Systematic Review and Meta-Analysis

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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 in patients treated with anti-CTLA-4 or anti-PD-1/PD-L1 alone. NLR level was superior in predicting OS if compared with LDH level in patients treated with anti-PD-1/PD-L1 + anti-CTLA-4. In subgroup analysis stratified by cutoff value, high NLR level was 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.[1] 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.[2] 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).[3] Immune checkpoint inhibitors (ICIs) such as ipilimumab (antibody against CTLA-4), pembrolizumab and nivolumab (both antibodies against PD-1) have demonstrated improved survival against melanoma.[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[7,8]; immune cell infiltration such as tumor-infiltrating lymphocytes (TIL) and "exhausted" T (Tex) cells in the tumor microenvironment[9,10]; tumor mutation burden (TMB)[11]; mismatch repair deficiency (MSI)[12]; and microbiomes.[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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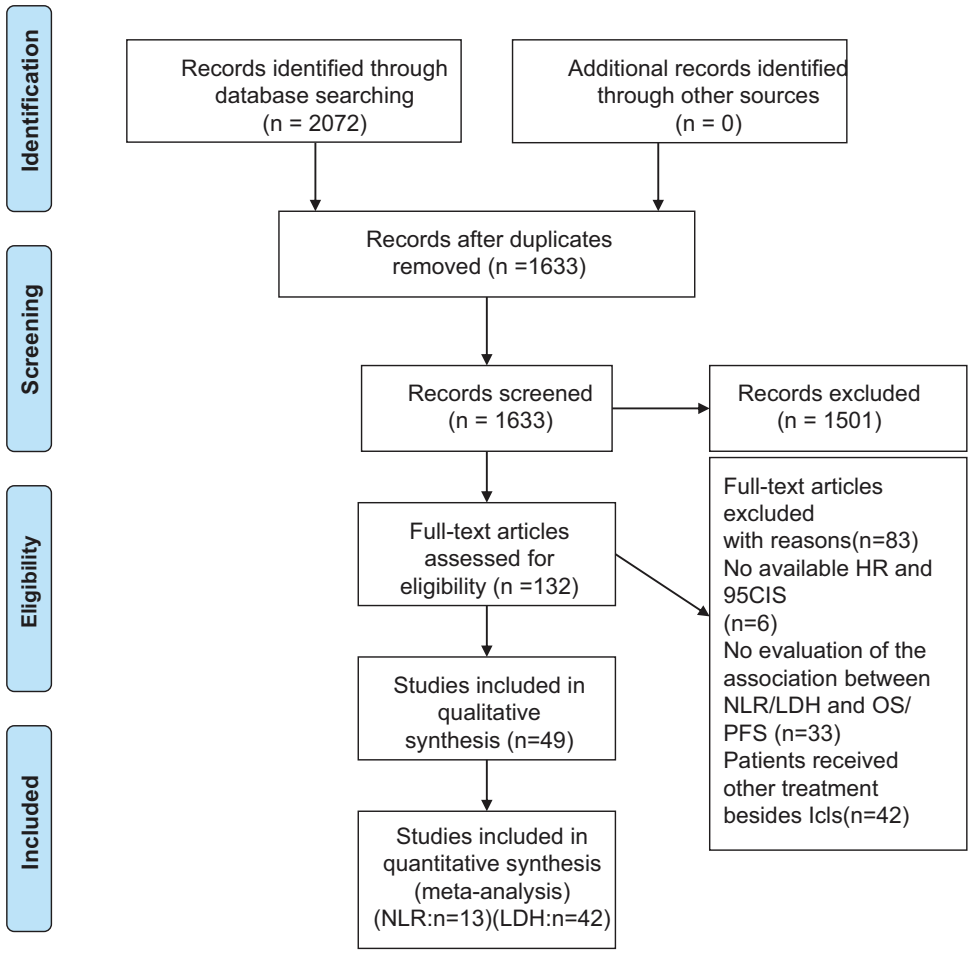

Figure 1. Flow chart of literature search and study selection. A total of 1633 articles were initially retrieved. After carefully reviewed 13 studies reporting NLR and 42 studies reporting LDH were included in the analysis. LDH = lactate dehydrogenase, NLR = neutrophil-lymphocyte ratio.

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[23]	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	8
Chasseuil 2018[30]	France	Nivolumab	87	3	OS/PFS	Multivariate	7
Ferrucci 2016[31]	Italy	Ipilimumab	720	5	OS/PFS	Multivariate	7
Jiyun Lee 2019	Korea	Nivolumab\ Pembrolizumab	152	2.1	OS/PFS	Multivariate	7
Khojia 2017	Canada	Ipilimumab	183	4	OS	Multivariate	7
Minkyu Jung2017	Korea	Ipilimumab	95	5	OS/PFS	Univariate	7
Rosner 2018[35]	United States	Nivolumab plus Ipilimumab	209	4.73	OS	Multivariate	7
Tsutsumida 2019[26]	Japan	PD-1/PD-L1 + CTLA-4	68	4	OS	Multivariate	7
Zaragoza 2016[36]	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.

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.[14–16] Inflammation responses play an important role in tumorigenesis, disease progression, and prognosis.[17] Systemic inflammation changes can be captured early by the level changes of NLR in peripheral blood.[18] The metabolic level of normal cells pale in comparison with highly proliferative cancer cells. Altered tumor cell

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[38]	UK	Ipilimumab	193	2 UNL	OS	Multivariate	6
Ascierto 2019[24]	Italy	PD-1	71	UNL	OS/PFS	Multivariate	7
Abu-Sbeih 2019[37]	United States	Nivolumab\Pembrolizumab	346	618 IU/L	OS/PFS	Multivariate	8
Arheden 2019[39]	Sweden	Nivolumab\Pembrolizumab	116	UNL	OS	Multivariate	6
Bhatia 2019[25]	United States	Ipilimumab	88	UNL	OS	Multivariate	7
Boudewijns 2016[42]	Netherland	Ipilimumab	48	UNL	OS	Univariate	7
Bocquet 2019[41]	France	Pembrolizumab	86	UNL	OS/PFS	Multivariate	-
Betof 2017	United States	Nivolumab\Pembrolizumab	254	UNL	OS/PFS	Multivariate	7
Balatoni 2018[27]	Hungary	Ipilimumab	47	1.5 UNL	OS	Multivariate	6
Bisschop 2019[40]	Netherlands	Pembrolizumab	147	2 UNL	OS/PFS	Multivariate	8
Chasset 2015[43]	France	Ipilimumab	45	500 IU/ML	OS	Multivariate	7
Chasseuil 2018[30]	France	Nivolumab	87	UNL	OS	Univariate	7
Damuzzo 2016[44]	Italy	Ipilimumab	44	UNL	OS	Multivariate	7
Delyon 2013[45]	France	Ipilimumab	73	2 UNL	OS	Univariate	6
Dick 2016[46]	Germany	Ipilimumab	86	2 UNL	OS/PFS	Multivariate	5
Diem 2015[47]	United States and Italy	Ipilimumab	128	UNL	OS	Univariate	8
Diem 2016[48]	United States and Spain	PD-1	66	UNL	OS	Multivariate	8
Failing 2017[49]	United States	Pembrolizumab	133	UNL	OS/PFS	Multivariate	7
Felix, 2016[50]	France	Ipilimumab	77	500 U/L	OS	Multivariate	7
Ferrucci 2016[31]	Italy	Ipilimumab	720	UNL	OS	Multivariate	7
González 2017	Spain	Pembrolizumab	67	UNL	OS	Multivariate	7
Heidelberger 2017[53]	France	PD-1	74	UNL	PFS	Multivariate	6
Heppt 2017[54]	Germany	PD-1 ± Ipilimumab	96	UNL	OS	Multivariate	5
Jiyun Lee 2019	Korean	PD-1	152	UNL	OS/PFS	Multivariate	7
Johnson 2015[55]	United States	Ipilimumab	35	UNL	OS	Multivariate	5
Jung 2017[32]	Korean	Ipilimumab	95	UNL	OS/PFS	Multivariate	7
Karydis 2016[56]	UK	Pembrolizumab	22	UNL	OS	Univariate	8
Kelderman 2014[57]	UK, Denmark And Netherland	Ipilimumab	230	UNL	OS	Univariate	7
Krajsova 2015	United States	Ipilimumab	196	UNL	OS	Univariate	5
Martens 2016[68]	International	Ipilimumab	209	2.3 UNL	OS	Multivariate	7
Nakamura 2016[59]	Japan	Nivolumab	98	UNL	OS	Multivariate	5
Nyakas 2019[60]	Norway	Ipilimumab	56	280 UI/ML	OS	Multivariate	8
Sade-Feldman 2016[67]	Israel	Ipilimumab	56	UNL	OS	Multivariate	5
Ridolfi 2020[66]	Italy	Nivolumab/Pembrolizumab	174	UNL	OS	Multivariate	7
Tsutsumida 2019[26]	Japan	PD-1/PD-L1 + CTLA-4	68	UNL	OS	Multivariate	7
Valpione 2015[61]	Italy	Ipilimumab	216	UNL	OS	Multivariate	7
Wagner 2018 cohort1[62]	Germany	Pembrolizumab	152	1.5 UNL	OS	Multivariate	7
Wagner 2018 cohort2[62]	Germany	CTLA-4 + Nivolumab	86	1.5 UNL	OS	Multivariate	7
Wang 2016[63]	United States	Nivolumab	221	UNL	OS	Multivariate	7
Wen 2017[69]	China	Ipilimumab/Pembrolizumab	52	UNL	PFS/OS	Multivariate	7
Weide 2016[64]	International	Pembrolizumab	615	UNL	OS	Multivariate	-
Yamazak 2017[65]	Japan	Nivolumab	24	UNL	OS	Univariate	6
Zaragoza 2016[36]	France	Ipilimumab	58	UNL	OS	Univariate	7

CTLA-4 = cytotoxic T-lymphocyte antigen 4, IU = international unit, LDH = lactate dehydrogenase, NOS = Newcastle-Ottawa Scale, OS = overall survival, PD-1 = programmed cell death 1, PD-L1 = programmed cell death ligand 1, PFS = progression-free survival, UNL = upper normal limit.

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," "l-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

(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 cutoff 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 by using Higgins I^2 statistic. $I^2 > 50\%$ 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 $I^2 < 50\%$. 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[24–26,30–32,34,36–69] 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.

3.2. Overall survival

Thirteen studies reported the prognostic value of NLR on OS, while the prognostic value of LDH on OS was evaluated in 42 studies. Overall, elevated NLR or LDH levels had an association with poor OS (NLR: HR = 1.71, 95% CI, 1.40–2.10, $P < .001$; LDH: HR = 2.03, 95% CI, 1.76–2.35, $P < .001$), with extensive heterogeneity (NLR: $I^2 = 90.0\%$; LDH: $I^2 = 86.2\%$) (Figs. 2 and 3). Next, stratified analyses of NLR and LDH levels were conducted upon different ICIs, cutoff value of NLR and LDH levels, and geographic regions (Tables 3 and 4). In subgroup analyses stratified by ICIs, elevated NLR and LDH levels were found to

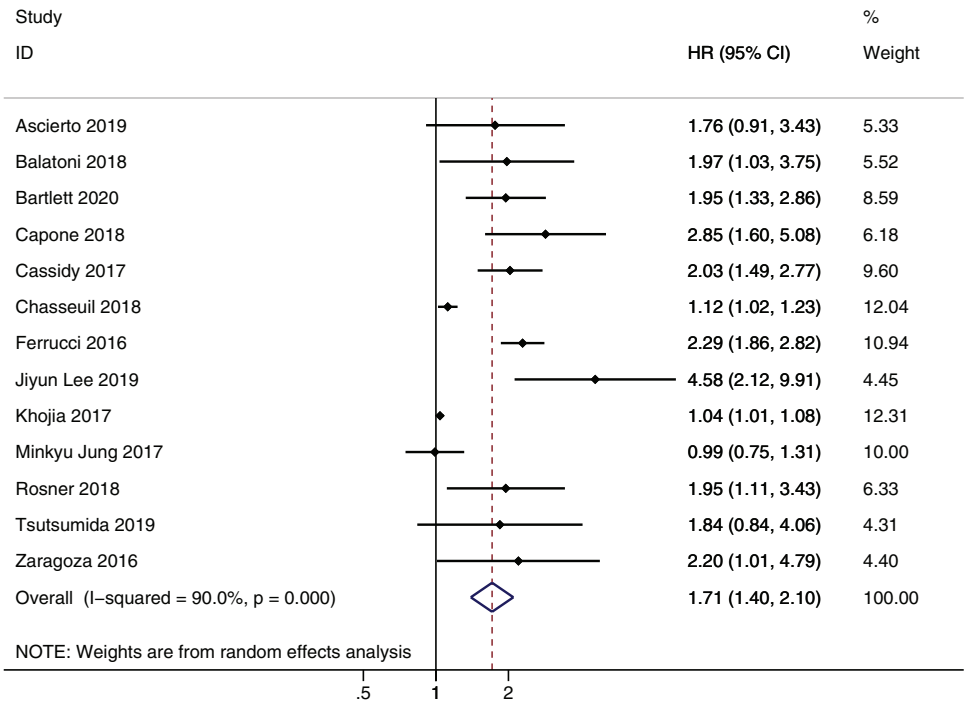

Figure 2. Forest plots of the relationship between NLR and survival outcomes in melanoma patients treated with ICIs. A random-effect model was used to evaluate the impact of NLR on OS. The pooled result indicated that high NLR was associated with poor OS. CI = confidence interval, HR = hazard ratio, 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; $P < .001$]).

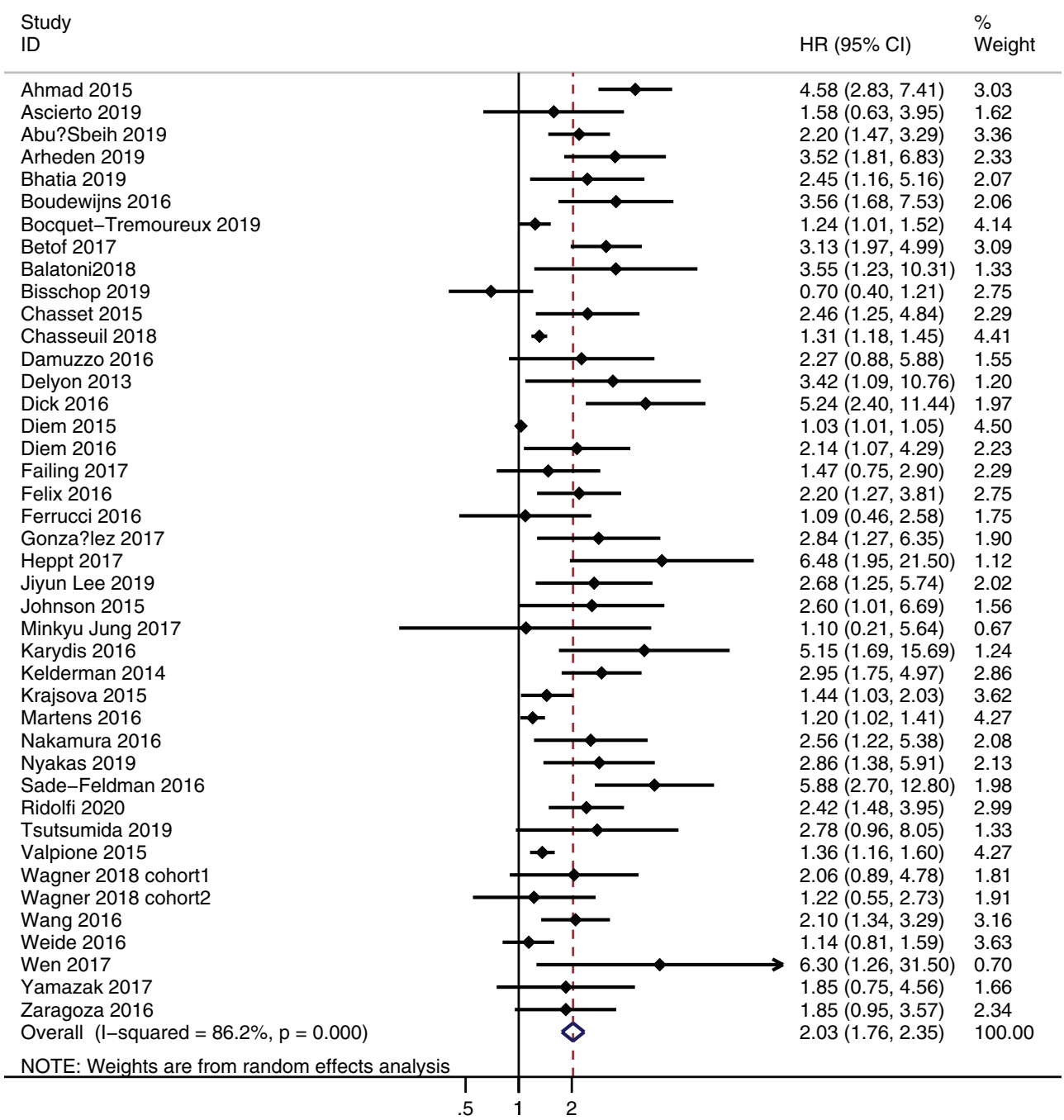

Figure 3. Forest plots of the relationship between LDH and survival outcomes in melanoma patients treated with ICIs. A random-effect model was used to evaluate the impact of LDH on OS. The pooled result indicated that high LDH was associated with poor OS. CI = confidence interval, HR = hazard ratio,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

Table 3

Subgroup analysis of NLR.

		OS				PFS		
		Association		Heterogeneity		Association		Heterogeneity
Analysis	N	HR (95% CI)	*P*	I^2	N	HR (95% CI)	*P*	I^2
Total	13	1.71 (1.40-2.10)	<.001	90.0%	8	1.83 (1.34–2.51)	<.001	89.9%
Agent								
PD-1/PD-L1	5	2.42 (1.68-3.50)	<.001	39.3%	4	1.93 (1.43–2.61)	<.001	0%
CTLA-4	6	1.45 (1.16–1.81)	.001	92.3%	3	1.66 (1.07–2.65)	.022	93%
PD-1/PD-L1 + CTLA-4	2	1.91 (1.21–3.03)	.006	0%	1	–	–	–
Cutoff Value								
=5	6	1.74 (1.22–2.47)	.002	82.9%	4	1.98 (1.69–2.31)	<.001	0
≠5	7	1.53 (1.24–1.88)	<.001	82.9%	4	1.70 (1.06–2.74)	.028	87.4%
Region								
Europe	6	1.90 (1.24–2.91)	.003	89.9%	4	1.68 (1.06–2.67)	<.001	92.5%
North America	4	1.64 (1.03–2.60)	0.036	90.7%	1	1.81 (1.33–2.46)	<.001	–
Asia	3	1.40 (0.87–2.44)	.166	86.2%	3	2.06 (1.48–2.86)	<.001	0%

CI = confidence interval, HR = hazard ratio, LDH = lactate dehydrogenase, NLR = neutrophil-lymphocyte ratio, OS = overall survival, PFS = progression-free survival.

Table 4

Subgroup analysis of LDH.

		OS				PFS		
		Association		Heterogeneity		Association		Heterogeneity
Analysis	N	HR (95% CI)	*P*	I^2	N	HR (95% CI)	*P*	I^2
Total	42	2.03 (1.76–2.35)	<.001	86.20%	12	1.65 (1.31–2.07)	<.001	61.30%
Agent								
PD-1/PD-L1	18	1.85 (1.52–2.26)	<.001	70.50%	1	–	–	–
CTLA-4	20	2.18 (1.73–2.74)	<.001	88.10%	1	–	–	–
Mixed	2	6.42 (2.45–16.79)	<.001	0	1	–	–	–
PD-1/PD-L1 + CTLA-4	2	1.71 (0.77–3.78)	.187	86.20%	1	–	–	–
Cutoff Value								
≠UNL	12	2.19 (1.51–3.17)	<.001	81.80%	3	1.89 (0.89–4.02)	.096	80.80%
UNL	30	1.97 (1.67–2.33)	<0.001	85.30%	9	1.59 (1.26–2.00)	<.001	50.90%
Region								
Europe	24	2.16 (1.76–2.64)	<.001	76.40%	6	1.62 (1.13–2.33)	.009	67.30%
North American	6	2.06 (1.154–2.74)	<.001	42.90%	3	1.82 (1.30–2.55)	<.001	49.10%
Asia	8	2.64 (1.94–3.58)	<.001	72.30%	3	1.53 (0.85–2.73)	.152	52.10%

CI = confidence interval, HR = hazard ratio, LDH = lactate dehydrogenase, NLR = neutrophil-lymphocyte ratio, OS = overall survival, PFS = progression-free survival.

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: $I^2 = 87.4\%$; LDH: $I^2 = 61.3\%$) (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, $P < .001$). But there was no significant association between elevated LDH and poor PFS if cutoff ≠ UNL group (HR = 1.89, 95% CI, 0.89–4.02, $P = .096$). And stratified analysis by geographic regions showed that elevated NLR was linked to poor PFS for patients in all areas included (Europe [HR = 1.68 95% CI, 1.06–2.67; $P = .028$], North America [HR = 1.81; 95% CI, 1.33–2.46; $P < .001$], and Asia [HR = 2.06; 95% CI, 1.48–2.86; $P = .001$]). In terms of LDH, the results of subgroups based on 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,

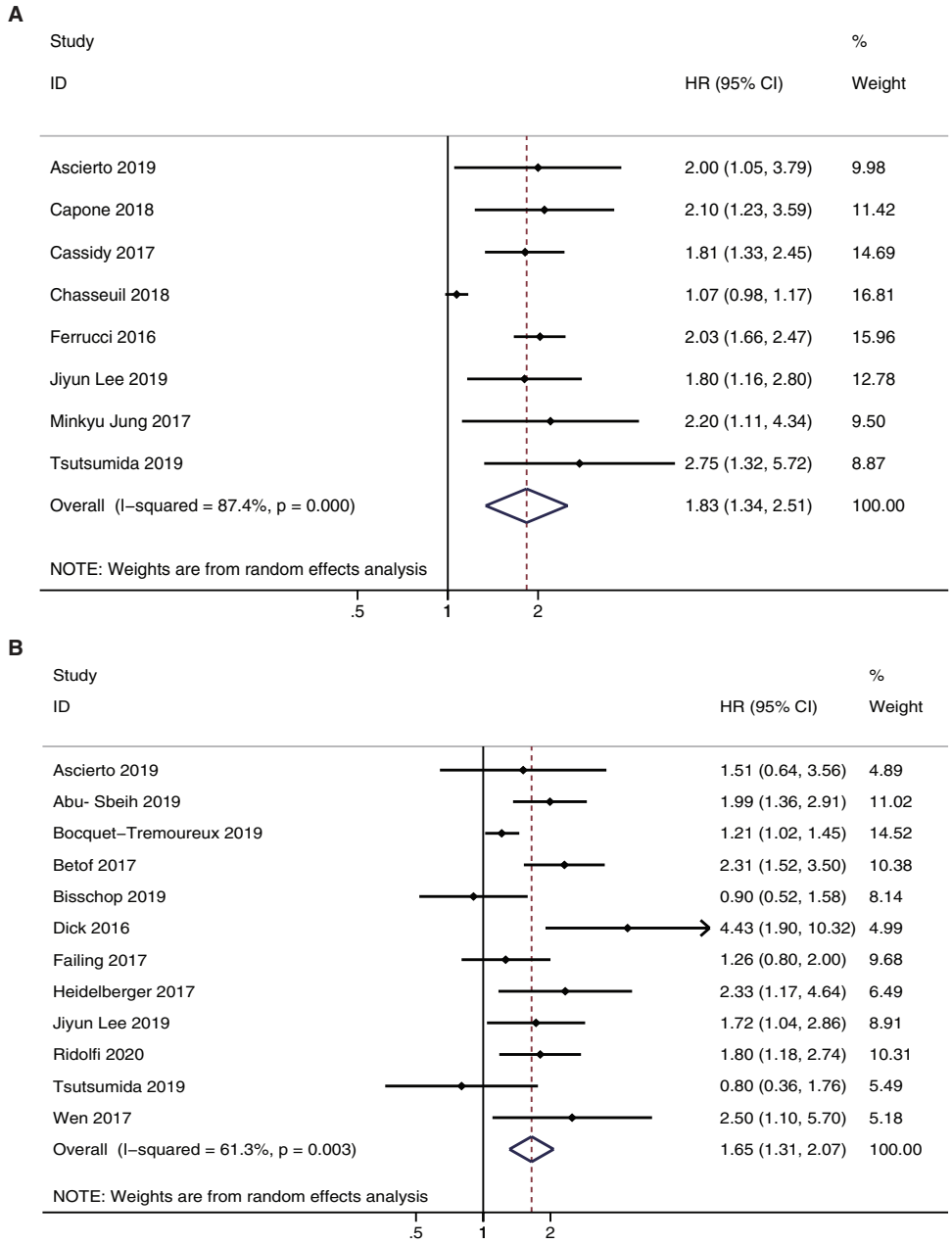

Figure 4. Forest plots of the relationship between NLR/LDH and PFS in melanoma patients treated with ICIs. (A) A random-effect model was used to evaluate the impact of NLR on OS. The pooled result indicated that high NLR was associated with poor OS; (B) A random-effect model was used to evaluate the 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.

$P < .001$) and LDH (Egger test, $P < .001$) (Fig. 5). 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; $P < .00001$).[20] Another meta-analysis which included 14 studies showed similar results.[21] 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

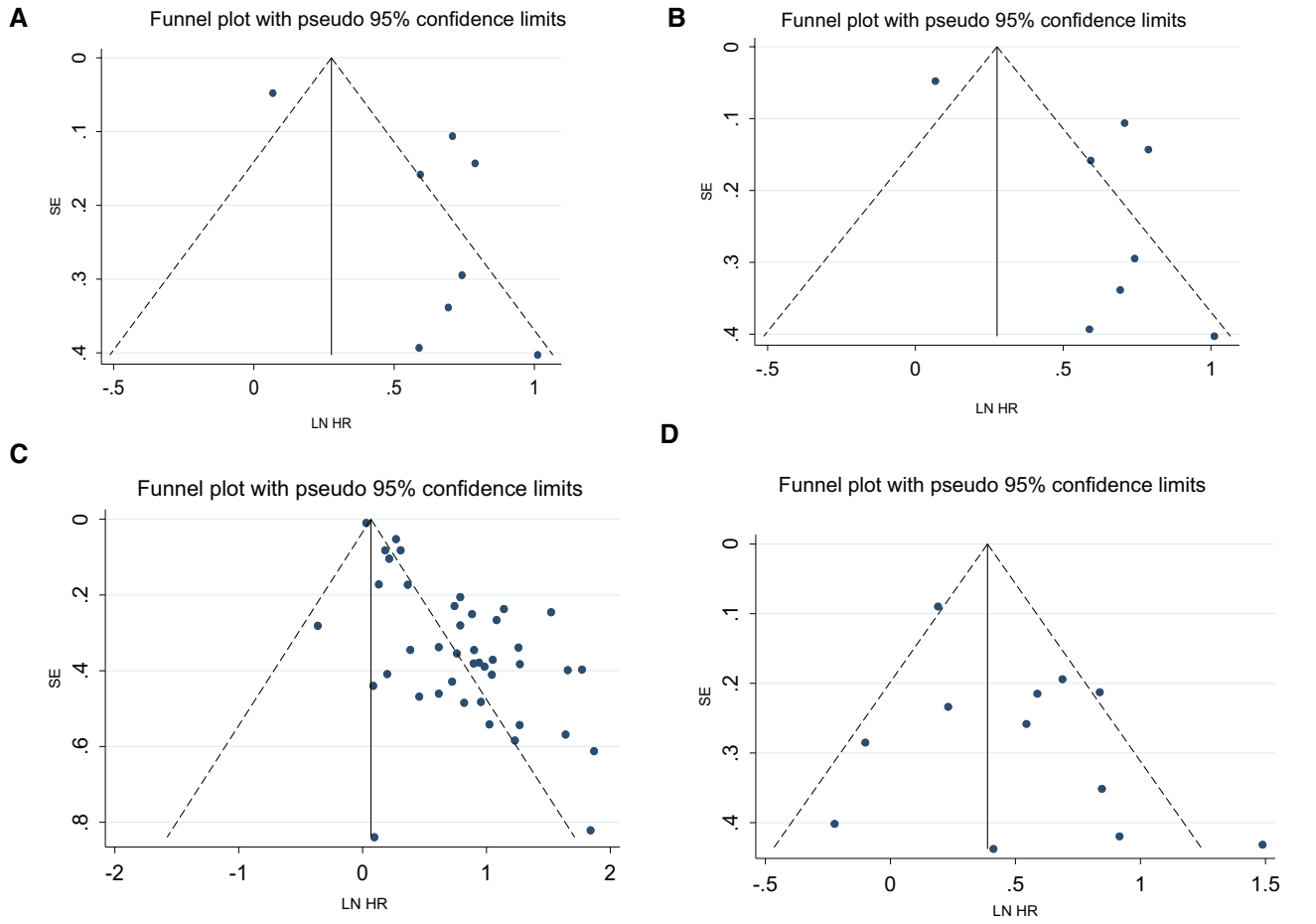

Figure 5. Funnel plots for assessing publication bias in survival outcomes. The funnel plots of the association between NLR and OS (A) and PFS (B) were not basically symmetrical; the funnel plot of the association between LDH and OS (C) was not basically symmetrical while PFS (D) was basically symmetrical. 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).[22] 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.[70–72] 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.[10,73]

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.[74] LDH as a key glycolytic enzyme plays a crucial role in pyruvate-to-lactate conversion, and the reproduction of oxidized nicotinamide adenine dinucleotide.[75] Melanoma cells with enhanced invasion and metastasis showed increased glucose uptake and lactate production.[76,77] 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-polarizated macrophages, which promotes tumor progression.[78] 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.[79] Lactate accumulation also inhibited tumor surveillance by decreased interferon-γ in T and natural killer cells in melanomas.[80] Dendritic cells were affected in a high LDH level. Increased lactic acidosis can compromise both the numbers and functions of dendritic cells.[81] Qiao et al[82] 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,[83,84] while cutaneous melanoma with higher

incidence of BRAF mutations is the predominant subtype in Europe and North America.[85] Second, the treatment models in Asian countries varies, 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.[86] 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[87] reported that a patient with uveal melanoma responds to ipilimumab plus nivolumab while developing autoimmune hepatitis with continuously rising LDH. In mixed group, there were 2 studies[54,69] including patients treated by anti-CTLA-4 alone, anti-PD-1/PD-L1 alone or anti-CTLA-4 + anti-PD-1/PD-L1. The association between LDH and OS was obtained in the whole cohort, rather than a certain monotherapy cohort. Although the 2 studies included 3 treatments, the percentage of patients treated with anti-CTLA-4 + anti-PD-1/PD-L1 were very low (14.8% and 19.2%). Thus, the low proportion of patients may less affect results of mixed group, which are consistent with anti-CTLA-4 alone group or anti-PD-1/PD-L1 alone group.

Although the prognostic roles of NLR and LDH levels in melanoma patients treated with ICIs have started to be recognized and investigated in recent years, there are open questions about the standardized use of NLR and LDH levels. First, a consensus of their cutoff value for prognosis prediction remains to be established. Although most of the eligible studies used NLR = 5 and LDH = UNL as cutoff values, our results demonstrated that NLR ≠ 5 and LDH ≠ UNL are associated with poor OS. Second, the definition of "baseline" is unclear. Eligible studies reported that the NLR and LDH levels were obtained from baseline, but potential risk of bias existed due to various timing of blood-test. Third, whether the treatment history has an impact on the prognostic value of NLR or LDH levels remains unclear. Thus, strictly designed clinical trials are warranted to further investigate the concerns above. Apart from NLR and LDH levels, some other biomarkers, such as S-100, have also been shown to have potential to predict prognosis of ICIs use. Therefore, NLR/LDH-based prognostic models, such as nomogram and other scoring systems, may be more appropriate to guide the use of ICIs.

There are several limitations in our study. First, most of the eligible studies were retrospective, whose results may not be very convincible. Second, the remarkable heterogeneity of involved studies might affect the stability and reliability of our analytical results. Third, the cutoff value for NLR and LDH levels varied in eligible studies. Therefore, a consensus of NLR and LDH cutoff values is needed.

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. WeiLi 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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