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The prognostic value of circulating tumor DNA in patients with melanoma: A systematic review and meta-analysis

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ABSTRACT

Background: Circulating tumor DNA (ctDNA) has been investigated as a potential prognostic biomarker to evaluate the therapeutic efficacy and disease progression in melanoma patients, yet results remain inconclusive. The purpose of this study was to illustrate the prognostic value of ctDNA in melanoma.

Objectives: To describe the clinical prognostic value of ctDNA for melanoma patients.

Methods: Searched for eligible articles from Pubmed, Web of Science and Embase. Pooled hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated to evaluate the association between ctDNA at baseline or during treatment and overall survival (OS) and progression-free survival (PFS).

Results: A total of 9 articles were obtained, involving 617 melanoma patients. The pooled HRs revealed that compared with baseline undetectable ctDNA patients, detectable ctDNA was highly correlated with poor OS (HR 2.91, 95% CI: 2.22–3.82; $p < 0.001$) and PFS (HR 2.75, 95% CI: 1.98–3.83; $p < 0.001$). A meta-analysis of these adjusted HRs was performed and confirmed that ctDNA collected at baseline was associated with poorer OS/PFS (OS: HR 3.00, 95% CI 2.19–4.11, $p < 0.001$/PFS: HR 2.68, 95% CI 1.77–4.06, $p < 0.001$). During treatment, a significant association was shown between ctDNA and poorer OS/PFS (OS: HR 6.26, 95% CI 2.48–15.80, $p < 0.001$; PFS: HR 4.93, 95% CI 2.36–10.33, $p < 0.001$).

Conclusion: Investigation and application of ctDNA will improve "liquid biopsy" and play a role in early prediction, monitoring disease progression and precise adjusting treatment strategies in melanoma patients.

Introduction

Malignant melanoma should be taken more seriously for its rapidly increased incidence worldwide and it accounted for highest mortality of all skin cancers. Although the development of targeted therapies (BRAF and MEK inhibitors) and Immune Checkpoint Inhibitor (anti-PD-1 antibodies alone or in combination with anti-CTLA-4 antibody) had significant improved both progression free survival (PFS) and overall survival (OS), ways of monitoring the therapeutic responses remained poor and clinical outcomes were difficult to predict [1]. Invasive tumor tissue biopsy and histological examination is still gold standard for diagnose, treatment and prognose of melanoma, such as Breslow thickness, ulceration status, and mitotic rate. According to updated version of the 8th edition American Joint Committee on Cancer (AJCC) melanoma staging system, lactate dehydrogenase (LDH) is the only serologic marker with significant prognostic value, however, its sensitivity and specificity are quite low [2]. S100B, C reactive protein (CRP), melanoma-inhibiting activity (MIA) protein and PD-L1 are limited and not sufficiently useful biomarker [2]. There is urgent need to identify biomarkers with sufficient predictive value to aid treatment and lifestyle decisions, monitor therapeutic response, and guide novel therapeutic strategy. Circulating tumor DNA (ctDNA)—a liquid biopsy tool [3,4], provides a promising minimally invasive way for prognosing melanoma patients [5]. ctDNA-genomic DNA fragments, which mainly origin from apoptotic or necrotic cell death [6], are proved that have a potential role in monitoring of the therapeutic response, prognosing clinical outcome and providing information for early intervention [7]. The fluctuations of ctDNA in cancer patients at different times are corresponded with tumor volume and can be effected by tumor location, vascularity and cellular turnover. In the

Abbreviations: PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; QUIPS, Quality In Prognosis Studies; HRs, hazard ratios; CIs, confidence intervals; OS, overall survival; PFS, progression free survival.

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Table 1
Risk of bias using QUIPS tool.

Author	Year	Overall risk of bias	Study participation	Study attrition	Prognostic factor measurement	Outcome measurement	Study confounding	Statistical analysis and reporting
Sanmamed	2015	Moderate	M	M	L	L	H	H
Schreuer	2016	Low	M	L	M	M	M	L
Lee	2017	Low	L	H	M	L	L	L
Ganzalez-Cao	2018	Moderate	L	L	L	L	M	M
Herbreteau	2018	Low	L	M	L	L	L	M
Lee	2018	Low	L	L	L	H	M	M
Forthun	2019	Low	M	H	L	M	L	L
Gorges	2019	Low	M	L	L	M	M	L
Seremet	2019	Moderate	L	H	M	L	M	H

L low risk of bias, M moderate risk of bias, H high risk of bias.
QUIPS Quality in Prognosis Studies.

past five years, a few literatures reported that ctDNA could effectively and well predict the conditions of advanced melanoma patients and the effects after receiving targeted therapy and immunotherapy. Compared with the undetectable ctDNA at baseline or during treatment, the detectable ctDNA (baseline or fluctuated during treatment) suggests a worse results of progression and overall survival, as well as the objective responses to therapies.

This review and meta-analysis summarized the latest information about ctDNA as a biomarker for advanced melanoma, and provided effective evidence for its clinical application in the future, especially the role of early detection and effective monitoring of tumor dynamics, which greatly improved prognosis and treatment of melanoma patients.

Materials and methods

Literature search strategy

Using Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) guidelines, a literature search was performed by two investigators in July 2020 without restriction to regions, publication types, or languages. The databases PubMed, Embase and the Web of Science were systematically searched using the search terms “ctDNA,” or “circulating tumor DNA,” and “melanoma.” Various alterations in spelling and abbreviations were applied due to the pronounced heterogeneity of this research field. At the same time, we manually screened the reference lists of the selected papers, including all of the relevant studies and reviews. For the data obtained from the published studies, no ethical approval and informed consent were required.

Study inclusion and exclusion criteria

The following inclusion criteria were used to select eligible studies: (a) the diagnosis of melanoma was pathologically confirmed; (b) clinical study populations examined for the prognostic value of ctDNA; (c) the prognostic values (hazard ratios (HRs) and 95% confidence intervals (95% CIs) of melanoma for overall survival (OS), progression-free survival (PFS) were reported. Accordingly, the exclusion criteria of the meta-analysis were as follows: (a) studies only reporting data on cell free DNA or circulating free DNA were not eligible; (b) meeting abstracts, reviews, review papers, or case reports; and (c) no sufficient data to estimate the HRs and 95% CIs. When multiple reported by the same institution and/or authors, either the one of higher quality or the most recent or complete was used.

Data extraction

Two investigators independently extracted data from the included studies using a predefined data extraction form. All data were verified for internal consistency, and disagreements were resolved by discussion between the two investigators. Details extracted from the studies included first author’s name, year of publication, country, sample size, number and gender of patients, median age, follow-up duration, tumor stage, cut-off value of positive expression, prognostic factor measurement, survival curves, and hazard ratios (HRs) and their 95% CIs. Data was extracted by the software “Engauge 4.0″ from survival curves if it was not shown in articles directly. Tumor survival rate was extracted for calculating corresponding HR using the formula recommended by Tierney et al. [8]. When multivariate analysis and univariate analysis results were both presented in one study, we chose the multivariate analysis results because they account for confounding factors and are more accurate.

Quality assessment

Quality In Prognosis Studies (QUIPS) tool, which was recommended for evaluating prognostic factor studies, was used to assess the quality of the selected studies [9]. This tool examines risk of bias across 6 domains: study participation,study attrition, prognostic factor measurement, outcome measurement, study confounding, and statistical analysis and reporting. Two investigators independently recorded statistical methods used and summary measures, however presented, including hazard ratios with confidence intervals, tests of significance (p values). We conducted a narrative (descriptive) synthesis with results structured by type of prognostic factor. Risk of bias findings are presented in Table 1. Overall risk of bias in the studies was judged to be low for 6 studies [10–15] and moderate for 3 studies [16–18]. Study participation and prognostic factor measurement were typically low. The domains most commonly at high risk of bias were study attrition (n= 3) [11,14,15] and statistical analysis and reporting (n = 2) [16,18].

Statistical analysis

The primary outcome of this study was to assess the association between baseline dectable ctDNA and OS/PFS in melanoma patients. Effect measures for the outcomes of OS, PFS were adjusted and/or unadjusted HRs and 95% CIs extracted from the published studies. When those data were not reported in the publications, we calculated the HR estimates and their 95% CIs using the abstracted survival probabilities in the Kaplan-Meier curve at specific time points according to the methods proposed by Parmar et al. [19]. The data was input into the spreadsheet published by Tierney et al. [8], which extracted from Kaplan-Meier curves through the software“Engauge Digitizer version 4.1″ (free software downloaded from http://sourceforge.net), and estimate censoring using the minimum and maximum follow-up or the reported numbers at risk, to obtain the HR, 95%CI, lnHR, V, and O-E by all possible methods. The log HR and its variance were pooled using an inverse variance weighted average, and the results were presented as HR and 95% confidence interval(CI). Both unadjusted and adjusted (if available) HR with corresponding 95% CIs were extracted from each study. Two separate analyses were performed: one included all the unadjusted and adjusted

HRs, the other one included adjusted HRs from each study. Considering unadjusted HRs with corresponding 95% CIs might be effected by multivariate factors, the rest of analyses only included the adjusted HRs. The cumulative hazard ratio (HR) was estimated after each study inclusion in chronological order. The heterogeneity across studies was tested by using the chi-squared Q test and the I^2 metric statistic. There was marked heterogeneity if p-value ≤ 0.10 and/or I^2 was >50%. A random-effects (RE) model was applied to pool results under significant heterogeneity; otherwise, a fixed-effects (FE) model was applied. A pooled HR ≥ 1 indicated poor survival for patients with a detectable ctDNA. The source for interstudy heterogeneity was explored using subgroup analysis. Publication bias was evaluated by assessing the asymmetry of the funnel plot. Furthermore, the Begg test and the Egger test for funnel plots, which provide quantitative evidence, were employed to search for publication bias between the studies. To examine the stability and the reliability of the overall meta-analysis results, we performed the sensitivity analysis by excluding one study in turn. The statistical analyses were performed using Stata 12.0 software (Stat Corp, College Station, TX, USA). All p-values were two-sided, and $P < 0.05$ was considered statistically significant.

Results

Search results

A total of 1128 articles were retrieved through the database search from PubMed, Embase and Web of Science, and 225 studies were excluded because of duplication. After title and/or abstracts were screened, 128 articles remained for full-text assessment, and 119 articles were excluded, including reviews, letters, meeting abstracts, and other articles irrelevant to this study. Finally, through full-text evaluation, 9 studies contained the data of OS or PFS, which were suitable for this meta-analysis. A flow diagram about the literature search and study selection process is presented in Fig. 1.

Features of included studies

In summary, a total number of 617 melanoma patients with 1621 samples were included in our current study. All the 9 articles dealt with clinicopathological factors. The characteristics and demographics of the 8 included studies are summarized in Table 2. The median or mean age of patients was 58 years old. The 9 included articles were published from 2015 to 2019. Among them, 8 studies contain OS data, and 7 studies contain PFS data. All samples were taken from plasma in each patient and the detection of ctDNA and mutations in tumor tissues were used a droplet digital PCR (ddPCR) specific.

Meta-analysis results

Effect of presence of ctDNA on the prognostic effect (OS and PFS)

Survival analysis was performed on HRs for both OS and PFS. The HR was measured by comparing the detectable baseline ctDNA with undetectable ctDNA in melanoma patients. HR > 1 implies a poor prognosis in detectable ctDNA groups. Data on OS and PFS, including the adjusted and/or unadjusted HRs were respectively available in 9 studies and 8 studies. The pooled HRs revealed that compared with baseline undetectable ctDNA patients, the presence of ctDNA was highly correlated with poor OS (HR 2.91, 95% CI: [2.22–3.82]; $p < 0.001$) (Fig. 2) and PFS (HR 2.75, 95% CI: [1.98–3.83]; $p < 0.001$) (Fig. 3), indicating that baseline detectable ctDNA increased the risk of both overall mortality and disease progression in patients with ctDNA. Obvious heterogeneity was not observed in both OS ($I^2 = 0\%$, $p = 0.602$) and PFS ($I^2 = 0\%$, $p = 0.404$) and the random effects model was conducted.

Given 7 studies reported adjusted HR for OS by multivariable analysis [10–14,16,17] and 2 studies reported unadjusted HR for univari-

Table 2
Characteristics of the included studies in this meta-analysis.

Author	Year	Country	Sample size	Age median (range)	Sex (male/female)	Stage	Treatment	Follow-up (months) Median (range)	Cutoff value	Survival analysis	Somatic mutation
Sanmamed et al	2015	Spain	19	50	13/7	IIIc/IV	Anti-BRAF	4.5	216 copies/mL	PFS/OS	*BRAF* V600E
Schreuer et al	2016	Belgium	245	52	12/24	IV	Anti-BRAF/MEK	3.7	571 copies/ml	PFS	*BRAF* V600
Lee et al	2017	Australia	263	65	52/34	IV	Anti-PD-1/Anti-CTLA-4	17.5	NR	PFS/OS	*BRAF,NRAS,KIT*
Ganzalez-Cao et al	2018	Spain	91	58(28–44)	32/34	IV	Mixed	NR	10.5pg/nl	PFS/OS	*BRAF*
Herbreteau et al	2018	France	262	64	29/24	IIIc/IV	Anti-PD-1	6.8	8 mutated copies/mL	PFS/OS	*BRAF,NRAS*
Lee et al	2018	England	150	52(19–87)	77/84	II/III	Surgery	>12	≥1 copy/2mL	OS/DFI/DMFI	*BRAF,NRAS*
Forthun et al	2019	Norway	50	63(29–77)	15/11	IV	Bevacizumab	NR	ctDNA fractional abundance <1%	PFS/OS	*BRAF,NRAS*
Gorges et al	2019	Germany	84	21–88	NR	III/IV	Mixed	21	0.5 ng/mL	OS	*BRAF,NRAS, KIT,MAP2K1*
Seremet et al	2019	Belgium	457	57(27–82)	37/48	I-IV	Anti-PD-1	>36	500 copies/ml	PFS/OS	*BRAF V600, NRASQ61/G12/G13*

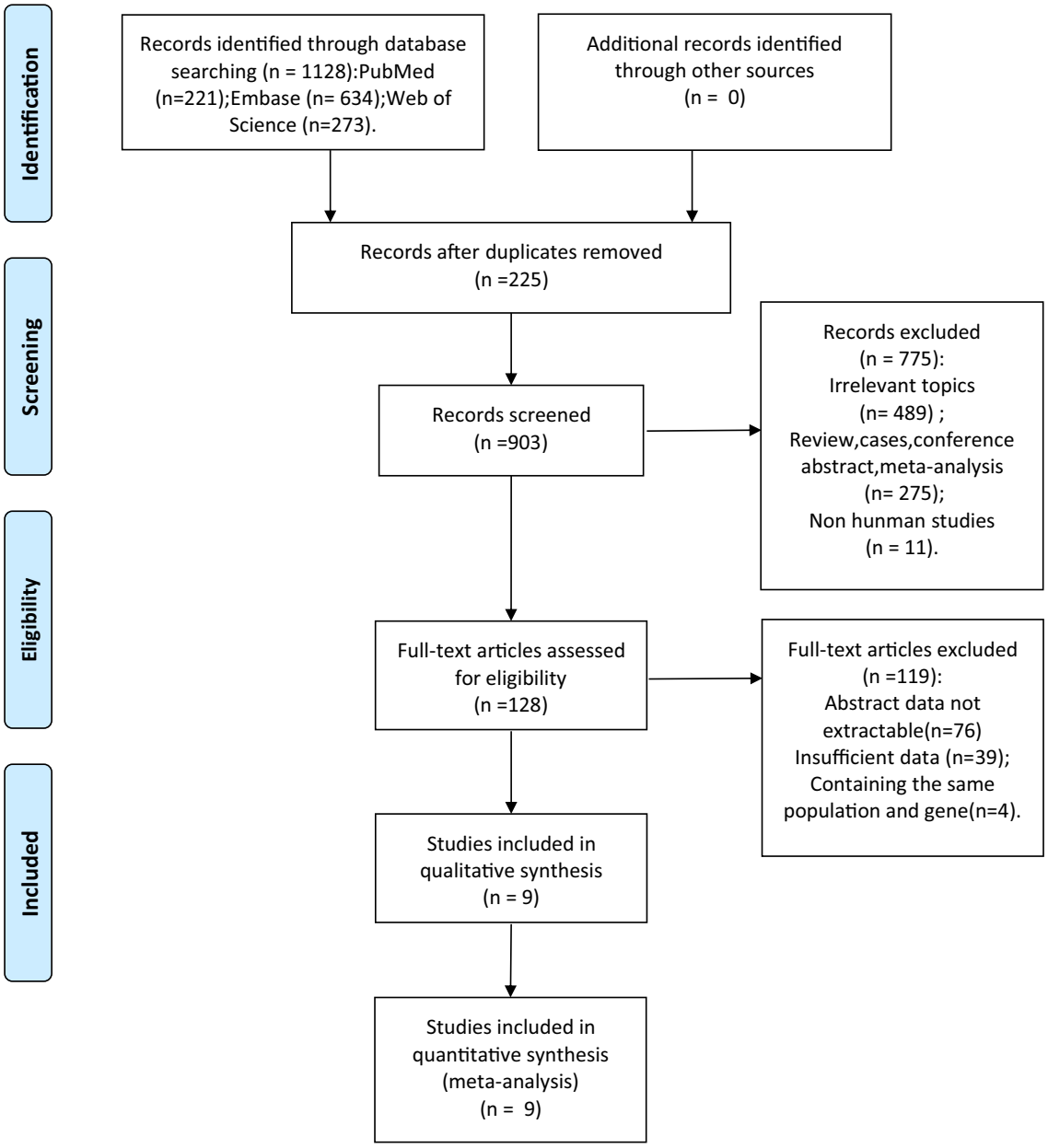

Fig. 1. PRISMA flowchart of the study. Selection process for study inclusion in the systematic review and meta-analysis according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA).

ate analysis [15,18], a meta-analysis of these adjusted/unadjusted HRs was performed (Fig. 2) and confirmed that ctDNA collected at baseline was independently associated with poorer OS (multivariable: HR 3.00, 95% CI 2.19–4.11, $p < 0.001$; univariable: HR 2.65, 95% CI 1.45–4.84, $p = 0.002$). Obvious heterogeneity was not observed in multivariate OS ($I^2 = 4.5\%$, $p = 0.392$) or univariate OS ($I^2 = 0\%$, $p = 0.937$).

For PFS, 5 studies reported adjusted HR by multivariable analysis [11,13,14,16,17] and 2 studies reported unadjusted HR for univariate analysis [15,18] was performed and a significant prognostic effect was confirmed in the analysis of studies that collected ctDNA at baseline (multivariable: HR = 2.68, 95% CI 1.77–4.06, $p < 0.001$; univarible: HR = 3.27, 95% CI 1.54–6.96, $p = 0.002$). Obvious heterogeneity was not observed in multivariate PFS ($I^2 = 21.4\%$, $p = 0.278$) or univariate OS ($I^2 = 0\%$, $p = 0.363$) (Fig. 3). A meta-analysis of 2 studies including melanoma patients undergoing treatments (immune-checkpoint inhibitors or bevacizumab) was performed and showed a obvious results, with a significant prognostic association between ctDNA and poorer OS and PFS (OS: HR 6.26, 95% CI 2.48–15.80, $p < 0.001$; PFS: HR 4.93, 95% CI 2.36–10.33, $p < 0.001$) with no significant heterogeneity (OS: $I^2 = 0\%$, $p = 0.881$; PFS: $I^2 = 0\%$, $p = 0.597$) (Fig. 4) .

Cumulative meta-analyses

A cumulative meta-analysis was conducted to evaluate the cumulative effect estimate over time, with the adjusted HRs for OS (Fig. 5a) and PFS (Fig. 5b), respectively. From 2015, a significant higher hazard ratio between baseline ctDNA and melanoma was observed. Many publications added cumulatively, resulted in an overall effect estimate became stable and narrowed the 95 percent confidence interval. This analysis confirmed the stability of the significant prognostic effect of ctDNA on effect.

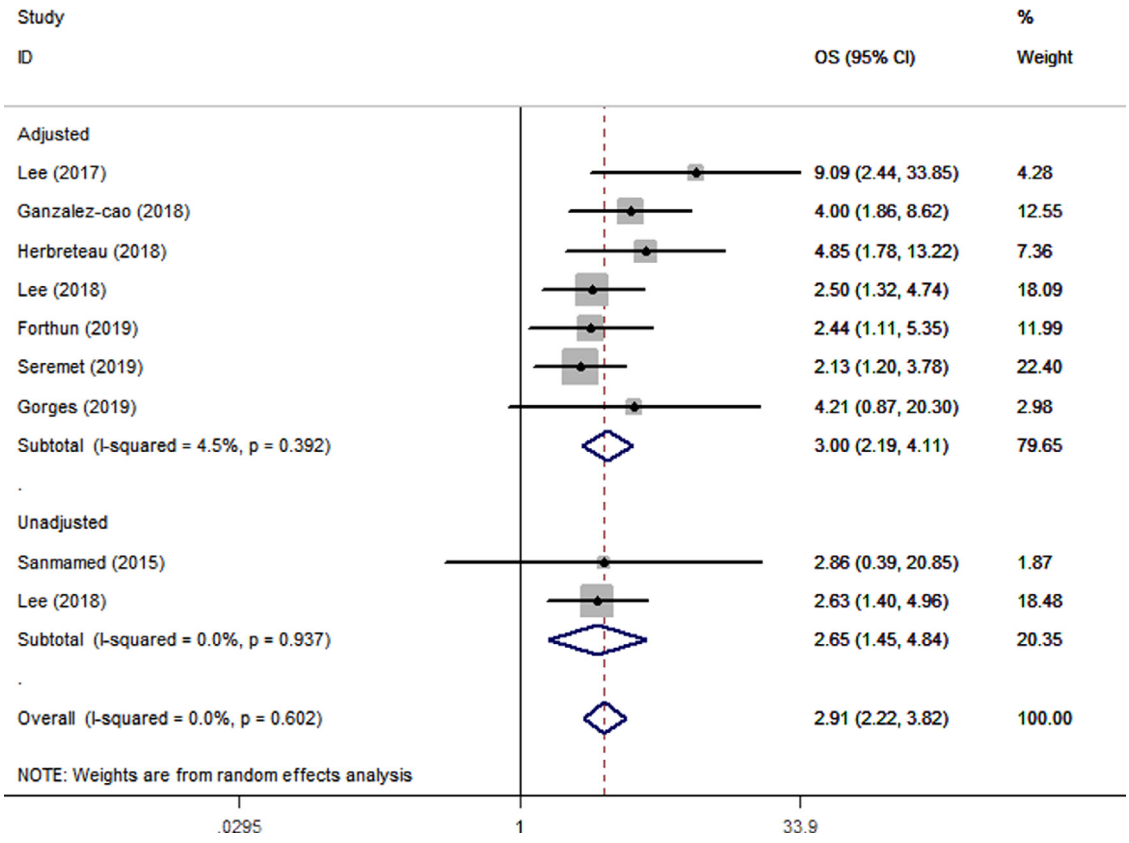

Fig. 2. Forest plot adjusted and unadjusted hazard ratio (HR) for the correlation between ctDNA and OS in melanoma.

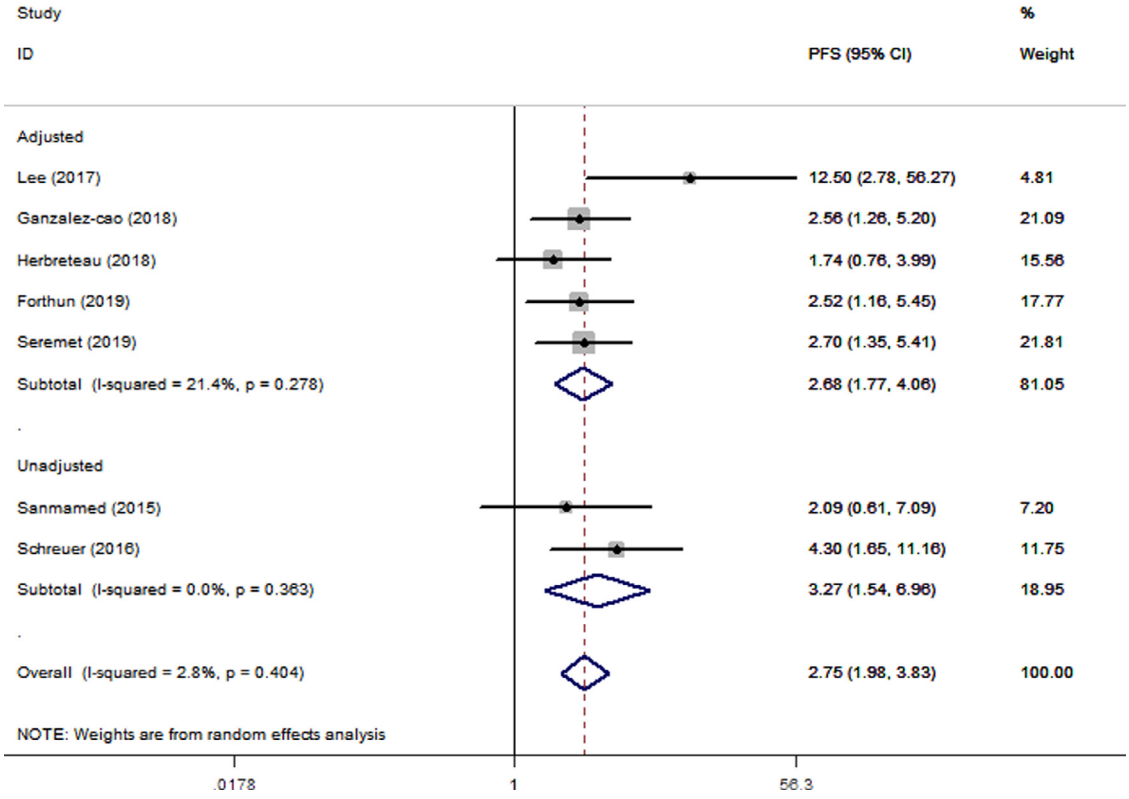

Fig. 3. Forest plot adjusted and unadjusted hazard ratio (HR) for the correlation between ctDNA and PFS in melanoma.

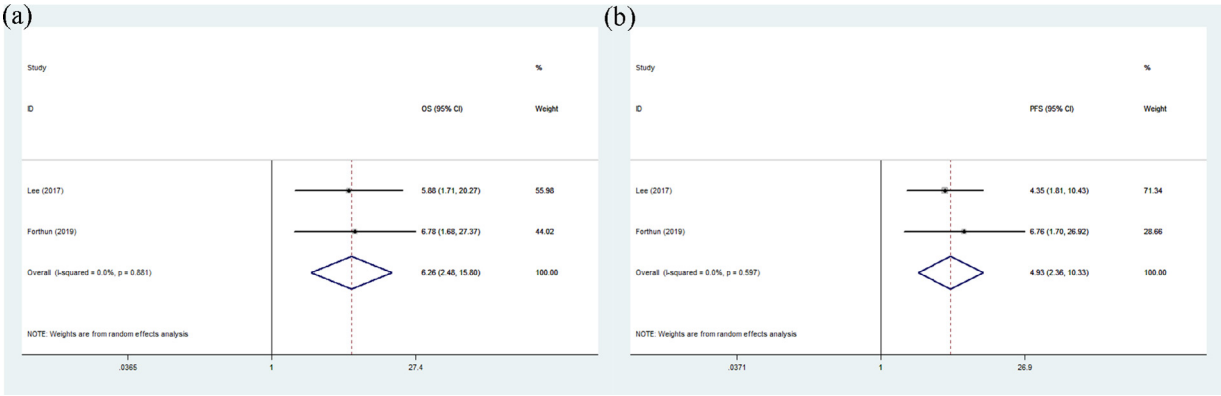

Fig. 4. Prognostic value of ctDNA in melanoma patients during treatment. (a) for OS; (b) for PFS.

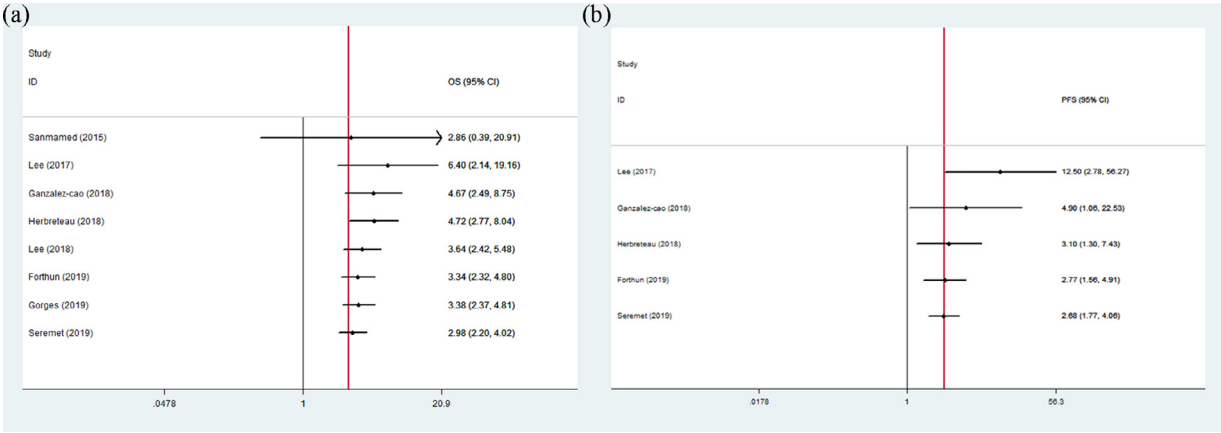

Fig. 5. Cumulative meta-analysis for OS (a) and PFS (b), based on year of publication.

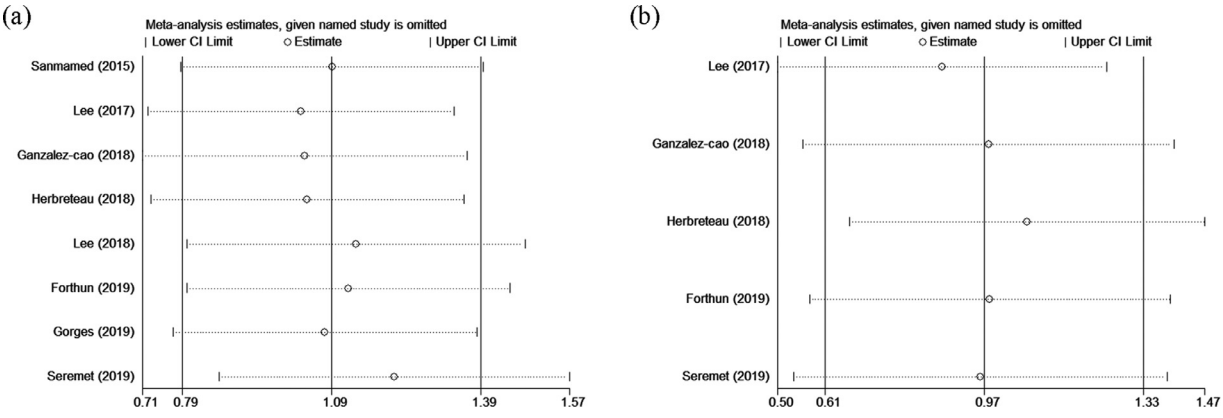

Fig. 6. Sensitivity analysis. (a) ctDNA for OS; (b) ctDNA for PFS.

Sensitivity analyses

Sensitivity analysis, in which one study was removed at a time, was performed to evaluate the stability of the results (Fig. 6). The results of the analysis demonstrated that no individual study significantly influenced the adjusted HRs with OS (Fig. 6a) and PFS(Fig. 6b), suggesting that the results of the present meta-analysis are credible.

Publication bias

Publication bias assessment of the studies were conducted by investigator using the Begg test [20] and the Egger test [21]. As shown in Fig. 7, no obvious asymmetry was observed in all of the groups. For OS, the *p*-value on the Begg test was 0.174 (Fig. 7a), and the *p*-value on the Egger test was 0.093 (Fig. 7c). For PFS, the *p*-value on the Begg test was 0.806 (Fig. 7b), and the *p*-value on the Egger test was 0.105 (Fig. 7d). No significant publication bias was observed among the current meta-analysis.

Discussion

To the best of our knowledge, this article is the first systematic review and meta-analysis to evaluate the prognostic capability of ctDNA in discriminating advanced melanoma. The results revealed that baseline level of ctDNA, and ctDNA fluctuations during treatment were significantly associated with outcome of melanoma patients [22]. Compared with patients with low levels of ctDNA, patients with dectable ctDNA

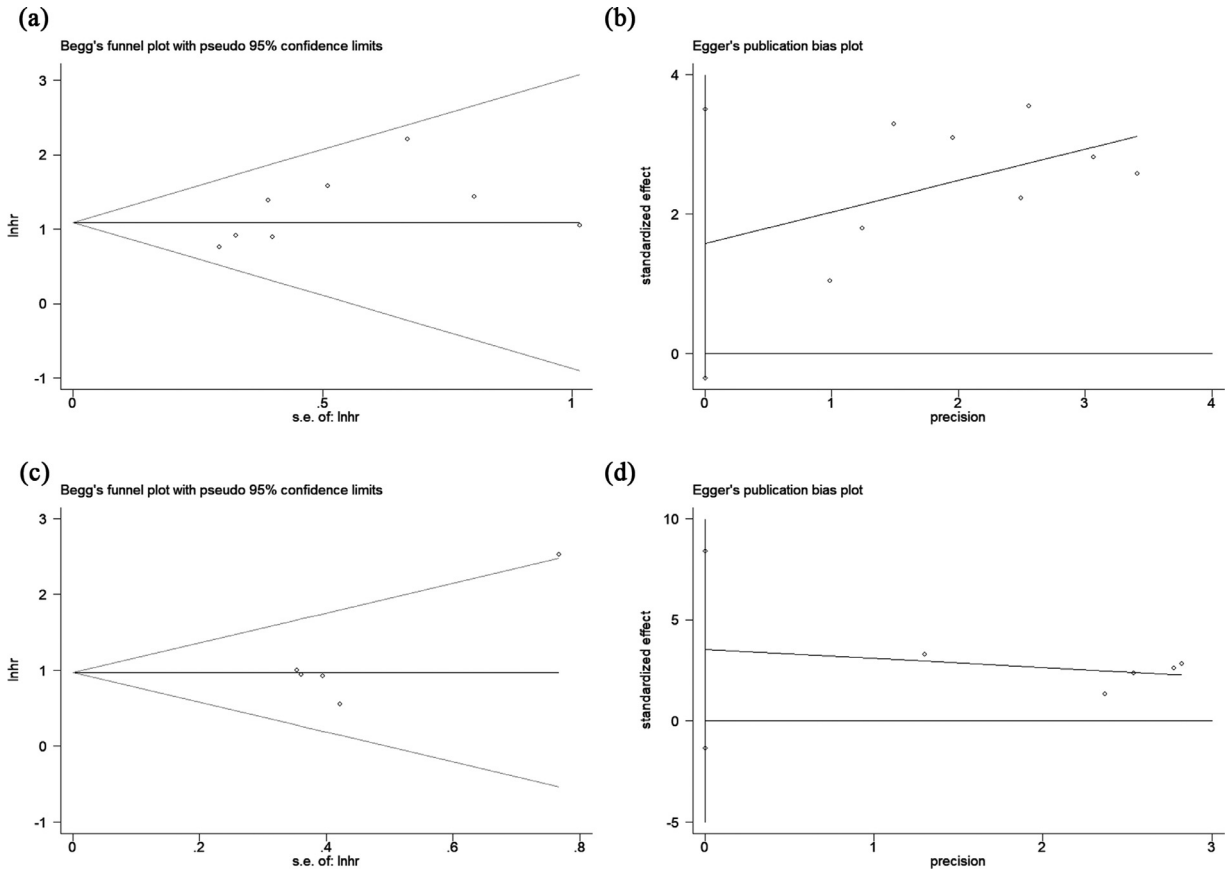

Fig. 7. Begg's funnel plot and Egger's test to evaluate publication bias. (a) Begg's test for overall survival (OS); (b) Egger's test for OS; (c) Begg's test for progression-free survival (PFS); and (d) Egger's test for PFS.

tend to have a unfavorable progression free survival. During treatment, the similar trend for OS and PFS can also be evaluated to monitor the therapeutic responses, although the number of reported studies was limited. No significant heterogeneity was observed among the included studies.

Malignant melanoma is a major lethal skin cancer. Since its high malignancy, early metastasis and easy relapse, melanoma accounts for huge economic burden on the patients and countries. In recent years, the immune checkpoint inhibitor (ICI) has achieved great success in the treatment of malignant cancers [23]. However, ICI treatment is only effective for a small number of melanoma patients. Although a number of candidate biomarkers for evaluating melanoma spring up, there are still limitations that may prevent the selection and routine use of biomarkers. Studies showed that compared to ctDNA, several common serological biomarkers, like LDH, Osteopontin, IL-8, YKL-40, MIA (Melanoma-inhibitory activity protein), would be associated with other biological processes such as infection, inflammation, autoimmune disease, which possibly translate to false-positive readouts and not specific to malignancy. Tyrosinase and Galectin-3 could not be ensured precise prognostic utility due to their unstable fluctuation. Except current serological biomarker, clinical pathological and radiological parameters, there are plenty of potential immunological markers have been investigated. As high-profile immune sentinels and molecules, Programed Death-ligand 1 (PD-L1) and tumor infiltrating lymphocytes (TIL) give more positive feedback about disease conditions and guiding significance for treatments, however, they also participate in other cancers and only benefit for part of melanoma patients [2]. Accurate biomarkers are urgently needed to evaluate the therapeutic efficacy and disease prognosis, as well as screening for combined immunotherapy [24]. Since there is a inefficient delay to use tissue biopsy when melanoma relapsed or in metastasis, molecular biology has been receiving an increasing attention [7]. As a minimally invasive approach, detection of ctDNA has been reported to be a potential method to predict survival in patients with advanced melanoma [25]. Furthermore, the development of more effective methods such as quantitative polymerase chain reaction (qPCR), droplet digital polymerase chain reaction (ddPCR) and NGS has allowed both screening and validation of genomic alteration in ctDNA, thus ensuring the availability of ctDNA detection [26–28].

The findings of studies support that baseline detectable ctDNA, which origins from different kinds of cancers, has ability to provide effective information for response to therapies, recurrences, metastasis or resistance mechanism [29]. Schreuer et al. observed that the fluctuations of ctDNA concentration were closely connected with disease condition during treatments. It revealed that ctDNA levels may be influenced by the proliferation of melanoma cells. A decrease of BRAF V600mut ctDNA could be detected in days after targeted therapy initiation, and a early increase occurred during disease progression or after discontinuation of targeted therapy [15]. It's critical to note that if the utilization of ctDNA can be approved in clinical setting in the near future, we can get the lead of carrying out early precise treatment to prevent or delay metastasis and recurrence [30]. In addition, continuous monitoring the fluctuation of ctDNA at different time points after curative intent surgery [12], during immune-checkpoint inhibitor(ICI) treatments or other decisions [17] in the daily clinical practice may be valuable for predicting clinical outcomes and adjusting further treatment strategies. What's more, as a minimally invasive tool, ctDNA not only relieve the pain for the melanoma patients, who spend lots of time and money on following up disease, especially in the progression of melanoma, from repeatedly invasive tissue biopsies or sometimes severe side effects and reduce financial burden on health service, but also bring reliable evidences for

accurate treatment. There is one study showing that ctDNA is regarded as valuable biomarker for identifying the differences between true progression and pseudoprogression in patients with melanoma receiving anti-PD1 antibody-based therapy. Early monitoring of ctDNA changes or variations during therapy could help clinicians quickly distinguish unresponsive patients, allow early adjustment to treatment strategies, and reduce exposure to ineffective and expensive treatments [31]. Atsuko et al. showed the point that ctDNA, as a useful biomarker, reflects independent impact of adverse events caused by systemic therapies for melanoma on tumor burden [32]. Further studies are needed to focus on the effect of ctDNA on the changes of condition of early stage melanoma in order to solidify our findings [29].

In most of meta-analyses with time-to-event data, the information about cut-off value was always difficult to handle with. Of the nine articles included in the study, six clearly put forward the cut-off value, but it could be seen after summarizing that four different measurement units were used respectively. In our study, after roughly converting the use unit conversion of ng/ml, pg/ml and copies/ml into unified units, a significant difference between these values could be found. It is assumed that the high or low level of ctDNA is mainly associated proliferative activity or status of tumor cells or tumor burden of the patients. Here, we meet a challenge at choosing a suitable cut-off value, and this problem must be faced during approving ctDNA as a biomarker in the clinical setting.

The in-depth study of gene mutations has gradually become clear. The rapid development of kits for detecting multiple genes and implying multiple mutations has also brought profound changes to the diagnosis, treatment and prognosis of melanoma. BRAF mutations account for the vast majority of melanomas, followed by NRAS, TERT promoter and other specific gene mutations [13,15,33,34]. There are also references in the literature that gene mutations in melanoma patients are related to drug resistance, such as BRAFV600E and NRASQ61K mutations [11]. Majority of studies regard great clinical significance of BRAF V600 specific mutation in ctDNA of melanoma. It has been confirmed that approximately 50% of melanoma patients have an activating mutation in the BRAFV600 gene on the long arm of chromosome 7. Based on this finding, the BRAF mutation test has been used to help stratify patients, which provides instructional information about receiving pathway inhibitor drugs. However, the test still has limitation on predicting the progression of disease. That is why ctDNA become a research hotspot, which possess the advantages of both diagnosing and predicting melanoma [15]. Moreover, ctDNA is very useful for real-time monitoring of the tumor genome and provides information about the response of malignant melanoma to immunotherapy and targeted therapy [35], thus supplementing the results of the usual tissue biopsy [28]. Therefore, ctDNA can monitor tumor genetic alteration in real time and provide more accurate information about patients with targeted therapy or immunotherapy [36].

Several possible limitations of this meta-analysis deserve to be mentioned. First, unavailable original data and data extracted from Kaplan-Meier curves may be the main identified barrier, which hinder the comprehensive investigation of the relations between ctDNA and the prognostic value for melanoma. Second, the number of studies included in this analysis is relatively small, which is to a limited extent due to the fact that the data extractions of Hazard Ratios (HRs) meet some difficulties. Third, the cut-off values varied at different time point and we meet a challenge on finding a precise threshold.

Despite these limitations, our study is of great value and essence. To start with, a thorough and scientific search was conducted; simultaneously, related articles and reference lists of these articles were also reviewed for additional studies. Then, after independent examination on the eligibility of studies and strict quality assessment by using QUIPS tools, the whole included studies were illustrated as high quality. Third, with clearly identifying the adjusted and unadjusted Hazard Ratios (HR), a whole group analysis and a subgroup analysis were performed in our study for exploring whether our results were affected by other confounding factors, in order to ensure more reliable results. Last but not least, some valuable information was integrated in our study, which would bring patients more convenience and good news if with further future studies.

Conclusion

In conclusion, our meta-analysis showed that ctDNA in baseline or during treatment can predict clinical outcomes and provide effective information for adjusting further treatment strategies. However, due to the several limitations, more studies including patients with diverse ethnicities are needed to solidify our findings.

CRediT author statement

Feng Sining: Conceptualization, Software, Formal analysis, Validation, Writing - Original Draft, Project administration

Wei Shanshan: Validation, Writing - Review & Editing, Project administration

Cen Xintao: Validation, Investigation, Resources, Data Curation

Tan Rui: Validation, Investigation, Resources, Data Curation

Sun Ledong: Validation, Writing - Review & Editing, Project administration

Statement of funding sources that supported the work

No funding.

Data availability statement

All data generated or analyzed during this study are included in this published article.

Author contributions

Feng Sining project development and manuscript writing. Wei Shanshan management and manuscript editing. Cen Xintao and Tan Rui data collection, analysis and management.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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