

Importance of the neutrophil-to-lymphocyte ratio as a prognostic factor in patients with spleen trauma: A single center experience

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Abstract. Abdominal trauma is a common diagnosis for patients admitted to the emergency room. Evaluation of the severity of such patients can sometimes be difficult due to rapid hemodynamic changes caused by the activation of inflammatory and anti-inflammatory regulatory mechanisms. To evaluate the prognosis of trauma patients, it is crucial to identify these changes and adapt various treatment strategies. Using the records of the Mures County Emergency Clinical Hospital, 126 patients were included in the present study with traumatic splenic injury admitted over 6 years. Blood parameters such as the neutrophil, lymphocyte, and platelet counts, the neutrophil to lymphocyte ratio (NLR), and the platelet to lymphocyte ratio (PLR) were determined, and the association between these and the severity of the traumatic injury [quantified as the injury severity score (ISS)] was calculated. We used linear and multiple regression analyses to identify and quantify the relationships. We found statistically significant associations between the ISS and the NLR ($P=0.002$), with an AUC of 0.816 and a cut-off value of $NLR=6.075$ (sensitivity=76.7%, specificity=74.3%), and between the ISS and hemoglobin ($P<0.0001$), hematocrit ($P<0.0001$), neutrophil count ($P<0.0001$), lymphocyte count ($P=0.0224$), and platelet count ($P=0.0163$). No association was found between the PLR and the severity of trauma. An elevated NLR at admission in trauma patients had significant predictive power for the severity of trauma. Patients with an NLR greater than the cut-off value of 6.075 were more likely to have suffered serious injuries and undergone deterioration.

Introduction

The spleen is an organ at high risk of injury during various abdominal traumas. Due to its fragile structure, the thin capsule enveloping it, and its position directly under the ribs, the spleen is commonly injured during abdominal trauma, especially through blunt forces (1) such as those caused by automobile accidents (2). After the initial trauma, patients usually present with various signs and symptoms ranging from hemorrhage to hemorrhagic shock. Depending on the severity, 'damage control' is a priority. A laparotomy is required, and if splenic damage is suspected, an immediate 'hemostatic' splenectomy should be performed (3). With the acknowledgment that a splenectomy increases the risk of serious infections in both pediatric (4) and adult patients (5), the number of post-traumatic splenectomies has steadily decreased in favor of non-operative spleen preservation techniques (6), aided by the use of more precise methods of exploration and surveillance such as angiography and selective angioembolization of small ruptured splenic branches (7). Thus, identifying the severity of spleen trauma plays a major role when choosing the appropriate treatment approach (8).

Although developed in 1989 by the American Association for Surgery of Trauma (AAST) (9) and eventually revised in 1994 (10), the current categorization of splenic trauma is still frequently used in practice. The AAST classification divides blunt splenic trauma into five categories based on the severity of the subcapsular and intraparenchymal hematoma and depth of the capsular laceration. Low-grade (I and II) injuries are often admitted to the surgical unit and treated conservatively. High-grade blunt splenic trauma patients (grades III, IV and V) are admitted to the critical care unit, their management being determined by the clinical state and development of the patient's condition.

According to the current literature, choosing the adequate paraclinical investigation to determine the severity of spleen trauma is crucial. The type of investigation depends on the patient's hemodynamic status (11). The most common

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investigation used for diagnosing spleen trauma is a FAST ultrasound and CT scan if the patient has a stable hemodynamic status. In cases of hemodynamically unstable patients, bedside ultrasound is the preferred method (12).

These imaging techniques have contributed to the advancement of non-operative management of splenic trauma (13). Although clinical criteria primarily dictate the decision to seek surgical interventions, CT findings support diagnostic accuracy and have been shown in multiple studies to be effective in successfully reducing excessive exploratory laparotomies (14).

Additionally, with image-based investigations when evaluating the severity of spleen trauma, it is crucial to take into consideration the activation of the immune system in response to the initial injury. Several studies have described a 'cytokine storm' and functional reprioritization of leukocytes after severe trauma (15-17). This is due to focused chemotaxis for leukocytes, cytokine release (including systemic expression of IL-6, IL-8, IL-1Ra, and IL-10), production of reactive oxygen species, leukocyte activation, and phagocytosis (18).

It is worth noting that the systemic inflammatory response syndrome (SIRS) not only includes several immune-system-activating factors but also significant suppressive factors that develop within minutes to hours after the initial traumatic injury (19). However, an initial injury of sufficient severity, combined with prolonged surgical intervention or hemorrhagic shock may cause an imbalance in the innate immune response, resulting in a dysfunctional cascade system (20). This imbalanced response to the initial injury can be observed even in routine bloodwork as an altered neutrophil to lymphocytes ratio (NLR) or platelets to lymphocytes ratio (PLR), recent studies suggest (21).

Several recent studies have shown that trauma patients with poor clinical outcomes evoke a more severe and sustained inflammatory response compared with those with better outcomes (22-24). These studies used blood collected at admittance and suggested the data acquired related to the immediate post-injury inflammatory response (22,25).

These findings, combined with various studies on the imbalance of pro- and anti-inflammatory factors in trauma patients, lead us to research the prognostic role of immune monitoring with the aim of identifying patients with a potentially poor outcome. Hence, we conducted this study to assess the importance of inflammatory biomarkers in evaluating systemic inflammatory syndrome in patients with spleen trauma. This study is based on the experience of the Mures County medical center in Romania (26-28).

Our primary hypothesis was that the NLR and PLR, when used as prognostic factors, may be associated with the evolution of patients with splenic trauma.

The primary aim of this study was to identify an association between the systemic inflammatory response of trauma patients and the severity of the traumatic injury. SIRS was indirectly evaluated using two parameters: NLR and PLR. The measurement of the severity of the initial traumatic injury was performed using the injury severity score (ISS).

The secondary aim of our study was to quantify the association between the NLR, PLR and ISS. The specific cut-off values for our SIRS parameters (NLR and PLR) were calculated, considering those the independent variables and the ISS score as the dependent variable.

Materials and methods

Study design. We carried out a retrospective observational study over 6 years. We used the database at the Mureş Emergency Clinical Hospital and studied all the observation sheets of hospitalized patients between January 1 2014 and December 31 2019. In total, 126 patients were admitted to all surgery clinics for spleen trauma as the main diagnostic condition. We followed the relationship between inflammatory markers determined at admission and the severity of the trauma, to identify an association between these studied parameters.

From our selected patients, we were mostly interested in the following clinical parameters: Age, sex, pathological antecedents, number of days of hospitalization, type of spleen trauma, other types of trauma and the severity of them, and blood count at admission. Specifically, from the complete blood count, we were mostly interested in the hemoglobin (Hbg), hematocrit (Hct), platelet count, leukocyte count, and differential white blood cell count.

For this study, we obtained approval from the Ethics Committee of the Clinical Emergency Hospital Mures (grant no. Ad.29366) to access the hospital database.

Inclusion and exclusion criteria. A total of 126 patients were included in the present study, including 90 males (71.43%) and 36 females (28.57%), with a mean age of 36 years and an age range of 3-87 years old. In this study, we included all patients admitted to the Mureş Emergency County Hospital with a primary diagnosis of spleen trauma under the S36 ICD-10 family code (29). This includes unspecified injuries of the spleen, minor and major contusion of the spleen, laceration of the spleen, and other splenic injuries. All patients included in this study survived the initial trauma, and were discharged with a healthy or significantly improved health status.

For this study, we excluded all patients with a preexisting, chronic inflammatory state such as in autoimmune disorders, cancer, or chronic infection. We also excluded trauma patients in need of urgent surgical interventions for different associated injuries, other than splenic trauma.

Data processing. The data obtained were processed using Microsoft Excel (Microsoft Corporation), SPSS (IBM Corp), and GraphPad Prism version 9 (GraphPad Software, Inc.). Patient data were entered into a table in Microsoft Excel, where the database was compiled. This database was then imported into GraphPad Prism 9 and SPSS and then statistically processed.

Data analysis. We indirectly assessed the severity of the systemic inflammatory response syndrome by computing the NLR and PLR. The NLR was calculated by dividing the neutrophil count by the lymphocyte count; similarly, for the PLR, we divided the platelet count by the lymphocyte count as shown by Russu *et al* (30). Using the data regarding the location and severity of the trauma from the clinical observational sheets at admission, we calculated the ISS to determine the relationship between the ISS and the inflammatory markers. The ISS was calculated according to the literature guideline as presented by Baker *et al* (31).

Table I. Age and sex of the patients.

Characteristic	No. of patients	Percentage
Sex		
Male	90	71.43
Female	36	28.57
Age, years		
<10	15	11.9
10-20	31	24.6
21-30	20	15.8
31-40	18	14.2
41-50	17	13.4
51-60	14	11.1
61-70	11	8.7

Overview of the statistical analysis. Statistical analysis included elements of descriptive statistics (mean, median, standard deviation) and inferential statistics. A Shapiro-Wilk test was applied to determine the distribution of the analyzed data series. Linear and multiple linear regressions were performed to determine the relationships between the analyzed variables, and a one-way ANOVA test to the model of the multiple regression analysis, to evaluate the statistical significance. To evaluate the accuracy, the area under the receiver operating characteristic (ROC) curve (AUC) was determined. To assess the performance of the diagnostic test over the range of possible cut-off points for the predictive variables, an ROC curve was used. For analysis, we selected a value of 6 for the ISS as our dependent variable. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Descriptive analysis of age, sex, and paraclinical investigation. Table I shows the age and sex analysis of the patients enrolled in our study. Out of the total 126 patients included in our study, the majority (71.43%) were male, with female patients accounting for 28.57% of the sample. We noted a peak frequency of patients belonging to the age group 10-20 years (28 patients) followed by an almost constant decrease towards 90 years of age, the curve possessing an almost Gaussian aspect with a slight increase towards the distal extremity.

Furthermore, we studied the descriptive analysis of the blood parameters and highlight their values in Table II. All of these parameters except for the Hct, platelet count, and PLR had a mean value outside of the normal range. We proceeded to calculate the ISS using the Excel formula provided in the literature (31), and the results are shown in Table III. The mean value of the ISS found in our study was 10.36, with a range of 1-25.

Regression analysis of the studied parameters. Since most of the studied data series did not pass the Shapiro-Wilk normality test, we proceeded to linear regression analysis to identify a possible association between the studied parameters. The results of the regression analysis are shown in Table IV.

From the results of our analysis, we found a statistically significant association between the ISS with Hbg, Hct, neutrophils, lymphocytes, platelets, and NLR. Of these findings, there was a directly proportionate relationship between the neutrophil count and ISS, and between the NLR and ISS. In other words, any increase in the neutrophil count or NLR was directly associated with a high ISS. In the case of the Hbg, Hct, lymphocyte levels, and platelet levels, as predictor variables, their relationship with the dependent variable ISS was inversely proportionate. This meant that any decrease in the former predictor variables was accompanied by a proportionate increase of the ISS.

We next performed multiple linear regression analysis to further quantify the strength of the association between the dependent and independent variables. The results are displayed in Tables V and VI.

As highlighted in Table VI, the only statistically significant association we found was between NLR and ISS, with a coefficient of 0.448. This meant that an increase in the NLR by one unit corresponded to an increase in the ISS by 0.448 units.

ROC curves of the NLR-ISS association. To further assess the performance of the diagnostic test (NLR) over the range of possible cut-off points for the predictor variable (ISS) we created an ROC curve (Fig. 1).

The AUC was found to be 0.816, and thus the accuracy of the prediction was very good ($P < 0.05$).

To directly measure the association between the NLR and the ISS, and to implement the knowledge of this study in clinical practice, we computed several cut-off values for NLR with various levels of sensitivity, specificity, and false positive rate. These values are shown in Table VII.

The cut-off values presented in the table are the averages of two consecutive ordered values observed in the hypothetical test (NLR).

In conclusion, the NLR was a reliable predictor of trauma severity in patients suffering from various traumatic spleen injuries.

Discussion

According to the current literature, when evaluating patients with splenic injury, the choice of paraclinical investigation is crucial (11).

The most common investigation used for diagnosing spleen trauma is a FAST ultrasound and CT scan if the patient has a stable hemodynamic status. In the case of hemodynamically unstable patients, bedside ultrasound is the preferred method for detecting intraperitoneal bleeding and guiding further surgical decisions. Such patients with a positive ultrasound need an exploratory laparotomy as soon as possible (32). According to some studies, the FAST ultrasound has a sensitivity of up to 98% (in optimal conditions) for detecting intraabdominal fluid collection (33). Its major limitation is user error, being an operator-dependent investigation. A systematic review revealed that a quarter of trauma-dependent intraperitoneal hemorrhages can be missed when using ultrasound alone as a diagnostic tool (34).

In comparison, CT scans offer a comparable sensitivity of 95-98% but have the advantage of a low rate (2.4%) of missed

Table II. Descriptive analysis of the Hbg, Hct, neutrophil, lymphocyte, platelet, NLR, PLR levels and length of hospital stay.

Parameter	Mean	Standard deviation	Lowest value	Highest value	Normal range
Hbg, g/dl	11.82	2.048	3.7	16.46	13.3-16.7
Hematocrit, %	34.89	5.72	11.2	45.7	39-55
Neutrophils, $10^3/\mu\text{l}$	14.49	6.664	3.14	35	1.7-6.1
Lymphocytes, $10^3/\mu\text{l}$	2.001	0.5917	0.3	4.82	1-3.2
Platelets, $10^3/\mu\text{l}$	249.7	96.21	50	786	143-332
NLR	9.154	6.048	1.359	28.83	0.53-6.1
PLR	155.4	98.73	32.26	714.5	44-332
Length of hospital stay	12.84	11.55	1	121	14-22

Hbg, hemoglobin; HCT, hematocrit; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio.

Table III. Descriptive analysis of the ISS scores.

ISS	n	%
0-5	34	26.98
5-10	28	38.8
10-15	19	16.6
15-20	18	11.9
20-25	14	5.5

ISS, injury severity score.

injuries (35). Granted, these high sensitivity rates are achieved when patients are admitted with severe trauma (ISS >15), but newer technologies such as machine learning and artificial intelligence combined with CT scanning aid the diagnosis and triage process of trauma patients, regardless of the severity of the trauma (36).

Nevertheless, using imaging techniques alone to evaluate the severity of spleen injury has certain limitations such as underestimating the degree and hemodynamic impact of certain splenic injuries, or having inadequate prognostic value for patients with complications such as delayed splenic hemorrhage.

Based on the findings of our study, we noticed that spleen trauma occurred primarily in the younger age group with a higher incidence in males, these data coinciding with the European average (37).

According to Harna *et al* (38) the average age of patients in their study was 33.4 with the highest incidence of trauma observed in patients aged between 20 and 40 years old, compared to our study, where the average age was 36 years. Male patients constituted 76% of their study population, with female patients accounting for just 24%. Reasons for the differences in the prevalence by sex may have to do with the cause of the trauma and the type of traumatic agent. Without having the possibility to analyze and quantify all causes of trauma, we have noticed a purely subjective prevalence of work-related accidents in construction domains in patients included in our study. This may be one of the reasons for a higher ratio of male patients included, our findings are in agreement with those of Harna *et al* (38).

To identify possible relationships between inflammatory markers and the severity of trauma patients, we have analyzed the relationship between the NLR and PLR with ISS.

A review of the literature revealed several studies on predictive factors for trauma patients, indicating that the prognostic capacity of some well-known scoring systems including the ISS at admission is not as powerful as the predictive capability of other physiologic-based scoring models including the Sequential Organ Failure Assessment (SOFA), Denver, Acute Physiology and Chronic Health Evaluation II (APACHE II), and the Trauma and Injury Severity Score (TRISS) (30). However, the use of such physiological-based models is difficult and cumbersome for everyday usage, as they require considerably more time to apply compared with ISS (21).

We did not find a statistically significant association between PLR-as an inflammatory marker and the severity of the trauma. However, this result may be caused by a sudden fluctuation in the number of platelets in the blood count. We also noted the heterogeneous distribution of our data for PLR with a wide standard deviation, which contributed to the lack of statistical significance. There is evidence that the proportion of neutrophils and platelets variably increases during any trauma due to systemic inflammation (39). Specifically, during splenic trauma, it appears that the platelet count is higher than in any other type of trauma. Reactive thrombocytosis is common following elective or urgent splenectomy, but there is a 20-30% incidence of thrombocytosis following major trauma regardless of splenectomy (40,41). The mechanism behind the increase in platelet counts after major spleen trauma relies upon the physiological role of the spleen. After a traumatic injury, due to the inflammatory and regenerative processes of the splenic parenchyma, its ability to clear platelets is impaired. Similarly, after post-traumatic splenectomy, a reactive thrombocytosis can be noticed over the following 2 to 20 days (40). Furthermore, studies have shown that thrombocytosis can occur even in patients with anemia due to the close relationship between erythropoietic and thrombotic growth factors (42). Such findings support our study since the mean Hbg value is well under the lowest range. These fluctuations in the platelet count are the most plausible cause of data alternation, preventing us from finding a relationship between the PLR and the ISS. There are several situations mentioned in the literature where there is a powerful association with NLR, whilst PLR and patient outcomes have no statistical significance (43).

Table IV. Linear regression analysis of the studied parameters.

Parameter	r coefficient	95% confidence interval	r ² coefficient	P-value
Age and ISS	-0.02797	-0.351	0.0007825	0.7568
Hemoglobin and ISS	-0.3514	-0.3091	0.1235	<0.0001
Hematocrit and ISS	-0.3724	-0.3039	0.1387	<0.0001
Neutrophils and ISS	0.3943	0.2349-0.5330	0.1554	<0.0001
Lymphocytes and ISS	-0.2041	-0.33704	0.04168	0.0224
Platelets and ISS	-0.2146	-0.33564	0.04604	0.0163
NLR and ISS	0.4931	0.3475-0.6154	0.2431	<0.0001
PLR and ISS	0.006319	-0.3513	0.00003993	0.9442

ISS, injury severity score; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio.

Table V. Multiple regression analysis of the studied parameters-model summary.

Model	R	R ²	P-value
1	0.611	0.373	<0.0001

Predictors: (Constant), PLR, hematocrit, neutrophils, platelets, lymphocytes, NLR, hemoglobin. Dependent variable: ISS.

Despite all of this we did find a proportioned, statistically significant association between NLR and ISS using regression analysis. Thus, we can state that any sort of increase in the NLR in trauma patients at admittance is directly associated with highly severe trauma and poorer outcome.

Furthermore, plotting an ROC curve showed the AUC was 0.816 suggesting a very good performance of our test. A similar article published on this matter in 2020 by Soulaïman *et al* (21) revealed comparable results for the predictive power of NLR at admission. Granted, the calculated AUC in their study was lower (AUC=0.633) with more modest levels of sensitivity and specificity. Yet, the main difference noticeable between the design of our study compared with the former is the level set for ISS as the dependent variable (ISS=15). We decided on a value of ISS=6 to differentiate between minor and moderate injuries since the majority of the patients included in our study suffered from less severe traumatic injuries, as can be noted from the distribution of the data (mean ISS=9.848).

After computing the cut-off values for NLR at a set ISS of 6, we were left with a choice of three separate values, all of them with various levels of sensitivity, specificity, and false positive rates. We preferred to take into account the cut-off value of NLR=6.075 due to its lower false positive rate (25.7%) and still potent capability of identifying patients suffering from aggressive trauma (76.7%). Thus, we could state that if a patient was admitted to the emergency care unit after trauma and the NLR was higher than the aforementioned value, it would be predictive of a poorer outcome, and hence we could adjust the therapeutic approach to improve prognostic outcomes.

The results on this topic in the medical literature are yet debated as authors find contradictory outcomes. According to a recent study, an increase in NLR throughout the first 48 h

after admission was linked to the occurrence of organ failure in male trauma patients (44). According to Dilektasli *et al* (45), who illustrated the predictive value of NLR on the second and fifth day when it came to estimating hospital fatalities in trauma patients when compared to the following days, the NLR throughout the first 24 h was not effective for predicting outcomes in the surgical intensive care unit. In this study, for the second and fifth hospital days, they found appropriate cut-off values of 8.19 and 7.92 with a sensitivity of 70.8% and a specificity of 61.9%; these results are similar to the cut-off values we found in our study.

We also found an association between HTC and ISS with a negative value according to the regression analysis. This indicates an inversely proportional relationship between the two parameters, and thus any decrease in the Hct count would suggest a higher severity of trauma. The hypothesis can also be inverted; thus, any patient with severe trauma, quantified by a higher ISS, will have a lower red blood cell count. This fact is to be expected due to the bleeding, both internal and external, after severe trauma.

The present study has a series of flaws, starting with the design of the study. It was retrospective and we analyzed a lengthy period, which represents fundamental restrictions of our work. Another limitation of our research is the fact that we analyzed patients admitted at only one hospital; thus, the data were limited by the quality of patient records and the inability to account for all factors. Third, our dataset lacked additional inflammatory indicators that may be used to define the inflammatory process's quality such as interleukins and interferons. Fourth, the data were limited to a single point in time, at admission; having more data points during the admission period, with a longer follow-up period may have provided more detailed information about the trend of these inflammatory cells in the earlier and even later phases, allowing for a more accurate interpretation of what drives these changes. Fifth, our study included a greater proportion of male patients, and hence the sex difference means our findings are exploratory at best. Finally, one important limitation of the study was the small sample size.

There are certain areas where the current study can be improved, such as the retrospective design of the study. We feel that a future prospective study where we analyze the same parameters not just at admittance, but daily, taking into

Table VI. Multiple regression analysis of the studied parameters-coefficients analysis.

Model	Unstandardized coefficients		P-value	95% confidence interval for B	
	B	Std. error		Lower bound	Upper bound
(Constant)	15.230	3.420	0.000	8.457	22.004
Hemoglobin	-0.050	0.710	0.944	-1.455	1.355
Hematocrit	-0.182	0.256	0.478	-0.688	0.324
Neutrophils	0.120	0.128	0.351	-0.133	0.372
Lymphocytes	-0.967	0.894	0.282	-2.737	0.803
Platelets	0.003	0.009	0.718	-0.014	0.020
NLR	0.448	0.194	0.022	0.064	0.831
PLR	-0.020	0.011	0.084	-0.042	0.003

Predictors: (Constant), PLR, hematocrit, neutrophils, platelets, lymphocytes, NLR, hemoglobin. Dependent variable: ISS. ISS, injury severity score; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio.

Table VII. Coefficients for the ANOVA of the studied parameters.

NLR	Cut-off value 5.44 (%)	Cut-off value 5.78 (%)	Cut-off value 6.075 (%)
Sensitivity	80	78.9	76.7
Specificity	65.7	68.6	74.3
False positive rate (1-Specificity)	34.3	31.4	25.7

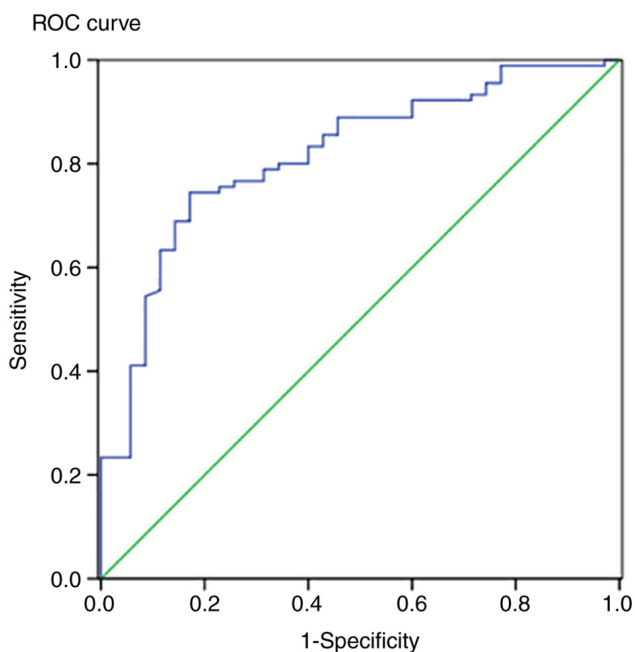


Figure 1. Graphical representation of the ROC curve.

account the variation of these observed values and the patients' evolution would be a suitable direction. Investigating these parameters over a longer period would also aid the quality of the study by increasing the number of patients that can be included. Yet, considering that this study was conducted in a Romanian reference medical center (a polytrauma center), we

consider our findings crucial for the further development of our country's deficient medical system. We hope that some of these findings will be used as a stepping stone toward developing new and adapted protocols for managing trauma patients. The least of our plans is to use this study in order to apply for financial support for further studies in order to better investigate the prognostic factors for the evolution of trauma patients, and furthermore develop a strategy for managing trauma patients.

In conclusion, elevated NLR in trauma patients at admittance had a high predictive power for the severity of the trauma. Patients with an NLR value higher than the cut-off value of 6.075 have a high probability of severe trauma.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

VV and BAS conceived the study. NB and IH designed the study. IGC and VV collected the data. DVG and IGC

performed the statistical analysis. CM was in charge of data curation and visualization. VV and IH wrote the original draft of the manuscript. BAS, CM and NB reviewed and edited the manuscript. VV, IGC and DVG confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Mures County Emergency Hospital.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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