Prognostic value of lactate dehydrogenase for melanoma patients receiving anti-PD-1/PD-L1 therapy
A meta-analysis

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Abstract
Background: Several studies indicate the level of pretreatment lactate dehydrogenase (LDH) may be associated with the prognosis of patients receiving immune checkpoint inhibitors targeting programmed death receptor-1 (PD-1)/programmed death ligand 1 (PD-L1) which had been reported to dramatically improve the survival of patients with advanced or metastatic melanoma; however, no consensus has been reached because the presence of controversial conclusions. This study was to perform a meta-analysis to comprehensively explore the prognostic values of LDH for melanoma patients receiving anti-PD1/PD-L1 monotherapy.

Methods: A systematic electronic search in the databases of PubMed, EMBASE and the Cochrane library was performed to identify all related articles up to April, 2020. The pooled hazard ratios (HRs) and 95% confidence intervals (CIs) were obtained to assess the prognostic values of pretreatment LDH in blood for overall survival (OS) and progression-free survival (PFS).

Results: A total of 22 eligible studies involving 2745 patients were included. Of them, 19 studies with 20 results assessed the OS and the pooled analysis showed that an elevated pretreatment LDH level was significantly associated with a worse OS (HR = 2.44; 95% CI: 1.95–3.04, P < .001). Thirteen studies reported PFS and meta-analysis also revealed that a higher pretreatment LDH level predicted a significantly shorter PFS (HR, 1.61; 95% CI, 1.34–1.92; P < .001). Although heterogeneity existed among these studies, the same results were acquired in subgroup analyses based on sample size, country, study design, cut-off of LDH, type of PD-1/PD-L1 inhibitors and statistics for HRs (all HRs > 1 and P < .05).

Conclusion: This meta-analysis suggests LDH may serve as a potential biomarker to identify patients who can benefit from anti-PD-1/PD-L1 and then schedule treatments.

Abbreviations: CI = confidence interval, CTLA4 = cytotoxic T-lymphocyte antigen 4, HR = hazard ratios, ICIs = immune checkpoint inhibitors, LDH = lactate dehydrogenase, NOS = Newcastle-Ottawa Scale, OS = overall survival, PD-1 = programmed death 1, PD-L1 = programmed death ligand 1, PFS = progression-free survival, ULN = upper limit of normal.

Keywords: lactate dehydrogenase, melanoma, prognosis, programmed death ligand 1, programmed death receptor-1

1. Introduction
Melanoma is the third most common type of skin cancer (after squamous cell carcinoma and basal cell carcinoma). Nevertheless, it represents the leading cause of skin cancer-related death, which may be partially attributed to its capacity to metastasize to distant organs. Although there were no curative options for unresectable or metastatic melanoma, the advent of agents targeting the immune system has been reported to dramatically improve the prognosis of patients. Clinically used immune checkpoint inhibitors (ICIs) include monoclonal antibodies against cytotoxic T-lymphocyte antigen 4 (CTLA-4) (i.e. ipilimumab), programmed death 1 (PD-1) (i.e. nivolumab and pembrolizumab) and programmed death ligand 1 (PD-L1) (i.e. atezolizumab, durvalumab and avelumab). Compared with ipilimumab, the anti-PD-1/PD-L1, as single agents, had higher clinical activity to improve the overall survival (OS), progression-free survival (PFS) and induce less adverse events. Also, the toxicity was increased in the combination group compared to either single agent anti-CTLA4 or anti-PD-1.
antibodies.\textsuperscript{[9,11]} Therefore, single agent PD-1/PD-L1 antibodies may be more cost-effective \textsuperscript{[12]} and acceptable \textsuperscript{[13]} for patients with advanced or metastatic melanoma. Unfortunately, there were still approximately 65% of patients who could not benefit from the anti-PD-1/PD-L1 monotherapy.\textsuperscript{[8,14]} Therefore, identifying predictive biomarkers for response to PD-1/PD-L1 inhibitors may be beneficial in guiding treatment selections.

Enhanced aerobic glycolysis (known as the Warburg effect) is the major pathway to provide the metabolic energy for cancer cells to achieve fast proliferation and metastasis. Lactate dehydrogenase (LDH) is an essential metabolic enzyme during the Warburg effect, which catalyzes the reversible conversion of pyruvate into lactate. Also, the accumulated lactate was proved to promote tumor immune escape by reducing the survival and cytolytic capacity of CD8+ T cells and natural killer cells.\textsuperscript{[15,16]}

Accordingly, we speculate that a high level of LDH in cancer patients may antagonize the effects of anti-PD-1/PD-L1 antibodies (which can prevent T-cell exhaustion by inhibiting the expression of PD-1/PD-L1)\textsuperscript{[17]} and lead to a poor prognosis.\textsuperscript{[18]} This potential prognostic value of LDH was verified in several studies on melanoma. For example, Chasseuil et al. reported that an increased pretreatment LDH level was significantly associated with a decreased OS [hazard ratios (HR) = 1.31; 95% confidence interval (CI): 1.18 – 1.45; \textit{P} = .01] and PFS [HR = 1.25; 95% CI: 1.13 – 1.38; \textit{P} = .01] in patients with advanced melanoma after treatment with nivolumab.\textsuperscript{[19]} The similar conclusion was also confirmed in the studies of Capone et al.,\textsuperscript{[20]} Rindolfi et al.,\textsuperscript{[21]} Ascierto et al.,\textsuperscript{[22]} Suo et al.,\textsuperscript{[23]} Cowey et al.,\textsuperscript{[24]} and Seremet et al.\textsuperscript{[25]} who investigated the association between LDH level and outcomes of nivolumab or pembrolizumab treatment. However, whether LDH can serve as a prognostic biomarker for melanoma patients treated with anti-PD-1/PD-L1 antibodies, remains uncertain because some evidence suggested no significant correlations between pretreatment LDH and OS/PFS.\textsuperscript{[26–28]}

These conflicting results may be associated with small sample sizes in each individual study or their heterogeneity in study designs.

The goal of this study was to perform a meta-analysis to re-assess the prognostic value of LDH for melanoma patients treated with anti-PD-1/PD-L1 antibodies. Meta-analysis of all evidence may overcome the limitation from the small sample sizes in individual studies and increase the statistical power and hereby, the resultant conclusion may be believable. Furthermore, the subgroup analysis was also performed for studies with consistent designs to further confirm the conclusion.

2. Materials and methods

2.1. Search strategies

A systematic electronic search in the databases of PubMed, EMBASE and the Cochrane library was performed to identify all related articles published up to April, 2020, in accordance with the Preferred Reporting Items for Systematic Review and Meta-analysis. The combined search terms were as follows: (“immunotherapy” OR “immune checkpoint inhibitors” OR “programmed death ligand-1” OR “programmed death-1 receptor” OR “PD-1 inhibitor” OR “PD-L1 inhibitor” OR “anti-PD-1 antibodies” OR “nivolumab” OR “pembrolizumab” OR “atezolizumab” “avelumab” OR “durvalumab”) AND (“lactate dehydrogenase” OR “LDH”). The reference lists of original studies and reviews were also manually searched for potential eligible publications. The need of ethical approval and patient consent is waived because of a meta-analysis of the published studies.

2.2. Inclusion and exclusion criteria

Studies were included if they fulfilled the following inclusion criteria:

1. patients were diagnosed as melanoma by histology;
2. patients were treated with anti-PD-1/PD-L1 antibodies as a single agent;
3. the associations of pretreatment LDH in blood with prognosis (including OS and PFS) were reported; and
4. the data of HRs and 95% CIs could be directly extracted or indirectly estimated from Kaplan-Meier curves.

The studies were excluded if they:

1. belonged to duplicated articles from different databases;
2. were case reports, reviews, cell or animal studies;
3. did not provide sufficient data to estimate HRs and 95% CIs;
4. were unrelated to the topic of interest;
5. had the patients who received combined treatment with other ICIs or chemotherapy simultaneously or after anti-PD-1/PD-L1 cycle; and
6. were unpublished or published in non-English language.

2.3. Data extraction

Two researchers independently extracted the relevant data from all eligible studies, and disagreements regarding the definition on the cut-off of LDH were resolved by careful reading the articles and discussion to reach consensus. The extracted information included the name of the first author, publication year, country, median age of patients, case number, study design, follow-up, type of PD-1/PD-L1 inhibitors, cut-off value of LDH, HRs with 95% CIs, SO/OS, and PFS and the method for HR calculation. HRs and 95% CIs were extracted preferentially from the multivariable analysis where available. If HRs and 95% CIs were not reported directly, they could be estimated from Kaplan-Meier curves using a digitizing software-Engauge Digitizer (version 4.1; http://digitizer.sourceforge.net/).

2.4. Quality assessment

Two reviewers independently assessed the methodological quality of all eligible studies according to Newcastle-Ottawa Scale (NOS).\textsuperscript{[29]} Studies with a NOS score $\geq$7 stars were defined as having a high quality.\textsuperscript{[30]}

2.5. Statistical analysis

The meta-analysis was performed using the STATA 13.0 software (STATA Corporation, College Station, TX). The pooled HRs and 95% CIs were used to assess the effects of elevated LDH levels on the prognosis in melanoma patients treated with PD-1/ PD-L1 inhibitors. A pooled HR of >1 indicated a poorer OS and PFS in patients with a higher pretreatment LDH level. The association between the level of LDH and the prognosis was thought to be statistically significant if the 95% CI did not overlap 1 and \textit{P}-value determined by Z-test $<0.05$. The statistical heterogeneity was evaluated by Cochrane Q test and \textit{I}^2 statistic.
A random-effects model was used for pooled estimates if a significant heterogeneity was identified ($P < .10$ and $I^2 > 50\%$); if not, a fixed-effects model was applied. To explore the source of heterogeneity, a subgroup analysis was also conducted according to the sample size, country, study design, cut-off of LDH, type of PD-1/PD-L1 inhibitors, statistics for HR and HR source. Publication bias was examined by Egger’s linear regression test. If publication bias was present ($P < .05$), the “trim and fill” algorithm was employed for adjustment. Sensitivity analysis was performed to further evaluate the influence of each study on pooled HR via removing 1 study in turn.

### 3. Results

#### 3.1. Literature search

Figure 1 illustrates the process of literature selection. A total of 2116 records were initially retrieved by searching the online databases with keywords; 618 of them were excluded due to duplicate reports. After reading titles and abstracts, 1471 articles were eliminated because they were either review (n = 15), case reports (n = 19), cell studies (n = 330), animal studies (n = 507), studies without prognostic outcomes (n = 168), unrelated to the current topics (n = 420) or with patients undergoing anti-PD-1/PD-L1 + other ICIs combined treatments (n = 12). The remaining 27 articles were screened by full-text reviewing. As a result, 5 of them were discarded for the following reasons: patients in 3 studies received adjuvant chemotherapy or other ICIs treatment after the use of anti-PD-1/PD-L1; two failed to provide the data to estimate HRs and 95% CIs. Eventually, 22 eligible studies involving 2745 patients were enrolled in our meta-analysis.

#### 3.2. Study characteristics

The characteristics of 22 included studies are shown in Table 1. These included studies were conducted in 11 countries (including Australia (n = 1), Belgium (n = 1), Canada (n = 1), France (n = 3), Germany (n = 2), Italy (n = 3), Japan (n = 3), Spain (n = 1), Sweden (n = 1), UK (n = 2), USA (n = 4)) and published from 2016 to 2020. The ICI agent for the treatment of these patients with advanced or metastatic melanoma was nivolumab in 7 studies; pembrolizumab in 8 studies; pembrolizumab or nivolumab in 7 studies. The data of most patients were retrospectively (19/22) collected from a single medical center (14/22). The study of Wagner et al [33] used 2 multivariate models to analyze the associations of LDH in blood samples with OS; thus, 19 studies with 20 results were used for OS meta-analysis. Thirteen studies reported the impact of LDH in blood samples on PFS. The cut-off of LDH was upper limit of normal (ULN) (although the value may also be different) in most studies; while some used the 1.5 ULN, 2 ULN or 2.5ULN as the threshold. HRs and 95% CIs were directly obtained from 17 studies (although some only used the univariate analysis), while indirectly estimated from the Kaplan-Meier curve in 5 studies. All studies had the NOS score ≥ 7, suggesting they were of high quality (Table 1).

#### 3.3. Correlation between pretreatment LDH level in blood samples and OS

The heterogeneity existed among the 19 studies with 20 results ($I^2 = 71.3\%, P < .001$), so a random-effect model was used to calculate pooled HRs. As shown in Figure 2, the pooled analysis suggested that an elevated pretreatment LDH level was significantly associated with a worse OS in patients treated with PD-1/PD-L1 inhibitors (HR = 2.44; 95% CI: 1.95–3.04, $P < .001$).

Subgroup analyses were then performed to explore the potential source of the heterogeneity. From the results in Table 2, we could see that melanoma patients with a higher LDH level had a poorer prognosis regardless of different sample sizes, countries, study designs, cut-offs of LDH, types of PD-1/PD-L1 inhibitors, statistical methods for HR and HR source, all with HRs > 1 and $P < .05$. But compared with overall estimates, the absence of a significant heterogeneity was seen in the analysis of studies with sample size > 100, non-European population, prospective-multicenter design, cut-off > ULN, pembrolizumab/mixed treatment and multivariate results ($I^2 < 50\%$, $P > .1$) which meant the influence of heterogeneity had been partially excluded.

#### 3.4. Correlation between pretreatment LDH level in blood samples and PFS

There was also evidence of a significant heterogeneity among the 13 studies ($I^2 = 57.3\%, P = .005$) assessing the relationship between the LDH level and PFS, so a random-effect model was utilized to estimate the pooled effect size. Similar to the results of OS, our meta-analysis revealed that a higher pretreatment LDH level of patients treated with PD-1/PD-L1 inhibitors predicted a significantly shorter PFS (HR, 1.61; 95% CI, 1.34–1.92; $P < .001$) (Fig. 3).
| Study            | Year | Country | Case No. | Median age | Disease status | Study design         | Median follow-up | Type of Anti-PD-1/PD-L1 | Cut-off of LDH | Survival endpoint | Statistical method for HR | HR source | NOS |
|------------------|------|---------|----------|------------|----------------|----------------------|------------------|-------------------------|----------------|------------------|---------------------------|-----------|-----|
| Falling JJ       | 2017 | USA     | 133      | 61 (18-90)| Metastatic     | Single-center, retrospective | 12 mo            | Pembrolizumab          | ULN            | PFS, OS (both non-significant) | PFS (UV), OS (MM)                | Reported  | 8   |
| Chasseuil E      | 2018 | France  | 87       | 71 (27-92)| Advanced      | Single-center, retrospective | 227 d           | Nivolumab              | ULN            | PFS, OS (both non-significant) | UV                         | Reported  | 9   |
| Wagner MB        | 2018 | Germany | 152      |            | Advanced      | Single-center, retrospective | 9.9 mo          | Pembrolizumab          | 1.5ULN         | OS (two MV models, one significant; one non-significant) | MV              | Reported  | 8   |
| Diem S          | 2016 | UK      | 66       | 56 (49-68)| Metastatic    | Single-center, retrospective | 9 mo            | Pembrolizumab or nivolumab | ULN            | OS (significant) | UV                         | Estimated  | 9   |
| Heidelberg V     | 2017 | France  | 63       | 65 (22-90)| Metastatic    | Single-center, retrospective | 7 mo            | Pembrolizumab or nivolumab | ULN            | PFS (significant) | MV              | Reported  | 8   |
| Aheden A        | 2019 | Sweden  | 116      | 66 (27-98)| Metastatic    | Single-center, retrospective | 17 mo           | Pembrolizumab or nivolumab | ULN            | OS (significant) | UV                         | Reported  | 8   |
| Capone M        | 2018 | Italy   | 97       | 61 (21-85)| Advanced      | Single-center, retrospective | -               | Nivolumab              | ULN            | OS, PFS (both significant) | MV              | Reported  | 7   |
| Riddi L         | 2030 | Italy   | 174      | 79 (75-93)| Metastatic    | Multi-center, retrospective | 8.97 mo         | Pembrolizumab or nivolumab | ULN            | OS, PFS (both significant) | UV                         | Reported  | 8   |
| Liu F           | 2019 | USA     | 359      |            | Advanced      | Multi-center, retrospective | -               | Pembrolizumab          | ULN            | OS (significant) | UV                         | Estimated  | 7   |
| Ascierto PA      | 2019 | Italy   | 71       | 61 (28-86)| Metastatic    | Single-center, retrospective | -               | Pembrolizumab or nivolumab | 2ULN           | OS, PFS (both non-significant) | MV              | Reported  | 7   |
| Wede E          | 2016 | Germany | 512      |            | Advanced      | Multi-center, retrospective | -               | Pembrolizumab          | 2.5ULN         | OS (significant) | MV              | Reported  | 7   |
| Suo A           | 2020 | Canada  | 143      |            | Advanced      | Multi-center, retrospective | 24 mo           | Pembrolizumab          | ULN            | OS, PFS (both non-significant) | MV              | Reported  | 8   |
| Covey CL        | 2018 | USA     | 168      | 66 (26-90)| Advanced      | Multi-center, retrospective | 10.5 mo         | Pembrolizumab          | ULN            | OS, PFS (both significant) | MV              | Reported  | 8   |
| Bocquet-Tremouleurs S | 2019 | France  | 87       |            | Metastatic    | Single-center, retrospective | 31              | Nivolumab              | ULN            | PFS              | MV              | Reported  | 8   |
| Namikawa K      | 2020 | Japan   | 14       | 60 (42-74)| Metastatic    | Single-center, retrospective | 15 mo           | Nivolumab              | 2ULN           | OS, PFS (both non-significant) | UV                         | Estimated  | 8   |
| Nakamura Y      | 2016 | Japan   | 93       | 67 (17-93)| Advanced      | Multi-center, retrospective | -               | Nivolumab              | ULN            | OS (significant) | MV              | Reported  | 7   |
| Gide TN         | 2019 | Australia | 27     | 67          | Metastatic    | Single-center, retrospective | -               | Pembrolizumab or nivolumab | ULN            | PFS (non-significant) | UV                         | Reported  | 7   |
| Serevet T       | 2019 | Belgium | 85       | 57 (27-82)| Metastatic    | Single-center, prospective  | 84 wk           | Pembrolizumab          | ULN            | OS, PFS (both non-significant) | UV                         | Reported  | 8   |
| Wang X          | 2016 | USA     | 221      | 59.2       | Advanced      | Single-center, prospective  | -               | Nivolumab              | ULN            | OS (significant) | MV              | Reported  | 7   |
| Yamazaki M      | 2017 | Japan   | 23       |            | Advanced      | Multi-center, prospective  | -               | Nivolumab              | ULN            | OS, PFS (both non-significant) | UV                         | Estimated  | 8   |
| Kandis I        | 2016 | UK      | 25       | 58 (32-83)| Metastatic    | Single-center, retrospective | 2.25 d          | Pembrolizumab          | ULN            | OS (significant) | UV                         | Estimated  | 7   |
| Gonzalez-Cao M  | 2016 | Spain   | 29       |            | Advanced      | Single-center, retrospective | -               | Pembrolizumab          | ULN            | OS (significant) | MV              | Reported  | 7   |

HR = hazard ratio, K-M = Kaplan-Meier curve, LDH = lactate dehydrogenase, MV = multivariate analysis, NOS = Newcastle-Ottawa Scale, OS = overall survival, PD-1 = programmed death receptor-1, PD-L1 = programmed death ligand 1, PFS = progression-free survival, ULN = upper limit of normal, UV = univariate analysis, wk = week.
Then, stratified analyses were conducted. The results also demonstrated that this significant prognostic potential of pretreatment LDH for PFS was not changed after subgroup analyses according to sample size, country, study design, cut-off of LDH, type of PD-1/PD-L1 inhibitors and statistics for HR (Table 3). However, no significant association was observed any more between pretreatment LDH and PFS in studies with the HR estimated from the Kaplan-Meier curve (HR, 1.98; 95% CI, 0.93–4.25; P = .079). The significant heterogeneity also disappeared in the subgroup analysis of studies with non-European population, prospective-multi-center design, cut-off of 2ULN and nivolumab/mixed treatment multivariate results (I² < 50%, P > .1), suggesting the results for them may be especially robust.

Table 2
Subgroup analysis on the association between LDH and OS.

| Comparison                        | Studies | HR (95%CI)          | Pₙ-value | Q² | Pₚ-value | Model |
|-----------------------------------|---------|---------------------|----------|----|----------|-------|
| Sample size                       |         |                     |          |    |          |       |
| <100                              | 10      | 2.51 (1.72,3.66)    | < .001   | 68.8| .001     | R     |
| >100                              | 10      | 2.43 (2.08,2.84)    | < .001   | 0  | .681     | F     |
| Country                           |         |                     |          |    |          |       |
| European                          | 13      | 2.46 (1.84,3.28)    | < .001   | 76.5| < .001   | R     |
| Non-European                      | 7       | 2.34 (1.88,2.90)    | < .001   | 0  | .623     | F     |
| Study design                      |         |                     |          |    |          |       |
| Retrospective                     | 17      | 2.40 (1.88,3.06)    | < .001   | 73.1| < .001   | R     |
| Prospective                       | 3       | 2.49 (1.75,3.56)    | < .001   | 0  | .462     | F     |
| Single center                     | 12      | 2.28 (1.69,3.07)    | < .001   | 66.5| .001     | R     |
| Multi-center                      | 8       | 2.57 (2.16,3.06)    | < .001   | 0  | .897     | F     |
| Cut-off of LDH                    |         |                     |          |    |          |       |
| ULN                               | 15      | 2.42 (1.88,3.12)    | < .001   | 73.7| < .001   | R     |
| 1.5ULN                            | 2       | 2.22 (1.22,4.03)    | .009     | 0  | .898     | F     |
| 2ULN                              | 2       | 2.61 (1.30,5.25)    | .007     | 0  | .966     | F     |
| 2.5ULN                            | 1       | 2.80 (2.01,3.91)    | < .001   | –  | –        | F     |
| Type of PD-1/PD-L1 inhibitors     |         |                     |          |    |          |       |
| Nivolumab                         | 6       | 2.02 (1.38,2.96)    | < .001   | 64.0| .016     | R     |
| Pembrolizumab                     | 9       | 2.55 (2.13,3.07)    | < .001   | 0  | .499     | F     |
| Mixed                             | 5       | 2.56 (1.86,3.35)    | < .001   | 0  | .892     | F     |
| HR source                         |         |                     |          |    |          |       |
| Reported                          | 15      | 2.38 (1.84,3.07)    | < .001   | 75.1| < .001   | R     |
| Estimated                         | 5       | 2.41 (1.83,3.18)    | < .001   | 0  | .645     | F     |
| Statistics for HR                 |         |                     |          |    |          |       |
| Multivariate                      | 11      | 2.48 (2.08,2.95)    | < .001   | 0  | .881     | F     |
| Univariate                        | 9       | 2.48 (1.72,3.59)    | < .001   | 77.3| < .001   | R     |

CI = confidence interval, F = fixed-effects model, HR = hazard ratio, F² = the degree of heterogeneity by F statistic, LDH = lactate dehydrogenase, OS = overall survival, PD-1 = programmed death receptor-1, PD-L1 = programmed death ligand 1, Pₙ = P-value for heterogeneity measured by Q-test, Pₚ = P-value for association determined by Z-test, R = random-effects model, ULN = upper limit of normal.
Table 3
Subgroup analysis on the association between LDH and PFS.

| Comparison              | Studies | HR (95%CI) | P-value | I² | P-value | Model |
|-------------------------|---------|------------|---------|----|---------|-------|
| Sample size             |         |            |         |    |         |       |
| <100                    | 11      | 1.55 (1.29,1.86) | <.001   | 51.7 | .023    | R     |
| >100                    | 2       | 1.80 (0.90,3.60) | .098    | 78.5 | .031    | R     |
| Country                 |         |            |         |    |         |       |
| European                | 7       | 1.56 (1.27,1.92) | <.001   | 62.3 | .014    | R     |
| Non-European            | 6       | 1.64 (1.15,2.33) | .006    | 42.9 | .119    | F     |
| Study design            |         |            |         |    |         |       |
| Retrospective           | 11      | 1.52 (1.28,1.81) | <.001   | 53.6 | .018    | R     |
| Prospective             | 2       | 2.47 (1.51,4.04) | <.001   | 0.0  | .455    | F     |
| Single center           | 9       | 1.44 (1.20,1.74) | <.001   | 51.1 | .038    | R     |
| Multi-center            | 4       | 1.98 (1.54,2.54) | <.001   | 0.0  | .644    | F     |
| Cut-off of LDH          |         |            |         |    |         |       |
| ULN                     | 11      | 1.57 (1.31,1.80) | <.001   | 61.1 | .004    | R     |
| 2ULN                    | 2       | 2.28 (1.16,4.50) | .007    | 0.0  | .958    | F     |
| Type of PD-1/PD-L1 inhibitors |     |            |         |    |         |       |
| Nivolumab               | 5       | 1.26 (1.16,1.37) | <.001   | 0.0  | .538    | F     |
| Pembrolizumab           | 5       | 2.04 (1.22,3.43) | <.001   | 69.4 | .038    | R     |
| Mixed                   | 5       | 1.76 (1.29,2.30) | <.001   | 22.6 | .271    | F     |
| HR source               |         |            |         |    |         |       |
| Reported                | 11      | 1.60 (1.32,1.93) | <.001   | 63.0 | .003    | R     |
| Estimated               | 2       | 1.98 (0.93,4.25) | .079    | 0.0  | .778    | F     |
| Statistics for HR       |         |            |         |    |         |       |
| Multivariate            | 6       | 1.80 (1.32,2.47) | <.001   | 63.9 | .016    | R     |
| Univariate              | 7       | 1.51 (1.14,1.90) | .004    | 52.7 | .048    | R     |

CI = confidence interval, F = fixed-effects model, HR = hazard ratios, I² = the degree of heterogeneity by $\chi^2$ statistic, LDH = lactate dehydrogenase, PD-1 = programmed death receptor-1, PD-L1 = programmed death ligand 1, PFS = progression-free survival, $P_{x} = P$-value for heterogeneity measured by $U$-test, $P_{y} = P$-value for association determined by $Z$-test, R = random-effects model, ULN = upper limit of normal.

3.5. Sensitivity analysis and publication bias

Although Egger’s linear regression test showed there was potential publication bias in the analysis of OS ($p < 0.001$) and PFS ($p = 0.014$), the adjusted results by the trim and fill method (Fig. 4) showed that the significant associations between an elevated LDH level and unfavorable OS (HR = 1.69; 95% CI: 1.37 – 2.08, $P < .001$) and PFS (HR = 1.38; 95% CI: 1.14 – 1.67, $P < .001$) were still present. Thus, the impact of publication bias on the pooled results may be weak.

Sensitivity analysis was performed to further assess the robustness of the pooled HR assessing the association between LDH and OS/PFS by omitting single study in turn. The results showed that no single study significantly influenced the summary HRs (Fig. 5), indicating the consequence of this meta-analysis was stable and reliable.

4. Discussion

Although there were several meta-analyses to investigate the prognostic values of pretreatment LDH level for cancer patients,[45–48] only 2 focused on the patients treated with ICIs: one was for non-small-cell lung cancer[18] and the other was for melanoma.[49]

Also, in the study of Petrelli et al,[49] the literatures involving all ICIs (including anti-PD-1/PD-L1 monotherapy, anti-CTLA4 monotherapy and combined therapy) were integrated together for meta-analyses. In the present study, we, for the first time, attempted to confirm the prognostic significance of pretreatment LDH for melanoma patients undergoing anti-PD-1/PD-L1 monotherapy. Compared with the study of Petrelli et al,[49] which searched the articles until 28 January 2018, our study newly enrolled some papers published in 2019 and 2020 to further increase the statistical power; thus, our conclusion may be more credible. This hypothesis has been confirmed in our analysis: overall estimate analysis using 19 and 13 publications showed that an elevated pretreatment LDH level was significantly associated with a poor OS (HR = 2.44) and PFS (HR = 1.61) in melanoma patients treated with anti-PD-1/PD-L1 single agents. The similar conclusions were also achieved in the subgroup analyses based on sample size, country, study design, cut-off of LDH, type of PD-1/PD-L1 inhibitors and statistics for HR. Sensitivity analysis and publication bias analyses also demonstrated the combined HR was stable. Accordingly, we got the conclusion that pretreatment LDH may serve as a potential prognostic biomarker for anti-PD-1/PD-L1 in patients with melanoma.

The potential mechanism to explain the inferior survival in the presence of elevated LDH levels is the activation of the Warburg effect (increased aerobic glycolysis) in advanced or metastatic melanoma. LDH is a key glycolytic enzyme responsible for pyruvate-to-lactate conversion, accompanied by the reproduction of oxidized nicotinamide adenine dinucleotide from reduced NADH for continued glycolysis. It had been widely observed that melanoma cells with suppressed proliferation, invasion and metastasis ability usually exhibited the characteristics of a decreased glucose uptake, lactate production, ATP generation, extracellular acidification rate, and an increased oxygen consumption rate.[50,51] The expression of genes encode for LDH was also increased in malignant melanoma compared with controls.[52–55] Knockout of LDH genes strongly reduced the LDH activity and lactate secretion as well as proliferation rates of melanoma in vitro and in vivo compared with their counterparts.[56] High serum LDH was found to be associated with a significant increase in LDH isoenzymes. Hereby, a high
concentration of LDH in blood may indirectly reflect the content and metabolism of LDH in the melanoma cells and predict the tumor progression and patient’s prognosis.

In addition, the prognostic potential of LDH for patients receiving PD-1/PD-L1 inhibitors may be attributed to the correlation between LDH/lactate and PD-1/PD-L1-mediated immune response. PD-1 is a surface receptor expressed on various activated immune cells, such as T cells, macrophages and dendritic cells. It can interact with its ligand PD-L1 and then reduce the survival of CD8+ T cells and macrophages (M1 type) and their cytotoxicity on tumor cells, thus evading the immune surveillance and promoting the progression of cancers.\(^{[17,57,58]}\) PD-1 expression on dendritic cells supported tumor growth by suppressing CD8+ T cell function.\(^{[59]}\) Lactate was demonstrated to upregulate vascular endothelial growth factor and arginase 1 via the transcription factor hypoxia-inducible factor 1α and then induce macrophages skewed to M2-polarized macrophages which represented a tumor-promoting state.\(^{[60]}\) LDH-associated lactate accumulation in melanomas also inhibited tumor surveillance by diminishing the production of interferon-γ in T and natural killer cells.\(^{[15]}\) Blood dendritic cells were dramatically depleted in melanomas, particularly in patients with a high LDH level. Exposure of lactic acidosis to dendritic cells impaired both the viability and functions of dendritic cells.\(^{[61]}\) Based on these findings, we speculate LDH may exert similar functions to the activation of PD-1/PD-L1 pathway and therefore, the presence of a high LDH level in melanomas may antagonize the therapeutic effects of PD-1/PD-L1 inhibitors and lead to a poor prognosis.

The present study had some limitations. First, considerable heterogeneity was identified among studies. However, the similar results were obtained in the subgroup and sensitivity analysis, suggesting our results are stable and credible. Without doubt, whether there were other potential factors (i.e. gender) that influence the heterogeneity may be still uncertain due to limited information in the articles. Second, most of included articles were retrospective cohort studies which may introduce some unavoidable bias (such as selection bias). Third, although the results were significant using all the cut-off value of LDH, which is the optimal, remains uncertain because the number of studies with cut-off of LDH > 1.5, 2 and 2.5 ULN was relatively small. Fourth, the HRs extracted from the survival curve may introduce potential errors, which may be a potential reason to explain the non-significant association between LDH and PFS in the estimated HR subgroup. Fifth, the associations between LDH and some therapeutic outcomes (such as the response rate and adverse effect events) were not investigated due to lack of sufficient data and controversial conclusions. Sixth, all the included studies explored the effects for anti-PD-1 antibodies and no studies of anti-PD-L1 antibodies were enrolled. Seventh, the relationship between the mRNA expression status of LDH gene and the prognosis of melanoma patients was not explored like other genes.\(^{[62–66]}\) Therefore, the significance of LDH for predicting the therapeutic effects in melanoma patients treated
with anti-PD-1/PD-L1 needs to be validated and updated in the future.

5. Conclusion

Our meta-analysis shows that a high pretreatment LDH level is significantly associated with poor OS and PFS of melanoma patients treated with anti-PD-1/PD-L1. LDH may serve as a potential biomarker to identify patients who can benefit from anti-PD-1/PD-L1 and then schedule treatments.

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