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Neutrophil to-lymphocyte and platelet-to-lymphocyte ratios as biomarkers for suicidal behavior in children and adolescents with depression or anxiety treated with selective serotonin reuptake inhibitors

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Abstract

Background: Both the neutrophil/lymphocyte ratio (NLR) and the platelet/lymphocyte ratio (PLR) have been proposed as biomarkers of suicidal risk in adults with depression. We examined whether these ratios may be considered biomarkers for suicidal behavior in young patients with major depressive or anxiety disorders before treatment with selective serotonin reuptake inhibitors (SSRIs), or as biomarkers for the adverse event of SSRI-associated suicidality.

Methods: Children and adolescents meeting criteria for major depressive or anxiety disorder were recruited. Serum levels of three pro-inflammatory cytokines (TNF-α, IL-6, IL-1β) were assessed; and NLR and PLR calculated, from blood samples collected at baseline and after 8 weeks treatment with SSRI. A Mann-Whitney test was performed to evaluate differences in NLR and PLR between children with and without a history of a suicide attempt prior to treatment. We compared hematological parameters before and after treatment, and between children who developed SSRI-associated suicidality versus children without treatment emergent suicidality.

Results: Among 91 children and adolescents (aged 13.9 ± 2.4 years), baseline NLR and PLR were significantly higher among those with a history of a suicide attempt versus those without such history. Statistically significant correlations were found for the suicide ideation subscale in the Columbia suicide severity rating scale with both baseline NLR and PLR. Baseline NLR and PLR were similar in children who did and did not develop SSRI-associated suicidality after 8 weeks. In the final logistic regression model (χ^2=18.504, df=4, p value=0.001), after controlling for sex, depression severity and IL-6 levels, NLR was significantly associated with a past

suicide attempt (β = 1.247, p = 0.019; OR [95% CI] = 3.478 [1.230-9.841]), with a NLR cut-off value of = 1.76 (area under the curve=0.75 (95% CI = 0.63-0.88, sensitivity = 73%, and specificity =71%, p value=0.003).

Conclusions: High NLR and PLR values may be associated with suicidal behavior in depressed and anxious children and adolescents. NLR appears as a better predictor of suicide attempt than PLR, and thus may be a useful biomarker of suicidality in young patients with depression or anxiety.

Keywords children, adolescents, NLR, PLR, suicidality

1. Introduction

Adolescent suicide is a serious public health problem, as suicide is the second cause of death globally among adolescents (WHO, 2021). In youth, a history of suicidal behavior (SB) is associated with increased risk of repeated suicidal attempts, and also an increased risk of subsequent death (Bergen 2012; Hawton et al, 2012). About one of three children and adolescents who initially attempt suicide at age 10-18 years eventually attempt a second time (Rosenbaum et al, 2017). Although most adolescent attempters do not die from the attempt, the event is almost always associated with significant deterioration in quality of life.

In investigations of potential biological markers of SB (Lin and Kim, 2011), the role of the immune system and inflammation has received particular attention; and a comprehensive model focusing on the influence of the immune system on the pathophysiology of SB was proposed (Courtet et al, 2016). Accordingly, the perception of threat that leads to contemplation of suicide may activate biological stress responses, including inflammatory responses. Abnormal levels of inflammatory cytokines such as IL-1β and IL-6 have been proposed as potential predictors of suicide attempt (SA) in individuals with major depressive disorder (MDD) (Black and Miller, 2015). Another inflammatory biomarker that has gained much attention is C-reactive protein (CRP), due to its long half-life and detectability at low levels (O'Donovnan et al, 2013).

Assessments of the neutrophil/lymphocyte ratio (NLR) and the platelet/lymphocyte ratio (PLR) in peripheral blood represent an easy method to evaluate inflammatory status (Marazziti et al, 2021). This is due to the ubiquitousness of these measures in basic blood work and the assessment without the requirement of external kits or new equipment. NLR has been proposed as a marker of low-grade inflammation and a predictor of clinical outcomes in several diseases including cardiovascular disease

(Azar et al, 2010), Alzheimer's disease (Rembach et al, 2014), Parkinson's disease (Akil et al, 2015) and MDD (Demir et al, 2015). NLR has also been proposed as a convenient and inexpensive marker of SB, and a biomarker of suicidal risk in individuals with depressive episode, both in bipolar disorder (Ivkovic et al., 2016) and unipolar MDD (Ekinci and Ekinci, 2017; Velasco et al., 2020; Vos et al, 2021).

PLR has been studied extensively in the context of mood disorders (Mazza et al, 2018; Bulut et al, 2021). This parameter has been suggested as a better predictor than NLR of the prognosis of major depression (Kayhan et al, 2016). The possible association of PLT with suicidality is less conclusive (Velasco et al, 2020).

Given the inflammatory mechanisms involved in the pathophysiology of SBs, and the relevant literature, the present study aimed to evaluate inflammatory ratios, namely NLR and PLR, in children and adolescents with MDD or anxiety disorder treated with selective serotonin reuptake inhibitors (SSRIs). The purposes of the current study were twofold. First, we examined whether NLR or PLR may be considered biomarkers for SB in adolescents with MDD or anxiety disorder before treatment. Second, we assessed whether NLR or PLR may serve as biomarkers for treatment response and for SSRI-associated suicidality.

2. Methods

2.1 Participants

For a comprehensive summary of the study design and the eligibility criteria, see Amitai et al, 2019. Briefly, children and adolescents with depression or anxiety disorders were recruited and treated with fluoxetine for 8 weeks.

2.2 Assessment

All the participants were assessed for socioeconomic and clinical data (sex, age, BMI). Response to treatment was measured with the Clinical Global Impressions–Improvement scale (Guy, 1976). Suicidality was assessed using the Columbia Suicide Severity Rating Scale (C-SSRS), at baseline (before starting treatment) and after 8 weeks of fluoxetine treatment. This questionnaire is designed to standardize the assessment of a broad range of SB, including the severity and intensity of suicide ideation (SI), SA, and SA lethality (Posner et al., 2011) Any elevation in score was considered worsening in suicidality.

Written informed consent was obtained from all the participants and their parents. The study received institutional approval.

Peripheral venous samples were collected between 8:00 and 9:00 a.m. in EDTA tubes. Complete blood counts were performed using a Sysmex XN-10/XN-20 Hematology Analyzer (Norderstedt, Germany). Complete blood counts included total white blood cells, neutrophils, lymphocytes, monocytes, and platelets. The NLR and PLR were calculated from complete blood parameters. Levels of three pro-inflammatory cytokines (TNFα, IL-6, IL-1β) were also assessed, as previously described (Amitai et al, 2019).

2.3 Statistical analyses

The data were analyzed using SPSS 18.0 (SPSS Inc., Chicago, IL). Data are presented as mean± standard deviation (SD) for numeric variables and as frequencies and percentages for categorical variables. Outliers (more than 2 SD away from the mean) in hematological values were removed from the final analyses. A Kolmogorov–Smirnov normality test was used to determine normal distribution of the variables. A chi-square (χ 2) test was used to compare categorical variables and frequencies. To analyze abnormally distributed variables, the Mann–Whitney U test was used for

independent variables, and the Wilcoxon test for dependent variables. Spearman's correlation test was used to correlate two independent variables. A receiver operating characteristic (ROC) curve analysis was performed to determine the cut-off level of biomarkers aimed to detect SA. The level of statistical significance was set at $\alpha = 0.05$ (two-sided). A stepwise logistic regression was performed to detect the independent variables associated with the risk of SA in the entire sample while using a history of SA (yes/no) as a dependent variable. Gender, NLR, CDRS-R, and IL-6 were inserted to the model as the independent variables (PLR and age were inserted due to high correlation with NLR). The model was run to assess the predictive value of IL-6 and NLR on SA.

3. Results

3.1 The study population

The final sample included 91 children and adolescents (56 females, 62%), aged 6-18 (13.9±2.42) years with DSM-5 MDD/anxiety diagnoses. The mean body mass index (BMI) at baseline was 20.34 ± 4.09 kg/m^2. Table 1 summarizes the complete blood parameters of the participants.

Table 1: Complete blood parameters and inflammatory ratios in the entire sample, before and after 8 weeks of fluoxetine treatment.

Mean ± SD	Baseline	After 8 weeks	P value (Wilcoxon test)
WBC count (X10^{3}cell/mm^3)	6.38 ± 1.87	6.26 ± 1.92	0.684
Neutrophil count (X10^{3}cell/mm^3)	3.44 ± 1.41	3.69 ± 1.61	0.819

Monocyte count ($X10^3$cell/mm^3)	0.40 ± 0.21	0.44 ± 0.19	0.140
Lymphocyte count ($X10^3$cell/mm^3)	2.12 ± 0.59	2.07 ± 0.74	0.989
Platelet count (Cell/mm^3)	270.90 ± 77.04	272.74 ± 64.71	0.420
NLR	1.70 ± 0.71	2.10 ± 1.53	0.841
PLR	136.59 ± 49.92	149.57 ± 68.58	0.925

Abbreviations: SD, standard deviation; BMI; WBC, white blood cell; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio.

Between baseline and 8-weeks post-treatment, mean NLR and PLR values increased but not statistically significant; from 1.70 ± 0.71 to 2.10 ± 1.53, and from 136.59 ± 49.92 to 149.57 ± 68.58, respectively.

Strong positive correlations were found between PLR and NLR, both at baseline (Spearman's correlation: $r=0.508$, $p<0.001$) and after 8 weeks treatment (Spearman's correlation: $r=0.598$, $p=0.001$).

A positive correlation was observed between baseline NLR and age (Spearman's correlation: $r=0.521$, $p=0.001$, corrected p value= 0.002). No such correlation was observed after 8 weeks treatment (data not shown). No correlation was observed between PLR and age.

NLR was higher in females than males; however, this difference was not statistically significant after false discovery rate correction (data not shown). A significant difference was not observed in PLR between females and males.

Statistically significant correlations were not found between NLR or PLR and BMI (data not shown).

3.2 NLR/PLR and depression scores

Children's Depression Rating Scale-Revised (CDRS-R) scores at baseline and at 8-weeks post-treatment were not found to correlate with NLR or PLR values at the respective times (data not shown). NLR and PLR values at baseline and after 8 weeks did not differ between responders and non-responders (Mann Whitney test, data not shown).

3.3 NLR/PLR and suicidality

Twenty-two participants (15 females and 7 males [65% of the total sample]) had a history of SA according to the C-SSRS. Statistically significant differences were not observed in age between children with and without a history of SA (data not shown). Compared to participants without a history of SA, among those with a history of SA, the mean depression severity (CDRS-R score) was higher: 68.37 ± 16.17 vs. 57.31 ± 17.86 [Mann Whitney: $p=0.016$]) and the C-SSRS SI intensity score at baseline was higher (18.09 ± 6.29 vs. 9.05 ± 8.57 [Mann Whitney: $p<0.001$]). Table 2 summarizes the mean ± SD of complete blood parameters and inflammatory ratios in participants with a history of a SA vs. those without a history of a SA.

Table 2: Complete blood parameters and inflammatory ratios in patients with versus without a history of a suicide attempt (SA) according to the C-SSRS evaluation at baseline.

Mean ± SD	No past SA	Past SA	p value (Mann-Whitney test)
WBC counts ($x10^3 cell/mm^3$)	6.28 ± 1.79	6.64 ± 2.11	0.671

Neutrophil count ($X10^3$cell/mm^3)	3.26 ± 1.32	3.95 ± 1.54	0.094
Monocyte count ($X10^3$cell/mm^3)	0.40 ± 0.23	0.40 ± 0.16	0.933
Lymphocyte count ($X10^3$cell/mm^3) (Mean ± SD)	2.19 ± 0.56	1.91 ± 0.64	0.076
Platelet count (cell/mm^3)	267.24 ± 81.28	281.24 ± 64.69	0.545
NLR	1.54 ± 0.62	2.16 ± 0.78	0.001**
PLR	128.37 ± 45.26	159.31 ± 53.98	0.044*

Abbreviations: C-SSRS, Columbia suicide severity rating scale; SA, suicide attempt; SD, standard deviation; WBC, white blood cell; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio.

A statistically significant correlation was found between the SI subscale in the C-SSRS and baseline NLR (Spearman's correlation: r=0.338, p value= 0.007, corrected p value=0.001 (Figure 1A). No correlation was observed between baseline NLR and CSSR-S at 8 weeks follow-up (Spearman's correlation: r=0.385, p value= 0.002). A positive correlation was observed between baseline PLR and the baseline SI subscale in the C-SSRS (Spearman's correlation: r=0.338, p value= 0.008) (Figure 1B).

The mean baseline NLR was lower among children with than without a history of SA: 1.64 ± 0.96 vs. 2.16 ± 0.78, p value=0.001, Mann-Whitney test (Figure 2A). The mean

baseline PLR was higher among those with than without a history of SA: 159.31 ± 53.98 vs. 133.56 ± 58.18, p value=0.044, Mann-Whitney test (Figure 2B).

According to the ROC curve analysis (Figure 3A), the optimal cutoff value for NLR in predicting SA was 1.76 (area under the curve [AUC] =0.75 (95% CI = 0.63-0.88, sensitivity = 73%, and specificity =71%, p value=0.003). The optimal cutoff value for PLR in predicting SA was 139.5 (AUC =0.64, 95% CI = 0.49-0.80, sensitivity = 69%, and specificity =64%, p value=0.09, see figure 3B). The multiplication of both values did not add further information to the prediction; the cut-off value was 199 (AUC =0.74, 95% CI = 0.62-0.87, sensitivity = 82% and specificity =62%, p value=0.003, see figure 3C).

3.4 NLR and PLR as biomarkers of SSRI-associated suicidality

Thirty-one children (34%) developed SSRI-associated suicidality after 8 weeks of treatment, according to the C-SSRS follow-up questionnaire. Four of them committed a SA. No differences were detected in NLR or PLR at baseline between children who developed SSRI-suicidality and those who did not (data not shown), and neither were they detected in the NLR or the PLR after 8 weeks of treatment between those with or without SSRI-associated suicidality.

3.5 Cytokines and NLR/PLR

Neither NLR nor PLR correlated with any of the three pro-inflammatory cytokines examined.

In a stepwise logistic regression model, gender, CDRS-S score at baseline, transformed IL-6 levels and NLR were inserted to the model. Only NLR and depression severity (as measured by the CDRS-R) were significant in the final model (χ^2=18.504, df=4, p value=0.001, Table 3).

Table 3: Logistic regression predicting suicide attempt in the final cohort.

	β	SE	Wald χ^2 df=1	OR (95% CI)	P value
constant	-7.29	1.940	14.106		<0.001
Gender	-0.166	0.818	0.041	0.847 (0.171-4.211)	0.767
CDRS-R	0.063	0.023	7.722	1.065 (1.019-1.114)	0.005
Transformed IL-6 levels	-0.016	0.325	0.002	0.984 (0.520-1.862)	0.961
NLR	1.247	0.531	5.520	3.478(1.230-9.841)	0.019

Abbreviations: NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio. SA – suicide attempt; CDRS-S, children's depression rating scale, revised.

4. Discussion

In this study we analyzed NLR and PLR in children and adolescents with depression or anxiety disorders treated with SSRIs. While most studies on this subject were conducted in the adult population, we showed the usefulness of the NLR and PLR in children and adolescents. An advantage of studying younger populations is that children lack many confounding factors that may affect NLR and PLR levels, such as drug addiction and physical illnesses. In addition, obesity and common medications that mediate immune function are also less frequent in children than in adults. Moreover, children usually experience first episodes of depression, in contrast to recurrent episodes in adults. Thus, in pediatric populations, neuroprogression is less relevant; i.e. changes occurring over time can create a new allostasis with regard to NLR and PLR levels with each recurrent

episode. These reoccurring episodes may increase markers associated with apoptosis, neurotoxicity, decreased neuroplasticity and increased oxidative damage (Anderson et al, 2015), which also affect NLR and PLR levels.

According to our findings, NLR and PLR, especially NLR, could represent longstanding markers of SB in individuals with depression and anxiety. Our results also suggest that NLR and PLR may be more closely related to suicidality than to depression, meaning that more severe inflammatory dysregulation may occur in suicidality. We report higher values of both NLR and PLR in suicidal vs. non-suicidal children and adolescents with depression and anxiety. Thus, these ratios may be a marker of SA in this population. Both PLR and NLR were correlated with the baseline SI subscale in the C-SSRS. Moreover, in a logistic regression, we showed that NLR is a better predictor of suicidality than IL-6, as NLR was identified as the main factor that predicts a SA in the study population (depression severity being the second variable).

Our results corroborate studies of individuals with MDD, which demonstrated higher NLR among those with than without a history of SA; and compared to healthy control groups (Ekinci and Ekinci, 2017; Velasco et al., 2020; Puangrsi et al, 2021). Our results are similar to those reported in adults by Valescto et al (2020), suggesting a cut-off value of 1.3 of NLR with high sensitivity (75%) but low specificity (35%). According to our data, NLR as a predictive biomarker for SA had a cut-off value of 1.76, with a sensitivity of 73%, and specificity of 71%. This indicates more clinical relevance in children and adolescent populations than in adults. PLR was also predictive of SA but with a lower area under the curve than NLR. PLR did not add predictive validity to the ROC curve model. Also, in a logistic regression, only NLR was regarded as an independent predictor of SB.

Elevated NLR suggests an imbalance in favor of innate immunity, as neutrophils are components of the first line of innate immune defense, while lymphocytes are primarily involved in the adaptive immune response. Therefore, greater activation of the innate immune response, with respect to adaptive immune response, is an indicator of current active, systemic inflammatory processes in circulation (or stemming from specific affected tissues and entering the circulation). Thus, changes in NLR reflect this balance between neutrophil and lymphocyte counts (Rimmele et al, 2016). Of note, NLR was originally conceptualized as a parameter that reflects the intensity of stress or systemic inflammation in critically ill patients (Zahorec et al, 2001). Thus elevated NLR might reflect the response of the immune system to the stress associated with the suicidal state; or alternatively, suggest a pathophysiological condition leading to immune system activation and suicidality.

The higher correlation of NLR than PLR with suicidality, despite the high correlation between the two ratios, is interesting and deserves further discussion. Velasco et al (2020) also found no association between PLR and suicide risk.

Another interesting point is the lack of correlations of cytokine levels with NLR and PLR in our sample. Cytokines are major mediators of inflammation (see reviews by Black and Miller, 2016; Kim et al, 2016). Cytokines are usually maintained at low levels under physiological conditions (Pitossi et al., 1997). However, when the microenvironment in the central nervous system is altered by an injury such as trauma, infection, or ischemic attack, cytokines are activated by glial cells (Kim et al, 2016). Of note, pro-inflammatory cytokines mediate changes in neurotransmission, in particular on serotonin (5-HT) synthesis and metabolism inducing synaptic plasticity dysfunction (Chou et al, 2016).

Pro-inflammatory cytokines have a crucial role in the pathophysiology of psychiatric illnesses such as MDD (Miller et al, 2009) and suicidality (Black and Miller, 2016). Levels of proinflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNFα) have been reported as increased in the plasma, serum, and cerebrospinal fluid of people who are suicidal (Serafini et al, 2013). Previously, we showed that IL-6 is a biomarker for SSRI-associated suicidality in children and adolescents treated with SSRIs (Amitai et al, 2019; Amitai et al, 2020). Thus, the lack of association between NLR and cytokine levels found in the current study was unexpected. However, it is important to note that NLR and PLR were correlated in this study with a history of SA and not with SSRI-associated suicidality. Thus, pro-inflammatory cytokines may be a state marker, indicating current immune activation, while the NLR may be a trait marker for suicidality.

This study has a number of strengths. To our knowledge, it is the largest cross-sectional study to compare peripheral inflammatory variables in children and adolescents treated with SSRIs. In addition to hematological markers, we analyzed pro-inflammatory cytokine levels, and administrated validated questionnaires, to analyze symptom severity and correlations with inflammatory markers. A strength of the research is the unique study group and the two time points analyzed.

A number of limitations should be discussed. We did not include healthy controls. Important variables related to the immune system, such as CRP, were not included in the analysis. Despite the levels of the three pro-inflammatory cytokines analyzed, other important cytokines were not included. Complex networks of cytokines, rather than individual cytokines, should be examined in the investigation of a complex and heterogeneous phenomenon such as suicidality. Other immune-inflammatory pathways should also be investigated, including changes in oxidative stress and neuroregulatory

tryptophan catabolites, which we did not assess in this study. Despite these limitations, we were able to suggest a cut-off with prognostic value.

5. Conclusion

In summary, the findings of this study suggest that NLR and PLR might be involved in SB of children and adolescents with depression and anxiety. This supports a role of NLR as a measure of inflammation in children and adolescent suicidality, and may contribute to better understanding of the mechanisms that link inflammatory processes to SBs. NLR appeared more predictive than PLR of SAs and may be a useful biomarker to predict suicidality in individuals with MDD, in an emergency setting, due to its accessibility and low costs. Our study also supports conceptualizing suicidality as a multi-system disorder. Much research remains to be undertaken to clarify the cause-and-effect relationship between suicidality and neuroinflammation.

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Figure legends

Figure 1: Correlations of baseline assessments of the suicide ideation subscale of the C-SSRS and inflammatory markers. (A) A position correlation was found between this subscale and the NLR (Spearman's correlation: r=0.385, p value= 0.002). (B) A positive correlation was found of this subscale with the PLR (Spearman's correlation: r=0.338, p value= 0.008).

Abbreviations: C-SSRS, Columbia suicide severity rating scale; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio.

Figure 2: Baseline inflammatory marker levels between children with a history of SA in the past and those without a history of a SA. (A) A statistically significant difference was found in baseline NLR levels between children with a history of SA (n=23) and those without (Mann-Whitney: no SA vs. SA: 1.64 ± 0.96 vs. 2.16 ± 0.78, p value=0.001), (B) The mean PLR level at baseline was higher among those with than without a history of a SA (Mann-Whitney: SA vs. no SA: 133.56 ± 58.18 vs. 159.31 ± 53.98, p value=0.044).

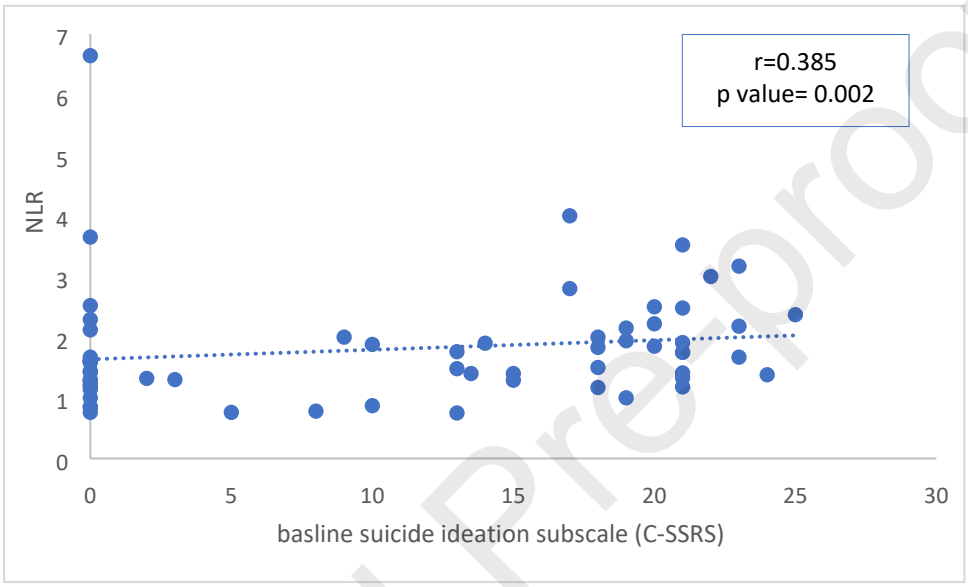
Abbreviations: NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio. SA – suicide attempt.

Figure 3: A ROC curve analysis with inflammatory markers. A. A ROC curve analysis with NLR. The optimal cutoff value for NLR in predicting SA was 1.76 (area under the curve =0.75 (95% CI = 0.63-0.88, sensitivity = 73% and specificity =71%, p value=0.003). B. A ROC curve analysis with PLR. The optimal cutoff value for PLR in predicting SA was 139.5 (area under the curve =0.64, 95% CI = 0.49-0.80, sensitivity = 69% and specificity =64%, p value=0.09). C. A ROC curve analysis with the multiplication of NLR*PLR. The multiplication of both values did not add further

information to the prediction with the cut-off value=199 (area under the curve =0.74, 95% CI = 0.62-0.87, sensitivity = 82%, and specificity =62%, p value=0.003).

Abbreviations: AUC, area under curve; ROC, receiver operating characteristic; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio.

A



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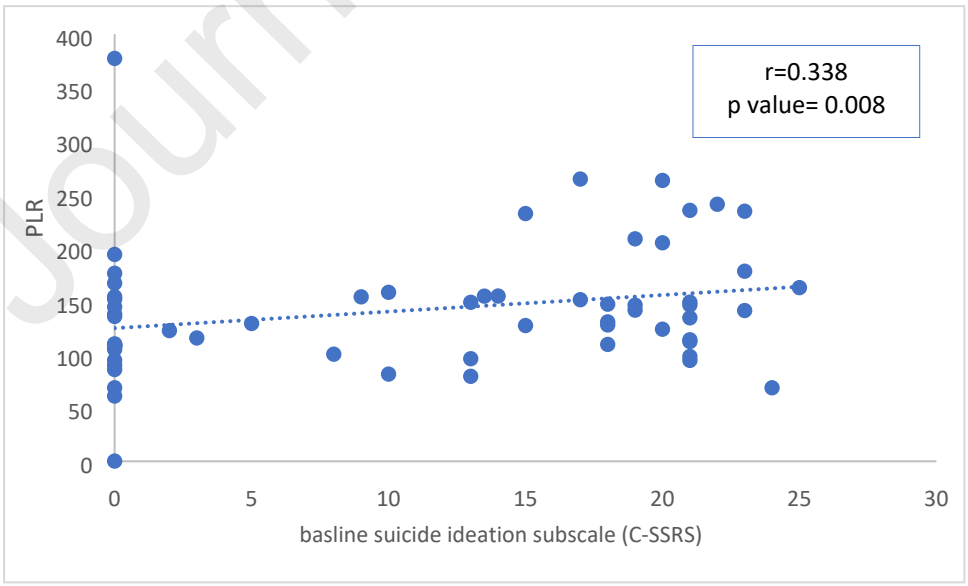

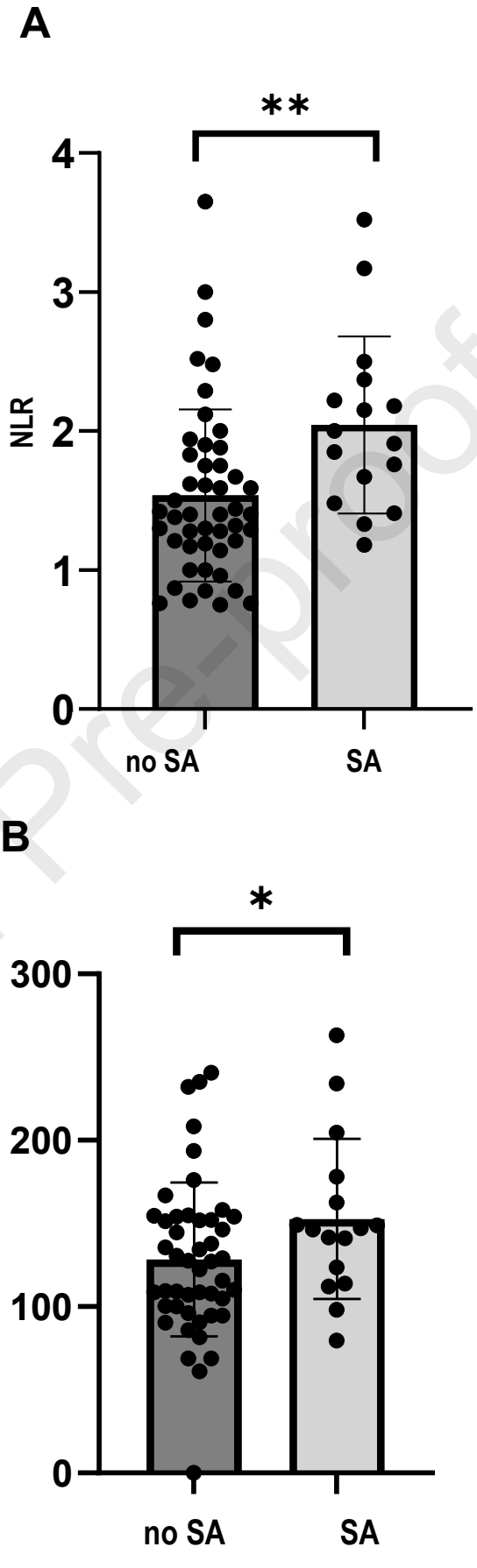

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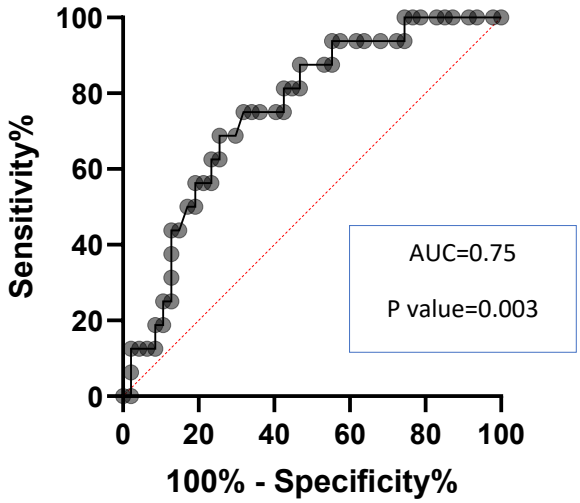

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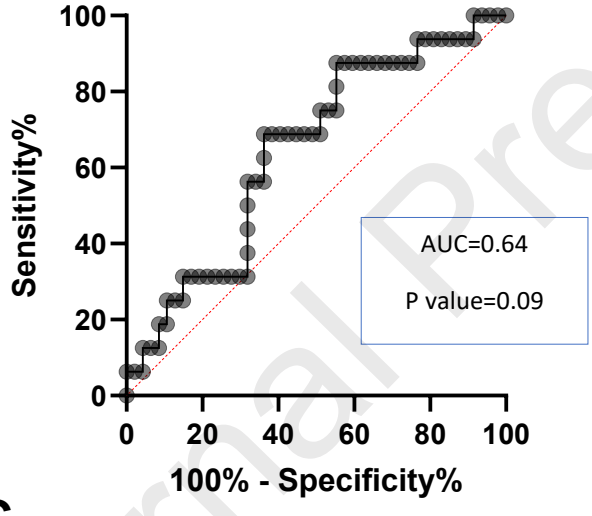

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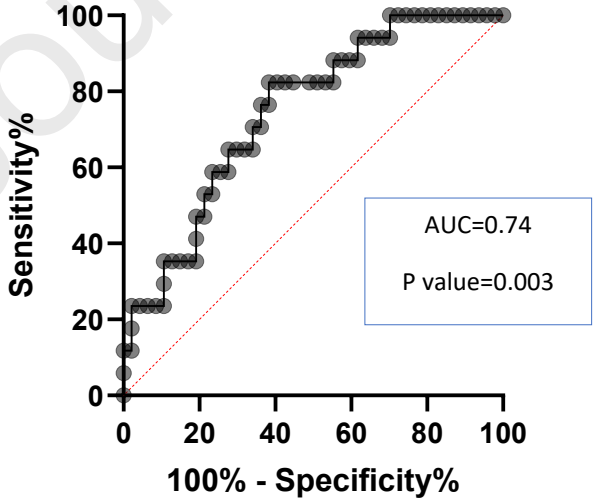

Highlights

- Neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios (NLR and PLR) were studied.
- We prospectively investigated children and adolescents with depression and anxiety.
- NLR associated more with suicide attempt than did PLR.
- NLR may serve as a biomarker of suicidality in youth with depression or anxiety.