Neutrophil-to-lymphocyte Ratio as a New Predictor of Sepsis in Critically-ill Stroke Patients

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Abstract

BACKGROUND: Several studies have reported that many severe stroke patients developed sepsis during their acute phase, which leads to poor outcomes. In stroke, there is a shift from predominant Th1 lymphocytes, which have proinflammatory characteristics, to predominant Th2 lymphocytes which activate anti-inflammatory responses that induce hyporesponsiveness of the immune system against an invasion of pathogen, known as stroke-induced immunodepression syndrome.

AIM: This study aims to examine whether the neutrophils-to-lymphocytes ratio (NLR) could predict the development of sepsis in acute stroke patients.

METHODS: Patients were admitted to Fatmawati hospital intensive care unit from September 2019 to May 2020.

RESULTS: The mean NLR of acute stroke patients during their stay in ICU was 16.8 ± 12.5. We performed Mann–Whitney test, which revealed that the mean rank of several NLR parameters, such as initial NLR, day-3 NLR, highest NLR, and dNLR in stroke patients at ICU, was associated with the incidence of sepsis. The median difference in day-3 NLR, highest NLR, and dNLR in the stroke group with sepsis differed from those of the non-sepsis group.

CONCLUSION: NLR is assumed to have potential as an early predictor to distinguish septic conditions from non-septic conditions, to prevent delay in establishing diagnosis and management of sepsis, especially in acute, critically-ill stroke patients.

Introduction

Sepsis remains a health problem in the world, with increasing incidence. At present, available data show that the morbidity and mortality of sepsis remain high. The mortality rate of sepsis varies widely, reaching as high as 60%. While the epidemiological data of sepsis in Indonesia are not yet available, real-world experience indicated that the number is not far from the worldwide data.

Early detection is a key to preventing the development of sepsis and achieving comprehensive sepsis management. However, implementation can be challenging especially in high-risk populations with maladaptive immune responses, such as patients with diabetes mellitus, chronic kidney disease, chronic obstructive pulmonary disease, malignancy, autoimmune diseases, patients who receive immunodepressants, and patients with acute stroke. Many patients with severe stroke are found to have sepsis during hospitalization. Failure to detect the progress of infection to sepsis in acute stroke resulted in inadequate management of sepsis, which will, in turn, impact the outcomes.

After the onset of stroke, there will be systemic inflammatory responses due to acute sympathetic drive.

Excessive catecholamine will disrupt the gut mucosal barrier and disrupts gut microbiota balance. This condition will be followed by a decrease in the immune response known as stroke-induced immunodeficiency syndrome (SIDS).

Neutrophils-to-lymphocytes ratio (NLR) describes the balance of innate and adaptive immune responses in the inflammatory process. The increase in neutrophils in early phase of inflammation such as in sepsis, stroke, or myocardial infarction will be followed by a decrease in lymphocytes, which is sometimes accompanied by a decrease in human leukocyte antigen (HLA)-DR monocytes expression that causes the release of anti-inflammatory cytokines (Figure 1) [1], [2].

Biomolecular studies have shown that lymphocytes, especially T-cells, play a role in secondary neuroinflammation after cerebral ischemia and cause poor outcomes after neurological injury. In stroke, there is a shift from predominant Th1, which has pro-inflammatory characteristics, to predominant Th2 which activates anti-inflammatory responses such as the release of IL-10 and IL-4 [4]. Meanwhile, the results of immunological studies show that microbiotas are a key in T-lymphocyte cell homeostasis. They play a role in the maturation of the immune system and maintain a balance between host and microbes. Benakis in
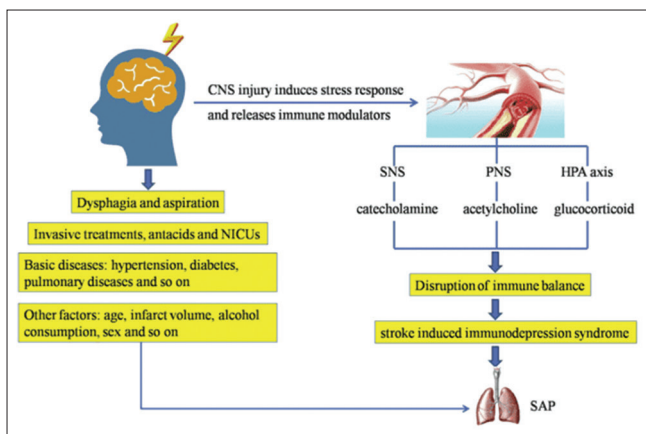
Figure 1: Immunodepression states observed after stroke are closely related to stroke associated pneumonia [3].

2016 explored the role of antibiotics in dysbiosis of an experimental animal model, in which antibiotics would provide a neuroprotective effect on stroke through increased Treg lymphocyte cells, decreased IL-17, and inhibited T-cells traffics from the lamina propria to the leptomeningens [5], [6].

Methods

Our study was a prospective and cohort analysis to examine the NLR parameters such as initial NLR, day-3 NLR, highest NLR, and delta NLR drawn from blood samples, in predicting sepsis and mortality in critically-ill acute stroke patients admitted to the intensive care unit (ICU) in Fatmawati Hospital from September 2019 to May 2020. During the study period, 42 critically-ill patients diagnosed with stroke were admitted, but only 37 people met the inclusion criteria and consented to participate as research subjects.

Results

About 65% of acute stroke patients treated in the ICU were male, aged 32–65 years, with an average of 53.7 ± 8 years. About 32% of respondents in this study had athletic habits. The most common type of stroke was hemorrhagic stroke (78%), followed by mixed type (14%) and ischemic stroke (8%). Almost all cases were first-onset. Most plausible indications for ICU treatment in this study (86%) were acute stroke patients after intervention to reduce intracranial pressure (ICP), either by ventriculoperitoneal shunt (VP shunt), craniotomy, or craniectomy. The mean APACHE II score in acute stroke patients admitted to the ICU was 19.57 ± 4.68, and the mean APACHE II score in stroke patients with sepsis was higher (21.29 ± 4.53) than

stroke patients who did not experience sepsis (17.31 ± 3.95) with p = 0.007; CI: −6.81−−1.14. A table was drawn below to compare the NLR between the sepsis and non-sepsis group (Table 1).

Table 1: Neutrophils-lymphocytes ratio comparison between sepsis and not sepsis

NLR Categories	Sepsis (n = 21)	Non sepsis (n = 16)
Initial NLR	19.45 ± 14.67	13.41 ± 11.19
NLR day 3	20.14 ± 13.31	13.58 ± 10.52
NLR max	28.60 ± 15.07	16.37 ± 12.09
dNLR	9.15 ± 11.39	2.97 ± 7.24

NLR: Neutrophils-to-lymphocytes ratio.

During their hospitalization, some of them developed severe infection and progressed to sepsis as indicated by infection markers and evidence of organ dysfunction measured using SOFA Score. Thirty-four out of 37 study subjects (91.9%) had infection, in which the most common cause was pneumonia (91.2%). The average incidence occurred on the 2nd day and progressed to sepsis on the 3rd day after the onset of stroke. Of 21 patients (56.7%) who had sepsis, 52.4% experienced sepsis in the 1st day of ICU admission. Acute stroke patients with sepsis also had longer length of stay compared to those without sepsis (6 ± 3 days vs. 4 ± 2.5 days, p = 0.04). In this study, we also observed several factors that affected gastrointestinal microbiota, such as diabetes mellitus and steroids. Both are often associated with dysbiosis and sepsis (Table 2).

Table 2: Characteristic distribution of acute stroke patients based on risk factors for sepsis

Subject characteristics	Sepsis (n = 21)	Non-sepsis (n = 16)	p	CI
Pneumonia	19 (90.5)	12 (75)	0.37	3.17 (0.5–20.03)
Diabetes Mellitus	12 (57.1)	4 (25)	0.05	4 (0.96–16.61)
Cardiovascular diseases	20 (95.2)	15 (93.7)	1.00	
Autoimmune diseases	1 (4.7)	0	1.00	
PPI	19 (90.5)	16 (100)	0.49	1.84 (1.36–2.49)
Statins	4 (19)	6 (37.5)	0.27	0.39 (0.09–1.74
Steroids	8 (38.1)	1 (6.2)	0.05	9.23 (1.01–83.94)
Angiotensin-receptor blockers	10 (47.6)	7 (43.8)	1.00	

The length of stay in ICU for acute stroke patients was 5.2 ± 2.86 days. Patients with sepsis were treated longer, up to 6 ± 3 days. Thirteen stroke patients died in ICU, all of whom experienced sepsis. In acute-stroke survivors who managed to move to a lower level of care, only 33% developed sepsis. Sepsis in acute stroke while being treated in the ICU appears to increase the risk of death by 2.6 times (CI: 1.52–4.53).

NLR is calculated by dividing absolute neutrophil count or neutrophil percentage by the absolute lymphocyte count or lymphocyte percentage. In this study, critically-ill acute stroke patients underwent NLR calculation twice or more, especially during feces specimen collection due to its relationship with brain-gut axis and dysbiosis. Then, those were monitored for the progression of infection to sepsis or improvement. The mean NLR of acute stroke patients during their stay in ICU was 16.8 ± 12.5. We performed Mann–Whitney test, which revealed that the mean rank of several NLR parameters, such as initial NLR, day-3 NLR, highest NLR, and dNLR in stroke patients at ICU,

was associated with the incidence of sepsis (Figure 2). The median difference in day-3 NLR, highest NLR, and dNLR in the stroke group with sepsis differed from those of the non-sepsis group. The p value, Hodges-Lehman, and the median range were p = 0.04; −5.59 CI (−15.23−−0.5); p = 0.005; −10 CI (−24.96−−2.980); p = 0.05; and −3.97 CI (−10.23−−0.0), respectively.
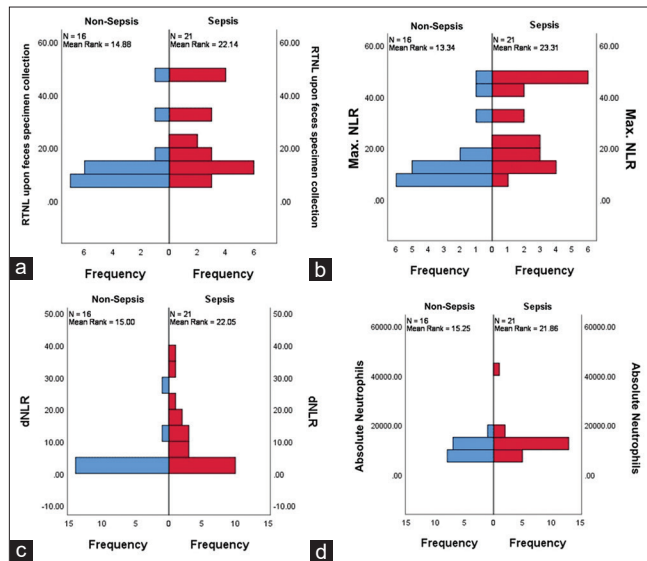
Figure 2: (a-d) Several neutrophils-to-lymphocytes ratio parameters and sepsis in stroke patients

The dNLR parameter had a significant mean rank for subjects in sepsis group who died in the ICU with p = 0.049; −5.17 CI (−11–0), as shown in Figure 3. The initial NLR, day-3 NLR, and the highest NLR did not differ significantly between the two groups.
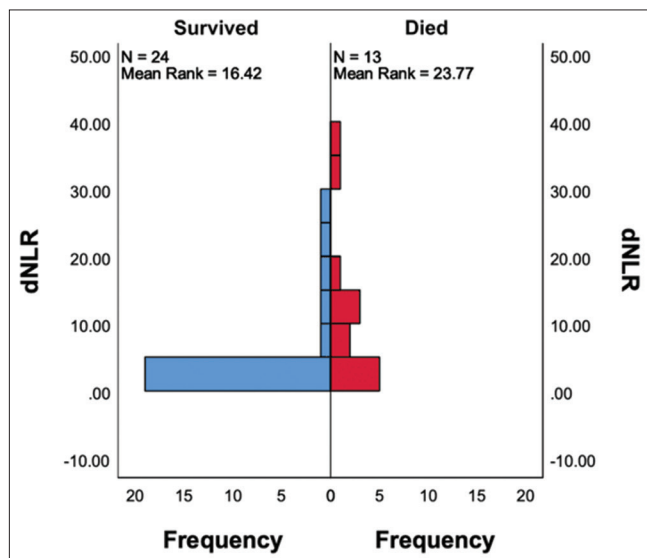
Figure 3: dNLR differences in stroke patients

In daily practice, a clinician will need NLR to be able to distinguish septic conditions from non-septic conditions to prevent delay in diagnosis and management of sepsis. A diagnostic test was performed on NLR data using receiver operating characteristic (ROC) analysis to determine the area-under-the-curve (AUC) and the optimal cutoff point sensitive and specific enough to

differentiate between septic and non-septic groups. The AUC was 0.610 (p = 0.07) and the optimal cutoff point was set at the NLR value of 11.9 with a sensitivity and specificity of 60% and 57%, respectively (Figure 4).

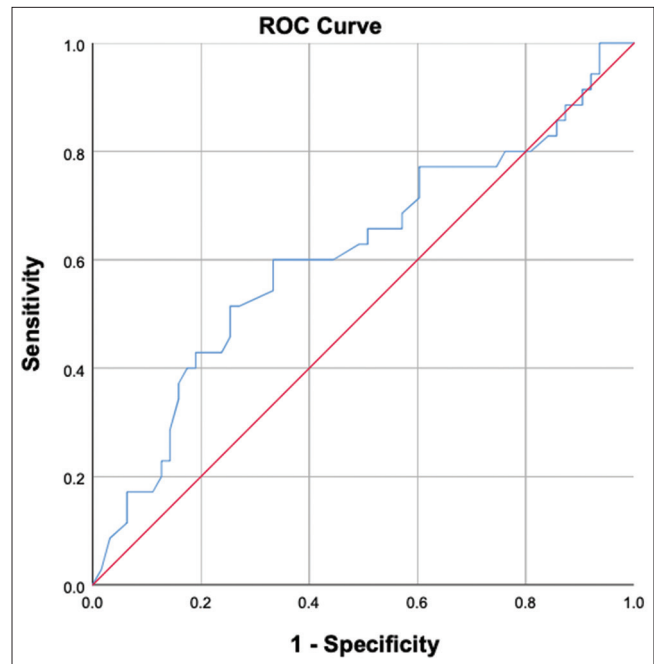*Figure 4: Neutrophils-to-lymphocytes ratio receiver operating characteristic curve*

Discussion

An increase in ICP due to bleeding or ischemia will result in brain-gut axis activation which induces inflammatory process. This condition will be followed by a compensatory anti-inflammatory response known as SIDS due to shifting of the adaptive immune response from predominantly Th1 to predominantly Th2.

Many evidence suggested that brain injury results in a severe inflammatory response due to the breakdown of the blood-brain barrier. Necrotic brain tissue, glial cells, and ischemic neuron cells will stimulate WBC migration, especially neutrophils. Weisenburger *et al.* observed hyperactivation of neutrophils in the plasma and expansion of the subset of neutrophils involved in phagocytosis, release of granular proteins (antimicrobials), free radical production, and NET-osis (neutrophil extracellular traps) within 6 h after stroke [7]. An increase in absolute neutrophil count as in NLR in stroke could serve as both a predictor of stroke severity and a prognostic determinant of stroke outcome.

Neutrophil-lymphocyte ratio (NLR) is a simple inexpensive biomarker of inflammation that often used by clinicians in daily practice. It is believed to describe the balance of innate immunity and adaptive immunity responses in infectious and inflammatory processes. The increase in neutrophils in the early

phase of inflammation will be followed by a decrease in the number of lymphocytes, where sometimes accompanied by decrease in the expression of HLA-DR monocytes that cause release of anti-inflammatory cytokines. This condition is found in sepsis as well as acute ischemic stroke or acute myocardial infarction. After subsequent events, it turned out that there is a decrease number of specific bacteria contributing to changes in gastrointestinal tract biodiversity that maintain physiological homeostasis of the digestive and immune system known as dysbiosis [8], [9]. There was an increase in T lymphocytes which produced IL-17, thereby increasing injury to ischemic brain tissue. However, the activation of regulatory T-cells (Treg) by IL-10 will suppress the production of IL-17 which aims to protect the brain from ischemia-reperfusion injury [10].

It is now known that the immune response and systemic inflammatory reactions are related to levels of neutrophils and lymphocytes. While neutrophil and lymphocyte activity is related to gut microbiota activity, NLR is potential to be used as a marker of dysbiosis in hospitalized patients [1], [2].

This study found a significant difference in the mean NLR between the stroke group with sepsis and non-sepsis group, especially in the maximum NLR parameter. This increase in absolute neutrophils is accompanied by a low absolute lymphocyte count due to SIDS. To distinguish between the stroke group with sepsis and the non-sepsis group, ROC analysis was performed, revealing an NLR cutoff point of 11.9 with sensitivity and specificity of 60% and 57%, respectively. Dividing the NLR based on the 11.9 cutoff points into two categories – namely, the <11.9 and >11.9 groups – could differentiate between the stroke group with sepsis and the non-sepsis group. These findings support the use of NLR as a marker of sepsis in critically-ill acute stroke patients. This finding is consistent with that of Gurol *et al.*, who determined NLR cutoff points based on procalcitonin value in 1468 sepsis cases, subsequently dividing NLR into the following categories: NLR <5 with procalcitonin (PCT) <0.05 indicating healthy individuals; NLR 5.68 ± 8.99 with PCT 0.05–0.5 indicating local infection; NLR 11.78 ± 11.04 to 13.16 ± 4.38 with PCT 0.5–10 indicating sepsis; and NLR 16.87 ± 9.55 with PCT >10 indicating septic shock. The dNLR data in this study showed significance (p = 0.05), supporting the potential use of dNLR as a simple parameter to periodically monitor the development of infection in critically-ill stroke patients treated in the ICU. However, further research with larger sample remains necessary.

Conclusion

NLR could be utilized as a clinical marker of decreased systemic immune response in stroke patients with lymphopenia. This study found a cutoff point of >11.9 for NLR as a marker of sepsis in acute stroke patients treated in the ICU. Delta NLR (dNLR), which reflects the difference between initial NLR and highest NLR, could be utilized as a predictor of poor outcomes and thus might be useful for guiding the management of sepsis.

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