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The Initial Neutrophil-to-Lymphocyte Ratio can Serve as a Biomarker for Outcome Prediction in Traumatic Brain Injury: A Systematic Review and Meta-Analysis

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ABSTRACT

Introduction: Inflammation plays an important role in traumatic brain injury (TBI) pathophysiology. Uncontrolled inflammation leads to secondary brain injury in TBI patients. The neutrophil-to-lymphocyte ratio (NLR) is a reliable and simple indicator of inflammation. The NLR is associated with poor outcomes in several central nervous system diseases. However, the role of NLR in TBI is still unclear. This study aimed to determine the possibility of NLR as a biomarker to predict outcomes in TBI through meta-analysis.

Methods: This was a systematic review and meta-analysis study. The systematic searching was performed using keywords (neutrophil to lymphocyte ratio) AND (traumatic brain injury) in the PubMed database. The outcome was measured using the Glasgow Outcome Scale (GOS) or GOS Extended (GOSE) and dichotomized into favorable (GOS 4 – 5, GOSE > 4) and unfavorable (GOS 1 – 3, GOSE < 5) outcomes. The mean difference (MD) of NLR level was compared between favorable and unfavorable outcomes using software Review Manager 5.3.

Results: Five articles met our inclusion and exclusion criteria. The random effect model was used because of the high heterogeneity between studies (I^2 = 99%, p < 0.00001). The MD of NLR between favorable and unfavorable outcomes was – 5.41 (95% CI -9.84 – 0.97, p = 0.02). The MD showed that favorable outcome tends to exhibit lower NLR levels compared to unfavorable outcomes. However, the result should be cautiously interpreted because of the high heterogeneity between studies.

Conclusion: The NLR could be used as a biomarker to predict the outcome of traumatic brain injury. Higher NLR levels in TBI patients predict unfavorable outcomes.

Keywords: biomarker, inflammation, Glasgow outcome scale, neutrophil-to-lymphocyte ratio, traumatic brain injury

Introduction

Traumatic brain injury (TBI) is a leading cause of mortality and morbidity in young adults with considerable cost impact on social and economic aspects.[1] Traumatic brain injury is mainly divided into primary and secondary TBI. Primary TBI is a direct injury due to mechanical forces applied to the head, while secondary TBI is an injury due to further consequences following primary TBI, such as metabolic cascade, and biochemical and inflammatory changes.[2]

Inflammation plays an important role in TBI pathophysiology. Inflammation reaction is beneficial in clearing pathological debris and initiating repair mechanisms.[3,4] However, uncontrolled inflammation can cause neuronal damage and lead to secondary brain injury in TBI. The initial response to injury is the responsibility of the innate immune system. Further, pro-inflammatory chemokine expression leads to the activation of the adaptive immune system. This process induces the adhesion of molecules on the blood-brain barrier (BBB) and co-stimulatory molecules expression on microglia. This local cellular reaction can be seen in pathological specimens, which show the migration of neutrophils into the injured area.[5]

The neutrophil-to-lymphocyte ratio (NLR) is a reliable and simple indicator of inflammation. The NLR is associated with poor outcomes in several central nervous system (CNS) diseases. However, the role of NLR in TBI is still unclear. This study aimed to determine the possibility of NLR as a biomarker for outcome prediction in TBI through meta-analysis.

Methods

This was a systematic review and meta-analysis comparing the mean difference (MD) of NLR between the favorable and unfavorable outcomes in TBI. We included all studies comparing NLR in TBI patients that were published in English. Other languages were translated using Google translate and decided by authors either included or not. The outcome of interest in this study was the MD of NLR between the favorable and unfavorable outcomes in TBI patients.

We performed systematic searching using keywords (neutrophil to lymphocyte ratio) AND (traumatic brain injury) in the PubMed database to find eligible studies. The study selection process was performed by two authors to decrease the chance of excluding relevant articles. In the occurrence of disagreement, another author's decision was applied. We removed duplicate articles and screening of titles and abstracts was performed initially. Passed articles were further evaluated for compliance according to inclusion and exclusion criteria.

The data collection process was performed using Joanna Briggs Institute (JBI) data extraction form by each author. Software Review Manager 5.3 was used to merge and manage the data. The collected data included the author's name, year of publication, study design, sample size, inclusion and exclusion criteria of the study, and mean and standard deviation (SD) of NLR in favorable and unfavorable outcomes. Articles that passed according to the inclusion and exclusion criteria of this review were assessed for their quality to ensure validity and reliability. Quality assessment was performed independently and matched with the study design using the JBI critical appraisal tool.

Favorable outcome was defined as Glasgow Outcome Scale (GOS) 4-5 and GOS Extended (GOSE) > 4. Meanwhile, unfavorable outcome was defined as GOS 1-3 and GOSE < 5. The outcome of interest in this study was the MD of NLR between favorable and unfavorable outcome. The mean and SD of NLR were pooled and analyzed. The outcome in the median was converted into mean ± SD using Luo and Wan methods. The mean difference was calculated using the software Review Manager 5.3. The random-effect model was used regardless of the heterogeneity between studies.

Results

As can be seen in **Fig. 1**, we performed systematic searching in the PubMed database using keywords and resulted in sixty articles. We did not find any additional articles from other sources. Twenty-three articles were screened and five studies were included in qualitative and quantitative synthesis. The flow of study selection can be seen in the PRISMA diagram **(Fig. 2)**.

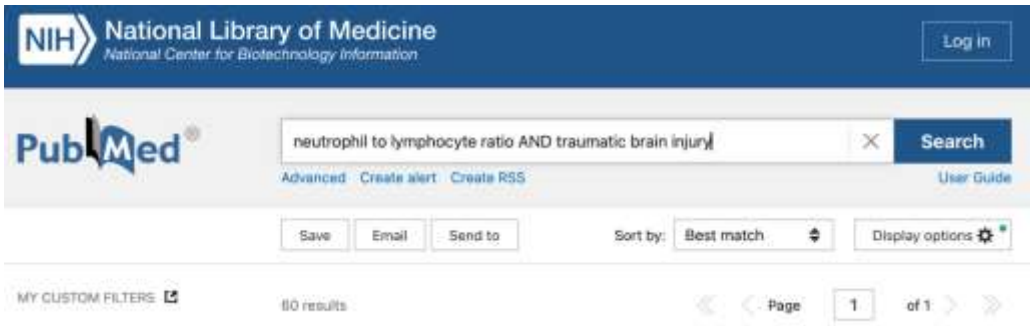

Figure 1. Record identified through systematic searching in PubMed database.

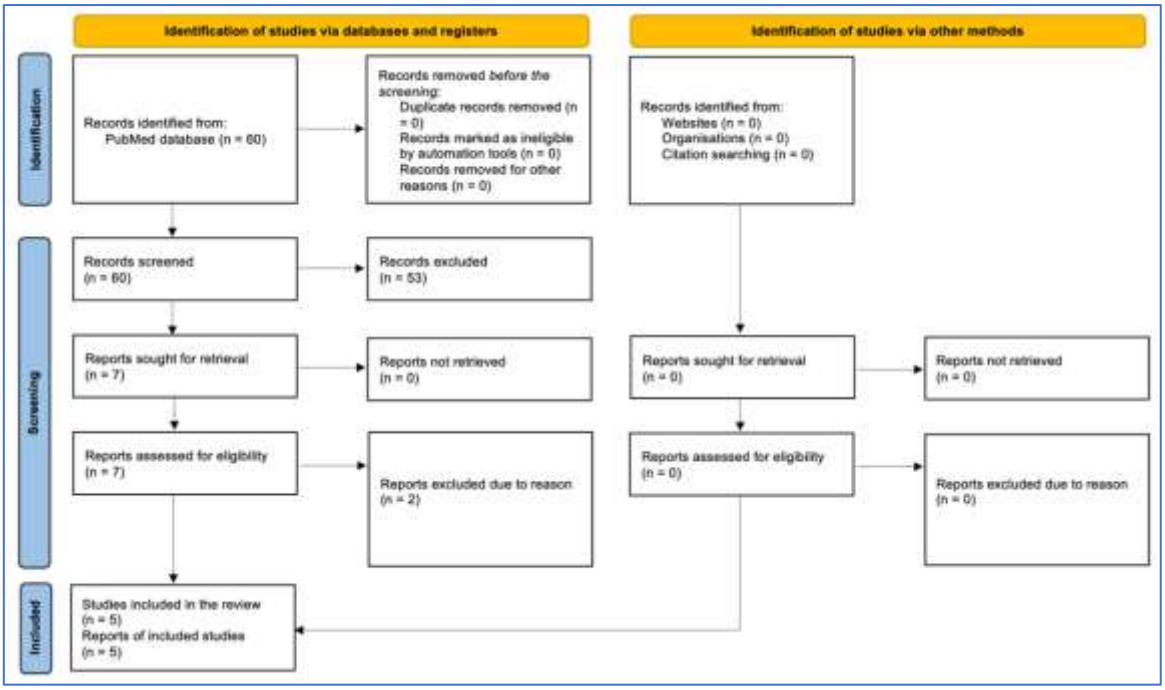

Figure 2. PRISMA diagram

Table 1. Summary of findings of included studies

Study author	Type of study	Subject condition	Total samples	Outcome
Bilgi *et al*.[6] 2021	Observational study, prospective cohort	Patients with moderate and severe isolated head injury (GCS 12 or less) admitted to the emergency department aged 18 – 60 years old.	Total = 96 cases; FO = 39 cases; UO = 57 cases	FO = 15.4 ± 10.4; UO = 15.5 ± 9.4
Chen W *et al*.[7] 2018	Observational study, retrospective cohort	Inclusion criteria: • Isolated head trauma • Posttrauma GCS score 8 or less • Time from injury to admission 6 hours or less.	Total = 688 cases; FO = 180 cases; UO = 508 cases	FO = 11.60 ± 4.05; UO = 15.07 ± 6.63
Chen J *et al*.[8] 2019	Observational study, retrospective cohort	Inclusion criteria: • Isolated head trauma • Admission GCS less than 9 • Age more than 16 years old • Time interval from injury to admission less than 24 hour	Total = 316 cases; FO = 59 cases; UO = 257 cases	FO = 11.55 (8.62 – 14.11); UO = 17.62 (13.08 – 20.89)
Corbett *et al*.[9] 2019	Observational study, retrospective cohort	Patients who underwent a DC after severe TBI requiring DC.	Total = 388 cases; FO = 237 cases; UO = 151 cases	FO = 6 (2 – 12); UO = 6 (3 – 11)
Zhao *et al*.[10] 2018	Observational study, retrospective cohort	Inclusion criteria: • Patients with TBI, confirmed with presence of TBI signs on head CT scan, such as EDH,	Total = 1291 cases; FO = 950 cases; UO = 341 cases	FO = 7.68 ± 6.54; UO = 24.71 ± 12.52

	SDH, IPH), contusion, and tSAH.
•	Patients were more than 14 years old.
•	Patients were admitted within 6 hours after injury.

CT: Computed tomography; **DC:** Decompressive craniectomy; **EDH:** Epidural hematoma; **FO:** Favourable outcome; **GCS:** Glasgow Coma Scale; **IPH:** Intraparenchymal hemorrhage; **SDH:** Subdural hematoma; **TBI:** Traumatic brain injury; **tSAH:** Traumatic subarachnoid hemorrhage; **UO:** Unfavourable outcome

The summary of the findings of the included studies can be seen in **Table 1**. Total pooled samples were 1465 and 1314 TBI cases in favorable and unfavorable outcome groups, respectively. Random effect model analysis with high heterogeneity (I^2 = 99%, p < 0.00001) showed MD of NLR between favorable and unfavorable outcomes in TBI was –5.41 (p = 0.02; 95%CI: -9.84–0.97). The pooled analysis, forest plot, and funnel plot can be seen in **Fig. 3**, **Fig. 4**, and **Fig 5**.

	Favourable			Unfavourable				Mean Difference
Study or Subgroup	**Mean**	**SD**	**Total**	**Mean**	**SD**	**Total**	**Weight**	**IV, Random, 95% CI**
Bilgi 2021	15.4	10.4	39	15.5	9.4	57	17.7%	-0.10 [-4.18, 3.98]
Chen J 2019	11.45	1.59	59	17.3	2.24	257	20.7%	-5.85 [-6.34, -5.36]
Chen W 2018	11.6	4.05	180	15.07	6.63	508	20.6%	-3.47 [-4.30, -2.64]
Corbett 2019	6.5	2.8	237	6.5	2.3	151	20.7%	0.00 [-0.51, 0.51]
Zhao 2018	7.68	6.54	950	24.71	12.52	341	20.3%	-17.03 [-18.42, -15.64]
Total (95% CI)			**1465**			**1314**	**100.0%**	**-5.41 [-9.84, -0.97]**
Heterogeneity: Tau^2 = 24.67; Chi^2 = 629.95, df = 4 (P < 0.00001); I^2 = 99%								
Test for overall effect: Z = 2.39 (P = 0.02)								

Figure 3. Pooled meta-analysis of mean difference of neutrophil-to-lymphocyte ratio between favorable and unfavorable outcomes in traumatic brain injury.

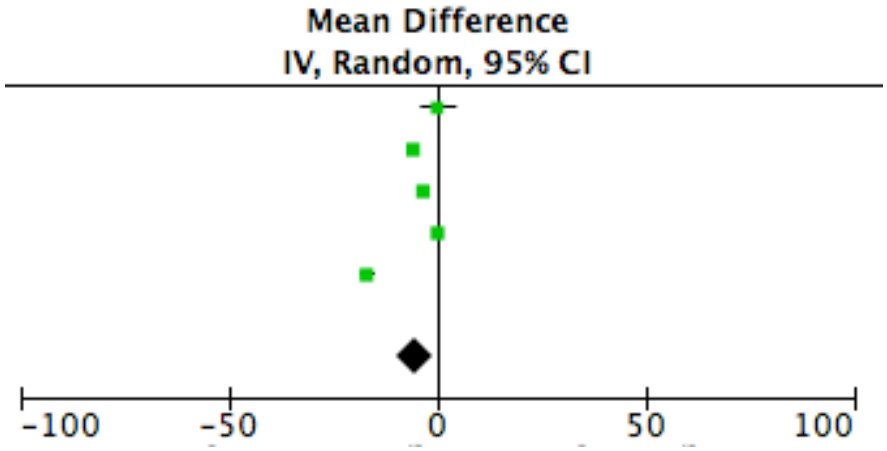

Figure 4. Forest plot analysis of mean difference of neutrophil-to-lymphocyte ratio between favorable and unfavorable outcome in traumatic brain injury.

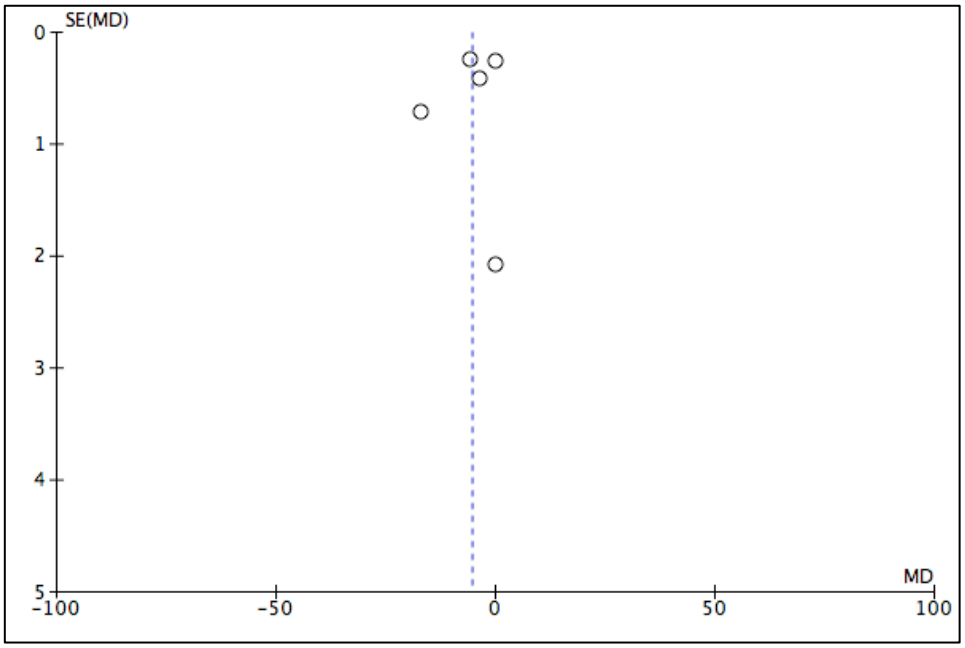

Figure 5. Funnel plot of mean difference of neutrophil-to-lymphocyte ratio between favorable and unfavorable outcome in traumatic brain injury.

Discussion

Neuroinflammation is the reaction of CNS and peripheral immune cells in responding to stimulus or injury, which aims to protect the CNS structure from the injury itself. However, it is also an essential initiator in secondary TBI pathophysiology.[11] Characteristics of neuroinflammation following TBI include increased expression of pro-inflammatory cytokines in the brain; glial cell activation (astrocytes and microglia); and recruitment and infiltration of peripheral white blood cells (WBC).[12] Inflammation response due to isolated TBI is not limited only to the CNS immune system but also triggers complex responses of the systemic immune system.[13]

Neutrophils and astrocytes have an important role in the neuroinflammation process as the main source of pro-inflammatory cytokines which exacerbate the inflammation cascade. Astrocytes are glial cells that play an important role in maintaining physiologic homeostasis in the CNS environment. Astrocytes are also essential as part of the structure in maintaining the integrity of BBB.[11] Astrocytes have been known as the source of important cytokines and chemokines during neuroinflammation.[14] Neutrophils are the main component of the innate immune system which have an important role in acute inflammation and can produce extensive tissue damage.[11]

Activation of microglia induces activation of endothelia followed by recruitment and infiltration of peripheral WBC into brain parenchyma.[15] Active microglia express pro-inflammatory mediators including IL-1β, IL-6, TNF-α, CXCL1-5, and CXCL8-10 which can recruit active neutrophils.[16] Expression of CXCL1, CXCL2, and GM-CSF can cause BBB disruption; initiate the process of neuroinflammation; and increase recruitment and infiltration of peripheral WBC.[17-19] Uptake of glutamate by astrocyte can be inhibited by cytokines IL-1β and TNF-α which lead to worsening of neuroinflammation.[20]

Neutrophils are the first peripheral WBC to respond to inflammation following TBI.[13] Activation and infiltration of neutrophils are important in eliminating pathogens through phagocytosis and degranulation. However, excessive reaction of this innate immune system may result in severe tissue damage and lead to secondary TBI. Active neutrophils can release chemokines and molecules in the process of secondary TBI.[11] Even in the absence of a danger signal, they are still difficult to stop due to their ability to strengthen activation via autocrine influence.[21-23] These abilities and processes could damage the brain tissue and cause severe secondary TBI. Neutrophils can also release reactive oxygen species (ROS), matrix metalloproteinase (MMP) 9, and lipocalin 2, which activate and amplify the effect of microglia.[24-26]

The activity of neutrophils can be modulated by external and internal factors. Trauma is classified as an external factor, while internal factors include granulocyte-macrophage-colony stimulating factor (GM-CSF) and granulocyte-CSF (G-CSF).[27-29] These factors activate neutrophils and are followed by differentiation and maturation processes. Increased expression of neutrophil membrane protein results in increased sensibility to danger signals. Increased sensibility of danger signals eventually leads to increased movement of neutrophils to inflamed tissue.[11] Expression of MMP, neutrophil elastase, myeloperoxidase, and neutrophils gelatinase-associated lipocalin are also increased due to activation of neutrophils. These granule proteins play an important role in the inflammation process.[30,31]

In one hour following TBI, neutrophils are attracted to the injured site in the brain and start to express pro-inflammatory mediators which leads to neuronal death.[32,33] Infiltration of neutrophils in meninges and perivascular space occurs in the early phase of injury.[33] Furthermore, in 48 hours following TBI, peripheral neutrophils increase and infiltrate the brain through the choroid plexus and meningeal blood vessels.[13,33,34] High amount of peripheral

neutrophils, eventually leads to damage of BBB and brain tissue, neuronal death, induces oxidative enzyme expression, and exacerbates inflammation reaction which causes further damage to the brain.[32,34,35]

The presence of BBB prevents neutrophils from infiltrating brain parenchyma via tight junction between endothelial.[36,37] However, in several pathological conditions such as trauma, disruption of BBB occurs and increasing infiltration of neutrophils to brain parenchyma.[38] Infiltration of neutrophils plays a major role in the pathophysiology of neuroinflammation following TBI. In TBI, damaged brain structures such as meninges, glial limitans, and parenchyma increase expression of pro-inflammatory cytokine (TNF-α, IL-1β, IL-6, and IL1) which leads to infiltration of neutrophils.[39] Infiltration of neutrophils through BBB is facilitated by the expression of adhesion molecules and the presence of pial microvessels surrounding the brain injured site.[40] Recruitment of neutrophils is induced by P_2X_7 and formyl-peptide receptor-dependent signaling in 1-3 hours after the injury.[33]

Infiltration of neutrophils occurred due to the direct effect of pro-inflammatory cytokine and BBB disruption. Pro-inflammatory cytokines including TNF-α, IL-1β, CXCL1, CXCL2, and CXCL5 are released due to danger signals from injured cells. These pro-inflammatory cytokines activate endothelial and neutrophils which results in increased infiltration of neutrophils into the brain.[41,42] In 12 hours, active neutrophils accumulate in the superficial area of the injured region and damage the integrity of BBB. In 24 hours, these neutrophils further infiltrate to injured brain parenchyma.[43] Blood-brain barrier disruption following TBI occurs due to the degradation of aggregated proteins such as vinculin, zonula occludens-1, β-catenin, and occludin; release of MMP; and ROS and nitric oxide (NO) release. The first two mechanisms cause hyperpermeability of BBB and increase infiltration of neutrophils.[11,44-47] The MMPs interfere with the cadherin-cadherin bond inducing hyperpermeability of BBB. Neutrophils can cause excessive production of ROS through the autocrine IL-17 pathway.[48] Released ROS and NO cause direct damage in the arrangement of claudin-5 and occludin in endothelium which leads to impairment of the integrity of BBB.[49,50] Blood-brain barrier disruption facilitates infiltration of immune cells into the brain to clear debris. However, immune cell infiltration can cause brain damage and secondary TBI.

Cerebral edema is a common problem in TBI patients. Excessive intracellular and extracellular fluid accumulation leads to an increased volume of brain tissue in rigid skull cavities. It causes high intracranial pressure and leads to insufficient brain perfusion and oxygenation. Decreased perfusion and oxygenation, eventually cause severe ischemic injury and lead to more severe cerebral edema.[51] Neutrophils can cause cerebral edema through two mechanisms, which are BBB disruption[52] and the direct effect of neutrophil granules.[53,54] As had been explained, BBB disruption causes hyperpermeability which allows infiltration of protein and intravascular fluid into interstitial space in the brain parenchyma.[52] Secondly, active neutrophils release granules such as lipoxin, elastase, and azurocidin which cause vascular hyperpermeability and lead to cerebral edema.[53,54] Blood-brain barrier injury is also exacerbated further by the presence of ROS and MMP-9, which change the expression of iNOS and NADPH oxidase.[55]

The balance between cerebral oxygen delivery and consumption can be interfered with by active neutrophils. In secondary TBI, oxygen consumption by neutrophils is higher due to the production and expression of hydrogen peroxide superoxide, NADPH oxygenase-related molecules, and antibacterial proteins such as defensins and cathelicidin to maintain the ability of phagocytic.[43,49,56,57] Decrease cerebral blood flow and oxygen saturation following TBI cause hypoxic conditions in the injured part of the brain.[11] Low oxygen level induced expression of NFκB and hypoxia-inducible factor-1α, which lead to prolonged neutrophil survival.[58] Furthermore, the duration and severity of cerebral hypoxia are associated with the clinical outcome of TBI.[59] Therefore, the presence of active neutrophils in secondary TBI causes more severe and longer cerebral hypoxia and eventually leads to worse clinical outcomes.

Lymphocytes also have an important role in the inflammation process. Lymphocytes can inhibit neutrophils after the digestion process.[11] T cell lymphocytes have been shown important in repairing inflamed tissue.[60] It is related to the modulation of microglial due to the expression of cytokines and growth factors.[60,61] Lymphocytes are known also as a cellular component of the cell-mediated immune system. Therefore, decreased lymphocytes are a sign of more severe brain damage and lead to poor outcomes.[62]

Cortisol hormone is released as a response to stress conditions such as trauma. High cortisol level leads to lymphopenia.[63] Higher lymphocyte count is a reflection of a stable immune or inflammatory response.[64] Therefore, the lymphocyte is a reflection of controlled inflammation and is less destructive to the surrounding cells.[65] Lower lymphocyte count following TBI is a sign of increasing damage due to inflammatory response.

By definition, NLR is a neutrophil and lymphocyte count ratio in peripheral blood.[66] The NLR is an objective, widely used, easily accessible, low-cost, and reproducible inflammation indicator. It is known as an indicator of cerebral tissue injury which caused by neutrophils and their by-products.[67] It reflects the inflammation response by neutrophils and the immune status of the host (lymphocytes). Increased NLR is a sign of high inflammatory cell recruitment and increased expression of pro-inflammatory cytokines.[68,69] Therefore, higher neutrophils and lower lymphocytes are signs of poor outcomes.[10,70]

Increased NLR on admission in severe TBI patients was reported associated with poor functional outcomes and mortality within one year after injury. The NLR was reported to provide a similar value to the GCS score in predicting poor outcomes in one year after injury in severe TBI patients. However, in predicting 1-year mortality, NLR was worse than the GCS score.[7] On the other hand, another study also reported that peak NLR was higher in severe TBI patients with poor outcomes. The NLR is usually achieving peak value on day 2 to day 4 and possibly associated with neutrophils and lymphocytes featured changes following trauma.[8]

Conclusion

The NLR could be used as a biomarker to predict the outcome of traumatic brain injury. Higher NLR levels in TBI patients predict unfavorable outcomes.

Conflict of interest

The authors declare no conflict of interest regarding the content of this manuscript.

Author contribution

Authors contribute equally to the preparation of this manuscript.

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