Prognostic value of neutrophil-lymphocyte ratio and lactate dehydrogenase in melanoma patients treated with immune checkpoint inhibitors
A systematic review and meta-analysis

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Abstract
Background: Immune checkpoint inhibitors (ICIs) showed promising therapeutic efficacy on melanoma. Neutrophil-to-lymphocyte ratio (NLR) and serum lactate dehydrogenase (LDH) showed predictive values on prognosis of various tumors, but not on melanoma yet. This meta-analysis was conducted to investigate the prognostic role of NLR and LDH levels in melanoma treated with ICIs.

Methods: A search was conducted for all reports published till March 2020 in PubMed, Web of Science, Cochrane Library, EMBASE, ClinicalTrials.gov, and the WHO International Clinical Trials Registry Platform (ICTRP). Studies were included if they investigated the association between pretreatment NLR/LDH and prognosis in melanoma patients treated with ICIs. Subgroup analysis, publication bias, and meta-regression were conducted to investigate heterogeneity.

Results: A total of 6817 melanoma patients were included. Overall, high pretreatment NLR and LDH were associated with poor overall survival (OS) ($P < .001$) and PFS ($P < .001$). Subgroup analyses revealed that elevated NLR and LDH levels were associated with poor OS and PFS regardless of cutoff value, but LDH works when cutoff value = upper normal limit (UNL). The predictive value of NLR and LDH levels on OS and PFS was partially compromised in the Asian populations, compared with the Western countries.

Conclusion: Blood NLR and LDH levels showed great potential to be used as early prognostic biomarkers in melanoma patients treated with ICIs.

Key Words: immune checkpoint inhibitors, lactate dehydrogenase, neutrophil-lymphocyte ratio, meta-analysis

1. Introduction

Incidence rates of melanoma continue to increase worldwide in 2019.\textsuperscript{[1,2]} Although the 5-year survival rate for melanoma is 92%, advanced melanoma, including unresectable stage III and stage IV melanoma, is associated with poor survival outcomes.\textsuperscript{[3]} Immunotherapy has had a great effect on treatment for various tumors in recent years. Immune checkpoint blockade enhances antitumor activity of immune cells by inhibiting down-regulators of immune system such as programmed cell death 1 (PD-1) or its ligand, programmed cell death ligand 1 (PD-L1), and cytotoxic T lymphocyte antigen 4 (CTLA-4).\textsuperscript{[4]} Immune checkpoint inhibitors (ICIs) such as nivolumab (antibody against CTLA-4), pembrolizumab and nivolumab (both antibodies against PD-1) have demonstrated improved survival against melanoma.\textsuperscript{[4–6]} Nevertheless, a significant portion of patients do not benefit from ICIs, creating an urgent need to identify biomarkers to predict which patients are most likely to benefit from the treatment.

Prognostic factors for melanoma patients treated with ICIs have received much publicity. To date, a variety of prognostic biomarkers have been discovered, including PD-L1 expression\textsuperscript{[7,8]}, immune cell infiltration such as tumor-infiltrating lymphocytes (TIL) and “exhausted” T (Tex) cells in the tumor microenvironment\textsuperscript{[9,10]}, tumor mutation burden (TMB)\textsuperscript{[11]}, mismatch repair deficiency (MSI)\textsuperscript{[12]}, and microbiomes.\textsuperscript{[13]}

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All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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However, prognostic values of these biomarkers vary from person to person. Notably, there is a great interest in identifying peripheral blood biomarkers associated with favorable response to immune checkpoint blockade in patients with advanced melanoma. Blood samples can be easily and safely collected at low-cost and peripheral blood biomarkers can be used to profile the systemic immune response in a way that tumor biopsies cannot.

Evidence supports the idea that neutrophil-to-lymphocyte ratio (NLR) and serum levels of lactate dehydrogenase (LDH) are associated with survival in various tumors. Inflammation responses play an important role in tumorigenesis, disease progression, and prognosis. Systemic inflammation changes can be captured early by the level changes of NLR in peripheral blood. The metabolic level of normal cells pale in comparison with highly proliferative cancer cells. Altered tumor cell

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**Table 1**

The main characteristics of studies included for NLR.

| Study            | Country       | Agent                      | Sample size | NLR cutoff | Survival analysis | Analysis model | NOS |
|------------------|---------------|----------------------------|-------------|------------|-------------------|----------------|-----|
| Ascierto 2019[24] | United States | Nivolumab/Pembrolizumab    | 71          | 5          | OS/PFS            | Multivariate   | 7   |
| Bartlett 2020[24] | United States | Pembrolizumab              | 224         | 5          | OS                | Multivariate   | 7   |
| Balatoni 2018[27] | Hungary       | Ipilimumab                | 47          | 4          | OS                | Univariate     | 6   |
| Capone2018[28]    | Italy         | Nivolumab                 | 97          | 5          | OS/PFS            | Multivariate   | 7   |
| Cassidy 2017[29]  | United States | Ipilimumab                | 197         | 5          | OS/PFS            | Multivariate   | 7   |
| Cassidy 2017[30]  | France        | Nivolumab                 | 87          | 3          | OS/PFS            | Multivariate   | 7   |
| Ferrucci 2016[31] | Italy         | Ipilimumab                | 720         | 5          | OS/PFS            | Multivariate   | 7   |
| Jiyun Lee 2019    | Korea         | Ipilimumab                | 152         | 2.1        | OS/PFS            | Multivariate   | 7   |
| Khoja 2017        | Canada        | Ipilimumab                | 183         | 4          | OS                | Multivariate   | 7   |
| Minkyu Jung2017   | Korea         | Ipilimumab                | 95          | 5          | OS/PFS            | Univariate     | 7   |
| Rosner 2018[32]   | United States | Nivolumab plus Ipilimumab | 209         | 4.73       | OS                | Multivariate   | 7   |
| Tsutsumida 2019[33] | Japan      | PD-1/PD-L1 + CTLA-4       | 68          | 4          | OS                | Multivariate   | 7   |
| Zaragoza 2016[34] | France        | Ipilimumab                | 58          | 4          | OS                | Multivariate   | 7   |

**Abbreviations:** OS, overall survival; PFS, progression-free survival; NLR, neutrophil-to-lymphocyte ratio; NOS, Newcastle-Ottawa Scale; PD-1, programmed cell death 1; PD-L1, programmed cell death ligand 1; CTLA-4, cytotoxic T-lymphocyte antigen 4.
metabolism can be reflected by serum LDH.\(^{[19]}\) For these reasons, NLR and LDH levels might serve as prognostic factors for patients with melanoma, among other tumors. Although there have been systematic reviews and meta-analysis investigated the prognostic value of NLR in cancer patients treated with ICIs\(^{[20,21]}\) or LDH in melanoma treated with immunotherapy,\(^{[22]}\) they did not focus on NLR and LDH in melanoma patients who received ICIs. Furthermore, new studies on NLR and LDH in melanoma patients treated with ICIs have been published recently.\(^{[23–26]}\) We thus performed this meta-analysis to investigate the correlation between baseline NLR or LDH levels and their prognostic value for melanoma patients treated with ICIs.

### 2. Materials and Methods

#### 2.1. Search strategies

The protocol for this systematic review was registered on PROSPERO (CRD42019147625). Institutional Review Board approval was not required because this is a meta-analysis. An electronic search was performed using PubMed, EMBASE, Cochrane Library, ClinicalTrials.gov, and the WHO International Clinical Trials Registry Platform (ICTRP) up to March 2020. The search strategy was based on the following key terms: “melanoma,” “neutrophil to lymphocytes ratio,” “NLR,” “lactate dehydrogenase,” “LDH,” “CTLA-4,” “PD-1,” “PD-L1,” “ipilimumab,” “nivolumab,” “avelumab,” “durvalumab,” “atezolizumab,” “pembrolizumab,” “immune checkpoint inhibitor,” “immunotherapy,” “prognosis,” “prognostic,” and “survival.” The references in the identified articles were also applied to trace other relevant studies.

### 2.2. Study selection criteria

The inclusion criteria for the study were as follows:

1. Patients had been pathologically confirmed with melanoma;
2. Studies involved the association of baseline NLR or LDH levels with OS or progression-free survival (PFS);
3. Sufficient data were provided to calculate the hazard ratio (HR) and 95% confidence interval (CI); and

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**Table 2**

The main characteristics of studies included for LDH.

| Study          | Country        | Agent                  | Sample size | LDH cut off | Survival analysis | Analysis model | NOS |
|----------------|----------------|------------------------|-------------|-------------|-------------------|----------------|-----|
| Ahmad 2015\(^{[16]}\) | UK             | Ipilimumab             | 193         | 2 UNL       | OS                | Multivariate 7 | 6   |
| Ascierto 2019\(^{[16]}\) | Italy          | PD-1                   | 71          | UNL         | OS/PFS            | Multivariate 7 | 7   |
| Abu-Sbeih 2019\(^{[17]}\) | United States  | Nivolumab/Pembrolizumab | 346         | 618 IU/L    | OS/PFS            | Multivariate 8 | 8   |
| Arheden 2019\(^{[18]}\) | Sweden         | Nivolumab/Pembrolizumab | 116         | UNL         | OS                | Multivariate 6 | 6   |
| Bhatia 2019\(^{[19]}\) | United States  | Ipilimumab             | 88          | UNL         | OS                | Multivariate 7 | 7   |
| Boudewijns 2016\(^{[20]}\) | Netherland     | Ipilimumab             | 48          | UNL         | OS                | Univariate 7  | 7   |
| Bocquet 2019\(^{[21]}\) | France         | Pembrolizumab          | 86          | UNL         | OS/PFS            | Multivariate 7 | 7   |
| Betel 2017       | United States  | Nivolumab/Pembrolizumab | 254         | UNL         | OS/PFS            | Multivariate 7 | 7   |
| Baltoni 2018\(^{[22]}\) | Hungary        | Ipilimumab             | 47          | 1.5 UNL     | OS                | Multivariate 6 | 6   |
| Bischoff 2019\(^{[23]}\) | Netherlands    | Pembrolizumab          | 147         | 2 UNL       | OS/PFS            | Multivariate 8 | 8   |
| Chasset 2015\(^{[24]}\) | France         | Ipilimumab             | 45          | 500 IU/L    | OS                | Multivariate 7 | 7   |
| Chasseul 2018\(^{[25]}\) | France         | Nivolumab              | 87          | UNL         | OS                | Univariate 7  | 7   |
| Damuzo 2016\(^{[26]}\) | Italy          | Ipilimumab             | 44          | UNL         | OS                | Multivariate 7 | 7   |
| Delyon 2015\(^{[27]}\) | France         | Ipilimumab             | 73          | 2 UNL       | OS                | Univariate 6  | 6   |
| Dick 2016\(^{[28]}\) | Germany        | Ipilimumab             | 86          | 2 UNL       | OS/PFS            | Multivariate 5 | 5   |
| Diem 2015\(^{[29]}\) | United States  | Ipilimumab             | 128         | UNL         | OS                | Univariate 8  | 8   |
| Diem 2016\(^{[30]}\) | United States  | PD-1                   | 66          | UNL         | OS                | Multivariate 8 | 8   |
| Failing 2017\(^{[31]}\) | United States  | Pembrolizumab          | 133         | UNL         | OS/PFS            | Multivariate 7 | 7   |
| Felix 2016\(^{[32]}\) | France         | Ipilimumab             | 77          | 500 U/L     | OS                | Multivariate 7 | 7   |
| Ferrucci 2016\(^{[33]}\) | Italy          | Ipilimumab             | 720         | UNL         | OS                | Multivariate 7 | 7   |
| González 2017    | Spain          | Pembrolizumab          | 67          | UNL         | OS                | Multivariate 7 | 7   |
| Heidellberger 2017\(^{[34]}\) | France        | PD-1                   | 74          | UNL         | PFS               | Multivariate 6 | 6   |
| Heppt 2017\(^{[35]}\) | Germany        | PD-1 + Ipilimumab      | 96          | UNL         | OS                | Multivariate 5 | 5   |
| Jiyou Lee 2019   | Korean         | Ipilimumab             | 152         | UNL         | OS/PFS            | Multivariate 7 | 7   |
| Johnson 2015\(^{[36]}\) | United States  | Ipilimumab             | 35          | UNL         | OS                | Multivariate 5 | 5   |
| Jung 2017\(^{[37]}\) | Korea          | Ipilimumab             | 95          | UNL         | OS/PFS            | Multivariate 7 | 7   |
| Karydis 2016\(^{[38]}\) | United States  | Pembrolizumab          | 22          | UNL         | OS                | Univariate 8  | 8   |
| Kelderian 2015\(^{[39]}\) | UK             | Pembrolizumab          | 230         | UNL         | OS                | Multivariate 7 | 7   |
| Kojsova 2015      | United States  | Ipilimumab             | 196         | UNL         | OS                | Univariate 5  | 5   |
| Martens 2016\(^{[40]}\) | International | Ipilimumab             | 209         | 2.3 UNL     | OS                | Multivariate 7 | 7   |
| Nakamura 2018\(^{[41]}\) | Japan         | Nivolumab              | 98          | UNL         | OS                | Multivariate 5 | 5   |
| Nyakas 2019\(^{[42]}\) | Norway         | Ipilimumab             | 56          | 280 IU/ML   | OS                | Multivariate 8 | 8   |
| Sade-Feldman 2016\(^{[43]}\) | Israel        | Ipilimumab             | 56          | UNL         | OS                | Multivariate 5 | 5   |
| Ridolfo 2020\(^{[44]}\) | Italy          | Nivolumab/Pembrolizumab | 174         | UNL         | OS                | Multivariate 7 | 7   |
| Tsutsuwimida 2019\(^{[45]}\) | Japan         | PD-1/PD-L1 + CTLA-4    | 64          | UNL         | OS                | Multivariate 7 | 7   |
| Valpinon 2017\(^{[46]}\) | Italy          | Ipilimumab             | 216         | UNL         | OS                | Multivariate 7 | 7   |
| Wagner 2018 cohort\(^{[47]}\) | Germany       | Pembrolizumab          | 152         | 1.5 UNL     | OS                | Multivariate 7 | 7   |
| Wagner 2018 cohort\(^{[48]}\) | Germany       | CTLA-4 + Nivolumab     | 86          | 1.5 UNL     | OS                | Multivariate 7 | 7   |
| Wang 2016\(^{[49]}\) | United States  | Nivolumab              | 221         | UNL         | OS                | Multivariate 7 | 7   |
| Wen 2017\(^{[50]}\) | China          | Ipilimumab/Pembrolizumab | 52          | UNL         | PFS/OS            | Multivariate 7 | 7   |
| Weide 2016\(^{[51]}\) | International | Pembrolizumab          | 615         | UNL         | OS                | Multivariate 7 | 7   |
| Yamazak 2017\(^{[52]}\) | Japan          | Nivolumab              | 23          | UNL         | OS                | Univariate 6  | 6   |
| Zaragoza 2016\(^{[53]}\) | France         | Ipilimumab             | 58          | UNL         | OS                | Univariate 7  | 7   |

CTLA-4 = cytotoxic T-lymphocyte antigen 4, IU = international unit, LDH = lactate dehydrogenase, NOS = Newcastle-Ottawa Scale, OS = overall survival, PFS = progression-free survival, UNL = upper normal limit.
(4) Articles were published in full texts, excluding the following:
   a. Case reports, letters, conference abstracts, editorials, and reviews,
   b. Studies with insufficient information to evaluate HRs and 95% CIs, and
   c. Studies that were not communicated in English.

2.3. Data extraction and quality assessment
Two investigators independently selected the studies that fulfilled our inclusion criteria and extracted the relevant information. Disagreements were resolved by discussion with an independent expert. The following information was extracted: first author’s name, publication year, country, sample size, treatment received, study design, the cut-off to categorize high and low LDH or NLR levels, HRs for OS and PFS, and 95% CIs. The Quality Assessment of Newcastle-Ottawa Scale (NOS) was used to evaluate the quality of studies. This scale consists of 3 parameters: selection, comparability, and outcome assessment. NOS scores > 6 is considered high-quality studies, which were assessed by 2 independent reviewers.

2.4. Statistical analysis
HRs with their 95% CIs from included studies were used to calculate pooled HR. Heterogeneity of pooled results was accessed using Higgins I² statistic. I² > 50% was defined significant heterogeneity. A fixed effect model or random-effect model was employed according to the heterogeneity of the studies. The data were synthesized using a fixed effect model with I² < 50%. Otherwise, a random-effect model was utilized. The sources of heterogeneity were evaluated by sensitivity, subgroup analysis, and meta-regression. Sensitivity analysis was used to appraise the stability of the outcome. Funnel plots and Egger test were constructed to evaluate publication bias. All statistical tests were 2-sided, and statistical significance was defined as \( P < .05 \). The pooled data were analyzed with STATA 16.0.

3. Results

3.1. Study selection and characteristics
The flow chart of the literature selection is shown (Fig. 1). Totally 2072 relevant records were initially retrieved from selected databases. There were 1633 records included after duplicates removed. Of these, 1501 were excluded by screening titles and abstracts (because they were either conference abstracts, letters, reviews, case reports, or irrelevant studies), leaving 132 potentially relevant full-text articles. Eventually selected were 13 studies\(^ {23-24,26-36} \) involving 2328 individuals and concerning NLR, and 42 articles\(^ {24-26,30-32,34,36-39} \) including 5907 patients with regard to LDH. Seven studies\(^ {24,26,30-32,34,36} \) reported both NLR and LDH associated with OS or PFS. As the study by Wagner et al\(^ {62} \) included 2 cohorts, in which patients were treated with different regimens and reported the HR and 95% CI, respectively, we termed them as “Wagner 2018 cohort 1” and “Wagner 2018 cohort 2.” The characteristics of the selected studies are summarized in Tables 1 and 2.

| Study ID     | HR (95% CI)          | Weight |
|--------------|----------------------|--------|
| Ascierto 2019| 1.76 (0.91, 3.43)    | 5.33   |
| Balatoni 2018| 1.97 (1.03, 3.75)    | 5.52   |
| Bartlett 2020| 1.95 (1.33, 2.86)    | 8.59   |
| Capone 2018  | 2.85 (1.60, 5.08)    | 6.18   |
| Cassidy 2017 | 2.03 (1.49, 2.77)    | 9.60   |
| Chasseuil 2018| 1.12 (1.02, 1.23)   | 12.04  |
| Ferrucci 2016| 2.29 (1.86, 2.82)    | 10.94  |
| Jiyun Lee 2019| 4.58 (2.12, 9.91)   | 4.45   |
| Khoja 2017   | 1.04 (1.01, 1.08)    | 12.31  |
| Minkyu Jung 2017| 0.99 (0.75, 1.31) | 10.00  |
| Rosner 2018  | 1.95 (1.11, 3.43)    | 6.33   |
| Tsutsumida 2019| 1.84 (0.84, 4.06)  | 4.31   |
| Zaragoza 2016| 2.20 (1.01, 4.79)    | 4.40   |
| Overall      | 1.71 (1.40, 2.10)    | 100.00 |

NOTE: Weights are from random effects analysis

Figure 2. Forest plots of the relationship between NLR and survival outcomes in melanoma patients treated with ICIs. A random-effect model was used to evaluate the impact of NLR on OS. The pooled result indicated that high NLR was associated with poor OS. CI = confidence interval, HR = hazard ratio, ICIs = immune checkpoint inhibitors, NLR = neutrophil-lymphocyte ratio, OS = overall survival.
be associated with poor OS in patients treated with anti-PD-1/PD-L1 or anti-CTLA-4 alone (NLR: HR = 2.42, 95% CI, 1.68–3.50, P < .001; HR = 1.45, 95% CI, 1.16–1.81, P = .001; LDH: HR = 2.18, 95% CI, 1.73–2.74, P < .001; HR = 1.85, 95% CI, 1.52–2.26, P < .001, respectively). In anti-PD-1/PD-L1 + anti-CTLA-4 subgroup, NLR is associated with poor OS (HR = 1.91, 95% CI, 1.21–3.03, P = .006), but there was no significant relevance between LDH and OS (HR = 1.71, 95% CI, 0.77–3.78, P = .187). In the mixed group which included anti-PD-1/PD-L1 alone, anti-CTLA-4 alone, and anti-PD-1/PD-L1 + anti-CTLA-4 regimen, high LDH was associated with poor OS (HR = 6.42, 95% CI, 2.42–16.75, P < .001). Stratified analysis based on cut-off value of NLR (cutoff = 5: HR = 1.74, 95% CI, 1.22–2.47, P = .002; cutoff ≠ 5: HR = 1.53, 95% CI, 1.24–1.88, P < .001) and LDH (cutoff = UNL: HR = 1.97, 95% CI, 1.67–2.33, P = .001; cutoff ≠ UNL: HR = 2.19, 95% CI, 1.51–3.17, P = .001; upper normal level [UNL]) indicated that high NLR and LDH were related to poor OS. Moreover, we performed stratified analysis by geographical region, which showed that elevated NLR led to poor OS for patients in Europe (HR = 1.90; 95% CI, 1.24–2.9; P = .003) and North America (HR = 1.64; 95% CI, 1.03–2.60; P = .036) with a possible exception of Asia (HR = 1.93; 95% CI, 0.75–4.95; P = .170). In terms of LDH, however, the results of subgroups based on region showed that high LDH led to poor OS for patients throughout the globe (Europe [HR = 2.16; 95% CI, 1.76–2.64; P < .001], North America [HR = 2.06; 95% CI, 1.54–2.74; P < .001], and Asia [HR = 2.64; 95% CI, 1.94–3.58; P < .001]).

### Table

| Study ID         | HR (95% CI) | % Weight |
|------------------|-------------|----------|
| Ahmad 2015       | 4.58 (2.83, 7.41) | 3.03     |
| Ascierto 2019    | 1.58 (0.63, 3.95) | 1.62     |
| Abu?Sbe 2019     | 2.20 (1.47, 3.29) | 3.36     |
| Artefen 2019     | 3.52 (1.81, 6.83) | 2.93     |
| Bharia 2019      | 2.45 (1.16, 5.16) | 2.07     |
| Boudeuwijn 2016  | 3.56 (1.68, 7.53) | 2.06     |
| Bocquet-Tremoureux 2019 | 1.24 (1.01, 1.52) | 4.14     |
| Betof 2017       | 3.13 (1.97, 4.99) | 3.09     |
| Balatini 2018    | 3.55 (1.23, 10.31) | 1.33     |
| Bisschop 2019    | 0.70 (0.40, 1.21) | 2.75     |
| Chasen 2015      | 2.46 (1.25, 4.84) | 2.29     |
| Chassell 2018    | 1.31 (1.18, 1.45) | 4.41     |
| Damuzzo 2016     | 2.27 (0.88, 5.88) | 1.55     |
| Delyon 2013      | 3.42 (1.09, 10.76) | 1.20     |
| Dick 2016        | 5.24 (2.40, 11.44) | 1.97     |
| Diem 2015        | 1.03 (1.01, 1.05) | 4.50     |
| Diem 2016        | 2.14 (1.07, 4.29) | 2.23     |
| Failing 2017     | 1.47 (0.75, 2.90) | 2.29     |
| Felix 2016       | 2.20 (1.27, 3.81) | 2.75     |
| Ferrucci 2016    | 1.09 (0.46, 2.58) | 1.75     |
| Gonzales 2017    | 2.84 (1.27, 6.35) | 1.90     |
| Hepton 2017      | 8.48 (1.95, 21.50) | 1.12     |
| Jiun Lee 2019    | 2.69 (1.25, 5.74) | 2.02     |
| Johnson 2015     | 2.60 (1.01, 6.69) | 1.56     |
| Minkyu Jung 2017 | 1.10 (0.21, 5.64) | 0.67     |
| Karydis 2016     | 5.15 (1.69, 15.69) | 1.24     |
| Kederman 2014    | 2.95 (1.75, 4.97) | 2.86     |
| Krajsa 2015      | 1.44 (1.03, 2.03) | 3.62     |
| Martens 2016     | 1.20 (1.02, 1.41) | 4.27     |
| Nakamura 2016    | 2.56 (1.22, 5.38) | 2.08     |
| Nyakas 2019      | 2.86 (1.38, 5.91) | 2.13     |
| Sade−Feldman 2016| 5.88 (2.70, 12.80) | 1.98     |
| Ridolfi 2020     | 2.42 (1.48, 3.95) | 2.99     |
| Tsutsuji 2019    | 2.78 (0.96, 8.05) | 1.33     |
| Vailpion 2015    | 1.36 (1.16, 1.60) | 4.27     |
| Wagner 2018 cohort1 | 2.06 (0.89, 4.78) | 1.81     |
| Wagner 2018 cohort2 | 1.22 (0.55, 2.73) | 1.91     |
| Wang 2016        | 2.10 (1.34, 3.29) | 3.16     |
| Weide 2016       | 1.14 (0.81, 1.59) | 3.63     |
| Wen 2017         | 6.30 (1.26, 31.50) | 0.70     |
| Yamazaki 2017    | 1.85 (0.75, 4.58) | 1.66     |
| Zaragoza 2016    | 1.85 (0.95, 3.57) | 2.34     |
| Overall (I² = 86.2%, P = 0.000) | 2.03 (1.76, 2.35) | 100.00   |

**NOTE:** Weights are from random effects analysis

Figure 3. Forest plots of the relationship between LDH and survival outcomes in melanoma patients treated with ICIs. A random-effect model was used to evaluate the impact of LDH on OS. The pooled result indicated that high LDH was associated with poor OS. CI = confidence interval, HR = hazard ratio, ICIs = immune checkpoint inhibitors, LDH = lactate dehydrogenase.
3.3. Progression-free survival

Evaluation of the correlation between pretreatment NLR or LDH and PFS were reported in 8 and 12 studies, respectively. Pooled data of HR showed that high NLR (HR = 1.83, 95% CI, 1.34–2.51, \(P < .001\)) and LDH (HR = 1.65, 95% CI, 1.31–2.07, \(P < .0001\)) were linked to poor PFS, with extensive heterogeneity (NLR: I² = 87.4%; LDH: I² = 61.3%) (Fig. 4).

Then, subgroup analyses were performed according to ICI regimen, the cutoff value of NLR and LDH, and geographic regions (Tables 3 and 4). The results of subgroup analyses based on ICIs found that a consistent significant association between high NLR and poor PFS. We did not conduct this analysis on LDH due to limited study numbers. In subgroup analysis stratified by cutoff value, there was a significant association between high NLR levels and poor PFS when cutoff = 5 (HR = 1.98, 95% CI, 1.69–2.31, \(P = .001\)) and cutoff ≠ 5 (HR = 1.70, 95% CI, 1.06–2.74, \(P = .028\)).

In terms of LDH, its elevation was associated with poor PFS if cutoff = UNL group (HR = 1.59, 95% CI, 1.26–2.00, \(P = .001\)) and cutoff ≠ UNL group (HR = 1.89, 95% CI, 0.89–4.02, \(P = .096\)). And stratified analysis by geographic regions showed that high LDH was associated with poor PFS for patients in Europe (HR = 1.62; 95% CI, 1.13–2.33; \(P = .009\)) and North America (HR = 1.82; 95% CI, 1.30–2.55; \(P < .001\)). While we did not find statistically significant differences in Asia (HR = 1.53; 95% CI, 0.86–2.73; \(P = .152\)).

3.4. Sensitivity analysis

We also performed sensitivity analyses for the OS and PFS to determine whether an individual study influenced the results; there was no significant influence. The combined HRs and its 95% CIs were not significantly altered when any study was excluded, suggesting that no single study held a significant impact on the polled results (see in Supplemental Digital Content 1, http://links.lww.com/MD/G929).

Meta-regression analysis was used for the detection of additional heterogeneity. ICIs regimen, study design, and cutoff value were incorporated as covariates, but neither of them changed the correlation between NLR or LDH and survival outcomes (data were not shown).

3.5. Publication bias

The funnel plot of all eligible studies involving NLR and LDH for OS indicated that obvious publication bias existed, the results were confirmed regarding the OS of NLR (Egger test,
For PFS, the funnel plots of NLR were not symmetrical, suggesting a high risk of potential publication bias in these studies, but the funnel plots of LDH suggested a low risk of potential publication bias in these studies. In addition, Egger test was done to further validate PFS (P = .018 and P = .127 for NLR and LDH, respectively) (see Supplemental Digital Content 2, http://links.lww.com/MD/G929).

### 4. Discussion

In this meta-analysis, we pooled the data of 2208 melanoma patients from 13 studies on NLR, and 5907 patients from 42 LDH studies to explore the prognostic roles of NLR and LDH levels in melanoma patients treated with ICIs. We found that elevated levels of NLR and LDH in peripheral blood may be able to predict poor OS and PFS in melanoma patients treated with ICIs.

Recently, the prognostic role of NLR has gained increasing attention in the cancer science community. Several meta-analyses revealed the significance of NLR in cancer patients who received immune checkpoint inhibitors. One publication found that patients with high NLR level had a significantly shorter OS (HR = 1.92; 95% CI, 1.29–2.87; P = .001) and PFS (HR = 1.66; 95% CI, 1.38–2.01; P < .0001). Another meta-analysis which included 14 studies showed similar results. However, these meta-analyses were targeted at various cancers, except melanoma. Our study incorporated all recent eligible studies on NLR in melanoma patients treated with ICIs and found that elevated NLR level in peripheral blood was associated with...

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**Figure 4.** Forest plots of the relationship between NLR/LDH and PFS in melanoma patients treated with ICIs. (A) A random-effect model was used to evaluate the impact of NLR on OS. The pooled result indicated that high NLR was associated with poor OS; (B) A random-effect model was used to evaluate the impact of LDH on PFS. The pooled result showed that elevated LDH was significantly correlated with inferior PFS. CI = confidence interval, HR = hazard ratio, ICIs = immune checkpoint inhibitors, LDH = lactate dehydrogenase, NLR = neutrophil-lymphocyte ratio, OS = overall survival, PFS = progression-free survival.
shorter OS and PFS. Another recent meta-analysis was focused on LDH levels as a potential prognostic and predictive factor in melanoma patients treated with immunotherapy and BRAF + MEK inhibitors, and showed that high baseline LDH levels were associated with poor OS (HR = 1.72; 95% CI, 1.6-1.85) and PFS (HR = 1.83; 95% CI, 1.53-2.2). Our study was the first meta-analysis to incorporate LDH and NLR levels as predictive factors for survival in melanoma patients treated with sole ICI and conducted various subgroup analysis, which provided more focused and detailed data on ICI.

The underlying mechanism between the high NLR blood level and poor prognosis of patients with melanoma treated with ICIs remained unclear. Current studies pointed out that tumor-infiltrating neutrophils promote cancer progression through the secretion of various inflammatory cytokines and the inhibition of host immune system via suppressing the activity of cytotoxic T cells. Circulating lymphocytes have long been considered one of the primary effector cells in antitumor response, and previous research showed that T lymphocytes were the major immune effector cells in the PD-1 pathway, and CTLA-4 inhibitor can strengthen responses by activating CD8+ T cell.

The underlying mechanism associated with poor survival in melanoma patients with high LDH levels may be related to enhanced aerobic glycolysis, which is termed as Warburg effect. LDH as a key glycolytic enzyme plays a crucial role in pyruvate-to-lactate conversion, and the reproduction of oxidized nicotinamide adenine dinucleotide. Melanoma cells with enhanced invasion and metastasis showed increased glucose uptake and lactate production. Moreover, LDH may help cancer cells suppress and evade the immune system. Previous study showed LDH-associated lactate can upregulate vascular endothelial growth factor and arginase 1 by hypoxia-inducible factor 1a, and then result in macrophages shifted to M2-polarized macrophages, which promotes tumor progression. M0 → M2 macrophage polarization is also accompanied by interchangeable glucose- or lactate-dependent tricarboxylic acid (TCA) cycle metabolism that directly drives histone acetylation, M2 gene transcription, and functional immune suppression. Lactate accumulation also inhibited tumor surveillance by decreased interferon-γ in T and natural killer cells in melanomas. Dendritic cells were affected in a high LDH level. Increased lactic acidosis can compromise both the numbers and functions of dendritic cells. Qiao et al evaluated the efficacy of using LDH inhibitor oxamate and pembrolizumab alone or in combination in an NSCLC humanized mouse model. They found that both oxamate and pembrolizumab monotherapy significantly delayed tumor growth. Furthermore, combination therapy exhibited better efficacy since oxamate increased the infiltration of activated CD8+ T cells in the tumor. This study showed that combined therapy of drugs targeting LDH and ICI is promising.

In addition, we have also found that NLR and LDH have dissimilar prognostic values for melanoma patients receiving ICIs in different global regions. This may be due to many factors. First, different melanoma subtypes exist in Asian patients compared to Western patients. In Asians, acral and mucosal melanoma with higher frequency of KIT mutations are the main subtypes while cutaneous melanoma with higher
incidence of BRAF mutations is the predominant subtype in Europe and North America.\cite{10} Second, the treatment models in Asian countries vary, which included anti-CTLA-4 alone, anti-PD-1/PD-L1 alone, or anti-CTLA-4 + anti-PD-1/PD-L1. Third, heterogeneity of lymphocytes exists between races. Previous study reported that Asian population have lower peripheral lymphocyte counts compared to Caucasian, African, and Latin-American populations.\cite{11} Finally, sample size was relatively small in our Asian subgroup, which may limit the statistical power to identify the prognostic value of NLR or LDH levels in melanoma patients.

In the subgroups of LDH based on agent, we have found that the prognostic roles of LDH vary in different treatment regimens. In anti-CTLA-4 + anti-PD-1/PD-L1 group, no association between LDH and OS may be due to high frequency of immune-related adverse events in responders. Elevated LDH were found in patients developing autoimmune hepatitis or colitis. Afzal et al\cite{12} reported that a patient with uveal melanoma responds to ipilimumab plus nivolumab while developing autoimmune hepatitis or colitis. These demonstrated the potential use of NLR and LDH levels for predicting immune-related adverse events. Thus, strictly designed clinical trials are warranted to further explore the potential of NLR and LDH for predicting immune-related adverse events.

5. Conclusion

Our study showed that elevated baseline NLR and LDH levels were associated with poor prognosis in melanoma patients treated with ICIs. Cutoff value, ICI regimen, and geographic region may affect the prognostic value of NLR and LDH levels. These demonstrated the potential use of NLR and LDH levels in guiding the proper use of ICIs to treat patients with melanoma, therefore saving valuable medical resources and benefiting patients.

Author contributions

Yongchao Zhang analyzed the data and wrote the first draft. Bozhi Liu analyzed the data. Wei Li designed the study, proof read and revised the submission. Sergei V. Kotenko proof read and revised the submission. All authors discussed the results and approved the final article.

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