



# Delta Neutrophil Index: in Search of an Early Indicator of Sepsis

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**Received:** 2 June 2020 ♦ **Accepted:** 15 June 2020 ♦ **Published:** 31 Aug 2021

**Citation:** Peneva P, Nikolova S, Bocheva Y. Delta neutrophil index: in search of an early indicator of sepsis. *Folia Med (Plovdiv)* 2021;63(4):496-501. doi: 10.3897/folmed.63.e55017.

## Abstract

**Introduction:** Sepsis is a significant cause of morbidity and mortality worldwide. Current clinical practice, however, lacks reliable diagnostic indicators for it and its prognosis.

**Aim:** The present study aimed to investigate the efficacy of delta neutrophil index (DNI), which reflects the proportion of circulating immature granulocytes, in predicting infections and sepsis.

**Materials and methods:** A prospective non-interventional single-center clinical follow-up study was performed in a Bulgarian ICU between January 1, 2017 and May 31, 2018. We analyzed adult patients: 45 patients met the sepsis criteria, as defined in SEPSIS-3, whereas 37 were infected patients fulfilling no criteria of sepsis. Logistic regression and Roc-curve analysis were used to evaluate the severity and prognostic value of DNI as a prediction marker in critically ill septic patients.

**Results:** The results have shown that at DNI values of 1.4 there is 73% sensitivity and 87% specificity (AUC 0.764, 95% CI 0.650–0.878,  $p=0.0001$ ) to assume the presence of sepsis. Additionally, DNI was significantly associated with the severity of the condition of patients, the organ dysfunction and the IL-8 marker.

**Conclusions:** DNI may serve as a useful marker for early diagnosis of sepsis and could support decision making process regarding its treatment at an early stage of a disease development.

## Keywords

DNI, infection, sepsis, septic shock

## INTRODUCTION

Rapid diagnosis of sepsis in critically ill patients remains a challenge for all physicians. Even though a number of indicators are monitored or used in practice to detect infections, none of them reliably indicates the risk of developing sepsis and septic shock. In the meantime, some of these tests are not available or consistently used in routine practice.

It has been established that during times of stress or infection, immature neutrophils enter the circulation and the number of immature forms increases. This condition is defined as left shifting and represents an increased ratio of immature cells to total granulocytes count or an increased number of immature neutrophils.<sup>1</sup>

A number of studies have found high susceptibility to infections in morphological changes in neutrophils (in 80%); toxic granulations, Döhle bodies and cytoplasmic vacuoles

have been tracked down.<sup>2</sup> Myeloid progenitor cells are also significantly high in infectious conditions.<sup>3</sup>

Immature granulocytes are also used as an indicator of sepsis. They could be a better indicator than the total count of leukocytes or neutrophils, or even that of immature neutrophils.<sup>4,5</sup> Therefore, a more reliable method for measuring immature granulocytes would have greater practical application.

In recent years, modern haematology analysers have been designed to provide information on leukocyte differentiation.<sup>6</sup> A haematology counter reports the various subpopulations of blood cells through flow cytometry-based processes. DNI represents an automated analysis of the fraction of immature granulocytes, obtained as the difference between the fraction of myeloperoxidase cells (Eo and Ne) and the fraction of mature polymorphonuclear leukocytes. The count of immature granulocytes includes promyelocytes, myelocytes, and metamyelocytes, without blasts.<sup>6,7</sup>

$$\text{Delta neutrophil index I} = (\text{Neu}\% + \text{Eo}\%) - \text{PMN}\%,$$

$$\text{Delta neutrophil index II} = (\text{Neu}\% + \text{Eo}\% + \text{LUC}\%) - \text{PMN}\%,$$

where LUCs are the large unformed cells which do not contain peroxide and remain unstained.

DNI assesses clinical severity and is a prognostic marker in critically ill patients. Elevated DNI has recently been associated with the diagnosis and prognosis of sepsis.<sup>6,8,9</sup> Its importance as a differentiating indicator of uncomplicated infections compared to those with rapid deterioration and development of sepsis and septic shock is gaining wider recognition.<sup>6,8,9</sup>

## AIM

We aimed to assess DNI values in patients with non-sepsis infections and sepsis and septic shock infections, and to determine its predictive value as a marker for distinguishing septic from non-septic patients, as well as to assess the risk of death.

## MATERIALS AND METHODS

We performed a prospective non-interventional single-centre clinical follow-up study. All participants – hospital patients, were included in the study after their written consent to participate. The study was conducted between January 2017 and July 2018, with the approval of the Ethics Committee for Clinical Trials at the Medical University of Varna.

Patients with infections of different localization, without sepsis criteria but meeting the SIRS criteria, were followed up.<sup>10</sup> The second main group were sepsis patients divided into two subgroups: with and without septic shock. All sep-

sis patients met the criteria according to the recommendations of the Third International Consensus Definitions for Sepsis and Septic Shock – SEPSIS-3 (2016), with the latest 2018 updates on early goal directed therapy for septic patients.<sup>11,12</sup>

Patients met common inclusion criteria: laboratory test results for constellation of symptoms for systemic inflammation, age over 18 years, with or without concomitant diseases. Exclusion criteria: pregnancy, age under 18 years and over 80 years of age, concomitant malignancies including haematological diseases in which DNI would have shown abnormal values even in the absence of a septic condition.

The study included peripheral blood tests and took into account the main haematomorphological parameters: haemoglobin, erythrocytes, leukocytes differential count, and platelets. Blood samples were obtained by vein punctures in 2 mL vacutainers, and then analysed within the first hour using ADVIA 2120i haematology analyser, after which DNI was calculated. Correlations and logistic regressions were performed to test for associations and whether DNI was a prognostic factor for sepsis and septic shock. Receiver operating characteristic (ROC) analyses was used to determine the cut-off values and the sensitivity and specificity of DNI in predicting sepsis in infected patients. All statistical analyses were performed using IBM SPSS v.25 for Windows.

## RESULTS

The calculation of DNI for all patients was performed on days 1 and 5, showing significant differences in the mean values in the three monitored groups.

The mean value in the group of patients with infections without sepsis was  $0.45 \pm 1.21$  on day 1, and  $0.8 \pm 1.7$  on day 5; in septic patients without shock:  $3.5 \pm 4.7$  on day 1, and  $3.6 \pm 4.2$  on day 5; the group of patients with septic shock showed values of  $7.90 \pm 11.6$  on day 1 and  $5.7 \pm 6.4$  on day 5.

To analyse the predictability of sepsis development, we used the method of multinomial logistic regression. Our control category – the group of patients with infections, was separately compared to the group of patients with sepsis without shock, and that with septic shock.

Analyses display DNI as a considerably significant marker in determining the severity of infection. Thus, in the group of infections without sepsis, its predictability was 7.5% (Exp (B) = 0.752,  $p=0.007$ ). The predictability of DNI was significantly higher in the group of sepsis without septic shock: 32% (Exp (B) = 1.329,  $p=0.007$ ). The highest DNI forecast values – 43% (Exp (B) = 1.430,  $p=0.001$ ), were exhibited for the development of septic shock. DNI indicates a substantially significant association with the development of sepsis and the severity of the condition ( $r=0.363$ ,  $p=0.001$ ).

Similarly, another marker that we followed – IL-8, showed a strong association with the septic status ( $r=0.461$ ,  $p=0.0001$ ). We found a correlation between the develop-

ment of organ dysfunction and DNI ( $r=-0.302$ ,  $p=0.008$ ), as well as with IL-8 ( $r=-0.245$ ,  $p=0.039$ ).

Even though DNI demonstrated its prognostic value in the detection of sepsis and organ damage, this marker did not reflect a prognostic value of 30-day mortality ( $R^2=0.147$ ,  $p=0.762$ ). We also tracked the ROC curve of DNI values above which sepsis development can most likely be assumed. ROC analysis revealed that at DNI values of 1.4 (the best cut-off value 1.4), at 73% sensitivity and 87% specificity (AUC 0.764, 95% CI 0.650–0.878,  $p=0.0001$ ), it is possible to expect and assume the presence of sepsis.

## DISCUSSION

The reasons for choosing DNI as a prognostic indicator are the simplicity, test speed and ease of calculation, literally within minutes, and its low price, along with the potential and applicability of the benefits. We aimed to determine whether DNI could be a diagnostic and prognostic marker for sepsis infections, as well as its value for mortality predictability. Normally, there are no immature granulocytes in the blood of healthy people, the difference is close to zero, and the value of DNI is approx. 0, respectively.

According to a systematic review and meta-analysis made by Park et al.<sup>8</sup>, the sum of Ne and Eo in healthy individuals was almost equal to the number of polymorphonuclear cells. However, in infections, because of the increased number of immature neutrophils, metamyelocytes, promyelocytes and myelocytes, PMN cells percentage in the nuclear density channel decreases. Thus, the difference between the total % (Ne + Eo) and PMN increases, i.e. with the development of infection/sepsis, DNI increases significantly.<sup>8</sup>

This indicator showed increased values in our patients with sepsis and septic shock. Whereas in infections without sepsis its mean values were  $0.45\pm 1.21\%$  on day 1, in septic patients without shock it was  $3.5\pm 4.7\%$ , and in the group of septic shock:  $7.90\pm 11.6\%$ .

DNI is an important marker for determining the severity of infection. DNI has a predictability of about 7% for the development of infection without sepsis (Exp (B)=0.752,  $p=0.007$ ), but this predictability increases significantly with the development of sepsis without shock: 32% (Exp (B)=1.329,  $p=0.007$ ), as well as in septic shock: 43% (Exp (B)=1.430,  $p=0.001$ ). In search for DNI values above which the development of sepsis can be expected (the best cut-off value), ROC analysis exhibited this value at 1.4%, with 73% sensitivity and 87% specificity (AUC 0.764, 95% CI 0.650–0.878,  $p=0.0001$ ).

Our follow-up study obtained results similar to Seok et al. who investigated this indicator as a differential diagnostic and prognostic marker in septic patients. They obtained mean DNI values of 0.8% for patients with SIRS, 3.4% for septic patients, and 18.6% for severe sepsis; the best cut-off value was 2.7%.<sup>9</sup>

Their values are similar to or slightly higher than those we obtained. We can account for this by the different criteria

for sepsis in 2012 and now, 2016 – 2018 (SEPSIS-3). At that time, patients with sepsis were categorized into three groups (SIRS, sepsis, and severe sepsis), rather than two (sepsis and septic shock), according to the current criteria.<sup>11</sup> Thereby, DNI values were divided and averaged over three separate groups; the more severe the condition, and the earlier it was examined in the course of sepsis, the higher the index. The time when the infection is identified is essential for the DNI values. So far, research data for this indicator have not specified the beginning of the infection and the day when the index was calculated. Conversely, the number of patients would also affect the results. That is to say, after the new sepsis criteria were adopted in 2016 (SEPSIS 3), there has been done no similar study.

Park et al. conducted a meta-analysis in view of using DNI as a predictive marker of infection and sepsis. Their results showed sensitivity of 67% (95% CI 0.62–0.71,  $I^2 = 86.0\%$ ), and specificity of 94% (95% CI 0.94–0.95,  $I^2 = 92.8\%$ ); ROC analysis reveals values below 0.89. As a mortality prognostic factor for patients with infections, DNI showed sensitivity of 70% (95% CI 0.56–0.81,  $I^2 = 0.0\%$ ), and a specificity of 78% (95% CI 0.73–0.83,  $I^2 = 26.6\%$ ), while the ROC curve values were below 0.84. Namely, this only confirms the need for further research.<sup>8</sup>

In another follow-up study, Zanaty et al. studied DNI as a prognostic marker for mortality. They compared DNI values, 6-hour lactate clearance, SOFA score and DIC score of sepsis survivors and deceased septic patients. The obtained DNI mean values for sepsis survivors were  $5.2\pm 1.2\%$ , respectively  $19\pm 3.2\%$  for the deceased septic patients ( $p=0.0001$ ). Similarly, DIC score for deceased patients was significantly higher (58.8% vs. 30.5%,  $p=0.005$ ). The team established a DNI value of 5.7% as critical for the development of sepsis (the best cut-off value). They concluded that DNI strongly correlates with SOFA score and mortality and that the index can be used as an early diagnostic and prognostic marker for the diagnosis of septic patients, as well as for the initiation of earlier and aggressive treatment.<sup>13</sup>

In contrast to their study, DNI values in our patients did not show significance as a prognostic marker for mortality ( $R^2=0.147$ ,  $p=0.76$ ), but only as a marker for predictability of sepsis.

DNI showed dependency to one of the key interleukins in septic conditions, namely interleukin 8. Interleukin 8 is an important regulator of neutrophil function, with a role in neutrophil activation and degranulation. Our results revealed significant amounts of IL-8 synthesized and released during sepsis. Interleukin 8 values in our septic patients were on average 6 to 10 times (in severe cases with short-term outcome of death – more than 30 times) higher than in those without infections without sepsis. Correlation analysis found a strong positive correlation between higher IL-8 levels and the development of sepsis ( $r=0.461$ ,  $p=0.0001$ ). Pearson's analysis also demonstrated a strong correlation between IL-8 and DNI ( $r=0.575$ ,  $p=0.0001$ ). This verifies that increase in both indicators goes alongside

with the development of sepsis. In addition, correlation between IL-8 and organ failure was statistically significant and negative ( $r=-0.245, p=0.039$ ). The correlation between organ failure and DNI was similar ( $r=-0.302, p=0.008$ ). Increase in IL-8 and DNI is associated with the development of sepsis, aggravation of organ damages and worsening of the condition. The most common organ involved in the course of sepsis was the lung ( $r=-0.437, p=0.0001$ ). In 90% of cases, it was the only one or accompanied by other organ dysfunctions – renal, hepatic or haematological damage.

In addition to the severity of infection, its impact on DNI and organ damage, IL-8 is also related to mortality. Our study established a strong correlation with lethality. We found that the higher the IL-8 levels were, the earlier severe complications and adverse outcome occurred ( $r=-0.422, p=0.045$ ). IL-8 had statistically significantly higher values in deceased septic patients than in survivors of sepsis ( $t$ -test:  $-1.703, p<0.054$ ). Higher and considerably more significant IL-8 mean values were observed in the septic group compared to that of non-septic patients ( $t$ -test:  $3.537, p<0.001$ ).

Yousef et al. published similar results after monitoring IL-8 levels in critically ill patients. In addition, they investigated genetic polymorphism. Their findings showed a statistical difference in the levels of IL-8 of deceased patients and sepsis survivors ( $t$ -test:  $-16.003, p<0.001$ ), with the deceased patients being with significantly higher IL-8 levels. Similarly, they established a significant difference in the mean IL-8 values of septic and non-septic patients ( $t$ -test:  $12.139, p<0.001$ ).<sup>14</sup>

In a study by Hyunjung et al., this indicator was used to monitor patients with severe pneumonia and acute upper respiratory tract infections. No significant difference between the groups in terms of total leukocyte count was found. However, the simultaneous monitoring of DNI, CRP and lymphocytes, which are significantly higher in severe pneumonia, has a high prognostic value. The fact that the total leukocytes and neutrophils counts were lower than that of DNI was also taken into account.<sup>15</sup>

Nahm et al. explored the severity of sepsis and found significantly higher DNI values in severe and fatal cases than in survivors. In patients with DNI over 40%, mortality was higher – 78.9%, and far exceeded the mortality rate in patients with DNI 5–10%. High DNI values were concurrent with the development of DIC syndrome, i.e. the patients met the criteria of the International Society of Thrombosis and Haemostasis. The incidence of DIC syndrome was significantly higher (52.6%) in patients with DNI over 40% than in patients with DNI 5–10%, for whom the incidence of DIC was 11.5%.<sup>6,13,16</sup>

Elevated DNI values in patients with sepsis and septic shock 12 hours before the onset of organ or circulatory dysfunction were reported in 82% of patients. This indicates that this marker could be useful in detection of a threatening septic condition as well. The index has the features of an independent predictive factor for 28-day mortality of patients with sepsis.<sup>6,8</sup>

DNI can also be useful in differentiating true bacteraemia from contaminated bacteraemia. In the “true” group – the one with positive blood cultures, DNI, CRP (C reactive protein) and PCT (procalcitonin) have significantly higher values than those in the contaminated group. Furthermore, in Gram-negative blood cultures, PCT value is higher and statistically significant; significantly higher DNI values correlate with increased mortality.<sup>17,18</sup>

It is noteworthy that an increased immature granulocyte count can be observed in both acute and chronic inflammatory processes, skin damages, acute haemorrhage, neoplasm and this can affect DNI values.<sup>19</sup> Therefore, an analysis of this indicator should be specified according to the patient’s condition.

## Limitations

Several limitations of the present study deserve consideration. First, the study had a relatively small sample size and was conducted at the Intensive Care Unit of one hospital. Secondly, patients were not selected randomly but purposefully and were further examined for changes in blood parameters during sepsis. Thirdly, the frequency and duration of the DNI follow-up were another limitation due to the differences in the DNI values at the beginning of the infection. Baseline values would more clearly shape the growth curve and would probably be useful at a very early stage of the infection – within hours or a day – sometimes enough time to unfold the picture of sepsis. Finally, not all patients with sepsis were included in the sample. Patients with some specific diseases were excluded from the study (i.e. patients with oncological diseases) because their DNI values were higher and were likely to mislead the average trend of the collected data.

## CONCLUSIONS

DNI analysis is an exceptionally fast and simple method that can be reliably used as a prognostic factor and would have major practical application. It could be valuable in assessing important decisions regarding the treatment of sepsis at a very early stage, even before significant biochemical and immunological abnormalities have occurred, well ahead of the cytokine storm.

Further studies could potentially enable a precision-method approach of matching changes in DNI with septic patient therapies during different cluster groups. It is quite reasonable to claim that this relatively cheap and fast indicator could become an essential diagnostic asset for the timely assessment of the septic condition.

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# Дельта-нейтрофильный индекс: в поисках раннего индикатора сепсиса

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**Дата получения:** 2 июня 2020 ♦ **Дата приемки:** 15 июня 2020 ♦ **Дата публикации:** 31 августа 2021

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**Образец цитирования:** Peneva P, Nikolova S, Bocheva Y. Delta neutrophil index: in search of an early indicator of sepsis. Folia Med (Plovdiv) 2021;63(4):496-501. doi: 10.3897/folmed.63.e55017.

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## Резюме

**Введение:** Сепсис является причиной значительной заболеваемости и смертности во всём мире. Однако в современной клинической практике отсутствуют надёжные клинические показатели для него и для его прогноза.

**Цель:** Настоящее исследование направлено на изучение эффективности дельта-нейтрофильного индекса (ДНИ), который отражает долю циркулирующих незрелых гранулоцитов в прогнозе воспаления и сепсиса.

**Материалы и методы:** Проспективное неинтервенционное одноцентровое последующее клиническое исследование было проведено в отделении интенсивной терапии Болгарии с 1 января 2017 г. по 31 мая 2018 г. Мы проанализировали взрослых пациентов: 45 пациентов соответствовали критериям сепсиса, определенным в SEPSIS-3, в то время как 37 пациентов были инфицированы, но не соответствовали критериям сепсиса. Логистическая регрессия и анализ кривой Roc были использованы для оценки тяжести и прогностической ценности ДНИ в качестве маркера-предиктора у пациентов с сепсисом в критическом состоянии.

**Результаты:** Результаты показали, что при значениях ДНИ 1.4 чувствительность составила 73%, а специфичность – 87% (AUC 0.764, 95% CI 0.650–0.878,  $p=0.0001$ ) при подозрении на сепсис. Кроме того, ДНИ был значительно связан с тяжестью состояния пациента, дисфункцией органа и маркером IL 8.

**Заключение:** ДНИ может служить полезным маркером для ранней диагностики сепсиса и может поддерживать процесс принятия решений относительно его лечения на ранней стадии заболевания.

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## Ключевые слова

ДНИ, инфекция, сепсис, септический шок

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