Malays J Pathol 2023; 45(1) : 77 – 85

ORIGINAL ARTICLE

Evaluation of mean neutrophil volume and immature to total neutrophil ratio as a biomarker for bacterial sepsis in adult patients

Siti Nur Azwa MD SHAHID[1], Azlin ITHNIN[1], Raja Zahratul Azma RAJA SABUDIN[1], Hafiza ALAUDDIN[1], Toh Leong TAN[2]*

[1]Department of Pathology, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur,
[2]Department of Emergency Medicine, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur.

Abstract

Introduction: Mean neutrophil volume (MNV) and immature to total neutrophil ratio (IT Ratio) has been found to support the detection of sepsis in elderly and neonates. This study aimed to assess the diagnostic significance of MNV and IT ratio in adult sepsis population. *Materials and Methods:* Sixty-four adult patients presented with suspected bacterial sepsis were included in this study. Relevant cultures and/or pertinent serology tests were performed. Full blood counts were analysed for MNV and IT ratio. *Results:* Fifty-one patients out of 64 recruited subjects were confirmed sepsis. Twenty-four patients had confirmed bacterial infection by cultivation and two were positive for leptospiral serology. MNV was very good in distinguishing sepsis from non-sepsis group (AUC = 0.80, 95% confidence interval (CI) = 0.69–0.91, Accuracy = 0.72, Kappa = 0.40) with a cut-off value of 153.5 (sensitivity = 67%, specificity = 92%). There was no significant difference in IT ratio between sepsis and non-sepsis group (p-value > 0.05). MNV was superior over IT ratio (AUC = 0.85, 95%CI = 0.76–0.95, and AUC = 0.70, 95% CI = 0.56–0.85, respectively) in diagnosing bacterial infection. The optimum cut-off value for MNV in bacterial infection was 154.5 (sensitivity = 67%, specificity = 89%) and for IT ratio was 0.035 (sensitivity = 45%, specificity = 67%). *Conclusion:* MNV appears to be a very good marker for diagnosing sepsis and bacterial infection. We recommend including MNV into sepsis workup in ED setting, since it can be determined without additional specimen.

Keywords: Neutrophils, bacterial infections, sepsis, blood cell count, emergency service

INTRODUCTION

Sepsis is considered as presence of two or more of the systemic inflammatory response syndrome (SIRS) criteria which is characterised by fever or hypothermia, tachycardia, tachypnoea and abnormal white blood cells count or immature forms, combined with any identified source of infection.[1] Bacterial infections are the most common cause of sepsis with more gram-positive infections compared to gram negative infections based on epidemiologic study of sepsis in United States in 1979 to 2000.[2] Meanwhile, estimated cases of sepsis from Emergency Department (ED) of Universiti Kebangsaan Malaysia Medical Centre (UKMMC) are around 14 000 cases annually and the prevalence in 2013 to 2014 is around 25-35% for those suspected sepsis age 18 years old and above.[3]

Increasing incidence of sepsis posed as a major health issue. However, it is treatable and curable with the current potent various antimicrobial agents. Therefore, an early and correct diagnosis is important to ensure that deserved medical managements are made.

Neutrophils function as the first line of defense against pathogens especially bacteria.[4] The morphologic changes seen in the reactive neutrophils and left-shifted granulocytes could be quantitatively evaluated by volume conductivity scatter (VCS) technology.[5] This technology is comparable to microscopic examination of peripheral blood smear but assesses more than a microscope.[6] The MNV which reflect neutrophil size had a better sensitivity than both total WBC count and the neutrophils percentage, proving it to be a promising indicator for diagnosing acute

**Address for correspondence:* Dr Toh Leong Tan, Department of Emergency Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, 56000 Bandar Tun Razak, Kuala Lumpur, Malaysia. Tel: +60125056775. Email: *sebastianttl@yahoo.co.uk*

bacterial infection.[7]

The presence of immature granulocytes indicates activation of bone marrow as seen in sepsis.[8] Thus, ratio of immature granulocytes to total neutrophilic cells (IT ratio) can also provide diagnostic information for sepsis although the test has never been well studied in adult population. A study on neonatal sepsis noted that higher degree of elevated IT ratio corresponds with greater risk of death.[9] Immature granulocytes also have been found in various other conditions including pregnancy, cancer, trauma, steroid therapy and myeloproliferative diseases.[10]

Even though there were previous studies done on MNV and IT ratio, most of the studies are based on hospital discharge diagnoses of sepsis which do not use the consensus definitions. Thus, by recruiting patients from emergency department using the above-mentioned criteria, hopefully the findings will be more useful especially for the treating physician in ED. Determination of MNV value is done during differential analysis and does not require additional specimen, as well as being not labour-intensive or time consuming. Whereas IT ratio just requires counts from blood smear examination. Thus, incorporation of these values in ED for sepsis algorithm may provide valuable information. However, adoption of the VCS technology for analysis of morphological changes within the cell population and IT ratio application in adult population of sepsis have never been well studied in Malaysia.

The outcome of this study is to determine the significance of MNV and IT ratio as diagnostic markers for adult patients with bacterial sepsis and to set the cut-off point for the most appropriate value to make a diagnosis. It would be expected that MNV and IT ratio has a high potential of becoming a significant marker in diagnosing sepsis as well as bacterial infection.

MATERIALS AND METHODS

Patient Recruitment

The study protocol was reviewed and approved by Universiti Kebangsaan Malaysia Research Ethics Committee (Approval code: FF-2015-116). This prospective study was carried out over a period of 8 months via convenience sampling (1st of May to 31st December 2015). All patients aged 18 years old and above, who presented to ED with a minimum of two SIRS criteria suspected of sepsis were recruited in this study. We exclude patients who were on steroid therapy, on antibiotics administration for more than 3 days, immunosuppressed patients, patients who sustained oncology diseases or any haematological malignancy and those who had trauma just prior to recruitment. The expected sample size for this study was 36 for each variable based on specificity of 97% based on study by Fernando Chaves, 2005 and sensitivity of 97% based on study by Thermiany AS *et.al*. 2008 with the prevalence of 13%. Written consent was obtained. Blood samples were collected for MNV and IT ratio quantifications. Relevant cultures and serology tests were carried out for all patients. Sepsis was defined as SIRS criteria with clinical suspicion of infection and/or documented positive culture or serology test. Bacterial infection was defined as clinical bacterial infection based on Horan *et al*. 2008[11] and/or positive bacterial culture, whereas clinical infection with negative bacterial culture and/or positive serology test for non-bacterial pathogen defined non-bacterial infection. Patients with indeterminate clinical outcomes and missing data were excluded. Control subjects include those who showed no symptoms or signs of infection i.e., no fever, WBC and differential counts within normal limit. Similar exclusion criteria are applied to this control group. All clinical information and reference standard results were blind to both the performers and assessors.

Determination of Mean Neutrophil Volume

Measurement of MNV was obtained from cell population data that was sent for Full Blood Count (FBC) in dipotassium Ethylene Diamine Tetraacetic Acid (EDTA) tube. The parameter was generated during the passage of each cell through the aperture and measured by the Unicel DxH 800 Coulter cellular analysis system (Beckman Coulter, Miami, Florida). VCS technology enabled various data from thousands of WBC to be obtained directly by using direct current impedance to measure cell volume (V) for accurate size of all cell types, radio frequency opacity to characterise conductivity (C) for internal composition of each cell, and a laser beam to measure light scatter (S) for cytoplasmic granularity and nuclear structure.

Determination of IT Ratio

Blood smears were made from EDTA sample within 4 hours of collection. The smears were stained using Wright's stain and examined manually using a light microscope. Calculation of IT ratio was performed by counting the proportion of immature granulocytes (i.e. promyelocytes, myelocytes, metamyelocytes,

bands) in 100 mature neutrophils. The stained slides were interpreted by two researchers independently and average count was obtained.

Statistical Analysis

Statistical analysis was executed using SPSS software version 23.0 (IBM, Armonk, Illinois). Since the data were non-parametric, they were presented using median and interquartile range. Area under the curve (AUC) were measured based on the receiver operating characteristics (ROC) curve that were plotted, to evaluate the power of both tests in distinguishing between sepsis and non-sepsis groups, along with bacterial and non-bacterial infection groups as well, and then subsequently determine the cut-off points of each variable. Value above cut-off point was considered as index test and value below cut-off was considered as reference test. The sensitivity and specificity were assessed based on the cut-off value. Moreover, the accuracy and kappa agreement for the parameters was determined using cross table.

RESULTS

Seventy-five patients were recruited during the study period of eight months. Of which, 64 patients were eligible for analysis after selection. A total of 11 patients were excluded for various reasons (three of them had malignancy, three were not consented, two had incomplete data, one patient was partially treated with antibiotic, one had HIV, and one had dengue infection). Six samples were not completely tested for both parameters (i.e. either MNV or IT ratio only). Only 51 of these recruited patients presented with sepsis though 24 of them were confirmed bacterial infections using cultures. Demographic data of the recruited subjects were tabulated in Table 1. Meanwhile, Table 2 demonstrated the detected bacterial organism by cultivation. A total of 97control subjects were recruited in this study. Measurement for MNV and IT ratio of this group were shown in Table 3.

The MNV levels were of non-parametric distribution in both groups of sepsis and bacterial infection. Median for MNV levels in the sepsis group (160.0 ± 9.96) were significantly higher as compared to the non-sepsis (150.5 ± 4.06) and control (142.0 ± 6.10) groups ($p = 0.002$ and $p = 0.000$ respectively, Mann-Whitney U test) (Fig. 1a and Table 3). Thus, MNV was able to discriminate between sepsis from non-sepsis group with a suggested cut-off point of 153.5. The MNV value had a specificity of 92% and sensitivity of 67%. The positive predictive value was 97% and the negative predictive value was 41%, making MNV a very good biomarker for sepsis (AUC = 0.80, 95% confidence interval (CI) = 0.69 – 0.91, Accuracy = 0.72, Kappa = 0.40). MNV levels for both bacterial and non-bacterial infection groups also showed statistically significant difference ($p > 0.001$) with a higher median in the bacterial group (160.0 ± 9.47) than non-bacterial infection group (149.0 ± 5.09) (Fig. 1b). We also observed a statistically higher level of MNV in bacterial cases as compared with control sample ($p>0.001$). Based on this study, we suggest a cut-off level of 154.5 to diagnose bacterial infection. The sensitivity for MNV was 67% while the specificity was 89%, whereas the positive and negative predictive values were 94% and 56%, respectively. MNV levels were found to have very good accuracy in diagnosing bacterial infection (AUC = 0.85, 95% confidence interval (CI) = 0.76 – 0.95, Accuracy = 0.73, Kappa = 0.46).

However, distribution of IT ratio was found to be not statistically significant across group of sepsis (0.015 ± 0.05) and non-sepsis (0.000 ± 0.016) ($p = 0.07$) (Fig. 2a). A cut-off level of 0.025 was not able to discriminate between the sepsis from non-sepsis group (sensitivity = 46.6%; specificity = 66.7%; positive predictive value = 84.4%; negative predictive value = 24.4%). Interestingly, IT ratio in control subjects with a median of 0.000 ± 0.005 was statistically significant as compared to sepsis group ($p > 0.001$) (Table 3). In addition, IT ratio was able to demonstrate a positive statistical significance in distinguishing bacterial infection (0.020 ± 0.050) with non-bacterial infection (0.000 ± 0.017) and control (0.000 ± 0.005) groups ($p = 0.014$ and <0.001 respectively) (Fig. 2b and Table 3). With the cut-off level of 0.035, IT ratio was able to differentiate between bacterial infection from non-bacterial infection group. Sensitivity and specificity for IT ratio were 83.3% and 21.6%, respectively, as well as the positive and negative predictive values each correspond to 73.3% and 33.3%. Thus, IT ratio was considered as a good biomarker to predict bacterial infection (AUC = 0.70, 95% confidence interval (CI) = 0.56 – 0.85, Accuracy = 0.73, Kappa = 0.35). Table 4 summarised both markers expression for the recruited patients. Fig. 3 shows ROC curves for both MNV and IT ratio according to diagnosis of sepsis and bacterial infection.

TABLE 1: Demographic data of recruited patients

	Patients (n = 64)
AGE (years; mean ± SD)	60.2 ± 19.4
GENDER	
Male	34 (53.1%)
Female	30 (46.9%)
RACE	
Malay	25 (39.1%)
Chinese	27 (42.2%)
Indian	10 (15.6%)
Others	2 (3.1%)
CLINICAL CHARACTERISTICS (mean ± SD)	
Systolic blood pressure	137 ± 34
Diastolic blood pressure	72 ± 22
Heart rate (per minute)	102 ± 22
Respiratory rate (per minute)	24 ± 6
Temperature (°C)	37.9 ± 1.3
Total white cell count (x 10^9/L)	13.5 ± 7.1
Absolute neutrophil count (x 10^9/L)	10.7 ± 6.2
SEPSIS	51 (79.7%)
NON-SEPSIS	13 (20.3%)
BACTERIAL CULTURE POSITIVE (BLOOD)	16 (25.0%)
BACTERIAL CULTURE POSITIVE (OTHERS)	8 (12.5%)
SOURCE OF INFECTION	
Respiratory	30 (57.7%)
Urinary	5 (9.6%)
Gastrointestinal	3 (5.8%)
Hepatobiliary	1 (1.9%)
Cardiac	1 (1.9%)
Cutaneous	6 (11.5%)
Musculoskeletal	1 (1.9%)
Blood/Catheter related	4 (7.7%)
Leptospiral serology	2 (3.1%)
Unknown	1 (1.9%)

DISCUSSION

Even though sepsis is considered as a clinical diagnosis, similarities of clinical signs and symptoms with other critical medical conditions can provide a difficult challenge in diagnosing sepsis in which laboratory investigations are useful in helping the clinicians to make an accurate diagnosis. The laboratory has a crucial role in providing parameters such as WBC count, C-reactive protein (CRP), procalcitonin (PCT) and blood culture results which are considered valuable information for the diagnosis of sepsis.

In addition, morphological changes in neutrophils are observed during sepsis (i.e. immature granulocytes tend to be larger and have lower nuclear complexity) as the bone marrow is stimulated by cytokine release to promote proliferation and differentiation of granulocytes progenitors.[12] Therefore, measurement of MNV which reflect the neutrophil size, as well as IT ratio are expected to have a diagnostic significance for sepsis together with bacterial infection patients thus providing clinicians with useful information to manage their patients.

This study demonstrated that MNV had high specificity and positive predictive value in distinguishing sepsis from non-sepsis group, as well as discriminate bacterial infection from non-bacterial infection group, making it an accurate marker for these purposes. This present study is in agreement with Lee *et al*. (2013) that

TABLE 2: Bacterial aetiology as detected via cultivation

Cultured Organism	Frequency
Escherichia coli	5
Escherichia coli ESBL	2
Klebsiella pneumoniae	1
Klebsiella species	3
Pseudomonas aeruginosa	2
Burkholderia cepacia	1
Aerococcus viridans	1
Enterobacter species	1
Proteus species	1
Salmonella species	1
Staphylococcus aureus	3
Methicillin-resistant Staphylococcus aureus	1
Streptococcus viridans	1
Streptococcus intermedius	1
Streptococcus milleri	2
Enterococuus faecalis	1
Diptheroides	1
Eggerthella lenta	1
Mycobacterium tuberculosis	3
Coagulase negative staphylococcus	1

stated MNV had better specificity for predicting sepsis, in addition to acute bacterial infection.[13] Several other studies also had demonstrated a significant elevation of MNV value in sepsis patient which correspond to our results.[7,14] Meanwhile, other observations among infected patients also produced similar findings.[15–17]

Besides the obvious advantages of MNV as compared to IT ratio as evident by our data, clinical application of MNV can provide several more benefits. First, the parameter is determined during WBC differential analysis. Thus, additional specimen is not required. Second, determination of the value is not labour-intensive or time-consuming, which is an issue with manual smear examination for IT ratio determination. Lastly, MNV value is more accurate and objective as the VCS technology evaluates automatically more than 8,000 WBCs rather than manual counts in IT ratio. MNV was also found to be superior to absolute neutrophil count in predicting sepsis and infection since the count might still be normal or even low in septic patients.[14–16] Therefore, MNV has the prospect to become a supplementary marker for predicting early sepsis and bacterial infection.

In comparison to MNV, IT ratio showed no significant difference in discriminating sepsis and non-sepsis group in this study. Since there is no study that has been done on adult population, no

TABLE 3: MNV and IT ratio for control, sepsis and bacterial infection groups

Parameters	Control (n=97)	Sepsis (n=51)	Bacterial infection (n=46)	P value[a]	
				Control vs Sepsis	Control vs Bacterial
MNV	142.0 ± 6.1	160.0 ± 10.0	160.0 ± 9.5	>0.001*	>0.001*
IT Ratio	0.00 ± 0.01	0.02 ± 0.05	0.02 ± 0.05	>0.001*	>0.001*

MNV, Mean neutrophil volume; IT Ratio, Immature to total neutrophil ratio;
*Statistically significant at $p < 0.05$, [a]Mann-Whitney *U* test

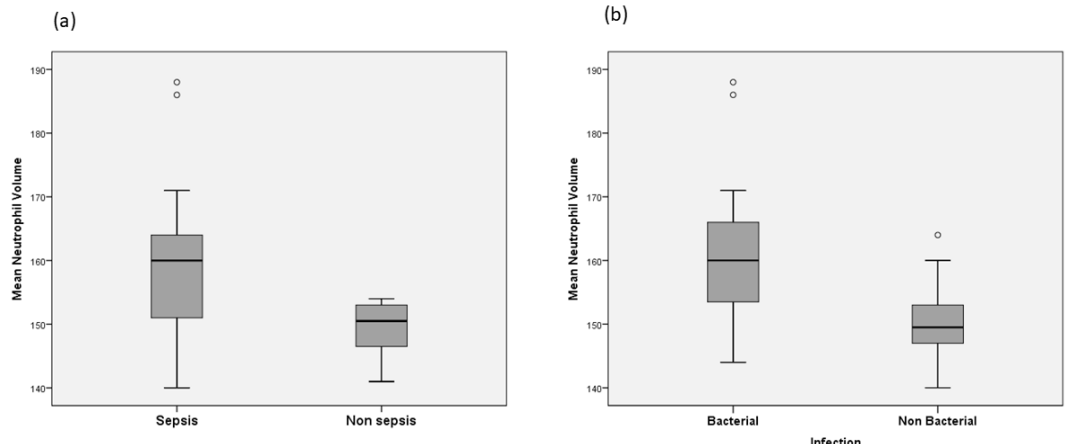

FIG. 1: Box-plot for MNV value in distinguishing the groups (a) sepsis and non-sepsis group (b) bacterial and non-bacterial infection. Boxes indicate the 25th-75th centiles, whereas whiskers denote the 10th and 90th centiles. Horizontal lines within the boxes demonstrate the median. Outliers are marked as circles.

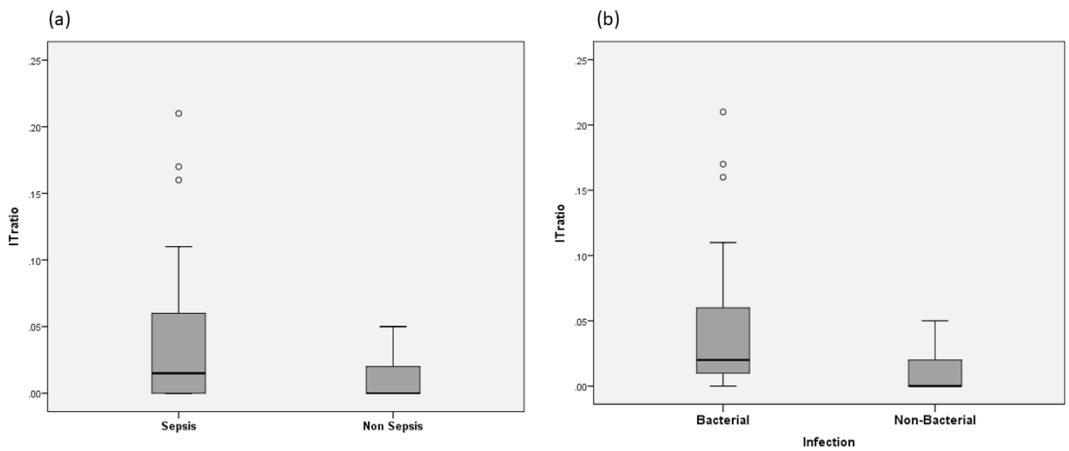

FIG. 2: Box-plot for IT ratio in distinguishing the groups (a) sepsis and non-sepsis group (b) bacterial and non-bacterial infection. Boxes indicate the 25th-75th centiles, whereas whiskers denote the 10th and 90th centiles. Horizontal lines within the boxes demonstrate the median. Outliers are marked as circles.

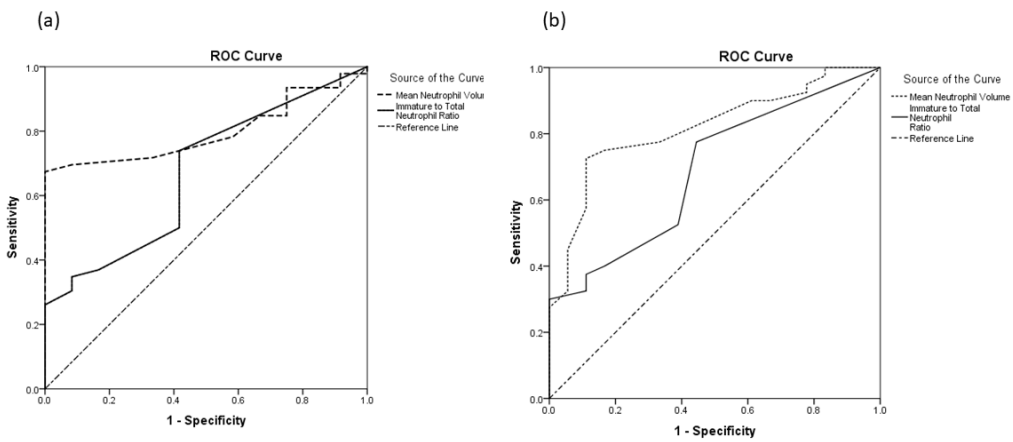

FIG. 3: ROC curves for MNV and IT ratio in diagnosis of (a) sepsis and (b) bacterial infection.

TABLE 4: MNV and IT ratio expression for recruited subjects

Parameters	AUC (95% CI)	Cut-off point	Sn (95% CI), %	Sp (95% CI), %	PPV (95% CI), %	NPV (95% CI), %	Accuracy (95% CI), %	Kappa	P value[a]
Sepsis versus non-sepsis patients									
MNV	0.80 (0.69 – 0.91)	153.50	67 (52-79)	92 (64-99)	97 (84-99)	41 (32-52)	72 (0.59-0.82)	0.40	0.002*
IT ratio	0.67 (0.51-0.83)	0.01	77 (63-87)	54 (25-81)	87 (78-92)	37 (22-54)	72 (0.59-0.82)	0.26	s0.070
Bacterial infection versus non-bacterial infection patients									
MNV	0.85 (0.76 – 0.95)	154.50	67 (52-81)	89 (65-99)	94 (81-98)	52 (41-63)	73 (0.61-0.84)	0.46	>0.001*
IT ratio	0.70 (0.56 – 0.85)	0.01	80 (66-91)	56 (31-78)	82 (73-89)	53 (35-69)	73 (0.61-0.84)	0.35	0.014*

MNV, Mean neutrophil volume; IT Ratio, Immature to total neutrophil ratio; AUROC, Area Under the Receiver Operative Curve; Sn, Sensitivity; Sp, Specificity; Positive Predictive Value; NPV, Negative Predictive Value; PLR, Positive Likelihood Ratio;
*Statistically significant at $p < 0.05$, [a]Mann-Whitney *U* test

comparison can be made on the result. However, IT ratio was found to be a good marker for predicting sepsis in neonate as well as paediatric population based on previous studies.[18–21] Based on our findings, IT ratio is not recommended to be inserted into investigation algorithm for suspected sepsis patient.

Manual peripheral blood smear examination can provide useful information on morphological changes (e.g., Dohle bodies, toxic granulation and cytoplasmic vacuolation) together with the differential counts of the neutrophils and immature granulocytes. The frequent qualitative changes seen in the sepsis group, as well as the band count, provided valuable methods in diagnosing bacterial infections.[22] Thus, it is not surprising that IT ratio was found to correlate with bacterial infection in this present study. Though, the cut off value in previous studies were higher with more sensitive and/or specific indicator in predicting bacterial infection.[19,23] In contrast to this study, Krediet *et al*. (1992) concluded that IT-ratio determination is of limited value as a diagnostic test in neonatal infection.[24] Even though most of the laboratory has the ability to examine blood smear preparation, IT ratio technique is nonetheless labour-intensive and time-consuming. Furthermore, it is associated with inter-observer and also intra-observer variation owing to the heterogenous distribution of leukocytes on the blood films.[25] Therefore, although our finding demonstrated that IT ratio was able to predict bacterial infection, it is not highly recommended since the sensitivity and specificity is not that great.

Up to date, there is still no single reliable test available for early confirmation or exclusion of sepsis.[26] Although blood culture is considered as the gold standard for diagnosing sepsis, this test shows low sensitivity[27] and also requires proper collection techniques with sufficient amount of blood, in which may result in delay in making the diagnosis. CRP has been widely used[28] but the value can never be diagnostic on its own since it is a non-specific protein produced as part of the acute phase response towards inflammation, infection and tissue damage.[29] Even though procalcitonin is considered as superior compared to other markers of infection since it can also provide prognostic information, there were also literatures that reports elevations in procalcitonin levels that were not related to bacterial infections.[30,31] Previous studies demonstrated that MNV showed comparable sensitivity and specificity with CRP and procalcitonin.[14,32]

Combination of several sepsis markers including MNV may have been more useful to improve the diagnostic accuracy in both sepsis as well as bacterial infection.

Limitations
This study is limited by its convenient sampling and small sample size since it is restricted to a certain time, researchers and financial constraint. Furthermore, adherence to a highly selected group recruitment protocol may have led to bias overestimating the diagnostic power of these investigated markers. However, these findings may still be applicable. Larger studies with probability sampling may be needed to further evaluate the clinical usefulness of these parameters. It might be interesting in the future to look at the association between MNV and IT ratio parameters with morbidity and mortality in sepsis patients. The findings may have prognostication or survival benefit that can facilitate physicians in management decisions.

CONCLUSION

Overall, MNV performance was found to be superior compared to IT ratio for sepsis and bacterial infection diagnoses in adults. Thus, it could be used either as singly or in combination with other markers for these purposes. We recommend that MNV should be incorporated into the diagnostic algorithm of sepsis in ED. This study finding may be able to facilitate clinicians in making decisions for administration of early antimicrobial therapy, improve risk stratification and promote the implementation of sepsis bundle.

Acknowledgements: The authors wish to thank Dr Christabel Kang Wan Li and Ms Nurul Saadah Ahmad for their contribution to the study.

Funding: This work was supported by Fundamental Fund, Faculty of Medicine, University Kebangsaan Malaysia (FF-2015-116), awarded to Dr Azlin Ithnin. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of Interests: The authors declare no competing interests.

Author's Contribution: Conceptualization: Azlin Ithnin and Toh Leong Tan; Research Methodology: Azlin Ithnin, Toh Leong Tan, Siti Azwa Md Shahid; Manuscript Writing: Azlin Ithnin, Toh Leong Tan, Siti Azwa Md Shahid; Review and Editing: Azlin Ithnin, Toh Leong Tan, Raja Zahratul Azma Raja Sabudin and Hafiza Alauddin.

REFERENCES

1. Chiesa C, Panero A, Osborn JF, Simonetti AF, Pacifico L. Diagnosis of neonatal sepsis: a clinical and laboratory challenge. Clin Chem. 2004;50:279-87.
2. Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. N Engl J Med. 2003;348:1546-54.
3. Tan TL, Ahmad NS, Nasuruddin DN, *et al*. CD64 and group II secretory phospholipase A2 (sPLA2-IIA) as biomarkers for distinguishing adult sepsis and bacterial infections in the emergency department. PLoS One. 2016;11:e0152065.
4. Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. Nat Rev Immunol. 2013;13:159-75.
5. Park DH, Park K, Park J, *et al*. Screening of sepsis using leukocyte cell population data from the Coulter automatic blood cell analyzer DxH800. Int J Lab Hematol. 2011;33:391-99.
6. Celik IH, Demirel G, Aksoy HT, *et al*. Automated determination of neutrophil VCS parameters in diagnosis and treatment efficacy of neonatal sepsis. Pediatr Res. 2012;71:121-25.
7. Chaves F, Tierno B, Xu D. Quantitative determination of neutrophil VCS parameters by the Coulter automated hematology analyzer: new and reliable indicators for acute bacterial infection. Am J Clin Pathol. 2005;124:440-44.
8. Nierhaus A, Klatte S, Linssen J, *et al*. Revisiting the white blood cell count: immature granulocytes count as a diagnostic marker to discriminate between SIRS and sepsis-a prospective, observational study. BMC Immunol. 2013;14:1-8.
9. Behrman RE, Christensen RD, Bradley PP, Rothstein G. The leukocyte left shift in clinical and experimental neonatal sepsis. J Pediatr. 1981;98:101-5.
10. Briggs C. Quality counts: new parameters in blood cell counting. Int J Lab Hematol. 2009;31:277-97.
11. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care–associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control. 2008;36:309-32.
12. Von Vietinghoff S, Ley K. Homeostatic regulation of blood neutrophil counts. J Immunol. 2008;181:5183-88.
13. Lee AJ, Kim SG. Mean cell volumes of neutrophils and monocytes are promising markers of sepsis in elderly patients. Blood Res. 2013;48:193-97.
14. Mardi D, Fwity B, Lobmann R, Ambrosch A. Mean cell volume of neutrophils and monocytes compared with C- reactive protein, interleukin-6 and white blood cell count for prediction of sepsis and nonsystemic bacterial infections. Int J Lab Hematol. 2010;32:410-18.

15. Suresh PK, Minal J, Rao PS, Ballal K, Sridevi HB, Padyana M. Volume conductivity and scatter parameters as an indicator of acute bacterial infections by the automated haematology analyser. J Clin Diagn Res. 2016;10:EC01-3.
16. Bagdasaryan R, Zhou Z, Tierno B, Rosenman D, Xu D. Neutrophil VCS parameters are superior indicators for acute infection. Lab Hematol. 2007;13:12-6.
17. Zhu Y, Cao X, Chen Y, *et al*. Neutrophil cell population data: useful indicators for postsurgical bacterial infection. Int J Lab Hematol. 2012;34:295-99.
18. Newman TB, Draper D, Puopolo KM, Wi S, Escobar GJ. Combining immature and total neutrophil counts to predict early onset sepsis in term and late preterm newborns: use of the I/T2. Pediatr Infect Dis J. 2014;33:798-802.
19. Aulia D, Sanjaya AI, Timan IS. The use of immatane to total neutrophil (IT) ratio to detect bacteriemia in neonatal sepsis. J Lab Med Qual Assur. 2003;25:237-42.
20. Frey B, Horton SB, Duke T, Shann F. The immature-to-total neutrophil ratio (IT ratio) is a sensitive indicator of sepsis after paediatric cardiopulmonary bypass. Schweiz Med Wochenschr. 2000;130:1572-75.
21. Thermiany AS, Retayasa W, Kardana M, Lila IN. Diagnostic accuracy of septic markers for neonatal sepsis. Paediatr Indones. 2008;48:299-305.
22. Zipursky A, Palko J, Milner R, Akenzua GI. The hematology of bacterial infections in premature infants. Pediatrics. 1976;57:839-53.
23. Hornik CP, Benjamin DK, Becker KC, *et al*. Use of the complete blood cell count in early-onset neonatal sepsis. Pediatr Infect Dis J. 2012;31:799-802.
24. Krediet T, Gerards L, Fleer A, van Stekelenburg G. The predictive value of CRP and I/T-ratio in neonatal infection. J Perinat Med. 1992;20:479-85.
25. Pierre R V. Peripheral blood film review: the demise of the eyecount leukocyte differential. Clin Lab Med. 2002;22:279-97.
26. Magudumana O, Ballot DE, Cooper PA, *et al*. Serial interleukin 6 measurements in the early diagnosis of neonatal sepsis. J Trop Pediatr. 2000;46:267-71.
27. Procop GW, Hartman JS, Sedor F. Laboratory tests in evaluation of acute febrile illness in pediatric emergency room patients. Am J Clin Pathol. 1997;107:114-21.
28. Povoa P, Coelho L, Almeida E, *et al*. C-reactive protein as a marker of infection in critically ill patients. Clin Microbiol Infect. 2005;11:101-8.
29. Van de Vyver A, Delport EF, Esterhuizen M, Pool R. The correlation between C-reactive protein and toxic granulation of neutrophils in the peripheral blood. South African Med J. 2010;100:442-4.
30. Sabat R, Höflich C, Döcke W-D, *et al*. Massive elevation of procalcitonin plasma levels in the absence of infection in kidney transplant patients treated with pan-T-cell antibodies. Intensive Care Med. 2001;27:987-91.
31. Schumm J, Pfeifer R, Ferrari M, Kuethe F, Figulla HR. An unusual case of progressive shock and highly elevated procalcitonin level. Am J Crit Care. 2010;19:93-6.
32. Zhu Y, Cao X, Zhang K, Xie W, Xu D, Zhong C. Delta mean neutrophil volume (ΔMNV) is comparable to procalcitonin for predicting postsurgical bacterial infection. J Clin Lab Anal. 2014;28:301-5.