Xu. Int J Pathol Clin Res 2015, 1:1
ISSN: 2469-5807

International Journal of
Pathology and Clinical Research

Review Article: Open Access

Clinical Applications of Leukocyte Morphological Parameters

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Abstract

Leukocyte morphological parameters, also known as cell population data (CPD), are measured by Coulter automated hematology analyzers with VCS technology. In recent years, clinical applications of CPD have been extensively investigated. The studies have demonstrated that diagnostic utility of neutrophil CPD in acute bacterial infection, particularly their roles in differentiating post-surgical bacterial infection versus systemic inflammatory response syndrome. Lymphocyte CPD show also specific changes in viral infection, providing a potential new hematological parameter for viral infection. The clinical application of these leukocyte morphologic parameters offers many advantages. These parameters are generated during automated differential analysis without additional specimen requirements. They are quantitative, more objective and more accurate than manual differential counts since over 8000 leukocytes are simultaneously evaluated. This so-called VCS technology is analogous to microscopic evaluation of a peripheral blood smear, but uses the most modern technology to refine the output for increased accuracy. These CPDs have potential usefulness in clinical environments.

Keywords

Hematology analyzer, VCS technology, Cell population data, Leukocyte morphological parameters

Introduction

Currently, the information about the peripheral blood leukocytes provided by the majority of hematology analyzers is the total white blood cell count with differentials (WBC-D). WBC-D, also known as leukocyte numerical parameters, has been widely accepted and used by clinicians and is generally considered to yield clinically useful information in health and disease state. Over years, the information derived from WBC-D along with erythrocyte parameters, such as hemoglobin concentration or mean corpuscular volume, as well as platelet parameters has become a foundation in laboratory hematology and is used for screening, diagnosing and monitoring hematologic and non-hematologic disorders. However, changes in leukocyte numerical parameters, such as neutrophil count, tend to be extremely variable and nonspecific, particularly in newborn infants, the elderly and myelosuppressed patients where response is less dramatic. Therefore, the neutrophil count can also be normal or decreased despite clinical evidence of an acute infection. In recent years, the leukocyte morphological parameters, also known as cell population data (CPD), have been described and their potential clinical applications have been extensively investigated.

Leukocyte Morphological Parameters

Leukocyte morphological parameters or CPD are originally used by service engineers to verify optimal setup for each of the four main WBC subpopulations: neutrophils, lymphocytes, monocytes and eosinophils. The CPD are measured by Coulter automated hematology analyzers, such as LH750 or DxH800, with VCS technology [1]. The VCS technology uses three independent energy sources simultaneously to evaluate the intrinsic biophysical properties of over 8000 leukocytes in their "near native states". Namely, it uses direct current impedance to measure cell volume (V) for accurate size of all cell types; radio frequency opacity to characterize conductivity (C) for internal composition such as cytoplasmic/nuclear ratio of each cell; and a laser beam to measure light scatter (S) for cytoplasmic granularity and nuclear structure. The VCS technology not only measures quantitatively the mean channels of V, C and S, but also the standard deviation of each of these parameters [1]. Therefore, there are six values displayed about the coordination of the four main WBC subpopulations in three dimensional spaces: volume mean and standard deviation; conductivity mean and standard deviation; and scatter mean and standard deviation [1]. This so-called VCS technology is analogous to microscopic evaluation of a peripheral blood smear, but uses the most modern technology to refine the output for increased accuracy.

Neutrophil CPD in Acute Bacterial Infection

During acute bacterial infection, the release of inflammatory cytokines, such as interleukin-1 and tumor necrosis factor, stimulates bone marrow stromal cells and T cells to produce increased amounts of colony-stimulating factors. These factors promote the proliferation and differentiation of committed granulocytic progenitors, resulting in a rapid increase in the egress of granulocytes from the bone marrow and a sustained increase in neutrophil production. Therefore, granulocytes usually not only increase in numbers of (leukocytosis or neutrophilia), but also exhibit a left shift maturation with increase in band forms and the presence of other immature neutrophils, such as metamyelocytes and myelocytes. In addition to these leukocyte numerical changes, there are other morphologic changes of reactive neutrophils, such as the presence of toxic granulation, toxic vacuolization and Dohle bodies in the cytoplasm. These left shifted granulocytes as well as reactive neutrophils, tend to be larger than their normal "resting" counterparts. Therefore, we expect the entire neutrophil population not only to increase in volume or size, but also to become less homogeneous, with increased variability of

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Citation: Xu D (2015) Clinical Applications of Leukocyte Morphological Parameters Int J Pathol Clin Res 1:002

Received: February 19, 2015: **Accepted:** March 23, 2015: **Published:** March 25, 2015

cell sizes and shapes. These morphologic changes seen in those left shifted and reactive neutrophils during acute bacterial infection could be quantitatively measured with VCS Technology.

We and others have demonstrated that the VCS technology could detect the morphologic changes seen in left-shifted and reactive neutrophils during acute bacterial infection [2-6]. The neutrophil CPD, particularly the mean neutrophil volume (MNV) and the standard deviation of the MNV, also known as neutrophil volume distribution width (NDW) that reflects the neutrophil size variability or neutrophilic anisocytosis, were significantly elevated in acute bacterial infection [2-4]. The increase in the MNV and the NDW was more pronounced in systemic infection than in localized infection [4] and observed even in infected patients with normal or low WBC [2,3]. On the other hand, the mean neutrophil light scatter, which reflects cytoplasmic granularity and nuclear structure, was significantly decreased compared to controls, suggesting left-shifted maturation [2]. The ROC analyses revealed that the MNV and the NDW were better parameters with the larger areas under the curve (AUCs=0.87 and 0.85, respectively) than those of C-reactive protein (CRP) (AUC=0.63), band count (AUC=0.73) and absolute neutrophil count (AUC=0.74), proving to be more sensitive and specific parameters for diagnosing acute bacterial infection [4]. The similar neutrophil morphologic changes described above were also documented in neonate and elderly patient populations with sepsis [7,8].

Most recently, the roles of neutrophil CPD, the MNV and NDW, in post-surgical bacterial infection have also been investigated [9-11]. As we know, post-surgical infection is a serious medical problem that can delay recovering from an operation, increase hospital stays, medical care costs, and even mortality. Timely diagnosing post-operative infection is critical for proper patient management. However, traditional parameters, such as WBC counts or body temperature, may not be adequate to detect the infection because non-infectious leukocytosis and fever are common phenomena in postsurgical period. The systemic inflammatory response syndrome induced by surgical trauma is a well-known entity, with resultant release of variety of inflammatory cytokines leading to fever and/or leukocytosis in spite of the absence of infection [12,13].

The studies demonstrated that the MNV and NDW in bacteria-infected patients were significantly increased after surgery compared to none-infected patients (157.5 ± 7.0 vs. 149.2 ± 4.4 and 26.5 ± 3.3 vs. 22.2 ± 2.4, respectively; reference range of MNV: 137-153; NDW: 16.9-22.0). Although WBC and percent neutrophils were also increased after surgery, there were no statistically significant differences seen between none-infected and infected patients (15.4 ± 2.4 vs.16.2 ± 3.04; p=0.115) and (84.5% ± 3.1 vs. 85.6% ± 3.4; p=0.08, respectively) [10]. The sensitivity and specificity of the MNV (90.3% and 88.4%) and NDW (88.3% and 76.3%) for predicting post-surgical bacterial infection are superior to those of WBC-D (29.0% and 88.1%) and CRP (83.8% and 56.6%), and comparable to procalcitonin levels (87.1% and 90.5%) [10,11]. These observations are particularly significant, suggesting the potential clinical usefulness of these neutrophil CPD in distinguishing post-surgical bacterial infection from systemic inflammatory response syndrome [9,11]. It will be interesting to further investigate the dynamic changes of these CPD parameters during disease course.

Lymphocyte CPD in Viral Infection

Viral infection induces lymphocyte activation, undifferentiated lymphocyte proliferation and antibody or cytokine/lymphokine secretion. An immune defense against a viral infection is more dependent on T cells and less dependent on antibodies. Cytotoxic T cells are important in killing virally infected cells. A number of cytokines, including γ-interferon and tumor necrosis factor, are secreted by the cytotoxic T cells [14]. It is conceivable that the activated lymphocytes may undergo not only morphologic changes, such as increase in size, but also alterations in cytoplasmic composition as compared to their normal "resting" counterparts. These morphological changes of reactive lymphocytes could be detected with VCS (volume, conductivity and light scatter) technology.

We have previously demonstrated that the lymphocyte CPD exhibit significant changes in response to hepatitis B virus (HBV) infection [15], which include increase in lymphocyte volume (LV: 94.2 ± 4.1 vs. 87.7 ± 2.9; reference range: 81.9-93.5), volume standard deviation (LV-SD: 20.1 ± 3.9 vs. 14.5 ± 1.3; range: 11.9-17.1) as well as decrease in lymphocyte conductivity (LC: 101.1 ± 8.1 vs. 107.6 ± 4.5; range: 98.6-116.6). These changes are more dramatic in active HBV patients than in HBV carriers [15]. The increases in LV, LV-SD and decrease in LC have also been observed in various viral infections including Epstein Barr virus, hepatitis A virus, hepatitis B virus, hepatitis C virus, herpes virus, cytomegalovirus, human herpes virus 8, Dengue virus and human immunodeficiency virus [16,17], indicating that these changes in lymphocyte CPD are not specific for hepatitis B virus infection, but for viral infection in general. Based on these observations, a simplified lymphocyte CPD was proposed, called Lymph-Index [15,17], which is calculated as [LV x LV-SD ÷ LC]. The Lymph-Index is significantly increased in various viral infections compared to normal controls (15.3 ± 1.8 vs. 10.9 ± 1.0, p<0.001; range: 9.0-12.9) and only minimally increased in acute bacterial infection (11.3 ± 1.1) [15,17]. Using a cut-off value of Lymph-Index as ≥12.92, the sensitivity of 91.7% and the specificity of 97.2% were achieved for diagnosing viral infection [17]. These findings may be important because they indicate that Lymph-index could be a potential hematological parameter for suggesting viral infection [17]. The morphological changes of lymphocytes in other infections and in lymphoproliferative disorders will briefly be described below.

Monocyte CPD in Various Infections

Circulating monocytes play also crucial roles against various infections. Early studies [18,19] have demonstrated that using the standard deviations (SD) of monocyte and lymphocyte volume/size, so called malaria factor, the sensitivity of 96.8% and the specificity of 82.5% for detecting malaria parasite (*P. vivax*) in peripheral blood were achieved. Recent studies [20,21] have also showed that mean lymphocyte and monocyte volumes and monocyte volume-SD incorporating mean corpuscular hemoglobin concentration and neutrophil percentage can distinguish malaria and dengue infection from other febrile illnesses with 85.1% of sensitivity and 91.4% of specificity [21]. Furthermore, incorporating platelet count and standard deviations of lymphocyte volume and conductivity can identify malaria with 90.4% of sensitivity, 88.6% of specificity, and incorporating platelet count, lymphocyte percentage and standard deviation of lymphocyte conductivity can identify dengue with 81.0% of sensitivity and 77.1% of specificity [21]. Besides the changes of monocyte CPD in malaria infection, the monocyte volume and volume-SD are also significantly increased in post-surgical bacterial infection compared to non-infected patients (unpublished observations). In addition, the similar monocyte CPD changes in acute hepatitis B viral infection as seen in those of lymphocytes were also evident [15].

Leukocyte CPD in Other Clinical Situations

Since leukocyte CPD reflects cellular structural characteristics, it is conceivable that any condition that may alter peripheral leukocyte morphology will likely affect CPD values. Several studies have demonstrated that the significant changes in CPD values occur in various hematologic abnormalities, such as myelodysplastic syndrome, chronic myelomonocytic leukemia [22], chronic myeloid leukemia [23] and lymphoproliferative disorders [16] as well as in non-hematologic conditions, such as vitamin B12 deficiency, parenteral lipid emulsions [24], storage [9] and exogenous growth factor administration [25]. In addition, chemotherapy in malignancy will likely alter the cellular morphology and affect CPD values. Therefore, given the clinical variability in these particular cases and the potential for false positive or false-negative results, no diagnosis or judgment should ever be made solely on the basis of a single laboratory test result since laboratory tests are not foolproof and can present an incomplete picture when not put into proper clinical context.

Discussion and Conclusion

The practice of manual leukocyte differential counts virtually has not changed in more than a century since the introduction of the Romanowsky dyes [26]. Despite the fact that manual differential count is imprecise, time consuming, and labor intensive, it has become widely accepted and used by clinicians. Although the modern hematology analyzer is able to provide accurate, fast and cost-effective automated leukocyte differential counts with greater statistical reliability, manual leukocyte differential counts, particularly manual band counts and other immature granulocyte counts, remain a common practice in most clinical hematology laboratories, especially on those cases triggered by laboratory review criteria based on instrument flags. Growing pressures for cost-containment, as well as shortages of trained medical technologists, have brought up the continuing debate about clinical usefulness of manual band counts [27-30] and increased the need to reduce the number of manual procedures [31,32].

In recent years, detection of leukocyte morphologic changes by automated hematology analyzers has gained more attention. Several instruments, such as Pentra DX Nexus by Horiba Medical or Sysmex XN-3000 by Sysmex America, are able to quantitatively analyze the immature granulocytes in the peripheral blood sample with increased sensitivity and specificity, which is useful for clinical management of patients with hematological disease, malignancy or infection. The clinical usefulness of the leukocyte morphologic parameters or CPD by Coulter hematology analyzers with VCS technology is the most studied. In addition to above mentioned applications, there are also many practical advantages. These parameters are generated during automated differential analysis without additional specimen requirements. They are quantitative, more objective and more accurate than manual differential counts since over 8000 leukocytes are simultaneously evaluated. In addition, they have shown a better diagnostic performance, particularly in post-surgical bacterial infection, than conventional parameters, such as total WBC, the percent neutrophils, manual band neutrophil count, absolute neutrophil count and CRP, which are traditionally used as indicators for acute infection. Furthermore, the leukocyte morphological parameters determined with VCS technology offer more robust turn-around-time and are more cost-effective. Since the hematology analyzers with VCS technology could determine not leukocytes numerical parameter, but also evaluate the morphologic features of these leukocytes, it will certainly improve the diagnostic accuracy if one combines both leukocyte numerical and morphological parameters to differentiate bacterial versus viral infections or other infections in clinical environments.

Most recently, we have demonstrated that the leukocyte CPD have much smaller overall within-subject and between-subject biological variations compared to WBC-D, suggesting that these morphological parameters are less variable around the homeostatic set point intra-individually and inter-individually. In addition, the index of individuality for all morphological parameters was low [33]. A low index of individuality indicates that conventional reference values for these parameters may be of little utility, particularly when deciding whether changes in an individual have occurred. We further documented the reference change value (RCV) for leukocyte CPD [34]. The RCV or critical difference is defined as the percentage change that should be exceeded given the analytical and biological variations inherent to a particular test, in that there is a significant difference between the two consecutive measurements. The RCV can be used as references to determine the statistical probability that a change in an individual's serial results is significant, and to generate objective delta-check values for use in quality management [35].

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