Relação Neutrófilo-Linfócito: Acrescentando um Biomarcador a uma Escala Preditiva de Pneumonia Pós-Acidente Vascular Cerebral

Neutrophil-to-Lymphocyte Ratio: The Role of Adding a Biomarker to a Predictive Post-Stroke Pneumonia Score

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Resumo:
Introdução: Avaliar a associação da relação neutrófilo-linfócito (NLR) e a incidência de pneumonia pós-acidente vascular cerebral (PSP), subtipo de acidente vascular cerebral (AVC), gravidade e prognóstico.

Material e Métodos: Foi realizado um estudo prospektivo observacional durante um período de 42 meses numa Unidade de AVC de um hospital terciário.Todos os doentes com AVC isquémico agudo (AIS) foram sequencialmente incluídos. O valor de NLR foi calculado na admissão. As características dos doentes como subtipo de AVC, gravidade e diagnóstico de PSP foram obtidos. A escala A2DS2 foi utilizada como preditor clínico de PSP.

Resultados: Foram identificados 521 doentes com AIS. A idade média foi 76,17 ± 10,16 anos, 46,9% eram homens. Verificou-se uma associação entre NLR, tipo e gravidade de AVC (p <0,01), persistindo em análise estratificada após exclusão de infeção concomitante. Doentes com NLR mais elevado apresentavam défice neurológico mais grave na admissão, maior mortalidade e maior grau de dependência na alta (p < 0,01). Foi realizada uma regressão logística para caracterizar a capacidade preditiva da NLR (≥3) e do A2DS2 (≥6) na probabilidade de desenvolver PSP (p < 0,005). O modelo explicou 17,1% (Nagelkerke R²) da variância nos diagnósticos de pneumonia, classificando corretamente 77,0% dos doentes com uma especificidade de 96,3%. Doentes com A2DS2 ≥6 (OR 8,36, p < 0,01) e doentes com NLR ≥3 (OR 2,35, p < 0,01) apresentaram um maior risco de desenvolver pneumonia.

Conclusão: NLR parece estar relacionado com a gravidade dos AIS, possivelmente como marcador de ativação neuroimune. Avanços na compreensão dos efeitos imunobiológicos da isquemia no cérebro poderão levar a desenvolvimentos terapêuticos futuros. Atualmente, sendo um biomarcador relativamente pouco dispendioso, talvez exista um papel da NLR na melhoria das escalas preditoras de PSP.

Palavras-chave: Acidente Vascular Cerebral/complicações; Linfócito; Neutrófilo; Pneumonia.

Abstract:
Introduction: To assess the association of neutrophil-to-lymphocyte ratio (NLR) with post-stroke pneumonia (PSP) incidence, stroke subtype, severity, and prognosis.

Material and Methods: Prospective observational study over a 42-month period in a Stroke Unit of a tertiary University Hospital. All patients with acute ischaemic stroke (AIS) were sequentially included. NLR was obtained at admission. Patient characteristics such as stroke subtype, severity and PSP diagnosis were ascertained. Score A2DS2 was used as clinical predictor of PSP.

Results: 521 patients with AIS were identified. The mean age was 76.17 ± 10.16 years years, 46.9% were men. Association was found between NLR and type and severity of stroke (p < 0.01), persisting in stratified analysis after excluding concomitant infection. Patients with higher ratio presented severer neurological deficits at admission, higher mortality, and dependency on discharge (p < 0.01). A logistic regression was performed to ascertain the predictive capacity of NLR (≥3) and A2DS2 (≥6) on the likelihood of developing PSP (p < 0.005). The model explained 17.1% (Nagelkerke R²) of the variance in pneumonia diagnoses, correctly classifying 77.0% of patients with 96.3% specificity. Patients with A2DS2 ≥6 were likelier to develop pneumonia (OR 8.36, p < 0.01). Moreover, patients with NLR ≥3 had higher odds of developing pneumonia (OR 2.35, p < 0.01).

Conclusion: NLR appears to be related to severity in AIS, possibly as surrogate of neuroimmune mediation. Advances in the understanding of the immunobiological effects of ischaemia in the brain may lead to future therapeutic developments. Presently, as a relatively inexpensive biomarker, there may be a potential role for NLR in improving PSP prediction scores.

Keywords: Lymphocytes; Neutrophils; Pneumonia; Stroke/complications.

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https://doi.org/10.24950/rspmi.2022.01.o
Introduction

Neutrophil-to-lymphocyte ratio (NLR) at hospital admission, defined as the quotient between both absolute values in a complete blood count, has been suggested to be a promising marker in cardiovascular ischaemic events, such as coronary artery occlusion. A higher NLR was independently associated with arterial stiffness and coronary calcium score in a large Korean study, proposing that NLR might be a useful additional measure of assessing cardiovascular (CV) risk. In fact, a high NLR despite a normal white blood cell (WBC) count is proposed to be predictive of atherosclerosis, and a more powerful predictor of CV disease than any leucocyte subtype, presumably due to the role of inflammation in the atherogenic process. It has also been associated with a poor short-term clinical outcome in patients with acute ischaemic stroke and hemorrhagic stroke, on par with other inflammatory biomarkers.

Post-stroke pneumonia (PSP) is defined as a lower respiratory tract infection complicating the first week after stroke onset, the period where pneumonia most frequently occurs in stroke patients. This fact probably reflects the period of highest risk in terms of dysphagia, immobility, impaired consciousness, and immunosuppression. It has an estimated incidence of 7% to 38% and has been consistently associated with a high attributable risk of early mortality, increased length of stay and medical cost. A high NLR despite a normal WBC count is proposed to be predictive of atherosclerosis, and a more powerful predictor of CV disease than any leucocyte subtype.

The A²DS² score – a ten-point score (age ≥75 years=1, atrial fibrillation=1, dysphagia=2, male sex=1, stroke severity, National Institutes of Health Stroke Scale NIHSS 0–4=0, 5–15=3, ≥16=5) developed from the Berlin Stroke Registry, is currently the most used tool. It was validated in the independent Northwest Germany Stroke Registry 34, and with external validation. A prospective multicentre comparison between A²DS², ISAN and AIS-APS scores (also commonly used to predict the risk of PSP), suggest that A²DS² might be the best score in the identification of patients at high risk of PSP, though none with a good positive predictive value. In fact, a A²DS² score ≥4 yields a sensitivity of 91% and specificity of 57% for the occurrence of PSP, while a A²DS² score ≥5 has a sensitivity of 83% and specificity of 72%.

The authors aim to assess the relationship between NLR and stroke severity, subtype, how it relates to mortality, and post-stroke pneumonia incidence.

Material and Methods

A prospective observational study over a 42-month period in a Stroke Unit of a tertiary University Hospital was conducted. All patients presenting with acute ischemic stroke during this period were included. Patients that underwent thrombolysis or mechanical thrombectomy were not included. Population and event characteristics were collected, namely: time of onset, type of event, Oxfordshire Community Stroke Project – OCSP, National Institute of Health Stroke Scale – NIHSS, mortality, mRankin at discharge and length of stay in days), risk factors such as chronic obstructive pulmonary disease, heart failure or active smoking, presence of respiratory tract infections, A²DS² and NLR at admission to the emergency department. NLR cut-off was selected based on previous published methodology.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS® statistical software version 24. Relevant variables were stratified in categories (A²DS² < 6, ≥ 6; NIHSS at admission <6, 6-13, >13; NLR <3, ≥3). When comparing two categorical variables the Pearson’s Chi-square test was used. A binomial logistic regression multivariate analysis was also performed. Applicability conditions were verified. The significance level was set at \( p < 0.05 \).

Results

A total of 606 patients were admitted to the Stroke Unit during the study’s period. Of these, 521 patients with acute ischemic stroke (AIS) were identified (Table 1). The remaining 85 patients were excluded due to being hospitalized with either a haemorrhagic event or a stroke mimic. Of the included 521 patients classified as AIS, 66 had a transient ischaemic attack (TIA).

Table 1: Diagnosis at admission

<table>
<thead>
<tr>
<th>Type of Event</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemic stroke</td>
<td>455</td>
</tr>
<tr>
<td>Transient ischaemic attack</td>
<td>66</td>
</tr>
<tr>
<td>Haemorrhagic stroke</td>
<td>21</td>
</tr>
<tr>
<td>Stroke mimic</td>
<td>64</td>
</tr>
<tr>
<td>Total</td>
<td>606</td>
</tr>
</tbody>
</table>

Concerning the included patients, the mean age was 76.17 ± 10.16 years (min. 47; max. 96 years old). Population demographics are summarized on Table 2. Missing data was characterized as unknown.

Association was found between NLR and type and severity of stroke according to OCSP classification – lower NLR in lacunar stroke and higher in total anterior circulation infarct (Table 3, \( p < 0.001 \)).

A NLR ≥3 was related to more severe neurological deficit as ascertained by the NIHSS scale ≥6 \( p < 0.001 \) and was associated with a higher mortality – from 26 patients that died,
77% had a NLR ≥ 3, vs 52% of those in the survival group (Table 3, p = 0.016). NLR ≥ 3 was also associated with higher disability as ascertained by mRankin score at discharge (Table 3, p < 0.0001). This association persisted after stratified analysis excluding infection (PSP).

The relationship between NLR at admission and the development of pneumonia is shown in Fig. 1. Patients with NLR ≥ 3 had higher odds of developing pneumonia (OR 2.531, 95%CI 1.654, 3.873).

Since a statistically significant relationship between NLR and post-stroke pneumonia was found, a logistic regression was performed to assess the predictive capacity of NLR (≥3) and A2DS2 (≥6) on the likelihood of developing post-stroke pneumonia. The model including both variables explained 17.1% (Nagelkerke R²) of the variance in pneumonia diagnoses, correctly classifying 77.0% of patients and showing a 96.3% specificity and a 25.6% sensibility (p < 0.005), with a better fitness than a model using A2DS2 alone (13.2%, Nagelkerke R2). The specificity and sensibility found in our...
A2 DS ≥ 6 was 8.36 times more likely to exhibit post-stroke pneumonia than A2 DS < 6 (p < 0.001), while NLR ≥ 3 had 2.349 higher odds (Table 4).

**Discussion**

There is little doubt that post-stroke inflammation is an important factor in brain injury. In fact, leucocytosis has...
been associated to a poorer clinical outcome in patients with AIS.40-43 Nevertheless, this inflammatory response is complex and involves several protagonists and immune pathways.17,38 NLR has been suggested as an interesting marker in patients with cardiovascular disease.44,45 It has been associated with a poor short-term clinical outcome in patients with acute ischaemic stroke7,8 and is also a marker of poor prognosis 3 months after AIS.46 In fact, the immune system might arise as a potential therapeutic target in patients with ischaemic brain lesion.47-49

In our study, there was an association between higher NLR, higher NIHSS and poorer outcome, with increased risk of mortality and a higher mRankin score at discharge. These results persisted after stratified analysis excluding infection.

Post-stroke pneumonia is a frequent but potentially preventable stroke complication, often associated with a significant increase in morbidity and mortality. It has been associated with a 4 times higher fatality rate in the first 30 days, 3 times higher length of stay and a significant increase in medical expenses.16,30 Our incidence of 21% falls within what has been reported in the literature.15,18-27 NLR is an established marker for inflammation and infection, having been studied as a predictor in pneumonia and bacterial infections50-53 However, its role in PSP is not as well supported. In a cohort of 1317 patients in a 2-center retrospective study, Nam K-W, et al report an association between NLR and an increased risk and severity of PSP.54 We also report an association between higher NLR and an increased risk of developing PSP.

Several clinical risk factors have previously been established for PSP, such as age, admission NIHSS score and stroke severity, presence of nasogastric tube, mechanical ventilation, and atrial fibrillation.55 However, the concomitant use of biomarkers in PSP assessment is, to the best of our knowledge, scarce.27,55,56 The PANTHERIS score included the total leukocyte count, but the lack of NIHSS evaluation seems to be a limitation.27 In our study, patients with NLR ≥ 3 were 2.35 times more likely to exhibit post-stroke pneumonia than NLR < 3 (p < 0.001) and NLR did appear to increase the fitness of the predictability of A²DS² in our logistical regression model, even though we did not find an increase in sensitivity or specificity in our sample.

The percentage of “unknown data”, namely in NIHSS, is attributed to logistical constraints in our practice setting: a tertiary centre involving two hospitals. Study inclusion was done at stroke unit admission in a different hospital from the emergency department (ED). When the details from the stroke physician’s evaluation were not charted at the ED, data was classified as unknown/missing.

Post-stroke inflammation is characterized by a rapid activation of resident cells (mainly microglial cells), followed by the infiltration of circulating inflammatory cells, including granulocytes (neutrophils), T cells, monocyte/macrophages, and other cells in the ischemic brain region.57-59 Microglial cell proliferation and proinflammatory mediator production, including IL-1β and TNF-α, has been documented within minutes of ischemia60 and appear to play a role in exacerbating tissue damage but may also protect the brain against ischemic and excitotoxic injury.61 In contrast, blood-derived leukocytes are recruited to the brain tissue with a delay of hours to a few days. Therefore, the timing of blood collection could affect NLR. NLR evolution during hospitalization was not evaluated due to concern of higher risk of bias due to infection, catheterization, and drugs. The NLR response in a severe cardiovascular event appears to be a surrogate of an immune-mediated response, not necessarily caused by infection but perhaps related to it (higher immune-dysregulation in more severe events).62

This, in our view, supports the current hypothesis of immune-mediated cell injury in stroke. Further studies are needed in determining the immunological mechanism of lesion and the possibility of therapeutic intervention.

**Conclusion**

NLR ≥3 is associated with a more severe stroke subtype, neurologic deficit, increased morbidity and mortality and higher rates of post-stroke pneumonia, though the relationships described do not seem to be attributable do the infection. NLR appears to be a surrogate of immunological dysregulation. Advances in the knowledge of the immunobiological effects of ischemia in brain may lead to future therapeutic developments. As an inexpensive, fast and widely available tool, NLR may have a role in identifying a subset of patients that may benefit in future immunomodulatory therapy trial.

Declaración de Contribución / Contributorship Statement:
D. Pedro, M. Narciso - Concepción e design, Análise e recoña de datos, Análise estatística e Interpretação de datos, Escrita do artigo, Pesquisa Bibliográfica, Revisão crítica do artigo, Aprovação final.

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Table 4: Variable analysis for the logistic regression for post-stroke pneumonia on NLR and A²DS².

<table>
<thead>
<tr>
<th></th>
<th>Odds ratio</th>
<th>p - value</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inferior</td>
<td>Superior</td>
<td>Inferior</td>
<td>Superior</td>
</tr>
<tr>
<td>A²DS² ≥6; &lt;6</td>
<td>8.358</td>
<td>&lt; 0.001</td>
<td>4.197</td>
</tr>
<tr>
<td>NLR ≥3; &lt;3</td>
<td>2.349</td>
<td>&lt; 0.001</td>
<td>1.504</td>
</tr>
</tbody>
</table>
Responabilidades Éticas
Conflicts of Interest: Os autores declararam a inexistência de conflitos de interesse na realização do presente trabalho.

Fontes de Financiamento: Não existiram fontes externas de financiamento para a realização deste artigo.

Confidencialidade dos Dados: Os autores declararam que os procedimentos seguidos estavam de acordo com os regulamentos estabelecidos pelos responsáveis da Comissão de Investigação Clínica e Ética e de acordo com a Declaração de Helsinki da Associação Médica Mundial. Provenienza e Revisão por Pares: Não comissionado; revisão externa por pares.

Ethical Disclosures
Conflicts of interest: The authors have no conflicts of interest to declare.

Financial Support: This work has not received any contribution, grant or scholarship

Confidentiality of Data: The authors declare that they have followed the protocols of their work center on the publication of data from patients. Protection of Human and Animal Subjects: The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki). Provenance and Peer Review: Not commissioned; externally peer reviewed.

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Received / Recebido: 11/11/2021
Accepted / Aceite: 12/01/2022
Published / Publicado: 22/03/2022

REFERENCES
RELAÇÃO NEUTRÓFILO-LINFÔCITO: ACRESCENTANDO UM BIOMARCADOR A UMA ESCALA PREDITIVA DE PNEUMONIA PÓS-ACIDENTE VASCULAR CEREBRAL

doi:10.1161/STROKEAHA.111.00598


