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The platelet-to-lymphocyte ratio (PLR) and the clinical impact on the outcome of stroke patients under previous aspirin therapy

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The platelet-to-lymphocyte ratio (PLR) and the clinical impact on the outcome of stroke patients under previous aspirin therapy

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À minha família, por sempre me incentivar a perseguir os meus sonhos e me reconfortar nas horas mais difíceis.

A todos, um sentido obrigada.

Sonhar, mesmo que seja impossível
Lutar, mesmo que o inimigo seja invencível
Suportar a dor, mesmo que seja insuportável
Correr, mesmo onde o bravo não ouse ir
Transformar no bem o que é mal,
mesmo que o caminho seja de mil milhas
Amar o puro e o inocente,
mesmo que seja insistente
Persistir, mesmo quando
o corpo não mais resista
E, afinal, tocar aquela estrela,
mesmo que seja impossível.

The platelet-to-lymphocyte ratio (PLR) and the clinical impact on the outcome of stroke patients under previous aspirin therapy

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The platelet-to-lymphocyte ratio (PLR) and the clinical impact on the outcome of stroke patients under previous aspirin therapy

Background: Platelets and lymphocytes play a critical role in the pathophysiological pathways of the early stages of acute ischemic stroke (AIS). Platelet-to-lymphocyte ratio (PLR) is an accessible parameter to evaluate inflammatory response as it can be calculated from a regular hemogram. Aspirin/acetylsalicylic acid (ASA) plays a central role in the secondary prevention of cardiovascular disease, however, its impact on PLR levels and consequent prognostics are not completely understood. Aims: Explore the relationship between aspirin and PLR and the consequent effects on mortality and stroke recurrence in patients with AIS. Methods: 424 patients were included in this observational study. PLR values were calculated from the first blood sample at admission. The effect of PLR on mortality and stroke recurrence was evaluated using Kaplan-Meier methodology, log-rank test, Cox proportional hazard models, and Bootstrap Analysis. Results: Our results indicate an association of high PLR (>170) with a poor overall survival on stroke patients (HR 1.880, 95%CI 1.176 – 3.005, p = 0.008), particularly in those with no record of aspirin therapy (p = 0.018). Modeling the risk of a second event (recurrence) in the timeframe of 36 months demonstrated a predictive capacity for PLR (p=0.004) confirmed by Bootstrap analysis (p = 0.001; 1000 replications). The inclusion of PLR in simulating models' equations provides a gain of nearly 20% in the predictive ability (C-index 0.779 increases to 0.946). Conclusions: The platelet-to-lymphocyte ratio (PLR) has a link to clinical impact with worse outcomes for stroke patients and this is firstly demonstrated related to previous aspirin therapy.

Keywords: Platelet-to-lymphocyte ratio; PLR; acute ischemic stroke; AIS; aspirin; acetylsalicylic acid; ASA; stroke recurrence; mortality

Introduction

Acute ischemic stroke (AIS) is a leading cause of death and disability-adjusted life-years (DALYs) throughout the world. [1] Ischemic stroke can be divided into what has been referred to as subtypes or etiologic categories: large-artery atherosclerosis,

cardioembolic, lacunar/small vessel occlusion, other specific causes (dissections, vasculitis, specific genetic disorders, and others), and of unknown cause. [2] The pathophysiological pathway of ischemic stroke leads to insufficient blood flow to perfuse cerebral tissue causing sudden impairment in the brain function of an affected area.

In the pathophysiology of AIS, platelets play a critical role in the development of atherosclerosis, inflammation, and coagulation which leads to thrombus formation and severely impacts the early stages of ischemic stroke. [3, 4]

The inflammatory process affects the ischemic region and produces a variety of damaging materials (reactive oxygen species, proteases, matrix metalloproteinase-9, cytokines), all of which lead to the exacerbation of cerebral damage. [5] Lymphocytes were proposed to represent a critical cellular subtype in determining the degree of neuroinflammation in acute brain injury, with intricate and varied impacts. [6]

Platelet-to-lymphocyte ratio (PLR) is a quick and efficient parameter to assess this inflammatory response as it can be readily calculated from a regular hemogram. Prior studies evaluating PLR noted a consistent association with unfavorable outcomes. Reports have shown that elevated PLR is associated with increased infarct volume, worsening grades of cerebral edema at 24 hours, worse functional outcomes, hemorrhagic transformation (HT) after AIS, intrahospital mortality of HT, unsuccessful reperfusion, post-thrombolysis early neurological deterioration, and incidence of stroke-associated infection. [5, 7-11]

In ischemic stroke, aspirin/acetylsalicylic acid (ASA) is widely used to prevent the recurrence and development of new cardiovascular disease. Aspirin can reduce the severity of a stroke by decreasing the size of the clot, the extent of the thrombosis, and the ensuing embolism. In addition, aspirin's anti-inflammatory and neuroprotective

properties may help enhance microcirculation in the ischemic penumbra by inhibiting platelet-derived vasoconstrictors.[12]

This study aims to explore the relationship between aspirin and PLR and the consequent effects on mortality and stroke recurrence in patients with AIS.

Material and Methods

Study Population

This observational study with post-hoc analysis includes 424 consecutive patients diagnosed with AIS admitted in the Department of Internal Medicine of Centro Hospitalar São João from September 2009 to April 2012. The inclusion criteria were patients over 18 years old admitted for AIS. Patients with acute haemorrhagic stroke or neurological deficits associated with space occupying lesions, whether primary CNS neoplasm or secondary unknown tumour, were excluded from the study.

Written informed consent was signed by all patients or their legal representatives. After recruitment, a follow-up protocol was set up. Ethical approval was obtained from the local Ethics Committee and conducted according to the principles of the Declaration of Helsinki.

Data collection

Diagnosis of AIS was based on clinical symptoms, signs, and image manifestations (computed tomography and magnetic resonance imaging). AIS was defined as focal neurologic symptoms lasting longer than 24 h and/or symptoms consistent with new lesions on computed tomography or magnetic resonance imaging.

The evaluated clinical parameters were retrieved from medical records, physical examination, and detailed patient history, and organized into a questionnaire. This

questionnaire included information regarding Diabetes Mellitus, hiperlipidemia, hypertension, heart failure, drug regimens, and tobacco use. Dose, timing, and adherence to treatment with aspirin were also evaluated. Body Mass Index was calculated through direct measurement of weight and height. Complete hemogram, biochemical and coagulation parameters were evaluated at admission, including blood glucose, creatinine, triglyceride, total cholesterol, HDL-C, LDL-C, activated partial thromboplastin time, prothrombin time, and fibrinogen.

The National Institutes of Health Stroke Scale, Oxfordshire Community Stroke Project (OCSP) Classification, and TOAST (Trial of ORG 10,172 in Acute Stroke Treatment) criteria were used to assess the severity of the stroke, type of ischemic stroke, and cause, respectively.

Statistical analysis

Statistical analysis was performed using the computer software IBM® SPSS® Statistics for Windows (version 22.0). The categorical variables are expressed in terms of frequency and percentage, and data were compared using a Chi-square test, using a 5% level of significance.

The first blood sample at admission was used to calculate the PLR, which was defined as the ratio of the absolute platelet count and absolute lymphocyte count. [13] Thereafter, PLR values were stratified into terciles, and all the patients were further divided into those with a PLR High level (PLR≥170 and PLR Low/Intermediate (PLR<170). Further ROC analysis confirmed PLR≥170 as the cutoff for the PLR High level.

The probabilities of survival and recurrence were calculated, and the mean life tables were computed using the product-limit estimate of the Kaplan–Meier method. The

curves were examined by the log-rank test. Cox proportional hazard models were used to assess Hazard Ratio/Mortality risk (overall, 3, 12, 24 months, 5 years) and risk of recurrence (12, 24 months). Posteriorly, it was validated using a bootstrap resampling to investigate the stability of risk estimates (1000 replications). A level of $p<0.05$ was considered statistically significant.

Subsequently, the relevance of hypothetical simulating models on the probability of a second stroke within the same timeframe was examined. The proposed simulation models' predictive performance was compared using Harrell's concordance (C-index) approach, with a C-index value of >0.5 regarded good prediction ability within a maximum of 1.

Results

A total of 424 consecutive patients with ischemic stroke were included in this study. An overall description of the population under study is given in **Table 1**. Ischemic stroke subtypes were distributed as follows: LACI (42.9%), TACI (25.5%), PACI (21.2%), and POCI (14.4%). Non-neurological causes of death were the most common (infection accounted for 57% of deaths). Prior to the AIS, 142 patients had been on aspirin medication, whereas 282 patients had not. The median PLR value among the individuals was 179.9 ± 166.8.

Our results reveal that patients with high PLR are associated with a poor overall survival on stroke patients (**Figure 1**). Further analysis demonstrated that high PLR values were associated with increased mortality (HR 1.880, 95%CI 1.176 – 3.005, p = 0.008), even when adjusted for age, AIS type and sex, at 3 months (HR 4.607, 95%CI 1.472 – 14.414, p = 0.009), 12 months (HR 2.295, 95%CI 1.125 – 4.679, p = 0.022), 2

years (HR 1.864, 95% CI 1.009 – 3.443, p = 0.047) and 3 years (HR 2.213, 95%CI 1.150 – 3.919, p = 0.006) (**Table 2**).

When evaluating the groups of patients with and without record of previous aspirin therapy separately, we discovered that high values of PLR are associated with poor overall survival on the patients with no record of aspirin therapy (p = 0.018, log-rank test, n = 230), but not on the patients with previous record of aspirin therapy (p = 0.31, log-rank test, n = 115) (**Figure 2**).

Interestingly, when we analyze the impact of PLR on recurrence risk, a significant association between high values of PLR and higher risk of recurrence can only be verified in patients under the age of 71, that have been submitted to previous aspirin therapy (p = 0.024, log-rank test, n = 142) (**Figure 3**).

The results of the predictive models for the effect of patients' PLR values at admission on mortality within 3 years for stroke patients are shown in **Table 3**. High PLR values maintained a statistically significant association with poor survival, even on the most complex model (HR 2.975, 95%CI 1.197 – 7.393, p = 0.019, p boot = 0.023).

Additionally, **Table 4** reveals the effect of patients' PLR values at admission on predictive models for recurrence risk within a 3-year timeframe. PLR values were associated with higher recurrence risk in patients under 71 years old with records of previous aspirin therapy. A gain of nearly 20% in the predictive ability was observed from the simplest model 1 (C-index 0.779) to the more complex model 4 (C-index 0.946).

Discussion

Our results demonstrate that patients with AIS presenting high PLR values at admission are associated with poor overall survival. Platelet-to-lymphocyte ratio is a quick and efficient parameter to assess inflammatory response as it can be readily calculated from a

regular hemogram. Prior studies evaluating PLR noted a consistent association with unfavorable outcomes. This is congruent with our results. Reports have shown that elevated PLR is associated with increased infarct volume, worsening grades of cerebral edema at 24 hours, worse functional outcomes, hemorrhagic transformation (HT) after AIS, in-hospital mortality of HT after AIS, unsuccessful reperfusion, post-thrombolysis early neurological deterioration, and incidence of stroke-associated infection. [5, 7-11]

Platelets are anucleate, small-sized cells (~4 μm), primarily associated with hemostasis. However, there is growing recognition of the wide variety of biological roles of platelets on immune and inflammatory responses, from atherosclerosis to infectious diseases, making them the most abundant circulating cell type that has an immune function (≈200 000/μL blood in humans). [14]

Due to the serious health consequences of atherosclerosis, much has been studied regarding platelet-endothelial cell interactions. Atherosclerosis is now known to be an inflammatory disease mediated by immune cell interactions with the vascular endothelium. [15] The sub-endothelium matrix, which is rich in pro-hemostatic proteins, is exposed when the endothelial wall is altered or disrupted. Platelet adhesion at the locations of lesions is induced when platelet surface receptors interact with these matrix proteins, activating a complex intracellular signaling mechanism. This results in the production and release of transcellular mediators, the exocytosis of adhesive and inflammatory proteins, and the provision of additional adhesive receptors as well as a procoagulant surface. [16] Early research into the pathophysiology of atherosclerosis revealed that platelets elicit an inflammatory response in endothelial cells and that platelet-derived mediators enhance endothelial permeability, allowing lipid entry into the artery wall and the progression of atherosclerosis. [17-20]

Platelets and other cells have a large number of potential interactions, both direct and indirect, as a result of which platelets can exert a wide range of inflammatory effects both locally and systemically. In ischemic stroke, circulating platelets served two key functions: first, promoting circulating arterial thrombosis and embolism; and second, functioning as a prime motor of the activators stored in platelet granules (e.g., chemokines and cytokines) that mediated other peripheral blood cells. [9] A platelet contains ~60 granules that store many molecules with immune functions. There are 3 types of platelet granules: alpha (α) granules, dense (δ) granules, and lysosomal granules. [14] Alpha (α) granules contain a wide variety of proteins, including adhesive proteins like thrombospondin, von Willebrand factor, and fibronectin; growth factors like insulin-like growth factor (IGF), transforming growth factor-beta (TGF-), and platelet-derived growth factor (PDGF); platelet factor 4 (PF4); and pro-inflammatory/modulatory chemokines and cytokines. P-selectin (CD62P) is expressed on the exterior surface of platelets as a result of α-granule exocytosis. Therefore, α-granules are implicated in inflammation, atherosclerosis, angiogenesis, wound healing, antimicrobial host defense, and platelet function in malignant hematological disorders. [16] ADP, ATP, calcium, and serotonin are abundant in dense (δ) granules, which play a vital role in hemostasis. Lastly, lysosomes secrete hydrolases after platelet activation. Platelets can stimulate dendritic cell maturation, NK cell activation, and monocyte/macrophage responses, all of which modulate T and B cell responses. Additionally, they also directly impact B cell isotype switching and CD8+ T cell proliferation. [21]

White blood cells are key components of our immune system that respond in an inflammatory immunological response. The control of inflammatory cell function is important in the healing and recovery of postischemic brain injury. The body's immediate response to infection or injury is acute inflammation, and inflammation plays a significant

role in the onset and progression of atherosclerosis. [22] Leukocyte infiltration into ischemic tissue is frequently the first step in the inflammatory process, which is aided by inflammatory cytokines and chemokines. [23] Peripheral leukocytes, on the other hand, may influence ischemic tissues. Lymphocyte counts are thought to have a neuroprotective impact and aid in the rehabilitation of neurological function. [8] The induction of inflammation may be required to repair brain damage following the commencement of a stroke. On the other hand, overactivation of inflammation can injure brain structures, leading to neurological degeneration, a reduction in circulating lymphocytes, and a higher risk of infection. [23]

PLR remains balanced if the process is in a relatively tolerable physiological range. Higher PLR readings could indicate an excessive inflammatory imbalance rather than protective regulation. [23]

Interestingly, the negative effect of high PLR values on survival depends on the patient's previous status regarding aspirin therapy. In patients who have not received aspirin therapy, high PLR is an independent factor of poor prognosis. However, this association cannot be verified in patients who have records of previous aspirin treatment. Consequently, aspirin therapy positively affects the survival of patients with high PLR who have suffered an AIS, neutralizing the added risk associated with the high PLR value.

To the best of our knowledge, the association between PLR and aspirin is not entirely clear and several lines of reasoning may be considered to explain our results. Arachidonic acid (AA) is released from platelet membrane phospholipids and consequently converted to thromboxane A2 (TXA2), a prothrombotic and vasoconstrictive substance. Classically, this pathway serves as the target for aspirin. [16] As for the mechanism of action, aspirin causes acetylation of the serine residues at positions 529 and 516 of COX-1 and COX-2 proteins, respectively. This results in

enzymatic inhibition and, eventually, platelet aggregation suppression due to non-conversion of the AA into TXA2. [24] Reactive oxygen species, inflammatory cytokines, and growth factor production are reduced due to platelet aggregation inhibition's secondary effects, which diminishes the inflammatory process and, as a result, improves endothelial function. [25, 26] Aspirin can minimize the severity of a stroke by reducing the size of the clot, the extent of the thrombosis, and the resulting embolism. Furthermore, by blocking platelet-derived vasoconstrictors, aspirin's anti-inflammatory and neuroprotective effects may assist improve microcirculation in the ischemic penumbra. [12, 27] However, aspirin showed no influence in vitro on either platelet count, volume, or mass [28], which suggests that aspirin exerts its effect in a way that is not directly correlated to platelet counts. Therefore, it is not expected for aspirin to alter platelet counts nor influence PLR value through this mechanism. This fact may explain the protective effect of aspirin in patients with high PLR values.

Regarding recurrence risk, high PLR values were associated with an increased risk of a second event in patients under 71 years old with records of previous aspirin therapy. This fact was confirmed through the analysis of the predictive models in Table 4, which interestingly revealed that both high PLR levels and previous aspirin treatment were independent negative prognostic factors of stroke recurrence in this patient subgroup. Despite continued aspirin antithrombotic medication, a significant number of patients are still at risk of recurrent vascular events. These results may be explained by different lines of reasoning.

Firstly, when evaluating the causes of recurrent ischemic events, around 50% are due to atherothrombotic disease of great vessels; 25% occur secondary to the occlusion of small vessels; 20% are caused by cardiac emboli and 5-10% are explained by other causes. [29] Atherothrombosis pathophysiology is complex, involving inflammation,

thrombosis, vascular biology, and hemodynamic considerations. [15] It is not totally clear whether ASA is effective in the prevention of ischemic events caused by non-atherothrombotic pathologies, which should be carefully studied and treated accordingly.

Secondly, aspirin is currently used in the secondary prevention of cardiovascular events, which logically implies the prior occurrence of an event. Hence, the record of treatment with aspirin in younger ages could indicate a heavier burden of cardiovascular risk factors which is not being effectively attenuated by ASA treatment solely. This could justify the vulnerability to the negative impact of PLR in patients who take aspirin under the age of 71 despite ASA treatment. It can be hypothesized that these patients should be approached as a distinct subgroup when planning secondary prevention of cardiovascular events, providing particular importance to the control of cardiovascular risk factors and thorough determination of stroke etiology, as it has a relevant impact on the therapeutic decision. Further studies are needed to evaluate the best approach to these patients.

Thirdly, the term ‘‘ASA resistance’’ (AR) or ASA-nonresponsive status has been used to describe the lack of expected pharmacologic effects of ASA on platelets and the association with poor clinical outcomes, such as recurrent vascular events, in patients under treatment with ASA. [30] In these patients, it should be thoroughly evaluated therapy compliance, tachyphylaxis, drug interactions, atherosclerosis progression, effective control of cardiovascular risk factors (HTN, dyslipidemia, obesity, smoking, etc.).[30, 31] Additionally, other sources of TXA2 production through the COX-2 pathway activation in monocytes, macrophages, and megakaryocytes are increased in inflammatory states such as diabetes, smoking, and dyslipidemia, leading to TXA2 formation from lipidic peroxidation of arachidonic acid with oxygen-free radicals. [32] This fact could explain stroke recurrence despite aspirin treatment. Nevertheless, intrinsic AR may explain the failure of aspirin therapy in some patients. Its etiology is thought to

be complex, involving both genetic and nongenetic origins, despite the fact that the precise mechanisms underlying AR are still unknown. In terms of genetic determinants, there is growing evidence that polymorphisms, particularly SNPs, can impact aspirin sensitivity. Numerous SNPs in cyclooxygenase, thromboxane, and platelet receptor-related genes have been identified as potentially associated with a decline in aspirin's antithrombotic effects, according to many genetic association studies. [33]

We consider our study to have limitations, including the small number of patients, the retrospective observational nature of the study, and the lack of a control group. More data about the patients, whenever possible, may add crucial input to the study and should be considered in future studies: data on previous strokes, statin use, reperfusion therapies used, and aspirin and anticoagulant use following the acute incident. Nevertheless, several lines of evidence support the biologic plausibility of our findings and appear promise for developing novel medicines or improving treatment strategies.

Conclusions

Our results suggest that patients with AIS presenting high PLR values at admission are associated with poor overall survival. The negative effect of high PLR values on survival depends on the patient's previous status regarding aspirin therapy. Aspirin therapy positively affects the survival of patients with high PLR who have suffered an AIS, neutralizing the added risk associated with the high PLR value. Regarding recurrence risk, high PLR values were associated with an increased risk of a second event in patients under 71 years old with records of previous aspirin therapy. Further studies are needed to clarify the influence of previous aspirin therapy in these patients.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Table 1. Baseline characteristics of patients with acute ischemic stroke

Table 2. Effect of patient's PLR values at admission: overall mortality risk (HR), within 3 months, 12 months, 2 years, and 5 years for stroke patients with PLR≥170.

Table 3. Predictive models for the effect of patient's PLR values at admission on mortality within 3 years for stroke patients.

Table 4. Predictive models for the effect of patient's PLR values at admission on the risk (HR) of a second event within 3 years for stroke patients.

Figure 1. Kaplan-Meier survival curves for 424 patients with acute ischemic stroke, according to the PLR value. Time measured in months ($p = 0.007$, log-rank test, $n = 424$).

Figure 2. Kaplan-Meier survival curves for 345 patients with acute ischemic stroke, according to the aspirin treatment and PLR value. Time measured in months. (a): comparison among patients with no record of previous aspirin therapy ($p = 0.018$, log-rank test, $n = 230$), (b): comparison among patients with previous aspirin therapy ($p = 0.31$, log-rank test, $n = 115$).

Figure 3. Kaplan-Meier recurrence curves for 424 patients with acute ischemic stroke under the age of 71 years, according to the aspirin treatment and PLR value. Time measured in weeks. (a): comparison among patients with no record of previous aspirin therapy ($p = 0.363$, log-rank test, $n = 282$), (b): comparison among patients with previous aspirin therapy ($p = 0.024$, log-rank test, $n = 142$).

Table 1: Baseline characteristics of patients with acute ischemic stroke

Characteristics	All	Aspirin therapy status at admission		*p* value
		Under aspirin therapy	No record of previous aspirin therapy	
Gender	424	142 (33.5%)	282 (66.5%)	
Male	56.1%	84 (35.3%)	154 (64.7%)	0.373
Female	43.9%	58 (31.2%)	128 (68.8%)	
Age	70.35 ± 12.7	73.38 ± 11.6	68.82 ± 12.9	<0.001
BMI	27.4 ± 4.4	27.1 ± 4.4	27.6 ±4.4	0.382
DM	38.0%	14.6%	23.4%	0.261
HTN	78.5%	29.6%	48.8%	0.140
Smoking	46.4%	13.9%	32.5%	0.485
NIHSS	7.6 ± 6.6	8.7 ± 6.9	7.0 ± 6.3	0.022
Hemoglobin	13.7 ± 1.8	13.5 ± 1.6	13.8 ± 1.9	0.067
Total WBC count	9.9 ± 7.1	9.9 ± 5.7	9.9 ± 7.8	0.892
Total cholesterol	181.6 ± 49.2	171.9 ± 47.6	186.49 ± 49.3	0.005
Triglycerides	119.4 ± 65.7	114.8 ± 65.2	121.8 ± 65.9	0.313
Platelet count	229.9 ± 96.2	216.7 ± 85.7	236.6 ± 100.6	0.044
PLR	179.9 ± 166.8	170.0 ± 112.8	184.9 ± 188.6	0.387
Glucose	138.0 ± 59.5	137.7 ± 55.6	138.19 ± 61.6	0.935

Table 2: Effect of patient's PLR values at admission: overall mortality risk (HR), within 3 months, 12 months, 2 years, and 5 years for stroke patients with PLR≥170

	Survival PLR high		
	HR	95%CI	p
Overall	1.880	1.176 – 3.005	0.008
Overall*	2.315	0.003 – 2.315	0.003
3 months	2.444	1.122 – 5.321	0.024
3 months*	4.607	1.472 – 14.414	0.009
12 months	1.674	0.950 – 2.949	0.075
12 months*	2.295	1.125 – 4.679	0.022
2 years	1.578	0.942 – 2.643	0.083
2 years*	1.864	1.009 – 3.443	0.047
3 years	1.856	1.145 – 3.008	0.012
3 years*	2.213	1.150 – 3.919	0.006

*adjusted for age, AIS type, sex

Table 3: Predictive models for the effect of patient's PLR values at admission on mortality within 3 years for stroke patients.

	HR*	95%CI	p	p boot**
Model 1				
PLR high	2.213	1.125 – 3.919	0.006	0.007
Age (<71 years)	0.229	0.112 – 0.467	<0.001	0.001
Sex (F vs M)	1.137	0.626 – 2.065	0.674	0.660
Type TACI	0.912	0.721 – 1.153	0.440	0.379
Model 2				
PLR high	2.192	1.510 – 4.180	0.017	0.021
Age (<71 years)	0.229	0.135 – 0.664	0.003	0.006
Sex (F vs M)	1.089	0.529 – 2.240	0.818	0.840
Type TACI	0.923	0.711 – 1.199	0.549	0.579
Previous aspirin	1.728	0.906 – 3.297	0.097	0.116
Previous statin	0.716	0.349 – 1.470	0.363	0.365
Hemoglobin <13g/dL	1.361	0.693 – 2.671	0.371	0.423
TG >150mg/dL	0.872	0.362 – 2.102	0.761	0.758
Model 3				
PLR high	2.975	1.197 – 7.393	0.019	0.023
Age (<71 years)	0.618	0.218 – 1.750	0.365	0.443

Sex (F vs M)	1.549	0.531 – 4.518	0.423	0.410
Type TACI	1.063	0.619 – 1.825	0.825	0.858
Previous aspirin	0.555	0.207 – 1.489	0.031	0.031
Previous statin	2.796	1.099 – 7.118	0.966	0.976
Hemoglobin <13g/dL	0.763	0.290 – 2.006	0.583	0.676
TG >150mg/dL	1.227	0.386 – 3.901	0.728	0.721
Active smoking	0.486	0.145 – 1.625	0.241	0.337
BMI>30kg/m2	1.178	0.365 – 3.797	0.784	0.793
NIHSS admission >10	13.395	4.695 – 38.217	<0.001	0.001

*Cox regression analysis: Hazard ratio (HR) adjusted for age, stroke type, sex. 95% CI, 95% confidence interval; **after bootstrap based on 1000 samples.

Table 4: Predictive models for the effect of patient's PLR values at admission on the risk (HR) of a second event within 3 years for stroke patients.

	HR*	95%CI	p	p boot**	C-index
Model 1					0.779
Previous aspirin	7.009	1.889 – 26.006	0.004	0.005	
Type TACI	0.694	0.154 – 3.122	0.634	0.497	
Previous AIS	1.283	0.154 – 3.122	0.391	0.437	
Model 2					0.886
Previous aspirin	7.870	1.399 – 44.282	0.019	0.001	
Type TACI	0.273	0.031 – 2.451	0.247	0.159	
Previous AIS	0.582	0.203 – 1.668	0.314	0.299	
Age (<71 years)	0.188	0.029 – 1.222	0.080	0.075	
Sex (F vs M)	2.356	0.421 – 13.184	0.329	0.330	
Previous statin	0.582	0.100 – 3.392	0.547	0.521	
Atrial fibrillation	0.995	0.165 – 6.009	0.995	0.995	
Model 3					0.880
Type TACI	0.515	0.061 – 4.381	0.543	0.318	
Previous AIS	0.634	0.276 – 1.460	0.284	0.262	
Age (<71 years)	0.243	0.056 – 1.048	0.058	0.035	
Sex (F vs M)	1.849	0.368 – 9.291	0.456	0.418	
Previous statin	2.477	0.595 – 10.312	0.213	0.206	
Atrial fibrillation	0.593	0.102 – 3.440	0.560	0.484	
PLR high	9.930	1.973 – 49.980	0.005	0.001	

Model 4					0.946
Previous aspirin	15.928	2.220 – 114,265	0.006	0.002	
Type TACI	0.145	0.012 – 1.727	0.126	0.046	
Previous AIS	0.437	0.146 – 1.308	0.139	0.150	
Age (<71 years)	0.283	0.042 – 1.901	0.194	0.151	
Sex (F vs M)	1.398	0.181 – 10.821	0.748	0.594	
Previous statin	0.728	0.117 – 4.513	0.733	0.687	
Atrial fibrillation	0.906	0.125 – 6.570	0.922	0.830	
PLR high	15.559	2.419 – 100.071	0.004	0.001	

*Cox regression analysis: Hazard ratio (HR) adjusted for age, stroke type, sex. 95% CI, 95% confidence interval; **after bootstrap based on 1000 samples.

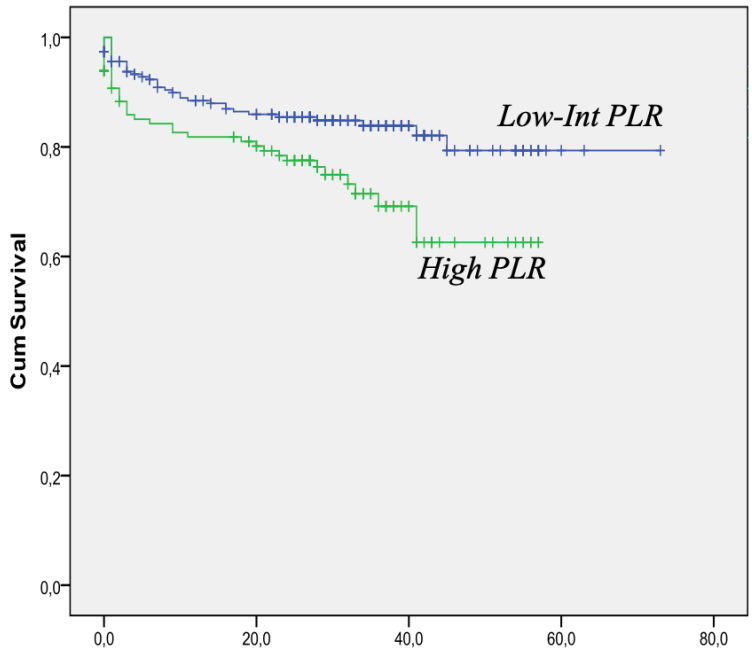

Figure 1: Kaplan-Meier survival curves for 424 patients with acute ischemic stroke, according to the PLR value. Time measured in months (p = 0.007, log-rank test, n = 424).

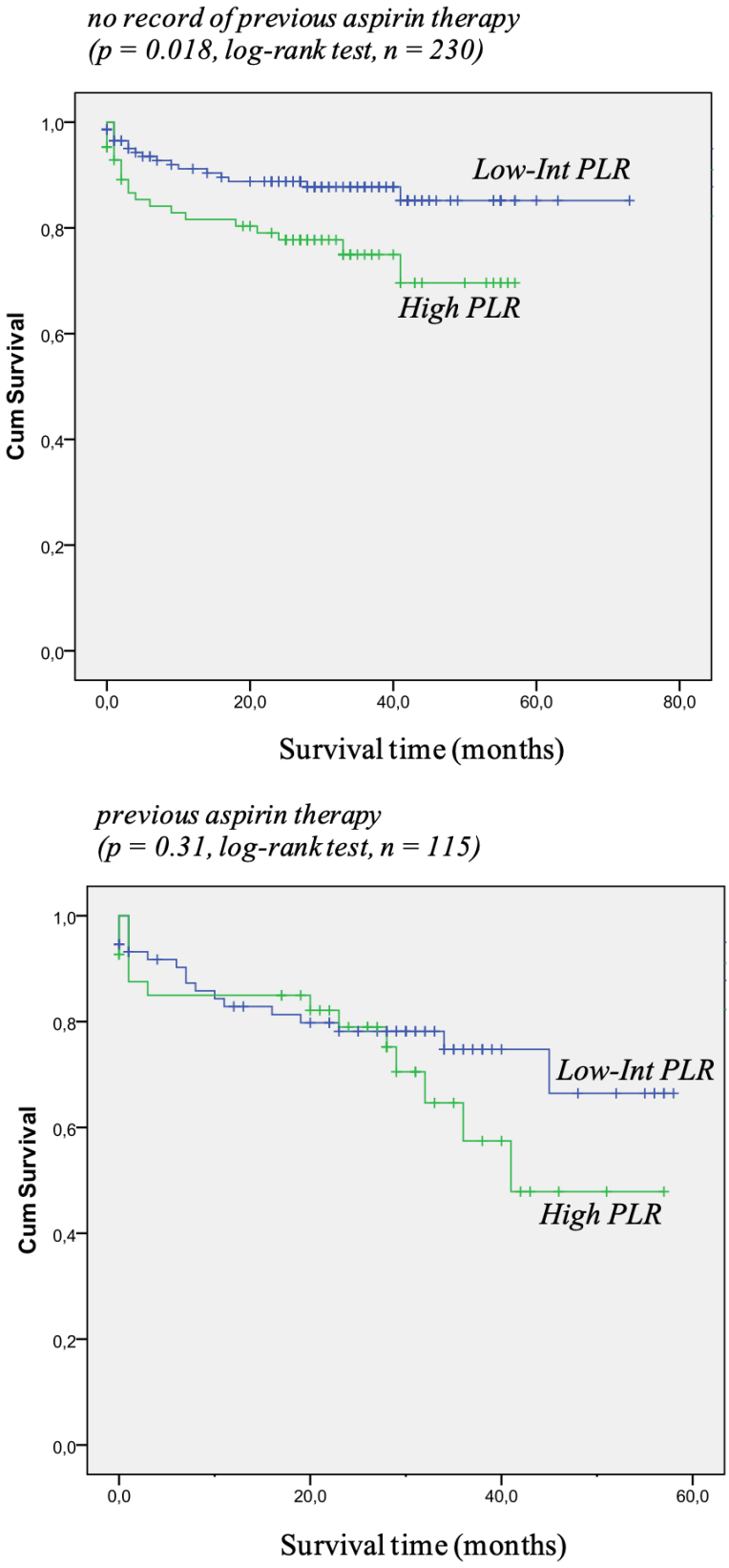

Figure 2: Kaplan-Meier survival curves for 345 patients with acute ischemic stroke, according to the aspirin treatment and PLR value. Time measured in months. (a): comparison among patients with no record of previous aspirin therapy (p = 0.018, log-rank test, n = 230), (b): comparison among patients with previous aspirin therapy (p = 0.31, log-rank test, n = 115).

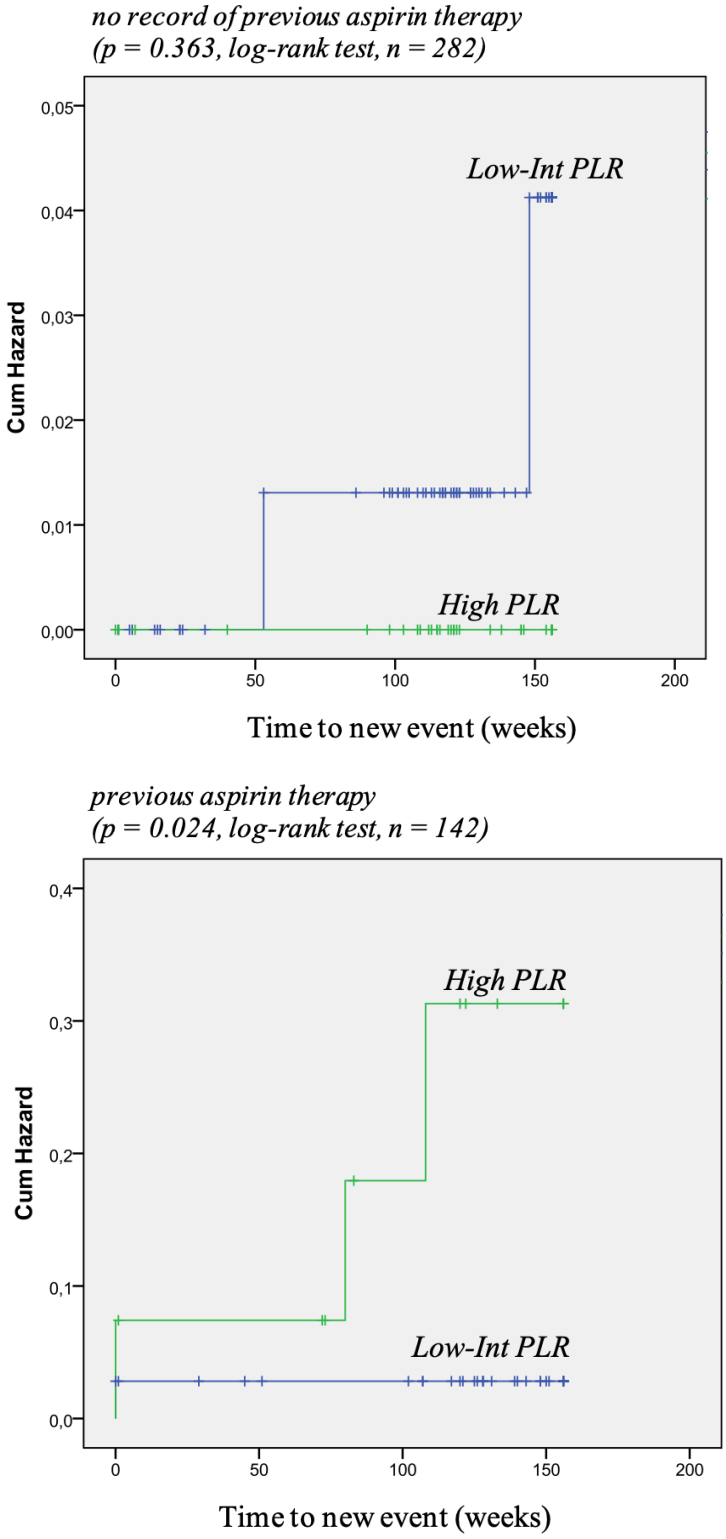

Figure 3: Kaplan-Meier recurrence curves for 424 patients with acute ischemic stroke under the age of 71 years, according to the aspirin treatment and PLR value. Time measured in weeks. (a): comparison among patients with no record of previous aspirin therapy (p = 0.363, log-rank test, n = 282), (b): comparison among patients with previous aspirin therapy (p = 0.024, log-rank test, n = 142).

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2	"424 patients were included in this observational study"
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2	"The effect of PLR on mortality and stroke recurrence was evaluated using Kaplan-Meier methodology, log-rank test, Cox proportional hazard models, and Bootstrap Analysis." "Our results indicate an association of high PLR (>170) with a poor overall survival on stroke patients"
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2, 3	"Prior studies evaluating PLR noted a consistent association with unfavorable outcomes. Reports have shown that elevated PLR is associated with increased infarct volume, (…) and incidence of stroke-associated infection." "Aspirin can reduce the severity of a stroke by decreasing the size of the clot, the extent of the thrombosis, and the ensuing embolism. In addition, aspirin's anti-inflammatory and neuroprotective properties may help enhance microcirculation in the ischemic penumbra by inhibiting platelet-derived vasoconstrictors."
Objectives	3	State specific objectives, including any prespecified hypotheses	3	"This study aims to explore the relationship between aspirin and PLR and the consequent effects on mortality and stroke recurrence in patients with AIS."
Methods				
Study design	4	Present key elements of study design early in the paper	3	"This observational cohort study with post-hoc analysis (…)"
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3	"This (…) study (…) includes 424 consecutive patients diagnosed with AIS admitted in the Department of Internal Medicine of Centro Hospitalar São João from September 2009 to April 2012."
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility	3	"The inclusion criteria were patients over 18 years old admitted for AIS. Patients with acute haemorrhagic stroke or neurological deficits associated with space occupying lesions, whether primary CNS neoplasm or secondary unknown tumour, were excluded from the study. Written informed consent was signed

		criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants		by all patients or their legal representatives. After recruitment, a follow-up protocol was set up."
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	4	"Prior to the AIS, 142 patients had been on aspirin medication, whereas 282 patients had not. The median PLR value among the individuals was 179.9 ± 166.8."
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	3	"Diagnosis of AIS was based on clinical symptoms, signs, and image manifestations (computed tomography and magnetic resonance imaging). AIS was defined as focal neurologic symptoms lasting longer than 24 h and/or symptoms consistent with new lesions on computed tomography or magnetic resonance imaging." "PLR, which was defined as the ratio of the absolute platelet count and absolute lymphocyte count."
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	3	"The evaluated clinical parameters were retrieved from medical records, physical examination, and detailed patient history, and organized into a questionnaire. This questionnaire included information regarding Diabetes Mellitus, (…) and fibrinogen." "The National Institutes of Health Stroke Scale, Oxfordshire Community Stroke Project (OCSP) Classification, and TOAST (Trial of ORG 10,172 in Acute Stroke Treatment) criteria were used to assess the severity of the stroke, type of ischemic stroke, and cause, respectively."
Bias	9	Describe any efforts to address potential sources of bias	3	"The evaluated clinical parameters were retrieved from medical records, physical examination, and detailed patient history, and organized into a questionnaire. This questionnaire included information regarding Diabetes Mellitus, hiperlipidemia, hypertension, heart failure, drug regimens, and tobacco use. Dose, timing, and adherence to treatment with aspirin were also evaluated. Body Mass Index was calculated through direct measurement of weight and height. Complete hemogram, biochemical and coagulation parameters were evaluated at admission, including blood glucose, creatinine, triglyceride, total cholesterol, HDL-C, LDL-C, activated partial thromboplastin time,

			prothrombin time, and fibrinogen."
Study size	10	Explain how the study size was arrived at	"This observational study with post-hoc analysis includes 424 consecutive patients diagnosed with AIS admitted in the Department of Internal Medicine of Centro Hospitalar São João from September 2009 to April 2012."

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	3	"PLR values were stratified into terciles, and all the patients were further divided into those with a PLR High level (PLR≥170 and PLR Low/Intermediate (PLR<170)."
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	3, 4	"The categorical variables are expressed in terms of frequency and percentage, and data were compared using a Chi-square test, using a 5% level of significance." "The probabilities of survival and recurrence were calculated, and the mean life tables were computed using the product-limit estimate of the Kaplan–Meier method. The curves were examined by the log-rank test. Cox proportional hazard models were used to assess Hazard Ratio/Mortality risk."
		(*b*) Describe any methods used to examine subgroups and interactions		*Not applicable – methods fully described previously*
		(*c*) Explain how missing data were addressed		*Not applicable – available data were thoroughly analysed*
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy		*Not applicable*
		(*e*) Describe any sensitivity analyses	4	"Posteriorly, it was validated using a bootstrap resampling to investigate the stability of risk estimates (1000 replications). A level of $p<0.05$ was considered statistically significant." "The proposed simulation models' predictive performance was compared using Harrell's concordance (C-index) approach, with a C-index value of >0.5 regarded good prediction ability within a maximum of 1."
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	4	"A total of 424 consecutive patients with first-ever ischemic stroke were included in this study"
		(b) Give reasons for non-participation at each stage		*Not applicable – written consent was obtained for all patients*
		(c) Consider use of a flow diagram		*Not applicable*
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	4	"An overall description of the population under study is given in Table 1. Ischemic stroke subtypes were distributed as follows: LACI (42.9%), TACI (25.5%), PACI (21.2%), and POCI (14.4%). Non-

				neurological causes of death were the most common (infection accounted for 57% of deaths). Prior to the AIS, 142 patients had been on aspirin medication, whereas 282 patients had not."
		(b) Indicate number of participants with missing data for each variable of interest		*Not applicable – available data were thoroughly analysed*
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)		*Not applicable*
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	5	"Further analysis demonstrated that high PLR values were associated with increased mortality (HR 1.880, 95%CI 1.176 – 3.005, p = 0.008), even when adjusted for age, AIS type and sex, at 3 months (HR 4.607, 95%CI 1.472 – 14.414, p = 0.009), 12 months (HR 2.295, 95%CI 1.125 – 4.679, p = 0.022), 2 years (HR 1.864, 95% CI 1.009 – 3.443, p = 0.047) and 3 years (HR 2.213, 95%CI 1.150 – 3.919, p = 0.006) (Table 2)."
		Case-control study—Report numbers in each exposure category, or summary measures of exposure		
		Cross-sectional study—Report numbers of outcome events or summary measures		
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	4	"high PLR values were associated with increased mortality (HR 1.880, 95%CI 1.176 – 3.005, p = 0.008), even when adjusted for age, AIS type and sex, at 3 months (HR 4.607, 95%CI 1.472 – 14.414, p = 0.009), 12 months (HR 2.295, 95%CI 1.125 – 4.679, p = 0.022), 2 years (HR 1.864, 95% CI 1.009 – 3.443, p = 0.047) and 3 years (HR 2.213, 95%CI 1.150 – 3.919, p = 0.006)"
		(*b*) Report category boundaries when continuous variables were categorized		*Not applicable – only dichotomic outcomes were evaluated*
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period		*Not applicable – results are already provided within a described timeframe*

Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	5	“When evaluating the groups of patients with and without record of previous aspirin therapy separately, we discovered that high values of PLR are associated with poor overall survival on the patients with no record of aspirin therapy (p = 0.018, log-rank test, n = 230), but not on the patients with previous record of aspirin therapy (p = 0.31, log-rank test, n = 115) (Figure 2).”
Discussion				
Key results	18	Summarise key results with reference to study objectives	9, 10	“Our results demonstrate that patients with AIS presenting high PLR values at admission are associated with poor overall survival.” “In patients who have not received aspirin therapy, high PLR is an independent factor of poor prognosis. However, this association cannot be verified in patients who have records of previous aspirin treatment.” “Regarding recurrence risk, high PLR values were associated with an increased risk of a second event in patients under 71 years old with records of previous aspirin therapy.”
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11	“We consider our study to have limitations, including the small number of patients, the retrospective observational nature of the study, and the lack of a control group. More data about the patients, whenever possible, may add crucial input to the study and should be considered in future studies: data on previous strokes, statin use, reperfusion therapies used, and aspirin and anticoagulant use following the acute incident. Nevertheless, several lines of evidence support the biologic plausibility of our findings and appear promise for developing novel medicines or improving treatment strategies.”
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	8	“Our results demonstrate that patients with AIS presenting high PLR values at admission are associated with poor overall survival. Platelet-to-lymphocyte ratio is a quick and efficient parameter to assess inflammatory response as it can be readily calculated from a regular hemogram. Prior studies evaluating PLR noted a consistent association with unfavorable outcomes. This is congruent with our results.”
Generalisability	21	Discuss the generalisability (external validity) of the study results	11	“Several lines of evidence reinforce the biologic plausibility of our results and seem to be promising to develop new treatments or improve treatment strategies.”
Other information				
Funding	22	Give the source of funding and the role of the	11	“The authors acknowledge the funding support of this work by the

	funders for the present study and, if applicable, for the original study on which the present article is based	Minister of Health of Portugal (CFICS-80/2007), and the Faculty of Medicine of Porto and Centro Hospitalar Universitário São João.”

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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