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Neutrophil-Lymphocyte Ratio (NLR) and Platelet-Lymphocyte Ratio (PLR) as neuroinflammatory biomarkers in Amyotrophic Lateral Sclerosis (ALS) progression

Razão Neutrófilo-Linfócito (RNL) e Razão Plaqueta-Linfócito (RPL) como biomarcadores neuroinflamatórios na progressão da Esclerose Lateral Amiotrófica (ELA)

Relación neutrófilos-linfocitos (RNL) y relación plaquetas-linfocitos (RPL) como biomarcadores neuroinflamatorios en la progresión de la esclerosis lateral amiotrófica (ELA)

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Abstract
Amyotrophic Lateral Sclerosis (ALS) is a rapidly progressing multisystemic and multifactorial neurodegenerative disease that affects upper and lower motor neurons. Neuroinflammation is an important factor in neurodegeneration, the increase of immune cells in the neural tissue and degranulation of these cells causes neuronal damage and death, thus playing an important role in ALS pathophysiology. Studies have sought to use the neutrophil-lymphocyte ratio (NLR) and the platelet-lymphocyte ratio (PLR) as biomarkers of the inflammatory response indicative of diagnosis, stratification, progression and response to treatment of several diseases, including neurodegenerative. Thus, the aim of this study was to evaluate NLR and PLR as possible biomarkers in the neurodegenerative process. This retrospective cross-sectional study was carried out with 43 ALS patients selected at the Dr. Henrique Santillo Rehabilitation and Readaptation Center (CRER), Goiânia, Goiás – Brazil. Neutrophil, platelet, and lymphocyte counts were collected from the patient's most recent hemogram. Statistical analysis was performed using SPSS software, version 26. The correlation between NLR and PLR according to clinical condition showed a significant difference in these parameters during the initial phase of ALS development ($p=0.01$), revealing a marked inflammatory, with subsequent decline as the disease progresses ($p=0.06$). Furthermore, the results indicated a moderate positive linear relationship between the two variables ($r=0.57$; $p<0.001$), showing a joint increase in these parameters. Therefore, NLR and PLR are important indicators of inflammation and can be useful due to their simplicity, high reproducibility and low-cost for routine use.
Keywords: Neurogenic inflammation; Neurodegeneration; Clinical rating; Prognosis.

Resumo
A Esclerose Lateral Amiotrófica (ELA) é uma doença neurodegenerativa multissistêmica e multifatorial de rápida progressão que afeta os neurônios motores superiores e inferiores. A neuroinflamação é um fator importante na neurodegeneração, o aumento de células imunes no tecido neural e a degranulação dessas células causam danos e

morte neuronal, desempenhando assim um papel importante na fisiopatologia da ELA. Estudos têm buscado utilizar a razão neutrófilo-linfócito (RNL) e a razão plaqueta-linfócito (RPL) como biomarcadores da resposta inflamatória indicativos de diagnóstico, estratificação, progressão e resposta ao tratamento de diversas doenças, incluindo as neurodegenerativas. Assim, o objetivo deste estudo foi avaliar a RNL e a RPL como possíveis biomarcadores no processo neurodegenerativo. Este estudo transversal retrospectivo foi desenvolvido com 43 pacientes portadores de ELA selecionados no Centro de Reabilitação e Readaptação Dr. Henrique Santillo (CRER), Goiânia, Goiás – Brasil. A contagem de neutrófilos, plaquetas e linfócitos foi coletada do hemograma mais recente dos pacientes. A análise estatística foi realizada por meio do software SPSS, versão 26. A correlação entre RNL e RPL de acordo com a condição clínica, mostrou diferença significativa nesses parâmetros durante a fase inicial do desenvolvimento da ELA (p=0,01), revelando um quadro inflamatório acentuado, com posterior declínio com o avanço da doença (p=0,06). Além disso, os resultados indicaram uma relação linear positiva moderada entre as duas variáveis (r=0,57; p<0,001), mostrando um aumento em conjunto desses parâmetros. Portanto, a RNL e a RPL são importantes indicadores de inflamação e podem ser úteis devido à sua simplicidade, alta reprodutibilidade e baixo custo para uso rotineiro.
Palavras-chave: Inflamação neurogênica; Neurodegeneração; Avaliação clínica; Prognóstico.

Resumen
La esclerosis lateral amiotrófica (ELA) es una enfermedad neurodegenerativa multifactorial y multisistémica que progresa rápidamente y que afecta a las neuronas motoras superiores e inferiores. La neuroinflamación es un factor importante en la neurodegeneración, el aumento de células inmunitarias en el tejido neural y la desgranulación de estas células provoca daño y muerte neuronal, por lo que juega un papel importante en la fisiopatología de la ELA. Los estudios han buscado utilizar la relación neutrófilos-linfocitos (RNL) y la relación plaquetas-linfocitos (RPL) como biomarcadores de la respuesta inflamatoria indicativos del diagnóstico, estratificación, progresión y respuesta al tratamiento de varias enfermedades, incluidas las neurodegenerativas. Por lo tanto, el objetivo de este estudio fue evaluar RNL y RPL como posibles biomarcadores en el proceso neurodegenerativo. Este estudio transversal retrospectivo fue realizado con 43 pacientes con ELA seleccionados en el Centro de Rehabilitación y Readaptación Dr. Henrique Santillo (CRER), Goiânia, Goiás – Brasil. Los recuentos de neutrófilos, plaquetas y linfocitos se obtuvieron del análisis de sangre más reciente del pacientes. El análisis estadístico se realizó con el software SPSS, versión 26. La correlación entre RNL y RPL según la condición clínica mostró una diferencia significativa en estos parámetros durante la fase inicial del desarrollo de la ELA (p = 0,01), revelando un marcado cuadro inflamatorio, con un declive posterior con el avance de la enfermedad (p=0,06). Además, los resultados indicaron una relación lineal positiva moderada entre las dos variables (r=0,57; p<0,001), mostrando un aumento conjunto de estos parámetros. Por lo tanto, RNL y RPL son indicadores importantes de inflamación y pueden ser útiles debido a su simplicidad, alta reproducibilidad y bajo costo para uso rutinario.
Palabras clave: Inflamación neurogénica; Neurodegeneración; Clasificación clínica; Pronóstico.

1. Introduction

Amyotrophic Lateral Sclerosis (ALS) is a rapidly progressing multisystemic neurodegenerative disease with a median survival of 3 to 5 years. The pathology affects upper and lower motor neurons in the cortex, bulb and spinal cord regions and presents symptoms such as muscular atrophy, dysarthria, dysphagia, and respiratory failure, which is the main cause of death (Brown & Al-Chalabi, 2017; Grollemund et al., 2019). ALS has a worldwide incidence of 1.55 to 1.96 for every 100,000 people, its etiology is undefined, being considered as a multifactorial pathology (Ferraiuolo et al., 2011; Marin et al., 2017; Su et al., 2016).

Despite the uncertainties in neurodegeneration, recent studies have demonstrated the important role of neuroinflammation in ALS. This mechanism, which is based on a complex innate immune response of the central nervous system (CNS) involving mainly macroglia and microglia, presents itself as an important factor in neurodegeneration due to the increased immune cells in neural tissue and degranulation of these cells releasing cytokines, eicosanoids, nitric oxide, and producing free radicals that cause lesions and death of neuronal cells (Prado et al., 2018b; Yang & Zhou, 2019). Further, it is known that increased cytokines levels released from astrocytes and microglia can also result in the permeability of the blood-brain barrier and the recruitment of various types of peripheral immune cells such as neutrophils, lymphocytes, monocytes, platelets, dendritic cells and fibroblasts to the CNS (Beers & Appel, 2019; Harlan et al., 2019; Spiller et al., 2018).

Various studies report the unbalance in the peripheral immune system in ALS through the increased and dysregulation

of immune cells in the blood and spinal cord, a fact that was associated with disease progression due to the pro-inflammatory and neurotoxic effects by these cells (Beers et al., 2017; McCombe & Henderson, 2011; Prado et al., 2018a). Therefore, the Neutrophil-lymphocyte ratio (NLR) and the Platelet-lymphocyte ratio (PLR) stand out as low-cost tools, calculated from blood count data, capable of evaluating the inflammatory process mediated by immune cells in various pathologies (Sargin et al., 2018). Alterations in NLR and PLR are associated with increased cytokines and pro-inflammatory mechanisms and are used as biomarkers in the diagnosis and prognosis of cancer (Arslan et al., 2018; Bilen et al., 2019; Najjar et al., 2018), cardiovascular diseases (Nam et al., 2018; Tokgoz et al., 2013), rheumatic (Gasparyan et al., 2019; Sargin et al., 2018) and degeneratives (Kalelioglu et al., 2017; Niazi et al., 2019).

Thereby, considering neuroinflammation as an important pathological mechanism in the neurodegenerative process, evaluating the NLR and PLR as possible biomarkers in the neurodegenerative process highlights its importance in the implementation of new technologies in prognosis, as a quick and loss-cost resource for clinical evaluation and, especially, as an aid for the development of new therapies based on the neuroinflammatory process.

2. Methodology

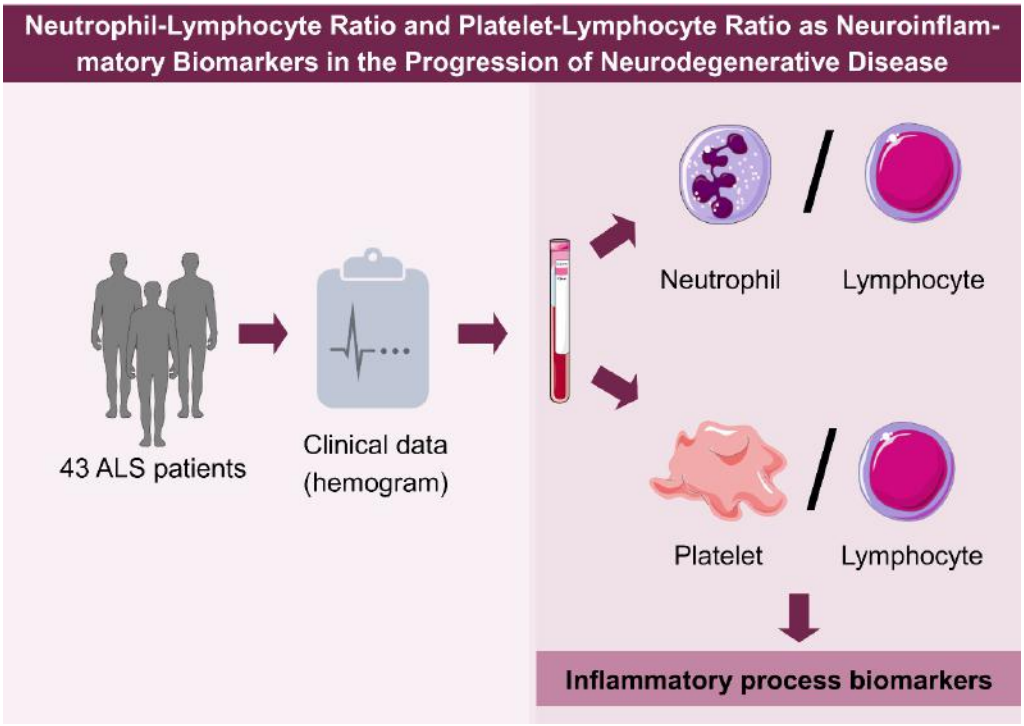
This is a cross-sectional retrospective study, carried out at the Dr. Henrique Santillo Rehabilitation and Readaptation Center (CRER), Goiânia, Goiás - Brazil. The study was approved by the Research Ethics Committee of the Federal University of Goiás (UFG) under protocol number CAAE 79593117.7.0000.5083 and conducted in accordance with the Ethical Principles for Medical Research involving Humans of the Declaration of the World Medical Association of Helsinki.

Clinical data were collected from 43 patients diagnosed with ALS from January 2014 to April 2019. Exclusion criteria were: patients who had not a confirmed diagnosis, with hematologic disorders, immunosuppressive drug users (steroids), and history of infection for less than two weeks.

The patients included had hemogram results consulted in the medical reporters, as well as clinical data such as age, gender, adherence to pharmacotherapeutic, presence of other pathologies and date of death. They were classified according to hematological changes (normal or altered) and severity of of the clinical status and neurodegeneration (initial or advanced), taking into account the respiratory insufficiency and the survival time after the diagnosis of the disease.

The count of neutrophils, platelets and lymphocytes were collected from the patient's most recent hemogram. NLR was defined by the absolute neutrophil count divided by the absolute lymphocyte count. Similarly, PLR was defined by the absolute platelet count divided by the absolute lymphocyte count (Figure 1). Several studies have used this methodology, these ratios are useful and easily accessible indicators, used as biomarkers that can help in the diagnosis, stratification, clinical evaluation, prognosis and pharmacological response of various pathologies (Acharya et al., 2019; Cai et al., 2020; Choi et al., 2020; Dong et al., 2019; Gasparyan et al., 2019; Leone et al., 2022; Wei et al., 2022).

Figure 1. Graphical summary of the research development stages.



Source: Authors (2022).

Statistical analysis was performed using SPSS software, version 26. For the characterization of continuous variables, mean and standard deviation were used. To determine the difference between the sample means, the Mann-Whitney test was used. The relation between NLR and PLR, as well as the correlation of these factors with the clinical profile was performed through Spearman's correlation. In all analyzes, the significance level of 5% ($p < 0.05$) was adopted.

3. Results and Discussion

The study sample consisted of 35 men and 8 women, of these 21 individuals were in the age group of 31 to 59 years and 22 individuals were in the age group of 60 to 81 years. Of the 43 patients selected, 29 had an early stage of ALS and 14 had an advanced stage of the disease. Table 1 shows the mean values of neutrophils, platelets and lymphocytes and the NLR and PLR found in the samples. It was observed that there was no significant difference between NLR and PLR in patients with early and advanced stages of the disease ($p=0.56$ and $p=0.45$, respectively).

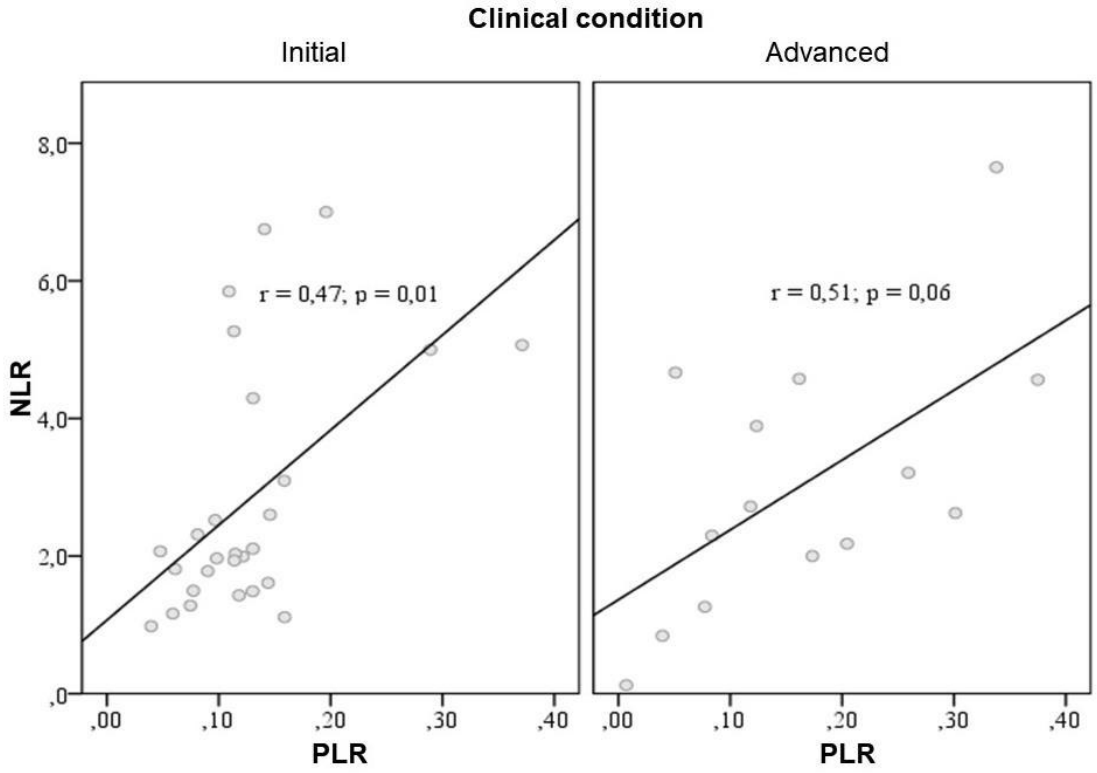
Table 1. Characterization of the samples in relation to the values of neutrophils, platelets and lymphocytes and the NLR and PLR.

	Clinical condition		p^a
	Initial	Advanced	
Mean ± Standard deviation			
Number of lymphocytes/mm	2288.10 ± 786.54	4264.07 ± 8173.84	0.69
Number of neutrophils/mm	7317.17 ± 9968.76	5675.79 ± 2076.47	0.79
Number of platelets mi/mm	348.24 ± 558.56	297.14 ± 115.46	0.14
NLR	3.71 ± 5.23	3.04 ± 1.93	0.56
PLR	0.16 ± 0.18	0.17 ± 0.12	0.45

[a]Mann-Whitney test. NLR: Neutrophil-lymphocyte ratio; PLR: Platelet-lymphocyte ratio. Source: Authors (2022).

The correlation between NLR and PLR according to clinical condition (Figure 2) showed a significant difference in these parameters during the initial phase of ALS development (p=0.01), revealing a marked inflammatory condition, with subsequent decline in inflammation as the disease advancement (p=0.06). While in figure 3, there is a correlation between the ratios, which indicates a moderate positive linear relationship between the two variables (r=0.57; p<0.001) and points to a joint increase in these parameters.

Figure 2. Correlation between NLR and PLR according to clinical condition.

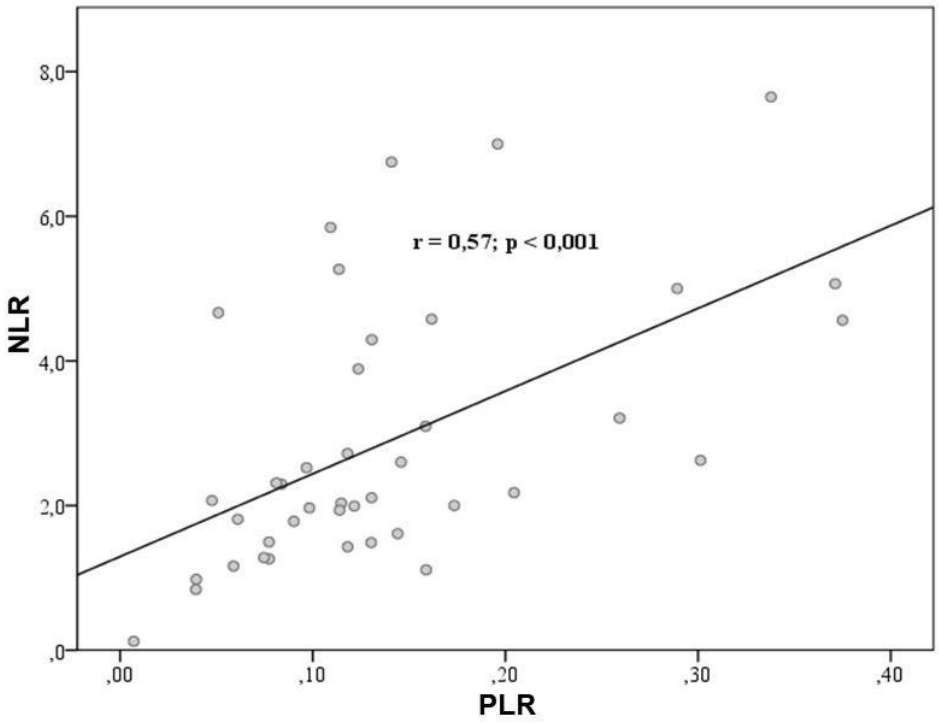


Source: Authors (2022).

NLR and PLR may be new inflammatory biomarkers and their increased values may be associated with the onset or prognosis of neurodegenerative diseases, such as Alzheimer's, Parkinson's and ALS (Akil et al., 2015; Choi et al., 2020; Dong et al., 2019; Rembach et al., 2014). Studies have reported a significant correlation between the increase in the number of

neutrophils in the peripheral blood of ALS patients and disease's progression (Choi et al., 2020; Murdock et al., 2017). Furthermore, neutrophils play a pro-inflammatory role in neurodegeneration, contributing to the disruption of the blood-brain barrier (Choi et al., 2020; Garbuzova-Davis & Sanberg, 2014).

Figure 3. Result of the correlation between NLR and PLR.



Source: Authors (2022).

Research further reveals that possibly neutrophils may also play a protective role in the initiation of neuronal repair. Short-lived neutrophils are generated in the bone marrow and released into the bloodstream, so the increased number of these cells in the blood may be due to bone marrow production rather than reduced CNS recruitment. Thus, it is assumed that the increase in these cells at the beginning of ALS development may reflect the exclusion of neutrophils after the onset of neuronal repair, suggesting that these changes may be caused by specific immune modulations of ALS pathogenesis (Choi et al., 2020; Kurimoto et al., 2013; Wei et al., 2022).

In addition, neuroinflammation is also regulated by increased microglial activation, exposing a complex association between different immune cells and the pathogenesis of ALS. Low T lymphocyte numbers have been associated with rapidly progressing ALS symptoms (Murdock et al., 2017; Wei et al., 2022). In mouse models this correlation was also observed, it was noted that regulatory T cells were responsible for preparing the immune system to protect the affected tissues and at the same time delay the progression of ALS (Beers et al., 2011; Wei et al., 2022). In summary, high NLR values are often associated with worsening survival (Scilla et al., 2017; Wei et al., 2022). However, the effects of this parameter on the progression of ALS remain uncertain, only four studies have evaluated NLR in ALS (Choi et al., 2020; Leone et al., 2022; Li et al., 2015; Wei et al., 2022) and, to our knowledge, no studies addressing PLR in ALS have been developed.

Despite recent advances in ALS research, there are no robust biomarkers that point to the diagnosis or progression of the disease in clinical practice (Ryberg & Bowser, 2008; Turner et al., 2009). Thus, peripheral blood becomes an adequate source for the search for biomarkers, the ease of access and handling allows multiple simple and low-cost tests. However, it

should be considered that blood composition can be affected by several biochemical changes as a result of pathological processes (Robelin & Aguilar, 2014). Thus, the NLR and PLR are important diagnostic and monitoring tools that are widely available and widespread, representing metrics that can provide independent and complementary information about various pathological conditions.

4. Final Considerations

In conclusion, routine peripheral blood parameters are easy to collect and detect, making them ideal potential biomarkers. Therefore, the method performed is simple and inexpensive, and can be used for diagnosis, stratification, clinical assessment, prognosis and response to pharmacos of various diseases, including neurodegenerative.

The limitations of this study need to be addressed in future investigations. First, we measured the ratios at a single point in time during the course of the disease. Additional longitudinal studies with sampling at different times are needed to understand how the NLR and PLR change during the course of the disease, before using it to monitor peripheral inflammation in ALS. Second, a small number sample of patients was analyzed, which did not allow for sub-analysis. Thus, future research with larger samples can clarify whether the changes in the NLR and PLR are due to some pathological process that influences the progression or if they are a secondary consequence of neuronal death.

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