, and MN-V-MO was significantly decreased. These results may be due to certain lymphocytes and monocytes in the circulating blood after bacterial infection being different from those influenced by G-CSF.

Although toxic granulation changes of neutrophils in patients with infection could be caused by increasing the

intrinsic G-CSF, differences in the volumetric parameters in lymphocytes and monocytes of patients with infection is distinct from the effect of increasing G-CSF. The clinical importance of these results is that G-CSF was used in patients that underwent chemotherapy or allogeneic SCT and may have a high risk of infection. This suggests that CPD parameters could be used as an indicator to discriminate the effects of G-CSF from acute bacterial infection, particularly when the patients received G-CSF.

In summary, our data show that several CPD of lymphocyte, monocytes, and neutrophils can be considered useful parameters for determining the effect of G-CSF. If these parameters are incorporated into a decision rule, they should help doctors in discriminating the effect of G-CSF from other inflammatory situations. Further studies aimed at verifying the CPD according to the concentration of G-CSF, and defining potential factors affecting leukocyte CPD are warranted.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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