Neutrophil Volume Distribution Width
A New Automated Hematologic Parameter for Acute Infection

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The complete blood cell count with differential is among the most commonly ordered tests for the diagnosis of acute infection. When performing a manual differential count on a peripheral blood smear, the medical technologist traditionally classifies cells using a series of visual criteria, such as size of the cell, density of the nuclear chromatin, presence of the nucleolus, nucleus-cytoplasm ratio, and presence of cytoplasmic granules. Automated hematology analyzers are now able to provide accurate and precise differential counts in a fast and cost-effective manner. Among these, the Coulter LH 750 (Beckman Coulter, Inc, Fullerton, Calif) uses a unique process of simultaneously collecting data from more than 8000 leukocytes, including direct current impedance to measure cell volume for accurate size of all cell types, radio frequency opacity to characterize conductivity for internal composition of each cell, and a laser beam to measure light scatter for cytoplasmic granularity and nuclear structure. The Coulter LH 750 measures not only the mean channels of cell volume, conductivity, and light scatter but also the SD of each of these parameters. 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.

Results. A significant increase in the NDW was observed in the bacteremic patients compared with the controls (24.7 ± 4.5 vs 19.0 ± 1.5; P < .001). Such increase was observed even in patients with white blood cell counts less than 11 000/μL (23.0 ± 5.6 vs 19.0 ± 1.5; P < .001) or with percentages of neutrophils less than 85% (24.0 ± 4.9 vs 19.0 ± 1.5; P < .001). The more dramatic increases were seen in patients with leukocytosis (25.7 ± 3.2, P < .001) or with neutrophilia (25.9 ± 3.4, P < .001). Using an NDW cut off of 23, a 100% specificity and a 69% sensitivity were achieved.

Conclusions. As a quantitative parameter, the NDW has potential for use as an additional indicator for diagnosing acute infection. (Arch Pathol Lab Med. 2006;130:378–380)

MATERIALS AND METHODS

Patient Selection

We retrospectively analyzed peripheral blood samples from 70 patients with positive blood cultures for bacteria. All samples were drawn within 2 days of the blood culture collection, and patients whose blood cultures yielded bacteria that were likely to be contaminants, such as coagulase-negative staphylococci, were excluded from the study. The patients’ medical records were analyzed for demographic data, neutrophil counts, and bacteremic status. A control group of patients was identified that was age- matched, but not bacteremic. Statistical analysis was performed using Student’s t test or Wilcoxon’s test.
were reviewed for clinical correlation and for exclusion of any concomitant hematologic diseases, such as leukemia or myelodysplasia, that would affect the leukocyte morphologic structure. Thirty-five control subjects were selected from among patients whose complete blood cell count and differential data were within normal limits (WBC count range, 4000–11000/µL; and percentage of neutrophils, <85%) and who had no signs of infection or hematologic disease.

**Data Collection**

Data collected included total WBC count, percentage of neutrophils, and the NDW. The NDW was calculated as the SD of neutrophil volume, measured by the direct current impedance of each cell passing through the aperture. Therefore, the NDW reflects the neutrophil size variability.1–2 Manual differential count was not performed in the present study. The study protocol was approved by the institutional review board.

**Statistical Analysis**

All analyses were performed using SPSS version 12.0 software (SPSS Inc, Chicago, Ill). Results were expressed as mean ± SD. Comparisons between means were performed by analysis of variance. Comparison between 2 means was performed by Student t test. P < .05 was considered significant.

**RESULTS**

**Clinical Data**

We retrospectively analyzed data from 70 patients with positive bacterial blood cultures, including 43 gram-positive and 27 gram-negative cases. The most common bacteria cultured were *Staphylococcus aureus* (n = 20), followed by *Escherichia coli* (n = 8), *Bacillus* (n = 6), and *Proteus* (n = 5); α-hemolytic streptococcus, *Enterococcus faecalis*, group B streptococcus, *Klebsiella pneumoniae*, and *Streptococcus pneumoniae* (n = 3 each); *Clostridium perfringens* and *Enterobacter cloacae* (n = 2 each); and β-hemolytic group G streptococcus, *Diphtheroid*, *Enterococcus*, group A streptococcus, group C streptococcus, group G streptococcus, gram-negative cocci—unidentified, gram-negative rods—unidentified, *Morganella morgani*, *Streptococcus viridans*, *Salmonella*, and *Serratia* (n = 1 each). The mean patient age was 52 years, and the male-female ratio was 1:2:1. The WBC count ranged from 1700/µL to 39200/µL (mean, 12700/µL). The percentage of neutrophils ranged from 29% to 97% (mean, 76%). All patients had clinical indications of acute infection. Thirty-five control subjects with normal complete blood cell counts with differential (WBC count range, 4100–10900/µL) were age matched (mean age, 51 years), with a mean WBC count and a mean percentage of neutrophils of 6930/µL and 61%, respectively.

**NDW in Acute Infection**

We initially investigated the changes in the overall NDW, which reflects the neutrophil size variability. As seen in the Figure, a significant increase in the NDW was observed in the bacteremic patients compared with the observed in the Figure, a significant increase in the NDW was observed in the bacteremic patients compared with the seen in the Figure, a significant increase in the NDW was observed in the bacteremic patients compared with the seen in the Figure, a significant increase in the NDW was observed in the bacteremic patients compared with the seen in the Figure, a significant increase in the NDW was observed in the bacteremic patients compared with the seen in the Figure, a significant increase in the NDW was observed in the bacteremic patients compared with the seen in the Figure, a significant increase in the NDW was observed in the bacteremic patients compared with the. The diagnostic value of the band count as an indicator of acute infection, however, is the subject of ongoing debate.8–14 In addition, the determination of band counts and other neutrophil morphologic changes, such as the presence of toxic granulation, toxic vacuolization, and Dohle bodies in the cytoplasm, is labor intensive and time consuming, as it requires manual ex-

**Evaluation of Sensitivity and Specificity in Predicting Acute Infection**

We then calculated the sensitivity and specificity of the NDW for predicting infection at designated cutoffs (Table). When we selected an NDW cutoff of 22 or higher, we achieved a sensitivity of 79% and a specificity of 94%. These results were slightly better than the sensitivity and specificity of the mean neutrophil volume using a cutoff of 150 or higher, which were 70% and 91%, respectively.3 In addition, this was significantly higher than the 55% sensitivity achieved using 11000/µL as the WBC count cutoff and the 33% sensitivity achieved using 85% as the percentage of neutrophils cutoff.5 We achieved 100% specificity and 69% sensitivity using an NDW cutoff of 23 (Table). Therefore, the NDW may be another reliable indicator for predicting acute bacterial infection.

**COMMENT**

The correct and timely diagnosis of severe acute infectious processes, such as septicemia, is critical for proper patient management. Laboratory tests most often ordered in this scenario are blood culture, complete blood cell count with differential, and manual differential count. For many years, WBC count, percentage of neutrophils or absolute neutrophil count, increased banded neutrophils, and immature-total neutrophil ratio have been used to predict acute infection.5–9 The diagnostic value of the band count as an indicator of acute infection, however, is the subject of ongoing debate.8–14 In addition, the determination of band counts and other neutrophil morphologic changes, such as the presence of toxic granulation, toxic vacuolization, and Dohle bodies in the cytoplasm, is labor intensive and time consuming, as it requires manual ex-
amnation by an experienced medical technologist. Furthermore, the results are subjective because they depend on human interpretation, and only a few hundred cells can be analyzed for any given sample. Although the blood culture is considered the gold standard for diagnosing septicemia, major pitfalls include low sensitivity, the need for proper collection techniques to avoid contamination, and the delay of at least a few days before results are available. Therefore, there is a need for a more objective and faster way to predict acute infection.

It was previously demonstrated that the morphologic changes of reactive neutrophils can be quantitatively determined by VCS parameters of the Coulter LH 750. Compared with controls, the neutrophils in septic patients had significant increases in the mean neutrophil volume. In addition, the mean channel of neutrophil light scatter, reflecting cytoplasmic granularity and nuclear structure, was significantly decreased in septic patients compared with controls.

In this study, we investigated the value of the neutrophil volume SD (the NDW), generated by VCS technology of the Coulter LH 750 hematology analyzer, as an additional predictor of acute infection. We demonstrated that, compared with controls, the neutrophil population of patients with infection is less homogeneous, with a wider variation of individual cell sizes, as shown by the increased NDW. Furthermore, change in the NDW has clinical value in the diagnosis of acute bacterial infection, as it was associated with positive blood cultures, higher WBC counts, and higher percentages of neutrophils and was present even in patients who did not have leukocytosis or neutrophilia. Because a significant proportion of patients with acute infection have unremarkable WBC count profiles, the ability of the NDW to predict infection even in these patients highlights at least 1 important possible clinical application of this new parameter. On the opposite end of the spectrum, the value of the NDW as a discriminator in the differential diagnosis between leukemoid reaction due to infection and chronic myelogenous leukemia is under investigation. Our preliminary results appear to indicate a more significant increase in the NDW of patients with chronic myelogenous leukemia compared with that in patients with infection and in controls (D.S.X., unpublished data, 2005).

The use of VCS parameters could have an important effect on the practice of laboratory medicine because their clinical application offers several 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 because more than 8000 leukocytes are simultaneously evaluated. In addition, the VCS parameters have shown better diagnostic performance than WBC count and percentage of neutrophils, which are traditionally used as indicators of infection. For example, using an NDW cutoff of 23, a 100% specificity and a 69% sensitivity were achieved (Table), compared with sensitivities of 55% achieved using 11 000/μL as the WBC count cutoff and 33% achieved using 85% as the percentage of neutrophils cutoff. The results obtained in this retrospective proof-of-concept pilot study are promising, considering that the clinical value of the WBC count, the percentage of neutrophils, and other traditional tests such as the band count has been the subject of much controversy in the medical literature. To further elucidate this matter, we are collecting data for a prospective study comparing the sensitivity and specificity of the neutrophil VCS parameters, including the NDW, with those of the band count and the C-reactive protein levels.

In conclusion, we believe that the NDW may be a sensitive and reliable VCS parameter for use in the diagnosis of acute infection. Its clinical value should be investigated in the differential diagnosis of several other conditions associated with neutrophilic leukocytosis, such as tissue infarction or ischemia (hypoxia), exercise or epinephrine medication, use of glucocorticoids, chronic inflammation, tumors, and myeloproliferative disorders. Use of the NDW is analogous to the approach used in the differential diagnosis of anemias, in which the mean corpuscular volume and the red blood cell distribution width can discriminate among the primary anemia subtypes and direct further diagnostic workup.

References