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Investigation of Neutrophil Volume, Conductivity, and Light-Scattering Parameters for Early Diagnosis of Bacterial Infections

Özlem DOĞAN [1], Emel ÇALIŞKAN [2], Asiye ALTINÖZ AYTAR [3]

ABSTRACT

Aim: Early diagnosis of bacterial infections is crucial in planning treatment. Thus, it is important to determine the gram staining property of bacteria as well as the presence of bacterial infection. The acute bacterial infection leads to morphological changes in neutrophils. In this study, we investigated the use of neutrophil volume, conductivity and light-scattering (VCS) parameters as potential indicators for rapid diagnosis of bacterial infections, and to distinguish infections caused by gram-positive and gram-negative bacteria.

Material and Methods: Patients with urinary tract infections, pneumonia, wound site infections and sepsis were included. The control group comprised patients without bacterial infections. The blood samples of the patients were examined for white blood cell and neutrophil counts; neutrophil VCS parameters were determined using a Coulter Analyzer. The VITEK 2 Compact System was used to detect microbial growth.

Results: The blood sample data of 472 patients sent to our laboratory for Complete Blood Count analyses from various clinics were analyzed in this study. A total of 370 samples showed significant growth in their bacteriological culturing, whereas the remaining 102 samples showed no growth. For the detection of bacterial growth, the specificities of median neutrophil volume (MNV) and median neutrophil conductivity (MNC) were found to be 96% and 99%, respectively. In addition, median neutrophil light scattering (MNS) was higher in the gram-negative bacterial group than in the gram-positive bacterial group.

Conclusion: The use of neutrophil VCS parameters is an effective and time-saving method to identify bacterial infections and distinguish between gram-positive and gram-negative bacterial infections.

Keywords: Bacteria; infection; neutrophil; VCS parameters

Nötrofil Hacim, İletkenlik ve Işık Saçılımı Parametrelerinin Bakteriyel Enfeksiyonlarda Hızlı Tanı Aracı Olarak Kullanımının Araştırılması

ÖZ

Amaç: Bakteriyel enfeksiyonların erken teşhisi, tedavinin planlanmasında çok önemlidir. Bu nedenle, bakteriyel enfeksiyonun yanı sıra bakterilerin gram boyanma özelliğinin belirlenmesi de önem arzeder. Akut bakteriyel enfeksiyon, nötrofillerde morfolojik değişikliklere yol açar. Bu çalışmada, bakteriyel enfeksiyonların hızlı tanısı ile gram pozitif ve gram negatif bakterilerin neden olduğu enfeksiyonları ayırt etmek için nötrofil hacim, iletkenlik ve ışık saçılımı (VCS) parametrelerinin potansiyel bir gösterge olarak kullanımı araştırıldı.

Gereç ve Yöntemler: Çalışmaya, üriner sistem enfeksiyonu, pnömoni, yara yeri enfeksiyonu ve sepsisi olan hastalar dahil edildi. Kontrol grubu, bakteriyel enfeksiyonu saptanmamış hastalardan oluşturuldu. Hastalarının kan örnekleri beyaz kan hücresi ve nötrofil sayımı için incelendi; nötrofil VCS parametreleri, bir Coulter Analizörü kullanılarak belirlendi. Üreyen bakteri türlerini tespit etmek için VITEK 2 Kompakt Sistemi kullanıldı.

Bulgular: Çalışmada, çeşitli kliniklerden tam kan sayımı için laboratuvarımıza gönderilen 472 hastanın kan örneği analiz edildi. Bu hastaların 370'inin bakteriyolojik kültürlerinde anlamlı üremesi mevcut iken, 102'sinin anlamlı bakteri üremesi yoktu. Bakteriyel enfeksiyonu saptamada, medyan nötrofil hacmi (MNV) ve medyan nötrofil iletkenliğinin (MNC) özgüllükleri sırasıyla %96 ve %99 olarak bulundu.

1 Department of Medical Biochemistry, Ankara Universitesi Tip Fakultesi, Ankara, Turkey.
2 Department of Medical Microbiology, Düzce Üniversitesi Tip Fakültesi, Duzce, Turkey.
3 Microbiology Laboratory, Keçiören Eğitim ve Araştırma Hastanesi, Ankara, Turkey.

Sorumlu Yazar / Corresponding Author: Özlem DOĞAN , e-mail: ozlemdogan@gmail.com.tr
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Ayrıca, medyan nötrofil ışık saçılımının (MNS), gram-negatif bakteri üremesi saptananlarda, gram-pozitif bakteri üremesi saptananlara göre daha yüksek olduğu görüldü.
Sonuç: Bakteriyel enfeksiyonları belirlemek ve gram-pozitif ve gram-negatif bakteriyel enfeksiyon varlığını birbirinden ayırt etmek için nötrofil VCS parametrelerinin kullanımı, etkin ve zaman kazandırıcı bir yöntemdir.
Anahtar Kelimeler: Bakteri; enfeksiyon; nötrofil; VCS parametereleri.

INTRODUCTION

Bacterial infections that are diagnosed early but not treated with the appropriate antibiotics on time can lead to serious clinical symptoms, such as sepsis. Bacterial infections can be definitively diagnosed with a culture positivity test over a period of 48–72 hours. Furthermore, any error incurred during sampling and transport of the sample contributes to incorrect results, leading to further prolongation of the diagnostic process. Therefore, different methods for the early diagnosis of bacterial infections are being investigated (1-3). Markers, such as leukocyte count and erythrocyte sedimentation rate, are crucial for the detection of bloodstream infections; however, their accuracy is not high (1). Procalcitonin (PCT), interleukins and C reactive protein (CRP) are among the other markers that have been used for the early and accurate diagnosis of bacterial infections (2).

The Coulter LH 780hematology analyzer, a peripheral enhancement technological innovation, provides detailed information in the examination of the volume, conductivity, and light-scattering parameters (VCS) of neutrophils (4). It analyzes >800 leukocytes and then calculates the mean value that enables us to distinguish cells like neutrophils, lymphocytes, monocytes, eosinophils, or basophils. Likewise, the Coulter Analyzer evaluates changes in the VCS parameters of reactive neutrophils (5). Although the neutrophil volume is higher in patients with bacterial infection, neutrophil conductivity and neutrophil light scattering may vary (4,5). Also, studies are conducted to determine the relationship between gram staining characteristics of bacteria and neutrophil VCS parameters (6).

Here, we investigated the use of the abovementioned VCS parameters as potential indicators for the early diagnosis of bacterial infections. We also aimed to briefly distinguish gram-positive and gram-negative bacterial infections using the VCS parameters.

MATERIAL AND METHODS

Study design, setting, and ethics

This prospective study was conducted at an education and research hospital in Ankara. Our hospital has 350 beds capacity in the internal clinic, surgical clinic and intensive care units. The study was approved by the Kecioren Education and Research Hospital Clinical Research Council (date, 03.26.2014; no. 532). The study protocol was in accordance with the ethical standards of the Helsinki Declaration.

Participants

Appropriate clinical cultivation and incubation of various clinical samples sent to the laboratory under sterilized conditions from various outpatient clinics and other services (internal departments, surgical departments, and intensive care units) were conducted 472 patient samples were randomly selected among all samples sent to the laboratory for culture method. The study group consisted of 370 patients with significant growth in their bacteriological cultures. The control group consisted of 102 patients with no growth in their bacteriological cultures, normal levels of complete blood count and differential data [WBC count <11,000/μL (<11.0 × 109/L) and neutrophil percent of <85% (0.85)]. Multiple Gram stain species were excluded from the study.

Bacterial evaluation

The samples were inoculated and spread onto blood agar and eosin methylene blue agar plates (Becton Dickenson, USA) and then plates were incubated in an aerobic atmosphere at 37°C for 18–24 hours. When bacterial growth was observed, the colonies were counted and it was stained with the gram staining method.

The automated identification system VITEK 2 Compact System (BioMérieux, France) or conventional methods were used to identify microorganisms. Urine samples with ≥105 CFU/ml of bacterial growth, deep tracheal aspiration (DTA) samples with ≥104 CFU/ml of bacterial growth, detection of growth in at least two of the several simultaneously sampled blood cultures of a patient, and the observation of several leukocytes and active bacteria in Gram-stained wound samples were considered meaningful results. Patients with bacterial growth in their urine cultures were diagnosed with urinary tract infection symptoms (e.g., dysuria, pollakiuria, urgency, fever).

The sampled blood was examined for the WBC and neutrophil content, and neutrophil VCS parameters using the Beckmann Coulter LH 780 Analyzer (Beckman Coulter, Fullerton, CA).

Statistical Analyses

The Statistical Package for the Social Sciences v.16.0 was used for statistically evaluating the variables. The suitability of the data to the normal distribution was evaluated by using Kolmogorov-Smirnov and Shapiro-Wilk tests. The Mann–Whitney U-test was used to compare non-normally distributed continuous variables between the samples with bacterial growth and those without. Receiver operating characteristics curves were plotted to assess the performance of the median neutrophil volume (MNV), median neutrophil conductivity (MNC), and median neutrophil light scattering (MNS) values to predict bacterial growth in the culture samples. To determine the discrimination performance of the VCS parameters, an ROC (Receiver Operating Characteristic) curve was created. The sensitivity, specificity, and likelihood ratios of these tests were calculated. $P < 0.05$ was considered statistically significant.

RESULTS

The blood sample data of 472 patients sent to our laboratory for CBC (Complete Blood Count) analyses from various clinics were analyzed in this study. A total of 370 samples showed significant growth in their bacteriological culturing, whereas the remaining 102 samples showed no growth. Of the samples showing positive growth, 265 (72%) were isolated from urine, 42 (11%) from wound sites, 43 (12%) from DTA, and 20 (5%) from blood cultures. Regarding the bacterial growth, the growth of 53 (14%) gram-positive and 317 (86%)

gram-negative bacteria was not ed. The distribution of the growth of the species of bacteria is shown in Table 1.

Table 1. The distribution of the growth of bacteria species according to the site of isolation of the sample

	Urine		**DTA**		**Wound site**		**Blood culture**		**Toplam**	
Bacteria	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Gram positive bacteri	**23**	**9**	**3**	**6**	**17**	**40**	**10**	**50**	**53**	**13**
Staphylococcus aureus	2	1	1	2	13	31	6	30	22	5
Enterococcus spp.	15	5	-	-	2	5	1	5	18	4
Streptococcus agalactiae	4	2	-	-	1	2	-	-	5	1
Streptococcus pneumoniae	-	-	2	4	-	-	-	-	2	1
Staphylococcus epidermidis	-	-	-	-	-	-	2	10	2	1
Staphylococcus saprophyticus	2	1	-	-	-	-	-	-	2	1
Streptococcus pyogenes	-	-	-	-	1	2	-	-	1	0
Staphylococcus capitis	-	-	-	-	-	-	1	5	1	0
Gram negative bacteria	**242**	**91**	**40**	**94**	**25**	**60**	**10**	**50**	**317**	**87**
Escherichiae coli	196	74	-	-	7	17	1	5	204	55
Acinetobacte r baumannii	5	2	24	56	9	21	2	10	40	13
Klebsiella pneumoniae	23	8	3	8	-	-	4	20	30	8
Pseudomonas aeruginosa	7	3	11	26	4	10	2	10	24	6
Proteus spp.	5	2	-	-	2	5	-	-	7	2
Enterobacter spp.	4	2	1	2	-	-	-	-	5	1
Morganella morganii	1	0	-	-	3	7	-	-	4	1
Serratia spp.	1	0	1	2	-	-	-	-	2	1
Stenotrophomonas maltophilia	-	-	-	-	-	-	1	5	1	0
Total	**265**	**100**	**43**	**100**	**42**	**100**	**20**	**100**	**370**	**100**

DTA: Deep trakeal aspiration

The WBC and neutrophil contents, the MNV were higher in the samples showing positive growth than in those showing no growth; the MNC and the MNS parameters were lower in the test group than in the control group. The comparison of the median WBC count, median neutrophil count, and median neutrophil VCS parameters based on the status of growth of the cultures is shown in Table 2.

In addition, for bacterial growth, the specificities of the MNV and MNC parameters were found to be 96% and 99%, respectively. The performance of the MNV, MNC, and MNS values in the prediction of bacterial growth in the culture samples is shown in Table 3.

When the patients were grouped according to the place of isolation of the samples, the MNC parameter was different among those with growth in DTA, blood samples, urine and wound site samples (respectively, $p<0,001$, $p=0,100$, $p<0,001$, $p=0,050$). While MNV parameters were higher in the patients with growth in DTA, blood samples, urine and wound samples than the control group ($p<0,001$), MNS parameters were lower in these groups than the

control group (respectively, p<0,001, p<0,001, p=0,001, p=0,016).

Table 2. The comparison of the median WBC count, median neutrophil count, median neutrophil percent, and median neutrophil VCS parameters according to the status of growth in cultures [(median(IQR)]

Culture	MWC	MNN	MNV	MNC	MNS
Bacterial growth detected	9.7 (33.1)	6.4 (29.4)	150.5 (134.3)	143.6 (48.1)	142.7 (48.1)
Bacterial non-growth detected	7.6 (5.0)	4.4 (3.4)	142.0 (24.1)	150.4 (18.1)	145.7 (28.8)
P-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

MWC = medians of number of white blood cells MNN = medians of the number of neutrophil; MNV= median neutrophil volume; MNC = median neutrophil conductivity; MNS = median neutrophil light scattering. IQR= Interquartile Range

Table 3. The performance of the MNV, MNC, MNS values to predict bacterial growth in culture samples

	CUT-OFF	**SENSITIVITY(%)**	**SPECIFITY(%)**	**LR^+**	**LR^-**	**AUC**	**ROC 95%CI**
MNV	150.8	49.4	96.0	12.6	0.53	0.762	0.721-0.800
MNC	145.1	45.6	99.0	46.6	0.55	0.712	0.669-0.753
MNS	142.1	52.7	71.6	1.9	0.66	0.642	0.597-0.686

AUC= area under the ROC curve; MNV = mean neutrophil volume; MNC = mean neutrophil conductivity; MNS=mean neutrophil light scattering; LR = likelihood ratios; CI = confidence interval.

The median WBC count, median neutrophil count, median neutrophil percent, and median neutrophil VCS parameters according to the place of isolation of the samples are shown in Table 4.

The WBC count, neutrophil count, median neutrophil percent, and MNV and MNC parameters were similar between the gram-negative and gram-positive bacterial groups, whereas MNS was higher in the gram-negative bacterial group than in the gram-positive bacterial group. The comparison of the mean WBC count, neutrophil count, neutrophil percent, and neutrophil VCS parameters based on the gram nature of the test samples is shown in Table 5.

Table 4. The median WBC count, median neutrophil count, median neutrophil percent, and median neutrophil VCS parameters according to the place of isolation of the sample [median(IQR)]

Culture	**MWC**	**MNN**	**MNV**	**MNC**	**MNS**
Urine	9.7 (25.9)	6.1 (25.0)	148.2 (67.0)	142.3 (46.6)	143.2 (43.8)
DTA	9.5 (17.8)	7.8 (18.4)	155.2 (58.6)	143.9 (38.4)	140.0 (44.3)
Wound site	9.9 (14.4)	7.4 (14.8)	151.8 (124.6)	147.5 (45.1)	143.3 (47.7)
Blood culture	11.1 (33.1)	9.0 (29.4)	160.9 (53.5)	145.5 (31.8)	133.9 (28.9)
Non-growth	7.6 (5.0)	4.4 (3.4)	142.1 (24.1)	150.4 (18.1)	145.7 (28.8)

MWC = medians of number of white blood cells MNN = medians of the number of neutrophil; MNV= median neutrophil volume; MNC = median neutrophil conductivity; MNS = median neutrophil light scattering; IQR= interquartile range; DTA: Deep tracheal aspiration

Table 5. The comparison of the median WBC count, neutrophil count, neutrophil percent, and neutrophil VCS parameters according to the gram nature of the bacterial growth [median(IQR)]

	MWC	**MNN**	**MNV**	**MNC**	**MNS**
Gram positive bacteria	9.7 (16.9)	6.4 (14.9)	149.3 (45.0)	144.4 (30.8)	139.0 (35.1)
Gram negative bacteria	9.8 (33.1)	6.4 (29.4)	151.0(134.3)	143.5 (48.1)	143.0 (45.1)
P-value	0.989	0.778	0.680	0.618	**0.034**

MWC = medians of number of white blood cells; MNN = medians of the number of neutrophil; MNV = median neutrophil volume; MNC = median neutrophil conductivity; MNS = median neutrophil light scattering; IQR= interquartile range

DISCUSSION

The applications of the neutrophil VCS parameters in life-threatening cases and in those needing a fast cure for sepsis or non-systemic infections are being tested (7,8). Chaves et al. compared their patient groups with positive (test) and negative (control) blood culture reports and found that MNV was higher and MNS was lower in the test group than in the control group, whereas MNC was similar between both the groups (3). Prohit et al. reported higher MNV in patients with sepsis than in control patients (9). Furthermore, Çeliket al. investigated the neutrophil VCS parameters during the early diagnosis of sepsis in newborns and found that MNV tended to be higher, whereas both MNC and MNS tended to be lower in the patient group than in the control group (4). Zhu et al. reported that the MNV in infected patients were significantly increased after surgery when compared with noninfected patients (10). In the present study, our patients not only had positive blood cultures but also some other infections, particularly urine infections; the applicability of the neutrophil VCS parameters for the early diagnosis of the infection was extensively investigated in the blood samples of these patients. High MNV specificity (96%), and LR+ (12.6) as well as MNC specificity (99.02%) and LR+ (46.59) values indicated that MNV and MNC together act as a powerful diagnostic tool for bacterial infection. MNV and MNC are extremely specific; they can quite precisely help rule out the possibility of diseases. Similar to that reported in other studies, in the present study, MNV was higher whereas MNC and MNS were lower in the test group than in the control group. Thus, the neutrophil VCS parameters may act as early diagnostic indicators not only for sepsis but also for other bacterial infections. In the acute bacterial infection, inflammatory cytokines such as interleukin-1 and tumor necrosis factor are released. This condition stimulates bone marrow stromal cells and T cells. These factors cause a rapid increase in granulocyte output from the bone marrow and an increase in bands and other immature neutrophils. During acute bacterial infection, an increase in the reactive neutrophil population is seen as MNV (10). In addition, Safak et al. reported that MNV values were higher in groups of patients having gram-negative bacteria than in groups of patients having gram-positive bacteria (6). We found that MNS was higher in gram-negative bacterial infections than in gram-positive bacterial infections. Thus, clarifications about the difference in MNS with more extensive investigations will contribute to the effectiveness of empirical treatment for early distinction between gram-positive and gram-negative bacterial infections. It is thought that more comprehensive studies are needed on the mechanism of altered neutrophil parameters in gram-negative bacterial infections.

Furthermore, some infection markers are routinely used in cases wherein rapid diagnosis is essential. Some studies compared the neutrophil VCS parameters with infection markers, such as CRP, PCT, and neutrophil counts. A study also compared the infectious markers across three groups of patients: those with localized infections, common infections, and no infections; the authors found that MNV was superior to CRP and neutrophil as an infection marker (11). Among MNV, CRP, and interleukin-6, Mardi et al. found that interleukin-6 was the most effective marker in indicating acute infection (12). In addition, Suresh et al. reported that MNV had similar values in sepsis and therefore the two could not be clearly distinguished (5). In contrast, Mardi et al. reported that MNV was higher in sepsis than in localized infections (12). The majority of our study subjects had urinary tract infections with MNV being higher in the control group; this suggests that MNV is an important marker not only for sepsis but also for non-systemic infections. Thus, our study highlights the importance of the neutrophil VCS parameters in the early detection of bacterial infections. However, the limitation of our study is that we could not determine the specific infection sites. Another limitation of the study is that biomarkers such as C-reactive protein, procalcitonin, which are known as infection markers, are not included.

CONCLUSIONS

In conclusion, the neutrophil VCS parameters were found to be useful indicators for the early diagnosis of bacterial infections. In the future, owing to their importance in leading empirical treatment, changes in the neutrophil VCS parameters based on the gram staining property of the bacteria need to be studied in detail using a broader base sample. More extensive studies involving microorganisms other than bacteria should also be undertaken.

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