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Original Research Article

Neutrophil to lymphocyte ratio and platelet to lymphocyte ratio as markers of early sepsis and mortality in pediatric burns: a prospective evaluation

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ABSTRACT

Background: Delay in the diagnosis of sepsis in pediatric burns results in advertently high mortality and morbidity. Our study aimed at evaluating the role of two upcoming biomarkers- neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR)- as predictors of early sepsis and mortality in this group of patients.
Methods: This was a prospective study conducted at a tertiary care burn centre of northern India over 18 months. 90 pediatric burn cases, aged 1-16 years, presenting within 24 hours of burns, with >10% body surface area of thermal burns/scalds were included in the study. Cell counts were measured on day 1, 3, 5 and 7 of burns. Patients were followed up till discharge, 30th post burn day or death, whichever was earlier.
Results: Sepsis was clinically present in 49 cases out of 90 (54.44%) with 30% median total body surface area (TBSA) of burns. Mortality was seen in 31cases out of 90 (34.44%) with 35% median TBSA burns. Higher PLR levels were seen in the sepsis group. NLR and PLR were also elevated in the survival group. Both parameters were found to be reliable markers of sepsis as well as mortality, particularly on days 5 and 7, in this cohort of patients.
Conclusions: Indices like NLR and PLR, which can easily be derived from complete blood count, have potential utility as determinants of both sepsis and mortality in children afflicted with thermal injuries.

Keywords: Complete blood count, Mortality, Neutrophil to lymphocyte ratio, Pediatric burns, Platelet to lymphocyte ratio, Sepsis

INTRODUCTION

The average global incidence of pediatric burns is estimated to be around 0.79%, with Asia and Africa accounting for the highest rates at 0.8% and 1.08% respectively.[1] The global pediatric burn mortality stands at approximately 2.5 per 100,000 cases, with higher incidence in low-income countries.[2] Sepsis is an important contributor to this mortality. Other factors influencing the vulnerability of children to burns include a requirement for precise fluid resuscitation, difficulty in venous access and avoidance of excisional burn surgery. Diagnosis of sepsis is often delayed due to reliance on clinical parameters with confirmation by blood cultures.[3] We believe identification of rapid and more sensitive biomarkers for diagnosing sepsis is needed, to ensure earlier commencement of antimicrobial therapy and improvement in overall outcomes.

A number of parameters like procalcitonin (PCT), C-reactive protein (CRP), neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR) etc. have been identified to diagnose sepsis in critically ill patients. However, an objective evaluation is needed to validate their utility in burn patients, who are in a state of chronic inflammation and hypermetabolism- a profile also seen in sepsis patients. Furthermore, the ability to predict sepsis and mortality can vary between adults and pediatric age-group for the same biomarker.

PCT has been shown to be a reliable marker for sepsis in both the adult, as well as the pediatric age groups.[4-14] On the other hand, while NLR and PLR have been demonstrated as prognostic indicators in community acquired pneumonia, critically ill patients with sepsis (both adult and pediatric age group) in various studies, there is paucity in literature as far as evaluation of these parameters in burns, especially pediatric burns, to predict sepsis and mortality, is concerned.[15-23]

The aim of this study was twofold- (1) to study the relation of NLR and PLR with the extent of burn injuries, and (2) to evaluate the role of these markers as predictors of sepsis and mortality in pediatric burn patients.

METHODS

This was a prospective study, carried out at Vardhman Mahavir Medical College and Safdarjung Hospital, a tertiary care burn center in northern India, from January 2019 to June 2020. Ethical clearance was taken from the institutional ethics committee. Written informed consent was obtained from the patients/authorized representatives of the patients.

Inclusion criteria of our study were as follows: (1) pediatric burns in the age group of 1-16 years, (2) cases presenting within 24 hours of burn injury, and (3) patients having >10% total body surface area (TBSA) of thermal burns or scalds. Electric and chemical burns were excluded from the study as were patients with severe malnutrition/debilitating disease.

Minimum sample needed for statistical significance was calculated a priori and found to be 65. However, we were able to recruit 90 subjects during our study period. All patients were resuscitated and treated as per standard operating protocols of the department. Complete blood count (CBC) was done using an automated analyzer (Pentra ES 60TM- Horiba Medical, Japan) on burn days 1, 3, 5 and 7. Total leucocyte count (TLC), differential leucocyte count (DLC) and platelet count (PC) were reported as part of the CBC results. NLR and PLR were calculated as per the following: NLR: ratio of absolute neutrophil count to absolute lymphocyte count. PLR: ratio of platelet count to absolute lymphocyte count.[15]

Patients were evaluated on days 1, 3, 5, 7 of burns for diagnosis of sepsis based on American Burn Association (ABA) sepsis criteria:[3] 1) Temperature: >102.2°F or <97.7°F. 2) Progressive tachycardia: >2 standard deviations (SD) above age specific norms (85% age adjusted maximum heart rate). 3) Progressive tachypnea: >2 SD above age specific norms (85% age adjusted maximum respiratory rate). 4) Thrombocytopenia (not applied until three days after initial fluid resuscitation): <2 SD below age specific norms. 5) Hyperglycemia (in the absence of preexisting diabetes mellitus): untreated plasma glucose >200 mg/dl. 6) Inability to continue enteral feedings >24 hours: a) abdominal distension; b) paralytic ileus; c) uncontrollable diarrhea.

Documented infection when any of these criteria were met: 1) positive culture, 2) pathologic tissue source, 3) clinical response to antimicrobials.

All patients were followed up till discharge, 30th burn day or death, whichever was earlier.

Statistical analysis

All collected data were analyzed using SPSS software (version 22.0, IBM Corp., Armonk, NY, USA). The statistical significance of quantitative variables between two groups (sepsis versus non-sepsis; survival versus dead) was done using non parametric Mann-Whitney test. The correlation of baseline NLR and PLR with TBSA burns was carried out using Pearson correlation coefficient. Efficiency of these parameters was assessed by calculating indices (sensitivity, specificity) and area under curve based on optimum cut off points determined by the receiver operator curve (ROC). Significance was set at $p<0.05$ with 95% confidence intervals.

RESULTS

A total of 90 cases were included in this study. The demographic characteristics of the study population have been depicted in Table 1.

Table 1: Demographic characteristics of study population (N=90).

Demographic variables		No. of cases (%)
Age (in years)	1-4	64 (71.1)
	4-8	11 (12.2)
	8-12	7 (7.8)
	12-16	8 (8.9)
Gender	Males	55 (61.1)
	Females	35 (38.9)
Nature of burns	Scalds	62 (68.9)
	Thermal burns	28 (31.1)

The TBSA of burns ranged from 10-95% (mean value 28.72±14.7%). Most of the pediatric burns i.e., N=36 (40%) had TBSA of burns between 10-20%.

Sepsis was clinically present in 49 cases of pediatric burns (54.44%). At the end of one month follow up, 59 (65.55%) cases survived. The study groups (sepsis versus non sepsis, survival versus dead) did not differ in gender distribution.

The median TBSA of burns was higher in sepsis group in comparison to non-sepsis group (30% versus 20%). The same was also higher in patients who had died vis-à-vis the survival group (35% versus 20%). In both scenarios, the differences were statistically significant ($p<0.05$). No strong correlation between day 1 NLR ($r=0.05$, $p=0.58$) and PLR ($r=-0.09$, $p=0.39$) was seen with TBSA of burns.

There was no statistically significant difference between the sepsis and non-sepsis groups with respect to mean NLR values (Table 2). In the sepsis group, NLR was highest on day 1 with lower values on days 3, 5 and 7. However, NLR values from day 3 to day 7 were progressively increasing. On the other hand, a falling trend was seen in the non-sepsis group (Table 3).

Mean NLR on day 5 and 7 was lower in the death group than in the survivor group (day 5 $p=0.044$, day 7 $p=0.002$) (Table 4).

In the survivor group, a rising trend in NLR was seen from day 3 to day 7. In patients who died, day 1 NLR was highest followed by a falling trend. A fall in NLR of 0.73 between day 3 and 7 in the expired group against a rise of 0.23 in the survival group was found to be statistically significant ($p=0.036$) (Table 5).

Table 2: Comparison of mean NLR and PLR levels between sepsis and non-sepsis group.

Days	Sepsis		Non-sepsis		P value	
	NLR	PLR	NLR	PLR	NLR	PLR
Day 1	2.65±2.2	101.06±49.9	3.6±3.2	106.7±65.2	0.384	0.997
Day 3	2.49±2.3	117.82±85.2	2.72±2.8	98.98±68.8	0.486	0.280
Day 5	2.51±2.6	116.36±66.3	2.47±2.5	81.91±71.6	0.656	0.009
Day 7	2.65±3.0	102.15±50.5	2.36±2.5	110.07±156.2	0.435	0.177

NLR- Neutrophil to lymphocyte ratio; PLR- Platelet to lymphocyte ratio

Table 3: Comparison of change in NLR and PLR levels between sepsis and non-sepsis group.

Range	Sepsis		Non-sepsis		P value	
	ΔNLR	ΔPLR	ΔNLR	ΔPLR	ΔNLR	ΔPLR
Day 1-3	-0.16	16.76	-0.94	-10.18	0.468	0.16
Day 1-5	-0.13	15.30	-1.19	-26.83	0.476	0.06
Day 1-7	-0.01	1.09	-1.35	-2.04	0.171	0.069
Day 3-5	0.02	-1.46	-0.24	-16.65	0.296	0.082
Day 3-7	0.15	-15.67	-0.40	8.13	0.217	0.874
Day 5-7	0.18	-14.21	0.69	24.79	0.874	0.120

Table 4: Comparison of mean NLR and PLR levels between dead and survival group.

Days	Dead		Survival		P value	
	NLR	PLR	NLR	PLR	NLR	PLR
Day 1	2.99±2.9	108.94±56.9	3.13±2.7	100.87±57.5	0.687	0.403
Day 3	2.31±2.7	120.79±102.7	2.74±2.4	103.54±62.8	0.187	0.430
Day 5	1.84 ±1.9	93.15±84.8	2.82±2.7	104.81±62.4	0.044	0.286
Day 7	1.61±2.0	65.77±61.3	2.97±3.0	125.27±123	0.002	0.000

Table 5: Comparison of change in NLR and PLR levels between dead and survival group.

Range	Dead		Survival		P value	
	ΔNLR	ΔPLR	ΔNLR	ΔPLR	ΔNLR	ΔPLR
Day 1-3	-0.75	7.95	-0.39	2.67	0.993	0.919
Day 1-5	-1.21	-18.79	-0.30	3.94	0.327	0.225
Day 1-7	-1.49	-47.41	-0.16	24.40	0.103	0.344
Day 3-5	-0.45	-26.74	0.08	1.26	0.393	0.150
Day 3-7	-0.73	-55.36	0.23	21.73	0.036	0.111
Day 5-7	0.29	-28.61	0.48	20.46	0.842	0.063

The levels of PLR varied significantly between sepsis and non-sepsis groups on day 5 (116.36±66.3 versus 81.91±71.6, p=0.009). In the sepsis group, day 1 PLR was the lowest with a further falling trend observed from day 3. Maximum fall was noted from day 5 to 7. In the non-sepsis group, variable trend was seen (Tables 2 and 3).

PLR on day 7 was found to be significantly lower in the dead group in comparison to the survivor group (65.77±61.3 versus 125.7±123, p=0.000). Differences in trends between the two groups were not statistically significant (Tables 4 and 5).

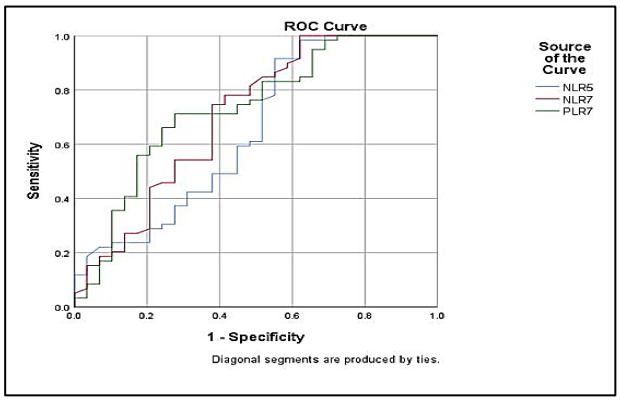

Figure 1: ROC Curve of day 5 NLR, day 7 NLR and day 7 PLR with survival.

Cut-off values: Day 5 and 7 NLR 1.36 and 1.25 (Sensitivity- 61%; Specificity- 51.7% and Sensitivity- 74.6%; Specificity- 62.1%); Day 7 PLR 60.28 (Sensitivity- 82.9%; Specificity- 67%). ROC- receiver operator curve; NLR- neutrophil to lymphocyte ratio; PLR- platelet to lymphocyte ratio.

ROC (Receiver operator curve) analysis of these biomarkers as predictors of survival in pediatric burns is shown in Figure 1 respectively.

DISCUSSION

Children with burns succumb more easily to sepsis than adult patients. Their body physiology is also different from adults. Biomarkers like PCT and CRP have shown to be reliable determinants of mortality associated with sepsis in both pediatric and adult burn patients.[4-14] In addition, NLR and PLR are two upcoming haematological parameters, which are very simple to calculate using the CBC report and may be useful, if found to have a role in predicting burn sepsis and mortality. Validation of these markers will decrease the delay in initiating antibiotic therapy caused by reliance on clinical features and blood culture sensitivity reports.

The mean age of our study population was 4.47±3.87 years with 71.1% (n=64) population belonging to age group 1-4 years. This highlights that the inquisitive nature of children, coupled with a lack of watchfulness by family members can result in accidental burns at home. Moreover, a higher proportion of scald injuries (68.9%), depict issues in developing countries like crowding, lack of access to electricity etc. (Table 1).

Overall mortality observed in our study was 34.4% (n=31) with 67.4% (n=21) of these cases having sepsis. Similar results were observed by Dhopte et al (mortality rate of 31.3%, with 71.3% harbouring infections).[24] Median TBSA afflicted by burns was found to be significantly higher in the sepsis group in comparison to non-sepsis group (30% versus 20%). Mean duration of stay in hospital was also higher in the sepsis group vis-a-vis non-sepsis group (11 days versus 7 days). A similar correlation was demonstrated by Hollen et al.[13] This indicates the increased risk of infection with an increasing TBSA of percentage of burns which in turn, reiterates the importance of prompt initiation of antibiotic therapy.

PCT is detectable 3-4 hours after an inflammatory stimulus, peaks at 14 hours and remains elevated for 24 hours, with a half-life in serum of 22-35 hours. However, it typically rises 6-12 hours after initial bacterial infection and falls only after the control of infection.[25] This makes it an important biomarker to distinguish inflammation from infection, as already proven by various studies.[26,27] CRP is an acute phase protein which rises over 2-3 days after stimulus, reaching peak at 50 hours and then falling according to the rate of disappearance of inflammation.[4,28] A few authors have shown PCT to be a more sensitive and specific marker for sepsis in children with burns than CRP.[29-31] However, both these parameters require enzyme backed assays for estimation, which are costly and may not be available in remote settings.

NLR and PLR are two new haematological biomarkers which are considered indicators of inflammation. There have been various studies correlating these two parameters with sepsis in critically ill patients (excluding trauma and burns) and as prognostic indicators in cancer, cardiac, and autoimmune diseases.[18-20,32-35] Systemic inflammatory response in burn injuries can lead to neutrophilic sequestration in organs with suppression of immune response causing lymphopenia.[18] According to Cato et al, platelet counts reach a nadir at day 2-5 of burns with peak values seen at day 10-18 in adult severe burns.[32] Very few studies, however, have evaluated these parameters as predictors of sepsis and mortality in the pediatric age-group. A recent retrospective study which collected 3-year data from septic pediatric patients in a pediatric intensive care unit (ICU) (not specific to burns) with a stay greater than 5 days excluding post-operative cases, found a rise in PLR (p=0.001) and NLR (p=0.026) to have prognostic value in predicting mortality. However, PLR had better overall accuracy (72.73%).[21] Another retrospective study of 670 critically ill pediatric ICU patients (not specific to burns), found only PLR to be a predictor of mortality (cut-off =3.9, p<0.001) with

no statistically significant role of NLR as a prognostic indicator.[17] NLR and PLR can be derived from a simple CBC report, which is an easily available test everywhere. This feature assumes significance in developing countries where health care cost is a major impediment to holistic patient care.

In our scenario, it was observed that the mean NLR level was lower in the expired group of patients, particularly on day 5 (p=0.044) and 7 (p=0.002) of burns. On the contrary, Ciftci et al, found higher NLR in patients who had died within a cohort of 366 adult burn cases.[23] These findings were upheld by Qiu et al who observed that higher day 3 NLR values predicted increased overall 90-day mortality in adults afflicted with thermal injuries.[37] Similarly, Angulo et al demonstrated a significantly higher association of day 1 and 7 NLR levels with mortality in adult burn injuries.[36] No correlation of NLR was seen with sepsis in our study. However, this was in sharp contrast to the observations by Fuss et al who noted a significantly higher NLR in adult burns with sepsis.[22] Due to the insufficient number of circulating neutrophils during the early stages of septic shock, there can be difficulty in mounting an effective innate response against the invading micro-organisms in burn patients. Increased neutrophil adhesion to the vascular endothelium, formation of leucocyte aggregates, micro-thrombi and endothelial damage can all lead to lower neutrophil counts with significantly poor outcomes, thus lowering NLR values in septic burn patients.[19]

Table 6: A summary of studies evaluating the role of NLR/PLR in burns.

Study/Year	Study population	Biomarkers	Study design	Sample size	Conclusion
Fuss et al[22] (2018)	Adult	NLR	Not mentioned	188	NLR can be used as a determinant of sepsis in adult burns
Ciftci et al[23] (2019)	Adult	NLR	Retrospective	366	Higher NLR associated with increased mortality in adult burns
Angulo et al[36] (2020)	Adult	NLR, PLR	Retrospective	88	NLR and PLR can predict survival in adults with thermal injuries
Qiu et al[37] (2021)	Adult	NLR	Retrospective	577	Higher NLR associated with significantly higher 90-day mortality
Lin et al[39] (2022)	Adult	PPR	Retrospective	590	Higher values indicate poor prognosis
Present study (2023)	Paediatric	NLR, PLR	Prospective	90	NLR and PLR are useful determinants of paediatric burn mortality and sepsis

NLR- Neutrophil to Lymphocyte Ratio; PLR- Platelet to Lymphocyte Ratio; PPR- Platelet distribution width to Platelet count Ratio

Average PLR was found to be higher in patients with proven infection and lower in those who showed mortality in our study. These differences were significantly higher on days 5 and 7 respectively. The cut-off value of mean PLR for survival on day 7 was found to be 60.28. A retrospective analysis of 88 adult burn cases by Angulo et al. also demonstrated higher PLR values in survivors with day 3 levels having a significant association with overall prognosis.[36] A recently published systematic review by Wang et al. to study the relationship between PLR and mortality in adult patients of sepsis (irrespective of the aetiology) concurred that the marker was indeed a significant determinant of survival with higher levels seen in non-survivors.[38] These differences in the findings of our study vis-à-vis others may be attributed to the age group of our study population. A brief summary of studies analysing the role of NLR/PLR in predicting sepsis/mortality and how they compare with ours is given in Table 6.

There were certain limitations to our study. First, it was a single centre research project. Therefore, results are not generally applicable and need further assessment. Second, there are a number of confounding factors which can affect the significance and power of our study design. Finally, while the study parameters (NLR and PLR) are continuous variables, we have observed only their cross-sectional values on alternate days in the first week of burns.

Having said so, our study also offers some key highlights. For instance, ours is a prospective evaluation and hence, free from selection bias. Secondly, our study cohort was homogenous in nature and sub-groups were similar demographically. To the best of our knowledge, this study is the first to assess two novel parameters viz. NLR and PLR in predicting sepsis and mortality in children afflicted with burns.

CONCLUSION

Markers like NLR and PLR, which can easily be derived from CBC values, have the potential to serve as simple as well as reliable determinants of sepsis and mortality in pediatric burn injuries. Multi-centric trials, with a larger sample size, are needed in order to further validate their utility in combination with other parameters and variables.

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