Delta Mean Neutrophil Volume (Δ MNV) is Comparable to Procalcitonin for Predicting Postsurgical Bacterial Infection

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Background: The Coulter LH750 (Beckman Coulter, Brea, CA) analyzer can determine intrinsic biophysical properties of white blood cell (WBC), known as cell population data. Previous studies have shown that mean neutrophil volume (MNV) was significantly increased in postsurgical patients with bacterial infection. To further validate its potential clinical usefulness, we investigate the changes in MNV before and after surgery, called Δ MNV. We also compare the Δ MNV with procalcitonin (PCT) and C-reactive protein (CRP) in terms of diagnostic sensitivity and specificity for postsurgical bacterial infection. **Methods:** Blood samples from 300 healthy controls, 219 cardiac surgical patients without postsurgical infection, and 31 cardiac surgical patients complicated with postsurgical bacterial infection were studied. **Results:** There are no statistically significant differences for WBC count

and neutrophil percentage prior to or after surgery between postsurgical noninfected and infected patients. However, the Δ MNV is significantly increased in postsurgical infected patients when compared with noninfected patients ($P < 0.05$). The receiver-operating characteristics analysis reveals the Δ MNV and PCT have largest areas under curves (0.92, 0.93 on the second day and 0.94, 0.99 on the third day postsurgery, respectively) compared to other parameters. **Conclusion:** Δ MNV shows comparable sensitivity and specificity to PCT and superior sensitivity and specificity to WBC or CRP for predicting postsurgical bacterial infection. The potential clinical application of this parameter merits further exploration in a larger prospective study. J. Clin. Lab. Anal. 28:301–305, 2014. © 2014 Wiley Periodicals, Inc.

Key words: neutrophil cell population data; delta mean neutrophil volume; postsurgical infection; procalcitonin

INTRODUCTION

Postsurgical infection is a serious medical problem that can delay recovery following surgery, increasing hospital stays as well as medical care costs. Timely diagnosis of postoperative infection is critical for proper patient management. However, traditional parameters, such as white blood cell (WBC) counts or body temperature, may not be adequate to detect the infection because noninfectious leukocytosis and fever are common phenomena in the postsurgical period. The systemic inflammatory response syndrome induced by surgical trauma is a well-known entity, with the resultant release of various inflammatory cytokines leading to fever and/or leukocytosis despite the absence of infection.

In recent years, procalcitonin (PCT) has been proposed to be an ideal biomarker for systemic bacterial infection or sepsis (1). PCT is the prohormone form of calcitonin and is produced by extra-thyroidal immune cells within 2–4 hr of a bacterial insult and/or inflammatory response. Elevated PCT is seen in septic patients and

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concentrations correlate with severity of disease. Increasing PCT over time is associated with poor prognosis, while decreasing concentrations correlate to good prognosis and/or response to antibiotic therapy (2).

It has also been previously demonstrated that cell population data (CPD), such as mean neutrophil volume (MNV) and neutrophil volume distribution width, are significantly increased not only in septic patients with high WBC, but also in those with normal or low WBC counts as well as in postsurgical patients complicated with bacterial infection (3–8). The CPD are determined by Coulter LH750 automated hematology analyzer (Beckman Coulter, Brea, CA) with VCS (volume, conductivity, and light scatter) technology. The VCS technology used in LH750 is able to generate the differential count based solely on the analysis of three parameters that are directly correlated to intrinsic biophysical properties of each WBC, using neither chemical reactions nor fluorescence. The volume or cell size is measured directly by impedance. The conductivity reflecting the internal cellular density is measured by the conduction of radio frequency waves across the cells, and the laser light scatter gives direct information regarding cytoplasmic granularity and nuclear complexity.

To further validate the clinical usefulness of CPD in postsurgical bacterial infection, in this study, we investigate the changes in MNV before and after surgery, called Δ MNV. We also compare the Δ MNV with PCT and C-reactive protein (CRP) in terms of diagnostic sensitivity and specificity for postsurgical bacterial infection.

MATERIALS AND METHODS

Study Population

In this retrospective case-control study, peripheral blood specimens were drawn into dipotassium Ethylene Diamine Tetraacetic Acid (EDTA) anticoagulant (for routine blood cell counts) and serum separate clot activator (for PCT and CRP measurements) in Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) and stored at room temperature. The subjects included 300 healthy controls (mean age: 37 years; men/women ratio [M/F] = 1.2:1), 219 cardiac surgical patients (mean age: 49 years; M/F = 1.2:1) without postsurgical infection, and 31 cardiac surgical patients (mean age: 51 years; M/F = 1.1:1) with postsurgical infection presenting with fever ($\geq 38.5^{\circ}\text{C}$) and elevated WBC and confirmed by positive culture for bacteria at the Second Affiliated Hospital of Nantong University between April 2009 and August 2012. There were 19 cases of Gram-negative bacteria, including *Escherichia coli* (8), *Klebsiella pneumoniae* (5), *Pseudomonas aeruginosa* (5), *Hemolytic streptococcus* (1), and 12 cases of Gram-positive bacteria, including *Staphylococcus aureus* (6), *Staphylococcus epidermidis* (4), and

Streptococcus pneumoniae (2). Prior to surgery, all patients had no fever (defined as maximum temperature $\leq 38.5^{\circ}\text{C}$), and their WBC counts and neutrophil (NEUT) percent were within normal reference ranges. Blood samples were collected on three consecutive days after surgery.

Hematological Data Collection

Neutrophil CPD, such as the MNV, was collected. The MNV was generated during automated differential analysis by each individual cell passing through the aperture and were optically and electronically measured using the Coulter LH750. The Δ MNV was calculated as follows: MNV after surgery – MNV before surgery. The conventional parameters, such as WBC count and NEUT%, were also collected. All specimens were analyzed within 2 hr after collection. Daily quality controls were run according to the manufacturer's instructions. Stability of MNV data was also tested, which showed no significant changes within 4 hr after specimen collection.

PCT Determination

PCT was measured using the electrochemiluminescent immunoassay on the Cobas e601 instrument (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's recommendations. The assay has a measuring range from 0.02 to 100 ng/ml. Imprecision varied between 2.7% and 3.9% at concentrations of 0.72–55.4 ng/ml. The reference range was up to 0.046 ng/ml.

CRP Determination

CRP was measured using a latex agglutination method (Shensuo Unf Medical Diagnostic Articles Company Limited, Shanghai, China) on Hitachi 7600–020 automatic analyzer (Hitachi Company, Tokyo, Japan) as per manufacturer's recommendations. This is a wide range CRP assay that covers from 0.1 to 300 mg/l. Imprecision was less than 5% at different levels. The reference range for healthy individuals was up to 3 mg/l.

Statistical Analysis

All analyses, including receiver-operating characteristics (ROC), were performed using the Statistical Package for the Social Science (SPSS) software, version 13.0 (SPSS, Chicago, IL). Imprecision was determined using coefficient of variation (CV). Comparison between two means was performed by the Student's *t*-test. Correlations were determined using Spearman's rank correlation coefficients. Results were expressed as the mean $\pm$ standard deviation (SD). A *P*-value less than 0.05 was considered significant.

TABLE 1. The Results of Test Parameters After Surgery

Parameters	Noninfected patients (<i>n</i> = 219)			Infected patients (<i>n</i> = 31)		
	First day	Second day	Third day	First day	Second day	Third day
WBC ($\times 10^9$)	15.98 $\pm$ 2.18	16.31 $\pm$ 2.30	16.12 $\pm$ 2.21	16.12 $\pm$ 2.64	16.95 $\pm$ 3.46	16.48 $\pm$ 2.56
NEUT%	85.02 $\pm$ 2.86	85.70 $\pm$ 2.66	85.68 $\pm$ 2.40	85.30 $\pm$ 2.73	86.38 $\pm$ 3.81	86.40 $\pm$ 3.09
Δ MNV (fl)	9.12 $\pm$ 0.80	8.56 $\pm$ 1.27	8.7 $\pm$ 0.72	9.41 $\pm$ 1.27	13.21 $\pm$ 4.05***	14.77 $\pm$ 5.16***
MNV (fl)	154.87 $\pm$ 3.55	154.32 $\pm$ 3.42	154.46 $\pm$ 3.66	155.56 $\pm$ 2.91	159.36 $\pm$ 6.13***	160.92 $\pm$ 6.42***
PCT (ng/ml)	0.12 $\pm$ 0.03	0.22 $\pm$ 0.06	0.13 $\pm$ 0.10	0.12 $\pm$ 0.04	1.04 $\pm$ 0.91*	1.21 $\pm$ 1.01*
CRP (mg/l)	93.18 $\pm$ 40.49	111.64 $\pm$ 55.72	96.86 $\pm$ 34.35	99.79 $\pm$ 36.42	148.87 $\pm$ 65.51*	159.74 $\pm$ 70.84*

P* < 0.05, compared with noninfected patients; *P* < 0.05, compared with the first day in infected patients.

WBC, white blood cell; NEUT, neutrophil; MNV, mean neutrophil volume; PCT, procalcitonin; CRP, C-reactive protein.

RESULTS

MNV Method Performance Characteristics

Using freshly collected 30 samples including 5 with low WBC counts of $\leq 4,000/\mu\text{l}$, 20 with normal WBC counts ($10,000/\mu\text{l} > \text{WBC} > 4,000/\mu\text{l}$), and 5 with high WBC $> 10,000/\mu\text{l}$, the mean within-run CVs were 1.37% (mean MNV of 143.2), 1.77% (mean MNV of 148.6), and 1.19% (mean MNV of 137.7) in normal, high, and low WBC groups, respectively. Three levels of hematology controls (Beckman Coulter) stored at 4°C were simultaneously determined. Replicate sample measurements for these controls showed mean MNV of 168.0, 149.1, and 142.5 with within-run imprecision of 0.93%, 0.57%, and 0.48%, respectively. Replicate sample measurements (*n* = 72) for three levels of hematology controls for 20-day period showed mean MNVs of 170.4, 148.7, and 143.3 with between-run imprecision of 2.91%, 2.68%, and 2.37%, respectively. These results have established that Coulter LH750 is a reliable analyzer to determine MNV.

WBC Count, MNV, PCT, and CRP in Postsurgical Infection

WBC count and NEUT% were within normal reference ranges for all patients prior to surgery. There were also no statistically significant differences in the MNV observed prior to surgery between postsurgical noninfected patients and infected patients confirmed by positive culture for bacteria (145.75 ± 3.66 vs. 146.15 ± 3.33 , *P* = 0.564). Although WBC count and NEUT% were both increased after surgery as expected, no statistical differences were seen between postsurgical noninfected and infected patients (*P* > 0.05). The MNV also did not show significant difference on the first day after surgery (Table 1). However, the MNV in infected patients was significantly increased when compared with noninfected patients on the second and third day after surgery (Table 1). Because of high interindividual variations of MNV values (the range of MNV before surgery: 138–154.6, for postsurgical non-

infected patients: 147.5–170.2, and for infected patients: 150.7–176.7), the Δ MNV (MNV after surgery – MNV before surgery) was introduced. As shown in Table 1, no statistical difference of Δ MNV was observed between postsurgical noninfected and infected patients on the first day after surgery. The Δ MNV was significantly increased on the second day and even greater on the third day (Table 1). In addition, the difference of Δ MNV between the first and the second day (*P* < 0.001) was larger than the MNV (*P* = 0.003) in infected patients, and the difference was more significant on the third day (*P* < 0.001). The PCT and CRP in infected patients were also significantly increased when compared with noninfected patients on the second and third day (*P* < 0.001). Furthermore, the Δ MNV was correlated well with PCT on the second and third day (*r* = 0.874, *P* < 0.01; Fig. 1) postsurgery.

Determination of Sensitivity and Specificity for Predicting Postsurgical Infection

The sensitivity and the specificity of the Δ MNV, mean MNV, PCT, and CRP for predicting postsurgical bacterial infection were next evaluated. The ROC curve analyses showed that the Δ MNV and PCT had the areas under the curves (AUCs) of 0.920 and 0.926, respectively, which were significantly greater than those of MNV (AUC = 0.747) and CRP (AUC = 0.720) on the second day after surgery. Using the cutoff points of ≥ 9.65 for Δ MNV and ≥ 0.411 for PCT, the sensitivities of 87.10% and 87.10% and specificities of 87.84% and 90.54% were achieved, respectively, which were significantly higher than the sensitivities and specificities obtained from MNV and CRP (Table 2, Fig. 2). Furthermore, on the third day, the Δ MNV (AUC = 0.936) and PCT (AUC = 0.988) had even greater AUC than those of MNV (AUC = 0.801) and CRP (AUC = 0.810; Table 2). These results suggested that Δ MNV is comparable to PCT as a sensitive marker to predict postsurgical bacterial infections.

Fig. 1. The scatter plot showed that the delta mean neutrophil volume (Δ MNV) was correlated well with procalcitonin (PCT) on the second (A) and third day (B) after surgery.

DISCUSSION

The presence of fever and leukocytosis has traditionally been utilized as the diagnostic marker of infection. However, several recent studies suggested that fever and leukocytosis are poor predictors for diagnosing postsurgical infection (9–11). Other commonly used laboratory tests include blood culture for bacterial microorganisms, CRP level, and erythrocyte sedimentation rate, but the diagnostic values of these parameters also vary greatly. Although microscopic evaluation of a peripheral blood smear can also detect the morphologic changes of reactive neutrophils, such as the presence of toxic granulation, vacuolization, and Dohle bodies in the cytoplasm, it is labor intensive, time-consuming, and requires manual examination by a trained medical technologist. In addition,

Fig. 2. Receiver-operating characteristics curve analysis showed that delta mean neutrophil volume (Δ MNV) and procalcitonin (PCT) had larger areas under the curve compared with other parameters on the second day after surgery.

the results are subjective because they depend on human interpretation, and only 100 or 200 cells are evaluated in a typical microscopic manual differential count, leading to imprecision and lack of confidence of the test results among clinicians (12, 13). Determination of serum PCT levels (2, 14, 15) and CD64 expression on peripheral blood leukocytes (16, 17) has recently been shown to improve diagnostic sensitivity and specificity for bacterial infection. These tests, however, require high technical support and are relatively expensive. Therefore, there is a clear need for a new and more cost-effective marker for acute bacterial infection.

In the present study, we have demonstrated that the neutrophil CPD, Δ MNV, is significantly increased in postsurgical infected patients when compared with noninfected patients although leukocytosis and neutrophilia were observed in both groups. In addition, Δ MNV shows comparable sensitivity and specificity to PCT and superior sensitivity and specificity to WBC or CRP for predicting postsurgical bacterial infection. It is conceivable that 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

TABLE 2. Sensitivity and Specificity in Predicting Postsurgical Infection

Parameters	The second day after surgery				The third day after surgery			
	Area under the curve	Cutoff value	Sensitivity (%)	Specificity (%)	Area under the curve	Cutoff value	Sensitivity (%)	Specificity (%)
Δ MNV (fl)	0.92	≥ 9.65	87.1	87.84	0.936	≥ 10.45	87.42	90.09
MNV (fl)	0.747	≥ 159.4	54.84	91.32	0.801	≥ 159.6	58.06	89.04
PCT (ng/ml)	0.926	≥ 0.411	87.1	90.54	0.988	≥ 0.301	96.77	94.52
CRP (mg/l)	0.72	≥ 90.95	83.87	56.62	0.81	≥ 86.95	90.32	55.71

MNV, mean neutrophil volume; PCT, procalcitonin; CRP, C-reactive protein.

produce increased amounts of colony-stimulating factors (18). 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 with increases in bands and other immature neutrophils (left-shifted), which tend to be larger in size as shown by increased MNV. Therefore, Δ MNV may provide additional information for clinicians to manage their patients, particularly for those patients clinically suspicious for bacterial infection, but negative culture result (false negative).

The clinical application of Δ MNV offers several additional advantages. The parameter is generated during automated differential analysis without additional specimen requirements. It is quantitative as well as more objective and more accurate than manual differential counts because more than 8,000 leukocytes are simultaneously evaluated. In addition, it has been shown to be a better diagnostic parameter than manual band neutrophil count, absolute neutrophil counts, and CRP (6), which are traditionally used as indicators for bacterial infection. Furthermore, the Δ MNV is comparable to PCT for diagnosing bacterial infection. However, the Δ MNV determined by the Coulter LH750 is more cost-effective. It has been demonstrated that patients with hematologic abnormalities, such as chronic myeloid leukemia, or other nonhematologic abnormalities, such as vitamin B12 deficiency or pediatric patients (19, 20), have altered MNV value that may not undergo similar changes after surgery when complicated with bacterial infection as has been observed in the current study. Therefore, the potential clinical application of Δ MNV for postsurgical infection in all populations merits further exploration in a larger prospective study.

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