



HEMATOLOGIC BIOMARKERS AS PREDICTORS OF IMMUNE-RELATED CUTANEOUS ADVERSE EVENTS IN PATIENTS TREATED WITH IMMUNE CHECKPOINT INHIBITORS

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ABSTRACT:

Purpose: The study aimed to evaluate the association between baseline hematologic parameters and the development of immune-related cutaneous adverse events (irCAEs) in patients treated with immune checkpoint inhibitors (ICIs). Identifying predictive biomarkers for cutaneous toxicity is essential for early risk stratification and improved patient management.

Material/Methods: This retrospective observational study included adult patients with solid malignancies who received ICIs (anti-PD-1, anti-PD-L1, or anti-CTLA-4) and developed irCAEs. Data were collected from two oncology centers between 2018 and 2024, including complete blood counts and inflammatory markers: absolute lymphocyte count (ALC), eosinophil count (AEC), neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR). Immune-related cutaneous adverse events were classified by their clinical morphology, and their severity was graded using the Common Terminology Criteria for Adverse Events (CTCAE).

Results: Higher AEC and ALC, along with lower NLR, were significantly associated with the incidence and severity of cutaneous irCAEs. AEC emerged as the strongest predictor ($p = +0.61$, $\beta = +0.52$), followed by ALC ($p = +0.38$, $\beta = +0.29$) and NLR ($p = -0.58$, $\beta = -0.41$). Regression analysis explained 47% of the variance in cutaneous toxicity, and receiver operating characteristic (ROC) analysis showed AEC (AUC = 0.83) and NLR (AUC = 0.76) had strong predictive value.

Conclusions: Baseline AEC, ALC, and NLR are reliable predictors for the development of immune-related cutaneous adverse events in ICI-treated patients. These hematologic markers can serve as accessible, practical tools for early toxicity prediction, allowing for personalized monitoring strategies and timely interventions.

Keywords: Immune checkpoint inhibitors, Cutaneous adverse events, Eosinophil count, Neutrophil-to-lymphocyte ratio, Predictive biomarkers,

INTRODUCTION

Immune checkpoint inhibitors (ICIs) have fundamentally transformed modern oncologic practice by improving survival across a broad spectrum of solid and hematologic malignancies. By targeting inhibitory immune pathways such as programmed cell death protein 1 (PD-1), programmed death-ligand 1 (PD-L1), and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), these agents restore anti-tumour T-cell activity and promote durable immune-mediated tumour control. Despite their clinical efficacy, enhanced immune activation frequently results in a distinct spectrum of immune-related adverse events (irAEs), which may affect virtually any organ system [1].

Among these toxicities, cutaneous immune-related adverse events (irCAEs) represent the most common complication of ICI therapy, occurring in up to 30–50% of treated patients. Dermatologic manifestations range from mild pruritus and maculopapular eruptions to lichenoid dermatitis, vitiligo-like depigmentation, bullous pemphigoid-like disease, and, rarely, severe life-threatening reactions such as Stevens–Johnson syndrome or toxic epidermal necrolysis. Although most cutaneous irAEs are manageable, they may significantly impair quality of life, necessitate systemic immunosuppression, or even lead to treatment interruption or discontinuation, potentially compromising oncologic outcomes [2].

Early identification of patients at increased risk for dermatologic toxicity remains challenging in routine clinical practice. Currently, reliable predictive biomarkers for irCAEs are lacking. In recent years, easily accessible hematologic and inflammatory parameters derived from peripheral blood counts have attracted attention as potential indicators of systemic immune activation. Markers such as absolute lymphocyte and eosinophil counts, neutrophil-to-lymphocyte ratio (NLR), platelet-to-

lymphocyte ratio (PLR), and other composite indices have been associated with both therapeutic response and the development of immune-related toxicities. These parameters may reflect the balance between pro-inflammatory and immunosuppressive mechanisms, thereby serving as surrogate markers of immune dysregulation during checkpoint blockade [3].

Several studies suggest that eosinophilia and lymphocyte expansion correlate with enhanced immune activation and may precede the onset of immune-mediated events, whereas elevated neutrophil-dominant inflammatory profiles have been linked to poorer outcomes and reduced immune responsiveness. Nevertheless, data specifically addressing the relationship between routine hematologic indices and the occurrence of cutaneous irAEs remain limited and heterogeneous [4].

Given the clinical relevance of early toxicity prediction and the accessibility of laboratory biomarkers, further investigation into hematologic predictors of skin-related immune complications is warranted [5]. Therefore, the present study aimed to evaluate the association between baseline and dynamic hematologic parameters and the development of immune-related cutaneous adverse events in patients receiving immune checkpoint inhibitors, with the goal of identifying simple and clinically applicable risk indicators.

MATERIAL AND METHODS

This retrospective observational study was conducted over a seven-year period between January 1, 2018 and December 31, 2024. Data were collected from two tertiary oncology centers. The study included adult patients with histologically confirmed solid malignancies who were treated with immune checkpoint inhibitors, including anti-PD-1, anti-PD-L1, and/or anti-CTLA-4 agents, and subsequently developed immune-related cutaneous adverse events.

For each patient, comprehensive demographic and clinical data were systematically collected. Laboratory evaluation included a complete blood count with differential leukocyte analysis, comprising absolute lymphocyte (ALC), eosinophil (AEC), monocyte (AMC), and neutrophil (ANC) counts, as well as platelet count (PLT). In addition, composite systemic inflammatory indices were calculated from these hematologic parameters, including the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and monocyte-to-lymphocyte ratio (MLR), which were used as surrogate markers of systemic inflammation and immune status.

Immune-related cutaneous adverse events were classified according to their clinical morphology, including pruritus, maculopapular eruptions, lichenoid reactions, vitiligo-like depigmentation, bullous dermatoses, psoriasiform eruptions, Stevens–Johnson syndrome/toxic epidermal necrolysis (SJS/TEN), alopecia, and nail changes. Severity grading was performed according to the Common Terminology Criteria for Adverse Events (CTCAE), version 5.0. Time-to-onset of the first immune-related cutaneous adverse event, temporal dynamics, and their asso-

ciation with duration of immune checkpoint inhibitor therapy were systematically evaluated.

Statistical analysis

Descriptive statistics were used to summarize demographic and clinical characteristics. Continuous variables were presented as mean $\pm$ standard deviation (SD), minimum and maximum values, whereas categorical variables were expressed as frequencies and percentages.

Comparative analyses included:

- Independent samples *t*-test for comparison of means between two groups;
- Chi-square (χ^2) test or Fisher's exact test for categorical variables, as appropriate;
- Cramer's V to measure the strength of association between categorical variables;
- Spearman's rank correlation coefficient (ρ , rho) to assess relationships between continuous or ordinal variables.

Spearman's correlation coefficient (ρ) was used to evaluate the strength and direction of monotonic associations between variables. The correlation coefficient (r or ρ) ranges from “-1 to +1, where values close to +1 indicate a strong positive association, values close to “-1 indicate a strong negative association, and values near 0 indicate no correlation. The strength of correlation was interpreted as moderate ($r = 0.5$ – 0.7), strong ($r = 0.7$ – 0.9), and very strong ($r > 0.9$). The direction of association was defined as positive (both variables increase together) or negative (one variable increases while the other decreases).

Regression analyses were performed to further evaluate predictive relationships between variables. The β coefficient (beta coefficient) represents the magnitude and direction of the effect of an independent variable on the dependent variable. A positive β value indicates a direct association, whereas a negative β value indicates an inverse association. The size of the β coefficient reflects the strength of the effect.

Receiver Operating Characteristic (ROC) curve analysis was conducted to evaluate the predictive performance of laboratory parameters. The ROC curve plots the true positive rate (sensitivity) against the false positive rate ($1 - \text{specificity}$) across different threshold values. The area under the curve (AUC) was calculated to quantify overall diagnostic accuracy. An AUC value closer to 1.0 indicates excellent predictive performance, whereas an AUC of 0.5 suggests no discriminative ability.

Statistical significance was defined as $p \leq 0.05$.

All statistical analyses were performed using IBM SPSS Statistics version 28 and GraphPad Prism version 10.

Ethical considerations

The study was conducted in accordance with the Declaration of Helsinki. Patient confidentiality was ensured through anonymization of all collected data.

RESULTS

The analysis of baseline hematologic parameters demonstrated significant associations between several inflammatory markers measured at the initiation of immune

checkpoint inhibitor therapy and the subsequent development of immune-related cutaneous adverse events.

Higher absolute eosinophil count and absolute lymphocyte count, as well as lower neutrophil-to-lymphocyte ratio, were significantly associated with both increased incidence and greater severity of cutaneous toxicity ($p < 0.01$).

Correlation analysis revealed the strongest positive association for AEC ($\rho = +0.61$), followed by ALC ($\rho = +0.38$), whereas NLR showed a pronounced inverse relationship with the occurrence of skin adverse events ($\rho =$

-0.58). These findings suggest that a relatively eosinophil- and lymphocyte-predominant immune profile at baseline may predispose patients to the development of immune-mediated dermatologic toxicity during ICI therapy.

In contrast, other hematologic parameters, including absolute monocyte count (AMC), platelet count (PLT), platelet-to-lymphocyte ratio (PLR), and monocyte-to-lymphocyte ratio (MLR), did not demonstrate statistically significant associations with the occurrence of irCAEs (Table 1).

Table 1. Association between baseline mean hematologic parameters and the development of immune-related cutaneous adverse events in patients treated with immune checkpoint inhibitors

Hematologic parameter	Category / Cut-off value	Patients with cutaneous adverse events (n=148)	Patients without cutaneous adverse events (n=275)	Correlation (ρ)	p-value
ALC (lymphocytes)	$\uparrow > 3.0 \times 10^9/L$	54 (36.5%)	58 (21.1%)	0.38	0.006
AEC (eosinophils)	$\uparrow > 0.3 \times 10^9/L$	78 (52.7%)	62 (22.5%)	0.61	< 0.001
ANC (neutrophils)	$\downarrow < 2.0 \times 10^9/L$	61 (41.2%)	48 (17.5%)	-0.44	0.004
PLT (platelets)	$\uparrow > 400 \times 10^9/L$	39 (26.4%)	55 (20.0%)	0.19	0.08
NLR (neutrophil-to-lymphocyte ratio)	$\downarrow < 3.0$	82 (55.4%)	63 (22.9%)	-0.58	< 0.001
PLR (platelet-to-lymphocyte ratio)	$\uparrow > 180$	36 (24.3%)	66 (24.0%)	-0.21	0.12
MLR (monocyte-to-lymphocyte ratio)	$\uparrow > 0.3$	27 (18.2%)	52 (18.9%)	0.11	0.29

An additional analysis using a multiple regression model was performed on the parameters AEC, NLR, and ALC, as these exhibited the highest statistical significance and strongest correlation with the occurrence of immune-related cutaneous adverse events. Patients with elevated eosinophil counts (AEC) and lymphocyte counts (ALC), as well as low NLR values, demonstrated a more than 60% increased likelihood of developing cutaneous toxicities. The strongest positive correlation was observed for AEC (r

$= +0.58$), followed by ALC ($r = +0.43$), whereas NLR showed a pronounced negative correlation ($r = -0.56$).

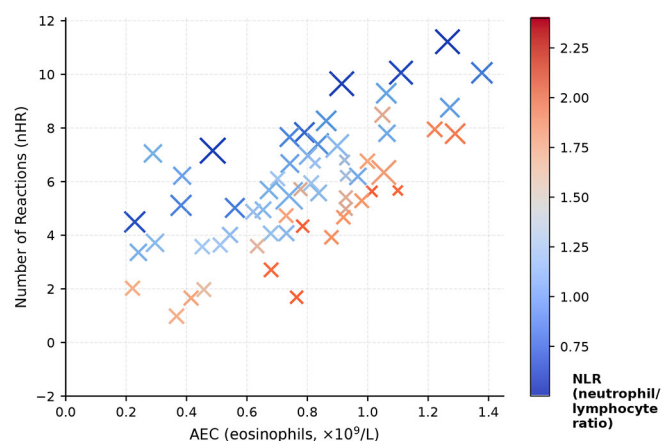
In a multiple linear regression analysis incorporating all laboratory parameters, the model explained approximately 47% of the variance in both the frequency and severity of cutaneous reactions ($R^2 = 0.47$; $p < 0.001$), indicating good predictive value. The most robust independent predictors were AEC ($\beta = +0.52$; $p < 0.001$), NLR ($\beta = -0.41$; $p = 0.004$), and ALC ($\beta = +0.29$; $p = 0.02$) (table 2).

Table 2. Additional multiple regression analysis of AEC, NLR, and ALC in patients with immune-related cutaneous adverse events and their association with the occurrence of irCAEs

Parameter	β coefficient	p-value	r (correlation coefficient)	Direction of association
AEC (eosinophils)	+0.52	< 0.001	+0.58	Strong positive
NLR (neutrophil-to-lymphocyte ratio)	-0.41	0.004	-0.56	Strong negative
ALC (lymphocytes)	+0.29	0.020	+0.43	Moderate positive
Model (R^2)	0.47	< 0.001	–	Good predictive value

The relationship between absolute eosinophil counts (AEC), neutrophil-to-lymphocyte ratio (NLR), and the number of irCAEs is further illustrated (Figure 1). The figure demonstrates a trend toward an increased number of cutaneous adverse events at higher AEC levels, whereas lower NLR values are associated with more pronounced skin toxicity

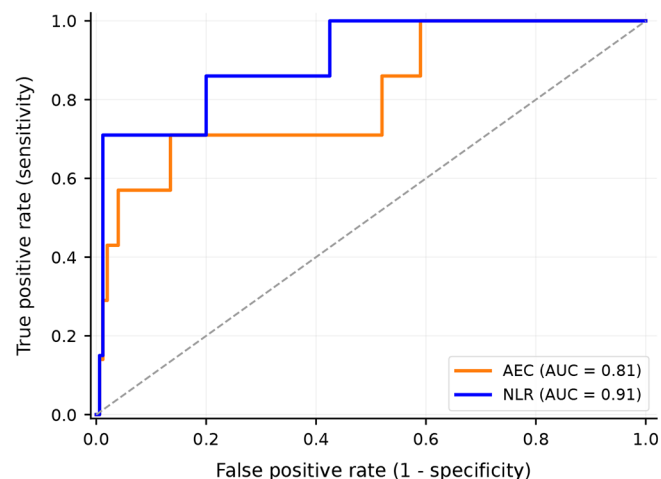
Fig. 1. Relationship between AEC, NLR, and the Number of Each Adverse Reaction



Additionally, the predictive value of ALC and NLR for the occurrence of immune-related cutaneous adverse reactions was evaluated (Figure 2). The ROC analysis showed that the eosinophil count (AEC) had the highest predictive value for the development of cutaneous adverse reactions (AUC = 0.83; $p < 0.001$), suggesting that elevated eosinophil levels may serve as a reliable early indicator of developing skin toxicity.

The neutrophil-to-lymphocyte ratio (NLR) also demonstrated good prognostic performance (AUC = 0.76; $p < 0.001$), with lower values being associated with a higher risk of immune-mediated skin manifestations.

Fig. 2. ROC curve for the predictive value of the laboratory parameters ALC and NLR for the occurrence of immune-related cutaneous adverse reactions during therapy with checkpoint inhibitors.



DISCUSSION:

In the present study, we demonstrated that baseline inflammatory hematologic parameters—particularly absolute eosinophil count, absolute lymphocyte count, and neutrophil-to-lymphocyte ratio—are significantly associated with the development and severity of immune-related cutaneous adverse events in patients treated with immune checkpoint inhibitors. Among all evaluated markers, AEC emerged as the strongest predictor, followed by NLR and ALC, while other commonly investigated indices, such as AMC, PLR, MLR, and platelet count, did not show significant associations.

Our correlation and regression analyses consistently identified a pattern characterized by elevated eosinophils and lymphocytes together with lower NLR as a risk profile for cutaneous toxicity. The strongest positive association was observed for AEC ($p = +0.61$; $\beta = +0.52$), suggesting that eosinophil predominance at baseline may reflect a pre-activated or Th2-skewed immune milieu predisposed to immune-mediated dermatologic reactions. In contrast, NLR demonstrated a robust inverse association ($p = -0.58$; $\beta = -0.41$), indicating that a relatively lymphocyte-dominant immune balance increases the likelihood of cutaneous irAEs.

Importantly, the multivariable regression model explained approximately 47% of the variance in the frequency and severity of cutaneous reactions ($R^2 = 0.47$; $p < 0.001$), underscoring the clinically meaningful predictive capacity of these routine laboratory parameters. Furthermore, ROC analysis confirmed the discriminative performance of AEC (AUC = 0.83) and NLR (AUC = 0.76), supporting their potential utility as early, accessible biomarkers for cutaneous toxicity risk stratification.

The role of peripheral blood inflammatory markers in predicting irCAEs remains controversial. While several studies have established NLR and PLR as prognostic markers of survival outcomes in melanoma, NSCLC, and gastrointestinal malignancies, their association with irCAEs has been inconsistent [6].

Some reports suggest that lower baseline NLR correlates with increased irCAE incidence, aligning with our findings. A lower NLR reflects relative lymphocytosis and reduced systemic inflammatory burden, indicating a more immunocompetent host environment [6]. Such an immune profile may enhance antitumor responses but simultaneously predispose to autoimmunity-like toxicities [7]. This concept supports the hypothesis that treatment efficacy and toxicity may represent two facets of the same immune activation process.

Conversely, other studies have reported elevated NLR as a predictor of certain organ-specific toxicities, particularly pneumonitis [8]. These discrepancies may stem from heterogeneity in tumor types, treatment lines, ethnic populations, timing of biomarker assessment (baseline vs post-treatment), and the diverse pathophysiology of different irCAEs [8]. Importantly, most prior investigations evaluated irAEs as a composite endpoint, whereas our study specifically focused on cutaneous manifestations, which may have distinct immunologic mechanisms.

Our finding that AEC is the strongest independent predictor is particularly noteworthy. Although eosinophilia has been sporadically associated with immune activation and improved immunotherapy outcomes, its specific relationship with skin toxicity has been less systematically evaluated [9]. Eosinophils are key effector cells in allergic and inflammatory skin conditions, capable of releasing cytotoxic granules, cytokines, and chemokines that amplify tissue inflammation [10]. Therefore, elevated baseline eosinophils may indicate a predisposition to exaggerated cutaneous immune responses following checkpoint blockade.

The pathogenesis of irCAEs is believed to involve loss of peripheral tolerance, expansion of autoreactive T-cell clones, cytokine dysregulation, and cross-reactivity between tumor and self-antigens. Checkpoint inhibition enhances CD8+ T-cell activation, but this activation is not tumor-restricted and may extend systemically [11].

From an immunological perspective [12]:

- Higher ALC may reflect a robust adaptive immune reserve capable of mounting strong effector responses.
- Lower NLR indicates a shift away from neutrophil-driven systemic inflammation toward lymphocyte-mediated immunity.
- Elevated eosinophils may signal a primed inflammatory microenvironment prone to cutaneous hypersensitivity-like reactions.

Together, these parameters depict an immune system that is highly reactive—beneficial for tumor control, but simultaneously increasing the risk of immune-mediated toxicity.

Currently, no validated, universally recommended biomarker exists for predicting irCAEs in routine clinical practice. Most candidate markers—including IL-6, CRP, LDH, cytokine profiles, HLA polymorphisms, gene signatures, and microbiome composition—remain investigational and are not widely accessible [13].

In contrast, AEC, ALC, and NLR are inexpensive, routinely available, and reproducible laboratory parameters. Our findings suggest that incorporating these markers into baseline risk assessment could help:

- identify patients at higher risk for cutaneous toxicity,
- Personalize dermatologic monitoring strategies,
- Educate high-risk individuals regarding early symptom recognition,
- Optimize benefit–risk discussions prior to ICI initiation.

Importantly, early detection of cutaneous irAEs may prevent progression to higher-grade toxicity and allow continuation of immunotherapy when clinically appropriate.

Strengths and Limitations

The strengths of our study include the focused evaluation of organ-specific (cutaneous) irAEs, the use of multivariable modeling to control for confounders, and the combined application of correlation, regression, and ROC analyses to assess predictive value.

However, several limitations must be acknowledged:

1. The retrospective design introduces potential se-

lection and information bias.

2. Confounding factors such as prior therapies, concomitant medications, and subclinical inflammatory conditions may have influenced baseline blood counts.

3. Other potentially relevant biomarkers (e.g., CRP, cytokines, autoantibodies, microbiome data) were not included.

4. External prospective validation is required before clinical implementation.

Future Directions

Prospective multicenter studies are needed to validate these findings and define clinically meaningful cut-off values for AEC and NLR. Future research should also explore composite predictive models integrating hematologic parameters with molecular, genomic, and microbiome data to improve predictive accuracy.

Given the growing use of ICIs across tumor types and treatment lines, the development of reliable, cost-effective predictive tools for irCAEs remains an urgent clinical priority.

CONCLUSION

In conclusion, our study demonstrates that elevated baseline AEC and ALC, together with lower NLR, are significant predictors of immune-related cutaneous adverse events during ICI therapy. Among these, AEC showed the strongest independent predictive value. These findings support the potential role of routine hematologic parameters as practical biomarkers for risk stratification of dermatologic toxicity in patients receiving immune checkpoint inhibitors.

The early identification of patients at increased risk may enable clinicians to implement closer and more individualized monitoring strategies. Such a risk-adapted approach could enhance patient safety, facilitate timely intervention, and ultimately contribute to improved treatment continuity and quality of life during immunotherapy.

Abbreviations:

irCAEs- immune-related cutaneous adverse events
 ICIs- immune checkpoint inhibitors
 PD-1 – Programmed Cell Death Protein 1
 PD-L1 – Programmed Death-Ligand 1
 CTLA-4 – Cytotoxic T-Lymphocyte-Associated Protein
 ALC- absolute lymphocyte count
 AEC- absolute eosinophil count
 ALC- absolute lymphocyte count
 AMC-absolute monocyte count
 ANC- absolute neutrophil count
 PLT- platelet count
 NLR- neutrophil-to-lymphocyte ratio
 PLR- platelet-to-lymphocyte
 MLR- monocyte-to-lymphocyte ratio
 CTCAE- Common Terminology Criteria for Adverse Events
 SJS- Stevens–Johnson syndrome
 TEN-toxic epidermal necrolysis
 SD- standard deviation
 ROC- Receiver Operating Characteristic
 AUC- area under the curve

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