STATISTICAL STUDY FOR THE PROGNOSTIC VALUE OF NLR VALUES IN PATIENTS WITH SEVERE BURNS: A STUDY IN THE SERVICE OF BURNS IN ALBANIA

VALEUR PRONOSTIQUE DU RAPPORT NEUTROPHILES/LYMPHOCYTES (RNL) CHEZ LES PATIENTS GRAVEMENT BRÛLÉS : UNE ÉTUDE DU CTB D'ALBANIE

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SUMMARY. The phases of burn care include initial resuscitation, definitive wound management, and rehabilitation of physical and psychological injuries. In severe burns, this response lasts for the first two to three days post-burn and burns larger than 15% of total body surface area lead to the initiation of systemic inflammatory response syndrome. Neutrophil-lymphocyte ratio (NLR) is an inflammatory biomarker that can indicate systemic inflammation. NLR is defined by the absolute number of neutrophils divided by the absolute number of lymphocytes. Many studies have been done in severe burn patients because they reflect changes in the systemic inflammatory response. This study aimed to investigate the prognostic value of NLR values in patients with severe burns and provide clinical guidance for sepsis and mortality. This was a retrospective cohort study conducted in the Service of Burns of the University Hospital Centre "Mother Teresa" in Tirana (UHCT), Albania. We compared NLR levels in burn patients with sepsis and without sepsis. Our data showed a significantly higher level of NLR in burn patients with sepsis, while the values of NLR on the 3rd day (NLR 3) have statistical significance ($p<0.0001$). From the logistic regression analysis, we found that only NLR 3 can predict mortality, whose cut-off (4.59) was determined with the help of ROC curve analysis and was accurately presented with the help of Kaplan-Meier curves with statistical significance. From multiple regression, we understand that mortality can be predicted based on TBSA%, age, presence of inhalatory burn, and NLR 3 together.

Keywords: NLR, severe burns, sepsis, NLR

RÉSUMÉ. *La prise en charge des brûlés se déroule en 3 phases : réanimation initiale, couverture cutanée et réhabilitation, physique comme psychologique. Les patients atteints sur plus de 15 % de SCT développent une réponse inflammatoire systémique qui dure 2 à 3 jours. RNL (rapport du nombre absolu des neutrophiles à celui des lymphocytes) est un biomarqueur de cette réponse, largement étudié chez les brûlés. Cette étude rétrospective se penche sur la valeur pronostique de RNL chez les brûlés sévères, en particulier vis à vis de la mortalité et de la survenue de sepsis. Elle a été conduite dans le CTB du CHU Mère Teresa de Tirana. Nous avons trouvé une augmentation significative de RNL chez les brûlés septiques, en particulier à J3 ($p < 0,0001$). En régression logistique, nous avons trouvé que RNL à J3 (RNL3) était le seul à être significativement associé à la mortalité. En analysant la courbe ROC et le diagramme de Kaplan-Meier, le seuil de 4,59 est le plus pertinent. En régression multiple, la surface brûlée, l'âge, l'inhalation de fumées et RNL3 sont des paramètres indépendants de mortalité.*

Mots-clés : *rapport neutrophiles/lymphocytes, brûlure grave, sepsis*

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Introduction

The phases of burn care include initial resuscitation, definitive wound management, and rehabilitation of physical and psychological injuries. There are two distinct phases of metabolic alterations following burn injury: the "ebb phase" occurs within the first 48 hours after injury, and the "flow phase" is characteristically associated with a hypermetabolic state. A decrease in tissue perfusion and metabolic rate characterizes the initial stress response to severe injury. This response lasts for the first two to three days post-burn in severe burns. In patients with burns larger than 15% of the total body surface area, because of the initiation of systemic inflammatory response syndrome, IV fluid resuscitation is necessary to prevent burn shock and death.

Numerous investigations have found NLR to be an independent risk factor for a variety of serious diseases, including burns, sepsis, cardiovascular disease and cancer.[1,2,3,4] Neutrophil-lymphocyte ratio (NLR) is an inflammatory biomarker that can indicate systemic inflammation but also indicates the presence of stress. NLR is defined by the absolute number of neutrophils divided by the absolute number of lymphocytes. Under physiologic stress, the number of neutrophils increases, while the number of lymphocytes decreases. The NLR combines these changes, making it more sensitive than either alone.

Burn shock results as an interplay of tissue injury from the causative agent, hypovolemia, and simultaneously the release of mediators of inflammation which impact microcirculation and vital organs. Many studies have been done in severe burn patients because they reflect changes in the systemic inflammatory response.[5,6,7] Severe burn injuries induce a systemic response described as systemic inflammatory response syndrome (SIRS) and is caused by an acute phase inflammation.[8,9,10,11]

This study aimed to investigate the prognostic value of NLR values in patients with severe burns and provide clinical guidance for sepsis and mortality.

Material and methods

Patients

This was a retrospective cohort study conducted in the Service of Burns of the University Hospital Centre "Mother Teresa" in Tirana (UHCT), Albania. The study includes adult, elderly and child patients with critical burns, hospitalized in the Intensive Care Unit of the service of burns during 2023. The inclusion criteria were all patients with burn injury (including flame, scalds, electrical, chemical and other causes of burns together with trauma as a concomitant illness together with burn).

The exclusion criteria were known pre-existing cardiac disease, kidney disease, hemolytic anemia, bone marrow arrest, or other inflammatory diseases. The study excluded non-burn-related admissions (Stevens-Johnson/toxic epidermal necrolysis, necrotizing fasciitis, and chronic wounds). The study was approved by the Institutional Board of the University of Medicine.

All patients were resuscitated in the first 48 hours with Parkland formula for adults and Shriner Galveston formula for children that was adjusted according to the physiological response according to American Burn Association (ABA) recommendations.[12]

During the disease, patients were diagnosed with sepsis or not according to the ABA Consensus Panel Publication for Infection and Sepsis.[13]

A diagnosis of sepsis will be made when >3 of the following criteria are met along with a positive bacterial culture or when a clinical response to antibiotics is detected:

- Temperature >39°C or <36°C.
- Progressive tachycardia: adults >110 beats per minute; children >2 SD above age-specific norms (85% of age-adjusted maximum heart rate).
- Progressive tachypnoea: adults >25 breaths per minute not ventilated or minute ventilation >12 L/minute for ventilated patients; children >2 SD above age-specific norms (85% of age-adjusted maximum respiratory rate).
- Thrombocytopenia: adults' platelet count <150x109/L [this will not be applied until 3 days after initial resuscitation]; children <2 SD below age-specific norms.
- Hyperglycaemia: untreated plasma glucose >200 mg/dL or insulin resistance as defined by either (1) >7 units of intravenous insulin per hour or (2) significant resistance to in-

sulin (>25% increase in insulin requirements over 24 hours).
- Feed intolerance: inability to continue enteral feeding >24 hours defined using (1) abdominal distension, (2) enteral feeding intolerance (adults, two times the feeding rate; children, residual >150 mL/hour), or (3) uncontrollable diarrhea (>2500 mL/day for adults or >400 mL/day in children).

Data collection

Each patient's demographic, clinical and laboratory data were obtained from the electronic medical records. The following criteria were collected: demographic and burn-related data including age, group ages (0-20 years were children and young patients and >20 years old were adults and elderly patients), gender, TBSA (total burn surface area %), presence of full-thickness, of inhalation injury, of infection and sepsis, the Abbreviated Burn Severity Index (ABSI), the cause of burns (scalds, flame, electrical, chemical and other causes, also trauma) and length of hospitalization (LOS). The laboratory examination was conducted at least every two days during the disease for the severe burn patients. Blood samples were analyzed by a fully auto hemocyte analyzer in the laboratory of the University Hospital Center. The outcomes were the presence of sepsis and mortality collected from the hospital medical records. From the complete blood count, we calculated NLR on admission (NLR 1), on the 3rd day (NLR 3), on the 5th day (NLR 5), and the 7th day (NLR 7) after the burn. All the parameters were compared between the sepsis and non-sepsis group. A normal range of NLR is between 1-2, values higher than 3.0 and below 0.7 in adults are considered pathological.[14]

Statistical analysis

SPSS 23.0 and Microsoft Excel were used for data analysis. Continuous data were presented as the mean value and standard deviation. Discrete data were presented in absolute value and percentage. Tables and graphs were used to present the data. To compare two mean sizes between two different groups, the Student's "t" test was used for two independent samples. Statistically significant differences between the proportions were analyzed by the Chi-square test. To answer our clinical question regarding the effect of NLR for predicting sepsis and mortality, we performed some statistical tests. Concretely for predicting sepsis, we performed relative risk and odds ratio to evaluate the impact of higher NLR values for the presence of sepsis. Logistic regression analysis is used for studies aimed at checking whether independent variables (NLR 1, NLR 3, NLR 5 and NLR 7) can predict a dichotomous dependent variable (mortality).

After that, we use this NLR as a variable in the receiver operating characteristic (ROC) curve analysis to estimate sensitivity, specificity, and areas under the ROC curve. The optimal cut-off value was obtained from the maximum value of Youden's index. The survival curves of the high and low NLR groups were calculated using the Kaplan–Meier method. A multiple regression was run to predict outcomes from TBSA%, age, presence of inhalatory burn, and NLR 3. Values were considered significant with $p<0.05$.

Results

During the study period, 154 patients with severe burns were hospitalized in the ICU of the Service of Burns. The participants' mean age was 36.88 (±29) years. In total, 65 (42.2%) patients were female and adults, and 62.3% of all patients were elderly (>20 years). The main causes were scalds and flame, with a mean burn surface area of almost 25% TBSA. The mean ICU stay length was 11.32±11.9 days and mortality was 9.1%.

Of the patients, 16.2% (n=25) were diagnosed with sepsis. The patient's age in the sepsis group was higher (56.08±27.11 years old vs. 33.1±9.8 years old) and predominately adults and elderly with higher TBSA% (33.2% vs. 23.3%) with statistical significance. LOS was higher at 27.2±19.6 days, and mortality rates were 36% (9 deaths in 25 patients with sepsis) vs. 3.8% (5 deaths in 129 patients without sepsis) with statistical significance respectively. The descriptive statistics for all variables for patients are shown in *Table I*.

Table I - Demographic, clinical and burn injury characteristics 2023 (n=154)

	All patients (n=154)	Patients with sepsis (n=25)	Patients without sepsis (n=129)	p-value
Age, mean (SD)	36.88(29.0)	56.08(27.11)	33.1(27.98)	0.0002
Gender, % female (n)	42.2(65)	40(10)	42.6(15)	0.81
Group ages				
Children (0-20y), %(n)	37.7(58)	16(4)	41.8(54)	0.0151
Adults and elderly (>20y), %(n)	62.3(96)	84(21)	58.1(75)	0.0148
Etiology of burns, %(n)				
Scalds	43.5(67)	20(5)	48.44(62)	0.0089
Flame	42.2(65)	56(14)	39.84(51)	0.135
Electrical	5.8(9)	4(1)	2.34(3)	0.634
Chemical	2.6(4)	8(2)	5.47(7)	0.623
Others and trauma	5.2(8)	12(3)	3.91(5)	0.097
TBSA%, mean (SD)	24.5(16.5)	33.2(18.1)	23.3(15.7)	0.0055
Full-thickness burn, %(n)	22.1(34)	41.8(14)	58.8(20)	0.118
Inhalation injury, Yes, %(n)	8.44(13)	32(8)	3.87(5)	<0.0001
LOS, mean (SD)	11.32(11.9)	27.2(19.6)	8.1(6.1)	<0.0001
ABSI, mean (SD)	5.9(2.81)	8.08(2.23)	5.47(2.81)	<0.0001
Mortality, %(n)	9.1(14)	36(9)	3.8(5)	<0.0001

Table II - NLR 1, NLR 3, NLR 5 and NLR 7 values for patients with and without sepsis

	Patients with sepsis (n=25)	Patients without sepsis (n=129)	p-value
NLR 1, mean (SD)			
All patients	6.37(4.0)	5.08(4.1)	0.154
Deceased	7.0(3.3)	5.7(3.6)	0.009
Survived	6.0(4.4)	5.0(4.1)	0.271
NLR 3, mean (SD)			
All patients	4.91(4.0)	2.28(2.4)	< 0.0001
Deceased	5.36(2.8)	4.72(2.1)	0.198
Survived	4.65(4.6)	2.19(2.4)	0.0001
NLR 5, mean (SD)			
All patients	4.56(3.6)	3.77(2.8)	0.22
Deceased	2.99(2.7)	5.7(3.1)	< 0.0001
Survived	5.45(3.9)	3.69(2.8)	0.0081
NLR 7, mean (SD)			
All patients	4.24(1.65)	4.56(2.04)	0.4615
Deceased	4.54(1.9)	6.25(2.28)	0.0006
Survived	4(1.4)	4.5(2.01)	0.2368

NLR 1: neutrophil to lymphocyte ratio on the 1st day; NLR 3,5,7: neutrophil to lymphocyte ratio on the 3rd, 5th and 7th day

Table III - Laboratory parameters on the 3rd day for patients with and without sepsis

	Patients with sepsis (n=25)	Patients without sepsis (n=129)	p-value
RBC (x10*12 /L), mean (SD)			
All patients	3.36(0.26)	4.34(0.76)	<0.0001
Deceased	3.34(0.3)	3.24(0.69)	0.478
Survived	3.43(0.1)	4.44(0.69)	<0.0001
HGB (mg/dL), mean (SD)			
All patients	10.9(2.39)	12.6(2.84)	0.005
Deceased	10.5(2.1)	10.7(2.7)	0.762
Survived	11.2(2.5)	12.8(2.7)	0.006
HCT (%), mean (SD)			
All patients	32.5(7.2)	36.7(8.9)	0.02
Deceased	31.7(6.8)	32(8.9)	0.87
Survived	33.2(7.6)	37.1(8.9)	0.04
WBC (x10*9/L), mean (SD)			
All patients	8.85(3.8)	9.81(5.66)	0.418
Deceased	8.85(3.7)	6(0.99)	<0.0001
Survived	9.0(5.6)	10.1(5.79)	0.383
PLT (x10*9/L), mean (SD)			
All patients	230(134.6)	235(107.2)	0.838
Deceased	218(123.04)	100(16.9)	<0.0001
Survived	266.5(218.5)	248(103.4)	0.511

RBC: red blood cells; WBC: white blood cells; PLT: platelets; HGB: hemoglobin; HCT: hematocrit

In *Table II*, we have presented the values of NLR 1, NLR 3, NLR 5 and NLR 7 for patients with and without sepsis. We noticed that in the comparison of all patients with and without sepsis, only the values of NLR on the 3rd day (NLR 3) have statistical significance ($p<0.0001$), while the others do not. There is statistical significance for deceased patients with sepsis (higher values of NLR 1 values), and also for those who survived (higher values of NLR 3 and NLR 5). Trending of NLR values during the disease are presented in *Fig. 1*. We see an increase in NLR values until the 5th day in deceased patients, which is accompanied by a decrease in NLR values in those who survived.

In *Table III* we have presented the values of complete blood count in sepsis patients. They have lower values of RBC, HGB and HCT with statistical significance. WBC and PLT values had statistical significance only for deceased patients ($p< 0.001$).

Analyzing outcomes (sepsis) in patients with higher NLR values on the third day (24 patients), we calculated the relative risk and odds ratio (*Table IV*). Those with overall higher NLR values on the third day had 3.0 times the risk for sepsis compared to those with normal values, accompanied by an odds ratio of 4.275 ($p=0.0036$).

To analyze the impact of NLR on mortality, we first analyze NLR values, and from the logistic regression analysis, aimed at checking whether independent variables (NLR 1, NLR 3, NLR 5 and NLR 7) have an impact on mortality. We found that only NLR 3 can predict the dichotomous dependent variable (mortality) with statistical significance, $p=0.0029$ (*Fig. 2*).

Taking into consideration NLR 3 values, we perform area under the curve ROC analysis. As shown in *Fig. 3*, the areas under the ROC curves of the 3rd day

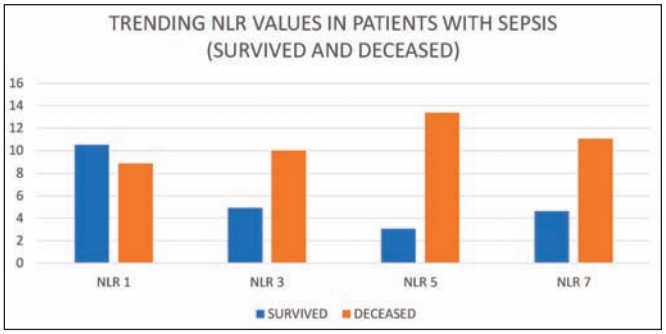
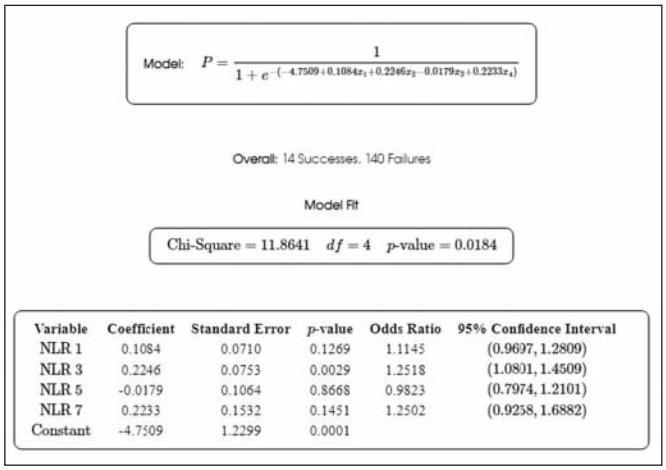


Fig. 1 - Trending of NLR values during the disease

Table IV - Relative risk for sepsis in patients with higher NLR values on the 3rd day

Sepsis	Relative risk (95% CI)	p	Odds ratio (95% CI)	p	Number needed to treat (95% CI)
(9 out of 24)	3.0469	0.0016	4.275	0.0036	3.969
(16 out of 30)	(1.5269 to 6.0800)		(1.6074 to 11.3693)		(10.378 to 2.454)

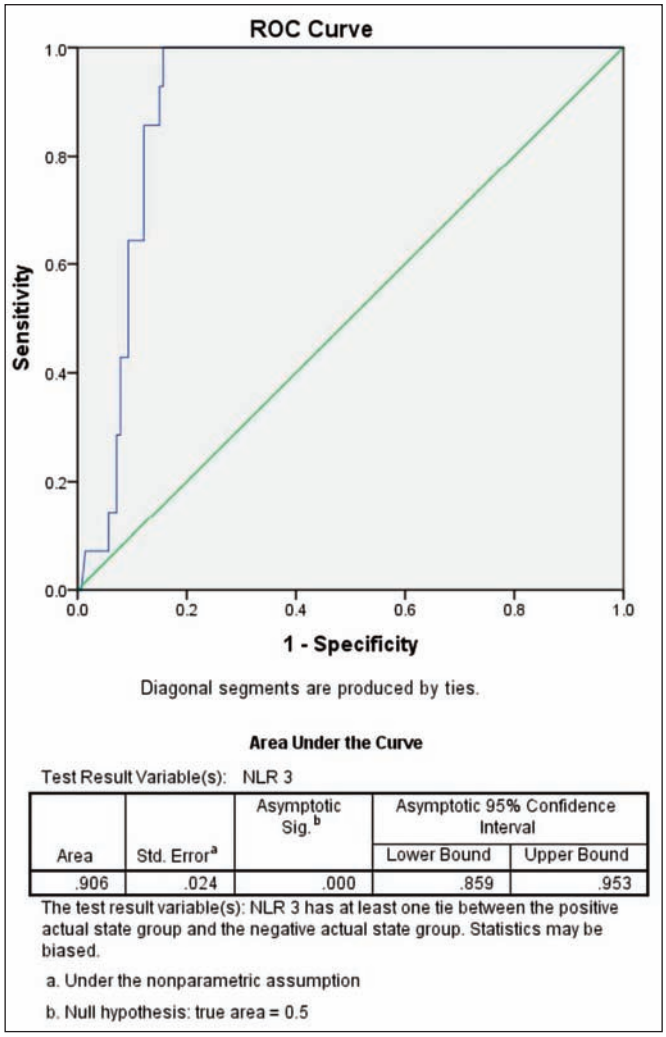


Variable	Coefficient	Standard Error	p-value	Odds Ratio	95% Confidence Interval
NLR 1	0.1084	0.0710	0.1269	1.1145	(0.9697, 1.2809)
NLR 3	0.2246	0.0753	0.0029	1.2518	(1.0801, 1.4509)
NLR 5	-0.0179	0.1064	0.8668	0.9823	(0.7974, 1.2101)
NLR 7	0.2233	0.1532	0.1451	1.2502	(0.9258, 1.6882)
Constant	-4.7509	1.2299	0.0001		

Fig. 2 - Logistic regression for NLR values on days 1, 3, 5 and 7 after burn injury and mortality

NLR for severe burn-delayed death prediction were 0.906 (95% CI, 0.859–0.953), and the optimal cut-off value of the 3rd day NLR was 4.59 [(64.00% (sensitivity),12.10% (1 - specificity)].

The 3rd day NLR was converted from a continuous variable to a categorical variable for the NLR >4.59 group and the NLR ≤4.59 group according to the optimal cut-off value. There was a significant difference in mortality between high and low NLR groups, with an overall mean survival time of 35.87±8.44 for patients with severe burn injury and NLR >4.59 versus 59.13±2.35 for patients with an NLR ≤4.59 (*Fig. 4*).



Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.906	.024	.000	.859	.953

The test result variable(s): NLR 3 has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Fig. 3 - Area under the ROC curves of the 3rd day NLR for severe burn–delayed death prediction

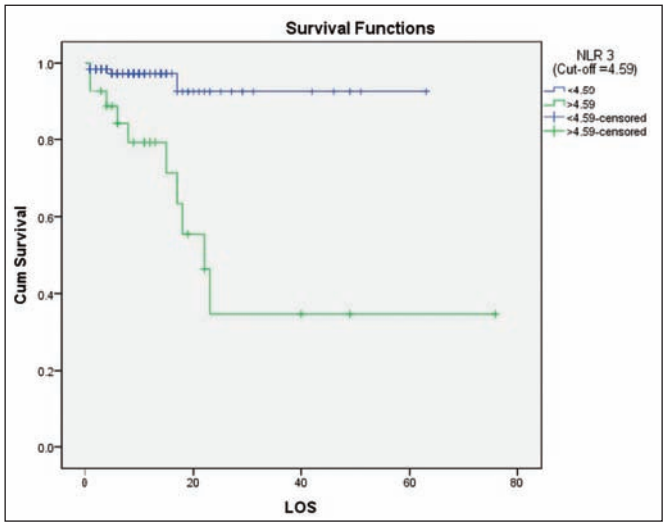


Fig. 4 - The survival curves of the high and low NLR groups calculated using the Kaplan-Meier method

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson
					R Square Change	F Change	df1	df2	Sig. F Change	
1	.629[a]	.396	.379	.227	.396	24.392	4	149	.000	1.867

a. Predictors: (Constant), AGE, NLR 3, TBSA (%), INHALATORY BURN

b. Dependent Variable: OUTCOME

ANOVA a

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	5.036	4	1.259	24.392	.000[b]
	Residual	7.691	149	.052		
	Total	12.727	153			

a. Dependent Variable: OUTCOME

b. Predictors: (Constant), AGE, NLR 3, TBSA (%), INHALATORY BURN

Coefficients a

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	-.110	.044		-2.513	.013	-.196	-.023
	TBSA (%)	.001	.001	.085	1.075	.284	-.001	.004
	INHALATORY BURN	.440	.082	.425	5.377	.000	.278	.602
	NLR 3	.016	.006	.168	2.577	.011	.004	.029
	AGE	.002	.001	.223	3.316	.001	.001	.004

a. Dependent variable: OUTCOME

Fig. 5 - Multiple regression to predict outcome (mortality) from TBSA%, age, presence of inhalatory burn, and NLR 3.

Multiple regression was run to predict outcome (mortality) from TBSA%, age, presence of inhalatory burn, and NLR 3 (*Fig. 5*). These variables statistically significantly predicted outcome (4, 149) = 24.392, p <.0001, R2 0.396. The last three variables (except TBSA %) added statistical significance to the prediction, p< .05.

Discussion

Many studies documented that NLR increases rapidly following acute physiologic stress within 6 hours, which is consistent with our study.[15,16,17] In our patients, there is a wide range of values of NLR 1 from 0.3 to 17.4, CI(95%) 0.66, Mean 5.29, SD 4.16. We found that on the 1st day, NLR was high, which could be explained by the excessive release of neutrophils followed by the intense stress of the burn. Endogenous cortisol and catecholamines may be major drivers of NLR. Increased cortisol levels are known to increase neutrophil count while decreasing lymphocyte count. According to a study, admission NLR values above 14 are associated with decreased survival in patients with extensive burns.[18] In our study, we had 7 patients with values of NLR 1 above 14 and all survived.

We compared the NLR levels in burn patients with sepsis and without sepsis. Our data showed a significantly higher level of NLR in burn patients with sepsis (deceased and survived ones separately), which is evidenced in many other studies.[19,20,21] Although in the comparison of all patients with and without sepsis, only the values of NLR on the 3rd day (NLR 3) have statistical significance (p<0.0001), while the others do not, we notice that in the cases that die, NLR 3 has no statistical significance. We think that this happens because deaths in severe burns are both in the period of combustion shock (in the first 2 days) and in sepsis, and NLR is an indicator of both stress and sepsis.

The 3rd day NLR may reflect better the immune response of the host. The authors showed that the circulating number of neutrophils was elevated within 24 hours of a burn injury, returned to normal levels on day 3, and elevated again on day 7.[22] On the other hand, studies have documented that lymphocyte populations were diminished on the 3rd-day postburn, and abnormal lymphocyte count at 72 hours postburn injury has been associated with adverse outcomes (infections, length of stay, intensive care unit length of stay, and ventilator days).[23,24] We found that the 3rd day NLR was 4.91±4.0 in the sepsis group versus 2.28±2.4 in the non-sepsis group, of which the difference was significant (p<0.0001). With the presence of infection, inflammation and immunosuppression, blood tests on the 7th day are affected by clinical therapy.

Analyzing sepsis and the risk of developing it in patients, we analyzed the relative risk of sepsis for NLR 3 values. With NLR values more than 3.0 on the third day, we observed a relative risk of 3.04, meaning that the chance of sepsis is three times higher if the patient presents with higher NLR values on the third day.

An OR of 4.27 indicates that the odds for sepsis are higher in the group with higher NLR values on the 3rd day (OR>1).

In absolute values, this would mean that the incidence of sepsis is three times smaller in the group with normal NLR values on the third day.

In our test, the NNT or benefit was 3.9, which highlighted the need for excellent monitoring and

surveillance of the 3 patients with higher NLR values on the 3rd day to prevent one bad outcome (sepsis).

From the logistic regression analysis, we found that only NLR 3 can predict mortality, whose cut-off (4.59) was determined with the help of ROC curve analysis and was accurately presented with the help of Kaplan-Meier curves with statistical significance.

From multiple regression, we understand that mortality can be predicted based on TBSA%, age, presence of inhalatory burn, and NLR 3 together. Multiple regression also allows us to determine the overall fit (variance explained) of the model and the relative contribution of each of the predictors to the total variance explained: concretely, values of NLR on the 3rd day have a contribution with statistical significance.

Conclusions

Our study showed that NLR is associated with the risk of sepsis and mortality in burn patients. Our data showed a significantly higher level of NLR in burn patients with sepsis, while the values of NLR on the 3rd day (NLR 3) have statistical significance ($p<0.0001$).

Interpretation of NLR depends on the clinical context. We can use NLR for detecting stress among patients with severe burns, because any cause of physiologic stress may increase NLR (hypovolemic or burn shock). We can perform prediction of outcome (mortality and sepsis) among patients with severe burns. We can provide important information for each patient with individual trajectories over time. Concretely, an NLR that decreases may be a favorable sign, whereas if the NLR increases this might suggest treatment failure.

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