Section A -Research paper

Neutrophils to Lymphocyte and Platelet to Lymphocyte Ratios as Diagnostic and Prognostic Marker in Sepsis at Pediatric Intensive Care Unit: Review Article

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Abstract:

Sepsis is a systemic syndrome induced by infection and leading to a widespread inflammation up to septic shock, multi organ failure, and death. Neutrophils are manly involved in the innate immune response and lymphocytes in the adaptive immune response. In sepsis, blood neutrophil count increases and the blood lymphocyte count decreases; thus, the neutrophil-to-lymphocyte ratio (NLR) has been suggested as an indicator of systemic inflammation. The NLR is advantageous in regard to simplicity, low cost, and availability compared to many other proposed biomarkers, which makes it promising for diagnosis of Severe sepsis. Platelet-to-lymphocyte ratio (PLR) is reported to be related to the outcome of intensive care unit (ICU) patients.

Keywords: Sepsis**,** NLR, PLR.

Introduction:

The neutrophil-to-lymphocyte ratio (NLR), is the ratio between the neutrophil and lymphocyte counts measured in peripheral blood, and used as a biomarker which includes two faces of the immune system: the innate immune response, mainly due to neutrophils, and adaptive immunity, supported by lymphocytes. Neutrophils are responsible for the first line of host immune response against invading pathogens, through different mechanisms, including chemotaxis, phagocytosis, release of reactive oxygen species (ROS), granular proteins and the production and liberation of cytokines. Neutrophils also play an important regulatory role in adaptive immunity and are the main effector cells during the systemic inflammatory response (SIRS). As regulators of innate immunity, neutrophils recruit, activate and programme other immune cells, secreting an array of pro-inflammatory and immunomodulatory cytokines and chemokines capable of enhancing the recruitment and effector functions of other immune cells, such as dendritic cells (DCs), B cells, NK cells, CD4, CD8 and γδ T cells, as well as mesenchymal stem cells **(1)**.

The elevated NLR and raise of isolated neutrophil count can be found in several conditions: bacterial or fungal infection, acute stroke, myocardial infarction, atherosclerosis, severe trauma, cancer, post-surgery complications and any condition characterized by tissue damage that activates SIRS. This is due to the early hyperdynamic phase of infection is characterized by a proinflammatory state, mediated by neutrophils and other inflammatory cells. SIRS is associated with the suppression of neutrophil apoptosis, which augments neutrophil-mediated killing as part of the innate response. Thus, NLR is often characterized by an increase in neutrophils and a decline in lymphocytes**(2)**.

NLR and the Pathophysiology of Inflammatory Disease Sespis

NLR considered a reliable marker for the diagnosis of bacteremia and sepsis. A recent study showed that a higher NLR was associated with poor prognosis in patients with sepsis (mean HR 1.75) and that NLR was higher in non-survivors than in survivors from sepsis (mean HR 1.18). Moreover, in a single-center prospective observational study of septic patients admitted to an intensive care unit (ICU), NLR values (9.53 ± 2.31) correlated with sepsis severity as calculated with the SOFA score (R = 0.65) and also with presepsin (R = 0.56), with a sensitivity of 47%, a specificity of 78% and an AUC of 0.631 ($p < 0.05$). The same study also showed that NLR was significantly higher in patients with septic shock (10.31 ± 2.32), suggesting the potential value of NLR in assessing sepsis severity, especially when its value is above 10 **(3)**.

Another study concluded that NLR and IL-6 were independent predictors of 28-day mortality in septic patients. Moreover, **Jang et al. (4)** found that age, NLR and delta neutrophil index (DNI) were the most strong predictors for sepsis status in all subjects, and in cluster-specific groups. These findings could contribute to suggest a strategy to screen sepsis patients without leukocytosis in the emergency care units, because NLR increase precedes WBC and CRP alterations, being the first sign of the activation of the immune system during sepsis. However, there are insufficient data about the role of NLR to identify the source of sepsis **(4)**.

Pneumonia

The community-acquired pneumonia (CAP) which characterized by high rates of mortality and morbidity is the most common causes of sepsis needing hospitalization especially in elderly patients. NLR has demonstrated a strong predictive value concerning short- and long-term mortality, need for ICU admission and re-hospitalization. Therefore, NLR correlated with post-CAP mortality better than traditional pneumonia scores (Pneumonia Severity Index, PSI; and Confusion, Urea, Respiratory rate and Blood pressure, aged 65 and older, CURB-65), WBC and CRP. Patients with NLR greater than 28.3 had the worst prognosis, so it could be hypothesized that they would require admission to a

respiratory ICU, while those with NLR less than 11.12 could be safely discharged and managed through an outpatient follow-up. Furthermore, 3-month likelihood of rehospitalization increases in parallel with NLR increase, with a rate >75% when NLR is >20 **(5)**.

Another study showed that NLR value and its incremental change can predict clinical instability and all-cause 30-day mortality, while NLR values on admission did not differ significantly between survivors and non-survivors, while those at 3–5 days differed and predicted more accurately 30-day mortality. This supposed that admission NLR values could not used as a mortality prediction tool in hospitalized patients for CAP or ventilator-acquired pneumonia (VAP), due to the better performance of serial measurements. Recently, in a post hoc analysis of a randomized clinical trial (RCT) investigating adjunctive dexamethasone treatment in CAP, patients with NLR ≥ 15.5 experienced benefit from dexamethasone treatment in terms of hospital stay shortening by 2 days **(6)**.

Regarding nosocomial pneumonia, which represent a problem, especially in ICU, in a retrospective observational study conducted with patients with hospital-acquired pneumonia (HAP), low NLR values were significantly correlated with multidrug-resistant (MDR) *Pseudomonas aeruginosa* (PA) (MDR-PA HAP) plus the worst prognosis. The authors concluded that one possible reason for this phenomenon was that the lower level of NLR may have been the consequence of a less virulent MDR-PA strain. Hence, it could be speculated that a decline in NLR (associated with other conventional tools, such as hemocultures) may indicate the involvement of a MDR strain. Further studies are needed, however, to confirm the prognostic role of NLR in patients with HAP and VAP **(7)**.

COVID-19 Pneumonia

COVID-19 pneumonia is a main cause of severe respiratory failure throughout the ongoing pandemic. recently, it was proved that the onset of SARS-CoV-2 infection is characterized by lymphocyte reduction and that the rate of this reduction inversely correlates with disease severity. In particular, T and NK cells, which are necessary for control of viral infection, were markedly decreased, while B cells were at the lower level of their normal range. Not only the number but also the function of NK and CD8+ T cells are compromised in COVID-19 patients. One of the reasons seems to be increased expression of the inhibitory receptor NKG2A in COVID-19 patients. Of note, NKG2A is a heterodimeric inhibitory receptor prominently expressed by cytotoxic lymphocytes, such as NK cells and CD8+ T cells **(8)**.

Cardiovascular Disease

Elevated NLR on admission, for particular values > 2.97 (sensitivity of 92.6% and specificity of 52.5%, AUC = 0.714, p = 0.001) was an independent predictor of all-cause 3-month mortality for hospitalized hypertensive patients older than

80 years. The reason for NLR elevation in cardiovascular diseases is strictly linked to the well-known role of inflammation and oxidative stress in the pathophysiology of atherosclerosis and endothelial dysfunction. In particular, the activation of the NPL3 inflammasome and consequent impairment in homeostasis between IL-1 and its antagonists was emphasized **(9)**.

Cancer

There is imprtant role for Inflammation in the pathophysiology of most solid and hematopoietic malignancies. Inflammation induces tumorigenesis as a chronic pathological response to chronic infection, to immune disorders and to aging in predisposed subjects. Tumor initiation switches on the so-called "cancer-elicited inflammation (CEI)", through a pro-inflammatory cytokine and chemokine storm, determine, in turn, the recruitment of immune cells, induction of angiogenesis and shifting to the promoting phase. Stimulation of tumor-associated macrophages (TAMs), to secrete IL-1β, and of tumor-associated neutrophils (TANs) causes metastatic progression and potentiates systemic neutrophilic inflammation. This supports the notion that it can be considered a reliable and cheap marker of ongoing cancer-related inflammation and a valid indicator of prognosis of solid tumors **(10)**.

Surgery

The preoperative values of NLR could be used as independent predictors for post-operative complications and as peri-procedural and post-procedural mortality, independently of the type of surgery (cardiac or abdomen surgery). However, patients undergoing surgery often show multimorbidity or multiple sources of stress, so that the real significance of NLR in these conditions could be misinterpreted **(11)**.

Platelet-lymphocyte ratio (PLR):

The interactions of blood cell are essential in the pathophysiology of inflammation, immune responses, hemostasis, and oncogenesis. These interactions are multifaceted, and it is often difficult to distinguish primary triggering signals and the specific roles of each cell type in the development and progression of disease states **(12)**.

Platelets are rich in proinflammatory agents and can release highly active microparticles, and they are intimately involved in the development and perpetuation of various inflammatory rheumatic diseases, which often manifest with arthritis and may be complicated by cardiovascular, metabolic, infectious, and lymphoproliferative, among many other, comorbidities. Fluctuations in platelet counts in rheumatic diseases reflect nonspecific inflammatory thrombopoiesis, with abundant release of reactive cells from the bone marrow to the bloodstream, their migration to and excessive consumption at inflammatory sites, and their destruction via binding to anti-platelet antibodies. Platelet counts correlate with platelet volume and reactivity and may indicate autoimmune disease activity, response to anti-

inflammatory therapies, and presence of various comorbidities **(13)**.

The high platelet count has a diagnostic value in elderly patients with temporal artery inflammation suggestive of giant-cell arteritis (GCA). Accordingly, a recent study found that a high platelet count predicts diagnosis of GCA even in the absence of characteristic temporal artery involvement. Interestingly, in children with systemic vasculitides, particularly Kawasaki disease (KD), platelet counts can predict disease outcomes at various stages. An increase in platelet count accompanied by an increase in lymphocyte count, a decrease in neutrophil count, and amelioration of immunoglobulin G, M, and A levels suggests a good response to intravenous immunoglobulin and aspirin therapy at the early, in-hospital stages of convalescence in KD. However, persistently high platelet counts may indicate a complicated course and the development of coronary aneurysms in KD. A high platelet count is also associated with renal involvement in Henoch-Schönlein purpura, another common systemic vasculitis in childhood**(14)**.

In healthy subjects, Platelets modulate the activities of neutrophils and monocytes by responding to stimulation of their platelet-bound Toll-like receptors (TLR) in a dose-dependent manner. Low doses of TLR agonists, such as lipopolysaccharide and fibroblast-stimulating lipopeptide-1, reduce CD66b expression on neutrophils and related granulocyte elastase secretion in platelet-neutrophil coculture. Platelets activated by fibroblast-stimulating lipopeptide-1 in coculture increase IL-6 and IL-10 and reduce TNF-α production **(15)**.

The activated platelets release microparticles which interact with neutrophils towards the expression of platelet-type lipoxygenase and activation of the eicosanoid pathway. Synovial neutrophils of patients with RA internalize platelet microparticles and thereby intensify synovial inflammation. Currently available anti-cytokinergic drugs, such as anti-TNFα, restrict the ability of platelets to bind to and activate leukocytes in RA, which may decrease the risk of thrombotic events **(16)**.

PLR in neoplastic, prothrombotic, and metabolic diseases:

PLR has confirmed as a universal laboratory marker for predicting various neoplastic, prothrombotic, and metabolic diseases. PLR fluctuations can be exolained in the context of the underlying multifaceted immune-inflammatory reactions. Shifts in this parameter correlate positively with other markers of systemic inflammation, particularly with NLR. PLR better predicts clinical outcomes in patients with systemic inflammation than either platelet or lymphocyte count. Basically, the magnitude of stress-induced hypercortisolemia with subsequent release of platelets into the bloodstream and transient lymphopenia influence the degree of elevation of PLR across numerous proinflammatory and prothrombotic disease states. Such a

nonspecific mechanism of PLR elevation can be counteracted by intensified platelet destruction or consumption at the sites of immune inflammation and thrombosis, necessitating cross-checks of all blood cell counts and other inflammatory and immune markers **(17)**.

PLR in inflammatory rheumatic diseases:

The PLR shifts were studied in four large retrospective studies of patients with RA. Patients with cardiovascular, endocrine, hematologic, neoplastic, gastrointestinal, autoimmune comorbidities, as well as those on corticosterioids were reportedly excluded to concentrate on potentially specific associations between PLR and rheumatoid activity. Only one study pointed to an exclusive association of PLR with RA, whereas the remaining studies considered PLR in combination with NLR as potentially valuable in the accurate evaluation of inflammatory activity **(18)**.

PLR in sepsis:

Sepsis is a complicated condition caused by a malfunction of the host's immune response to infection, which cause an uncontrollable inflammatory response and immunosuppression. It develops due to infections acquired both in the community and in the healthcare system, particularly in intensive care units (ICUs), where it is the leading cause of death, responsible for more than half of all ICU deaths. So, sepsis is seen as a global health problem with significant economic effects. As a result, identifying prognostic and diagnostic biomarkers is critical in order to avoid adverse outcomes and reduce mortality by initiating treatment before irreversible damage occurs. A delay of one hour in sepsis treatment was associated with a 7–10 percent increase in sepsis-related death **(19)**.

Complete blood count (CBC) metrics is considered another sepsis biomarkers including the neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR), could be valuable tools. Undoubtedly, CBC has many advantages: (i) it is inexpensive, (ii) it has a quick turnaround time (TAT), (iii) it is accessible in all health centers, (iv) it is simple to perform, (v) clinicians regularly request CBC as part of patient management, and (vi) it is the most commonly ordered laboratory test in all medical settings, from the ICU to the emergency department (ED) **(20)**.

The most common causes of death globally is Sepsis and septic shock are with high treatment expenses. Mortality prediction is a significant issue in sepsis management. In sepsis patients, laboratory parameters or biomarkers are utilized to diagnose and predict the clinical outcomes. Multiple biomarkers have been evaluated in the hopes of aiding prognosis and diagnosis. Still, none of them have proven accurate enough to be utilized in routine daily clinical cases. During recent years, lymphocyte and platelet counts have been discovered to play essential roles within the inflammation reaction. As a result, PLR has been studied as a possible biomarker of inflammation in a number of disorders, particularly sepsis. For example, the PLR has been correlated to the

diagnosis, monitoring, and prognosis of tumors in the digestive, reproductive, and respiratory systems **(21)**.

Platelets are generated in the bone marrow by mature megakaryocytes. Recent research reported that cytokines such as thrombopoietin (TPO), IL3, IL6, IL9, IL11, and stem cell factor (SCF) can increase megakaryocyte production. These factors are found to be increased in septic patients. This may link the increased PLR to sepsis severity **(22)**.

The levels of IL-6 are increased in septic patients, and can be used as a predictor of survival. IL-6 also allowing the conversion of megakaryocytes to platelets and is implicated in neutrophil recruitment. Levels of IL-3, another inducer in megakaryocytes production, are also higher in patients with sepsis and correlate with the severity of disease. Also, Froeschle et al. reported a higher level of IL-9 in neonatal septic patients. This cytokine plays a crucial role in neonatal sepsis **(23)**. The imbalance between the two cells is reflected in the PLR change and an increase in the PLR suggests an imbalance in the proinflammatory and anti-inflammatory reactions. This immune response imbalance causes numerous organ failures, metabolic problems, immunodeficiency, and a mismatch between oxygen supply and demand, all of which lead to mortality. In sepsis, the immune response involves both pro- and anti-inflammatory activities simultaneously, and the immune response is often separated between a main cytokine-mediated hyperinflammatory stage and a secondary immuno-suppressive stage **(24)**.

NLR and PLR in sepsis:

The main cause of sepsis which is a systemic inflammatory reaction syndrome is microbial infection, the pathogens include bacteria, viruses, fungus, and protozoon. Neonatal sepsis (NS) is a common cause of neonatal death. It causes high morbidity especially in newborns, with approximately 3 million cases globally and a mortality rate ranging from 11–19%. Sepsis falls into early onset sepsis (EOS, Septicemia that occurs within 72 h after delivery) and late-onset sepsis (LOS, Septicemia that occurs more than 72 h after delivery) according to the time of onset. The condition of infants with sepsis changes rapidly and the treatment remains intractable, leading to a high mortality rate. Early diagnosis and timely intervention are of great importance for improving the prognosis of NS newborns**(25)**.

The early stages of neonatal sepsis has atypical symptoms and signs. The gold standard diagnosis for NS is blood culture, which needs a long culturing time with a low positive rate, making the early diagnosis is difficult. There is an necessary need for a rapid biomarker and high specificity to help the early identification of NS before getting a positive blood culture. However, there is no excellent biomarker to be used in predicting NS. Numerous biomarkers have already been investigated for the early detection of sepsis. The classification of these markers includes risk prediction,

diagnosis, monitoring, and outcome. Procalcitonin and CD14 are demonstrated to be effective markers, while the costs of detection are often unaffordable for low- and middle-income nations like Brazil **(26)**.

Martins et al., (27) showed that Neutrophil-To-Lymphocyte Ratio (NLR) and Platelet-To-Lymphocyte Ratio (PLR) could be used as biomarkers for NS. The normal ranges of NLR and PLR do not have been unified, which depends on the age and health status of the neonates. NLR and PLR is applicable, feasible, and affordable approaches for rapid diagnosis of NS, with great significance for the early diagnosis, treatment, and prevention of NS. However, whether NLR or PLR is a better indicator for the diagnosis of neonatal sepsis and its diagnostic accuracy is still debated **(27)**.

Neutrophil, lymphocyte and platelet counts were reported as clinical indicators in blood analysis, which requires a quick and accessible laboratory investigation. Indicators of NLR and PLR generated from blood analysis have received interest in the study of inflammation-related disorders in recent years **(28)**.

The increase of PLR in the inflammatory response contributed to the alteration of body microcirculation, the permeability of blood vessels is increased, platelets are activated, and a large number of platelets are accumulated, which in turn aggravates the inflammatory response of the body **(29)**. NLR is considered a highly sensitive indicator for microbial infection. It rises rapidly after being infected and is often associated with disease severity **(30)**. There have been many studies demonstrating that NLR and PLR have a clinical significance for the diagnosis of NS and associated with the severity and prognosis of the disease(**31)**.

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