Prognostic relevance of tumor-infiltrating lymphocytes in residual tumor tissue from patients with triple-negative breast cancer following neoadjuvant chemotherapy: A systematic review and meta-analysis

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Abstract. Further adjuvant chemotherapy treatment can provide benefits to certain patients with triple-negative breast cancer (TNBC) that fail to achieve pathological complete response (pCR) after the administration of a neoadjuvant chemotherapy (NAC) regimen. However, biomarkers suitable for identifying patients likely to experience poor prognostic outcomes after undergoing additional adjuvant chemotherapy are currently lacking. Accordingly, the present meta-analysis was conducted to explore the relationship between tumor-infiltrating lymphocytes (TILs) or TIL subtypes ($CD4^+$ or $CD8^+$) in residual tumor (RT) tissue following NAC and TNBC patient prognosis. Relevant studies published through March 2023 were identified in Pubmed, The Cochrane Library, Embase and Web of Science databases. After excluding irrelevant studies, data were extracted from the remaining reports, while study quality was analyzed with the Newcastle-Ottawa Scale. Subsequent analyses were performed with Stata 14.0 and Review Manager 5.3. In total, seven relevant studies incorporating 1,202 patients were identified, all of which were retrospective cohort studies. Pooled analyses demonstrated that those patients exhibiting higher levels of RT TIL infiltration following NAC exhibited significantly improved recurrence-free, metastasis-free and event-free survival (RFS/MFS/EFS) compared with patients with lower RT TIL infiltration levels, together with an improved distant recurrence-free interval (DRFI) [hazard ratio (HR)=0.52; 95% confidence interval (CI)=0.39-0.69; P<0.00001]. In addition, patients exhibiting high RT TIL infiltration exhibited improved overall survival (OS) and breast cancer-specific survival (BCSS; HR=0.49; 95% CI=0.38-0.65; P<0.00001). Additional subgroup analyses revealed that patients with higher TIL infiltration levels or TIL subtype ($CD4^+$ or $CD8^+$) infiltration exhibited improved RFS/MFS/EFS/DRFI as compared with patients with lower levels of overall TIL or TIL subtype ($CD4^+$ or $CD8^+$) infiltration in RT tissue (HR=0.35, 95% CI=0.20-0.59, P<0.0001; HR=0.49, 95% CI=0.33-0.71, P=0.0002). Consistently, the OS/BCSS of patients exhibiting high levels of overall TIL or TIL subtype ($CD4^+$ or $CD8^+$) infiltration was increased compared with patients with lower levels of such infiltration (HR=0.33, 95% CI=0.19-0.59, P=0.0002; HR=0.55, 95% CI=0.41-0.76, P=0.0002). These data thus demonstrate that levels of overall TIL infiltration or infiltration by $CD4^+$ or $CD8^+$ TILs in RT following NAC can be used as a biomarker to reliably predict prognostic outcomes in patients with TNBC, in addition to highlighting possible targets that may guide the further immunotherapeutic management of these patients.

Introduction

Triple-negative breast cancer (TNBC) cases account for 15-20% of total breast cancer incidence, but patients with TNBC face a worse prognosis in due to the fact that these tumor cells do not express traditional therapeutic targets, including hormone receptors and human epidermal growth factor receptor-2 (1). In addition, TNBC cases tend to be more aggressive and associated with a higher risk or recurrence, contributing to a higher overall mortality risk (2,3). As endocrine and targeted therapy strategies fail to effectively treat TNBC, patients primarily undergo chemotherapeutic and surgical treatment. No differences in survival rates have been observed between patients with TNBC who undergo adjuvant chemotherapy and those that undergo neoadjuvant chemotherapy (NAC), such that the latter has emerged as the preferred systemic treatment regimen for this cancer type (4). NAC enables the direct assessment of tumor responses to chemotherapeutic drug administration, allowing clinicians to gauge patient prognosis and to opt for further adjuvant chemotherapy as appropriate (5). Given that TNBC tumors are generally more sensitive to cytotoxic drugs, higher pathological complete response (pCR) rates tend to be

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observed following NAC, which is noteworthy given that those patients who achieve pCR after NAC exhibit a better prognosis compared with those who do not (6). When residual tumor (RT) tissue is evident after NAC, patients face higher recurrence rates and shorter overall survival (OS) (7). The CREATE-X trial determined that those patients with TNBC and RT who post-operatively undergo intensified adjuvant capecitabine therapy experience improved outcomes (5). However, the administration of further cytotoxic drugs can also contribute to a higher risk of toxic side effects with the potential to impact patient quality of life, including persistent peripheral neuropathy and an elevated risk of cardiac events (8). Moreover, a subset of these patients with RT fail to relapse, suggesting that some of these individuals exhibit a good prognosis (9). Thus, there is a pressing need to identify novel biomarkers capable of reliably predicting patients with RT outcomes in order to guide the identification of high-risk patients that are most likely to benefit from the administration of further adjuvant chemotherapy. Such biomarkers would provide an effective approach to the individualized treatment of patients with TNBC by minimizing the risk of unnecessary treatment-related toxicity while maximizing the odds of better therapeutic outcomes.

Malignant tumor development is strongly influenced by the composition of the tumor microenvironment (TME), which consists of extracellular matrix components and a range of cell types. Tumor-infiltrating lymphocytes (TILs) within the TME are particularly relevant, given that they comprise the majority of this niche and are also vital to the effective control of tumorigenesis (10,11). TILs include a range of mononuclear immune cell populations including natural killer cells, dendritic cells, $CD4^+$ T cells and $CD8^+$ T cells (12,13). TIL infiltration has been reported in up to 75% of TNBC tumors, of which ~20% exhibit particularly high levels of such infiltration (14). In one recent meta-analysis focused on patients with TNBC undergoing NAC, a higher level of total TIL or TIL subtype ($CD4^+$ or $CD8^+$) infiltration prior to treatment was associated with higher pCR rates and improved post-treatment OS and recurrence-free survival (RFS) (15). Cytotoxic drug treatment can contribute to the mobilization and activation of TILs within the TME, thus reflecting TME responses to chemotherapy. Accordingly, analyses of TILs present in RT tissue can potentially offer additional prognostic information beyond that provided by analyses of pre-treatment TILs (16). The evaluation of RT TILs is also currently recommended by both the International Immuno-Tumor Biomarkers Working Group (14) and the International Immuno-Tumor Biomarkers Working Group.

There have been relatively few studies focused on the clinical relevance of TIL infiltration in RT tissue following NAC in patients with TNBC, and the prognostic value of such infiltration is still subject to controversy. As such, the present meta-analysis focused on those patients with TNBC that failed to achieve pCR after NAC in order to assess the prognostic value of TILs in RT tissue.

Materials and methods

Search strategy. Relevant studies published as of March 2023 were identified by searching the Pubmed (https://pubmed.ncbi.nlm.nih.gov/), Embase (www.embase.com/), The Cochrane Library (https://www.cochranelibrary.com/), and Web of Science (https://www.webofknowledge.com/) databases using the following search terms: 'Triple negative breast cancer', 'tumor-infiltrating lymphocytes', 'neoadjuvant chemotherapy' and 'residual tumors'. Subject headings combined with free words were used for study retrieval.

Inclusion and exclusion criteria. Studies eligible for inclusion were: i) Published in English; ii) studies enrolling patients with TNBC that did not achieve pCR following NAC; iii) studies assessing the association between RT TILs and TIL subtypes ($CD8^+$ or $CD4^+$) in RT tissue and patient prognosis; iv) original research studies of any design; and v) studies in which prognosis-related hazard ratios (HRs) and 95% confidence intervals (CIs) were provided or could be calculated indirectly. Studies were excluded if they were: i) Case reports, systematic reviews or literature reviews; ii) articles enrolling patients that achieved pCR following NAC; iii) studies lacking outcome indicators; or iv) studies for which HRs and 95% CIs could not be established.

Data extraction and quality analyses. Study screening was independently performed by two investigators, who initially conducted title, abstract and full-text review based on the established inclusion and exclusion criteria. Discrepancies were resolved by discussion and consensus. Relevant data were extracted from eligible studies, including: First author, publication year, country, sample size, TILs subtype, high infiltration threshold, detection method, outcome indicators, HRs and 95% CIs.

Literature quality evaluation. Included study quality was assessed using the Newcastle-Ottawa Quality Assessment Scale (NOS) (17). Possible NOS scores were between 0 and 9, and high-quality studies were those with scores of ≥6.

Statistical analysis. Stata 14.0 (version 12.0; StataCorp LP) and Rev Man 5.3 (RevMan 5.3; Cochrane) were used to conduct this meta-analysis as per the Cochrane International Collaboration Network Systematic Review Guidelines. Pooled effect sizes for survival data were calculated using HRs and 95% CIs, and $P<0.05$ was selected as the cut-off for statistical significance. The I^2 statistic and Cochrane's Q test were used to assess the heterogeneity of included studies, with $P<0.10$ or $I^2>50\%$ being considered indicative of significant heterogeneity. Random and fixed effect models were used when significant heterogeneity was and was not detected, respectively (18). Additional analyses of heterogeneity were performed by specifically analyzing results pertaining to TILs and TILs subtypes ($CD8^+$ or $CD4^+$). Egger's test and funnel plots were used to gauge publication bias, with $P<0.05$ as the cut-off for publication bias detection. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Study selection and characteristics. The initial study search yielded 208 potentially relevant studies, of which seven enrolling 1,202 patients with TNBC were ultimately included in this analysis (Fig. 1).

Table I. Baseline characteristics of included studies.

First author	Year	Country	Sample size	Type of TILs	Threshold	TILs evaluation method	Outcome	(Refs.)
Dieci *et al*	2014	France	278	iTILs and/or sTILs	60%	H&E	MFS/OS	(27)
Miyashita *et al*	2015	Japan	101	$CD8^+$	Not specified	IHC	RFS/BCSS	(36)
Luen *et al*	2019	Australia	375	sTILs	20%	H&E	RFS/OS	(19)
Pinard *et al*	2019	France	109	$CD8^+$	0.164	IHC	DRFI	(32)
	2019	France	109	$CD4^+$	0.066	IHC	DRFI	(32)
Wang *et al*	2021	China	109	sTILs	30%	H&E	RFS/OS	(29)
Da Silva *et al*	2022	Brazilian	134	$CD8^+$	Not specified	IHC	EFS	(31)
	2022	Brazilian	134	$CD4^+$	Not specified	IHC	EFS/OS	(31)
Lejeune *et al*	2023	Spain	96	$CD4^+$	Not specified	IHC	RFS/OS	(28)

TILs, tumor-infiltrating lymphocytes; iTILs, intratumoral tumor-infiltrating lymphocytes; sTILs, stromal tumor-infiltrating lymphocytes; H&E, hematoxylin-eosin staining; IHC, immunohistochemistry; MFS, metastasis-free survival; RFS, recurrence-free survival; DRFI, distant recurrence-free interval; EFS, event-free survival; OS, overall survival; BCSS, breast cancer-specific survival.

All studies eligible for inclusion in the present meta-analysis were retrospective cohort studies. TILs were classified into intratumoral TILs (iTILs) and stromal TILs (sTILs) based on their distributions. $CD4^+$ and $CD8^+$ T cells were the primary TIL subtypes, as distinguished through immunohistochemical staining, and the thresholds used to define high TIL or TIL subtype ($CD4^+$ or $CD8^+$) infiltration varied across studies (Table I).

Analyses of study quality. All seven of the included studies exhibited NOS scores >7, and were thus classified as high-quality studies (Fig. 2).

Association between RT TIL infiltration and RFS/MFS/EFS/DRFI. In total, the seven included studies enrolled 1,202 patients. A high degree of heterogeneity was detected among these studies (I^2=74.2%; P=0.0001), and results were thus analyzed with a random effects model. In sensitivity analyses, the iterative omission of individual studies from pooled analyses did not impact the overall results, indicating that this selected random effects model yielded stable and reliable results. Pooled analyses revealed that those patients with high levels of total TIL or TIL subtype ($CD4^+$ or $CD8^+$) infiltration in RT tissue following NAC exhibited significantly improved recurrence-free survival (RFS)/metastasis-free survival (MFS)/event-free survival (EFS)/distant recurrence-free interval (DRFI) as compared with patients with lower infiltration levels (HR=0.52; 95% CI=0.39-0.69; P<0.00001) (Fig. 3A).

When subgroup analyses for this endpoint were conducted for studies analyzing total TILs and TIL subtypes ($CD4^+$ or $CD8^+$), a total of three studies were included that provided results for overall TIL infiltration. Pooled analyses of these studies were conducted with a random effects model owing to a high degree of heterogeneity (I^2=83%; P=0.002), and the exclusion of the study by Luen *et al* (2019) (19) eliminated this heterogeneity (I^2=0%; P=0.39). When results were analyzed with a fixed effects model for this subgroup, patients with high levels of TIL infiltration exhibited improved RFS/MFS/EFS/DFRI compared with patients with low TIL infiltration (HR=0.35; 95% CI=0.20-0.59; P<0.0001) (Fig. 3B). In addition, four studies reported results for the TIL subtype ($CD4^+$ or $CD8^+$) subgroup, and a random effects model was used owing to moderate heterogeneity (I^2=58%; P=0.04). Pooled analyses for this subgroup similarly confirmed that patients with high levels of TIL infiltration exhibited improved RFS/MFS/EFS/DRFI compared with patients with low levels of TIL infiltration (HR=0.49; 95% CI=0.33-0.71; P=0.0002) (Fig. 3B). Sensitivity analyses revealed no changes in the combined effect size when alternating between fixed and random effects models, confirming that these results are robust.

Association between RT TIL infiltration and OS/BCSS. In total, six of the included studies incorporating 1,093 patients reported on OS/BCSS outcomes. As a high degree of heterogeneity was detected (I^2=77%; P=0.0007), a random effects model was selected. Sensitivity analyses indicated that the study conducted by Luen *et al* (19) had the greatest effect on this heterogeneity, which was reduced following the exclusion of this study (I^2=36%; P=0.18). A fixed effects model was thus selected for pooled analyses based on this reduction in heterogeneity. This approach revealed that patients with high levels of total TIL or TIL subtype ($CD4^+$ or $CD8^+$) infiltration exhibited significantly improved OS/BCSS relative to patients with lower levels of such infiltration (HR=0.49; 95% CI=0.38-0.65; P<0.00001) (Fig. 4A).

Subgroup analyses of studies assessing TILs and TIL subtypes ($CD4^+$ or $CD8^+$) were additionally conducted for this endpoint. Just two studies were included in the TILs subgroup, and pooled analyses of these studies were performed with a fixed effects model owing to an absence of heterogeneity (I^2=16%; P=0.27). The present analysis demonstrated that patients with higher levels of RT TIL infiltration following NAC exhibited better OS/BCSS as compared with patients with low levels of infiltration (HR=0.33; 95% CI=0.19-0.59;

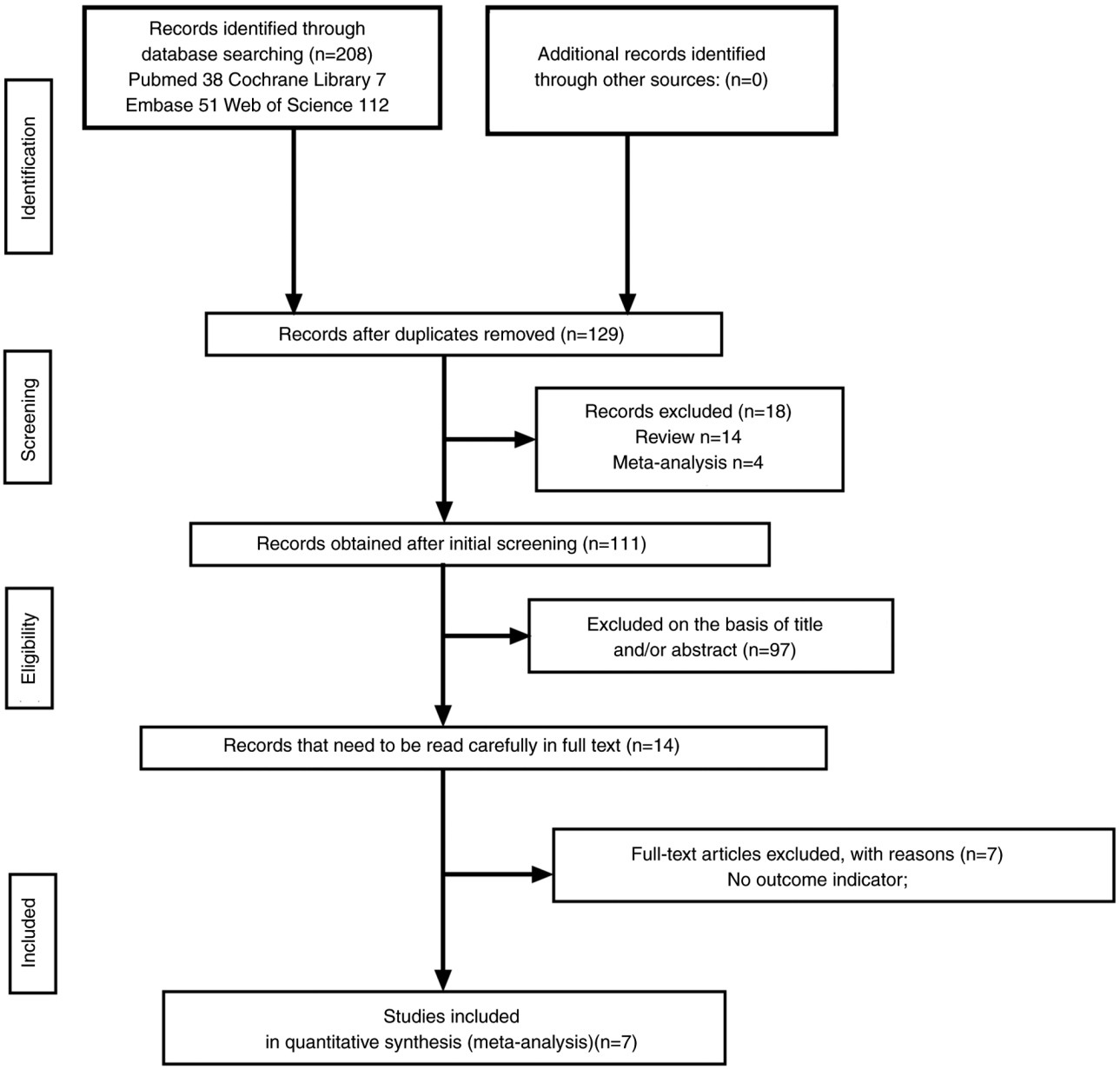

Figure 1. Study selection flow chart.

P=0.0002) (Fig. 4B). In addition, three studies reported results for TIL subtypes ($CD4^+$ or $CD8^+$), and these pooled analyses were also performed with a fixed effects model owing to the absence of significant heterogeneity (I^2=26%; P=0.26). As aforementioned, those patients with higher levels of TIL ($CD4^+$ or $CD8^+$) infiltration exhibited significantly better OS/BCSS as compared with patients with lower levels of such infiltration (HR=0.55; 95% CI=0.41-0.76; P=0.0002) (Fig. 4B). Sensitivity analyses revealed no changes in the combined effect size when alternating between fixed and random effects models, confirming that these results are robust.

Publication bias. Generated funnel plots were asymmetrical, and Egger's test yielded evidence of significant publication bias (P<0.0001 and P=0.002) (Fig. 5). This suggests that these results are subject to some degree of publication bias. This may be attributable to the choice to only include English language studies, contributing to language bias. In addition, negative research results often go unpublished, contributing to further bias in the literature. These findings suggest that the present meta-analysis results are not stable, underscoring the need for further research focused on this experimental topic.

Discussion

NAC has emerged as the standard approach to TNBC management, with most patients receiving a combination of anthracyclines, alkylates and taxanes while undergoing NAC treatment (20). The administration of these cytotoxic drugs can modulate lymphocyte infiltration of the TME in a manner that induces a robust antitumor immune response (21,22). Adaptive immunity can also be triggered in response to tumor-specific antigens, promoting the infiltration of lymphocytes engaged in immune surveillance whereupon they can destroy target tumor

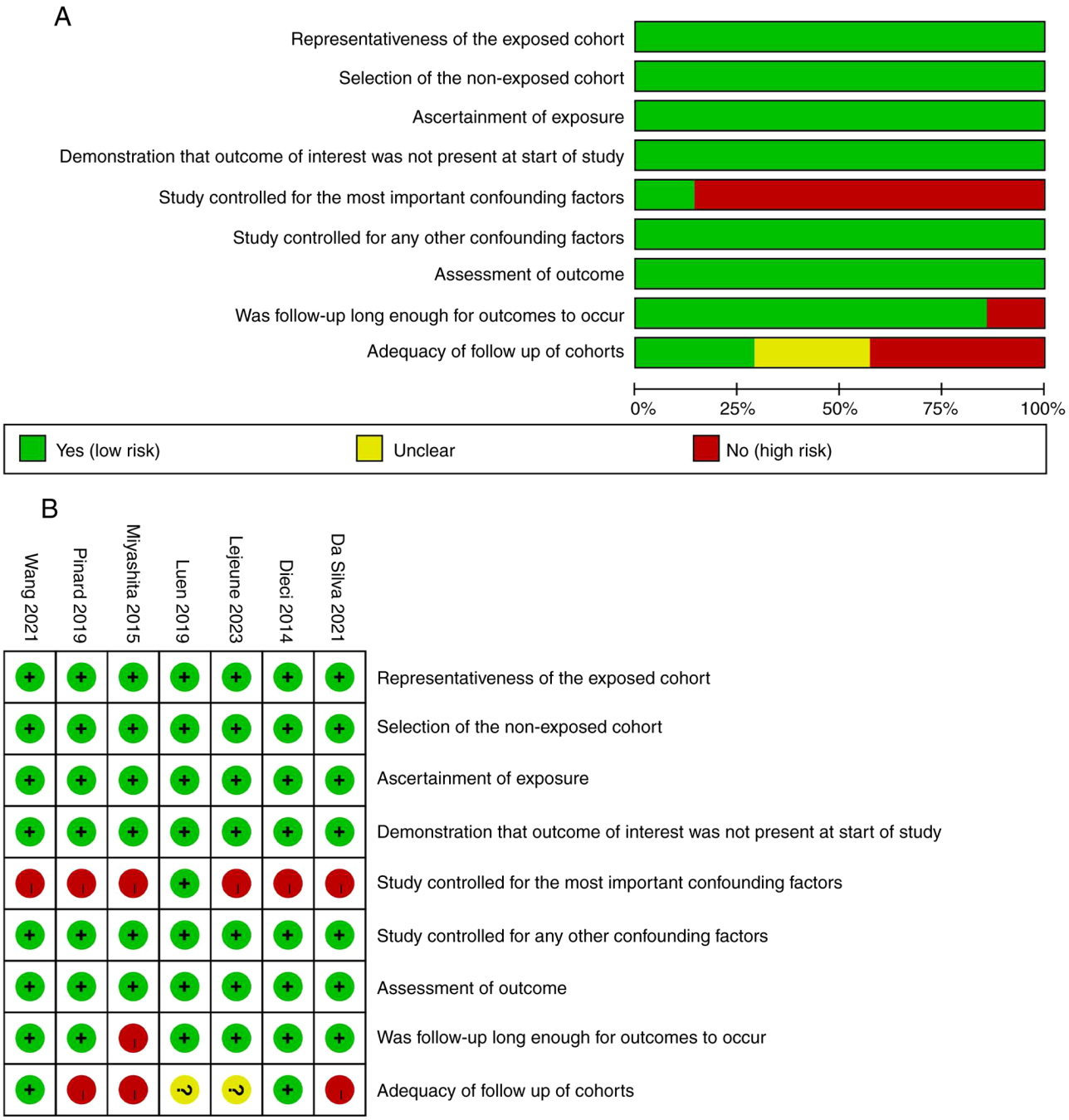

Figure 2. (A) A graph presenting the risk of bias for each of the indicated items in the form of percentages of the included studies. (B) A summary of the risk of bias for each included study.

cells (23). TIL infiltration can impact the effects of cytotoxic drugs on tumor cells, while also offering value as a biomarker associated with treatment outcomes and the overall prognosis of patients with cancer, providing guidance for treatment planning efforts. A number of studies to date have confirmed that TIL infiltration is significantly correlated with TNBC patient prognostic outcomes and treatment responses (24-26). While numerous studies have explored the association between TIL infiltration and pCR following NAC, there is also some evidence that a subset of patients who fail to achieve pCR can also achieve better outcomes (19,27).

Several recent studies have documented an association between TILs present in RT tissue following the prognosis of patients with NAC and TNBC (28,29). While this suggests the existence of a relationship between TILs and patient survival, systematic high-quality studies focused on this topic are lacking at present. As such, a meta-analysis was conducted in the present study as a means of systematically reviewing published studies to explore the prognostic implications of RT TIL infiltration levels. Owing to limitations with respect to the outcome indicators used in different studies, RFS/MFS/EFS/DRFI were utilized as short-term endpoints. Pooled analyses indicated that patients exhibiting higher levels of TIL infiltration experienced better postoperative RFS/MFS/EFS/DRFI compared with patients with lower levels of such infiltration (HR=0.52; 95% CI=0.39-0.69).

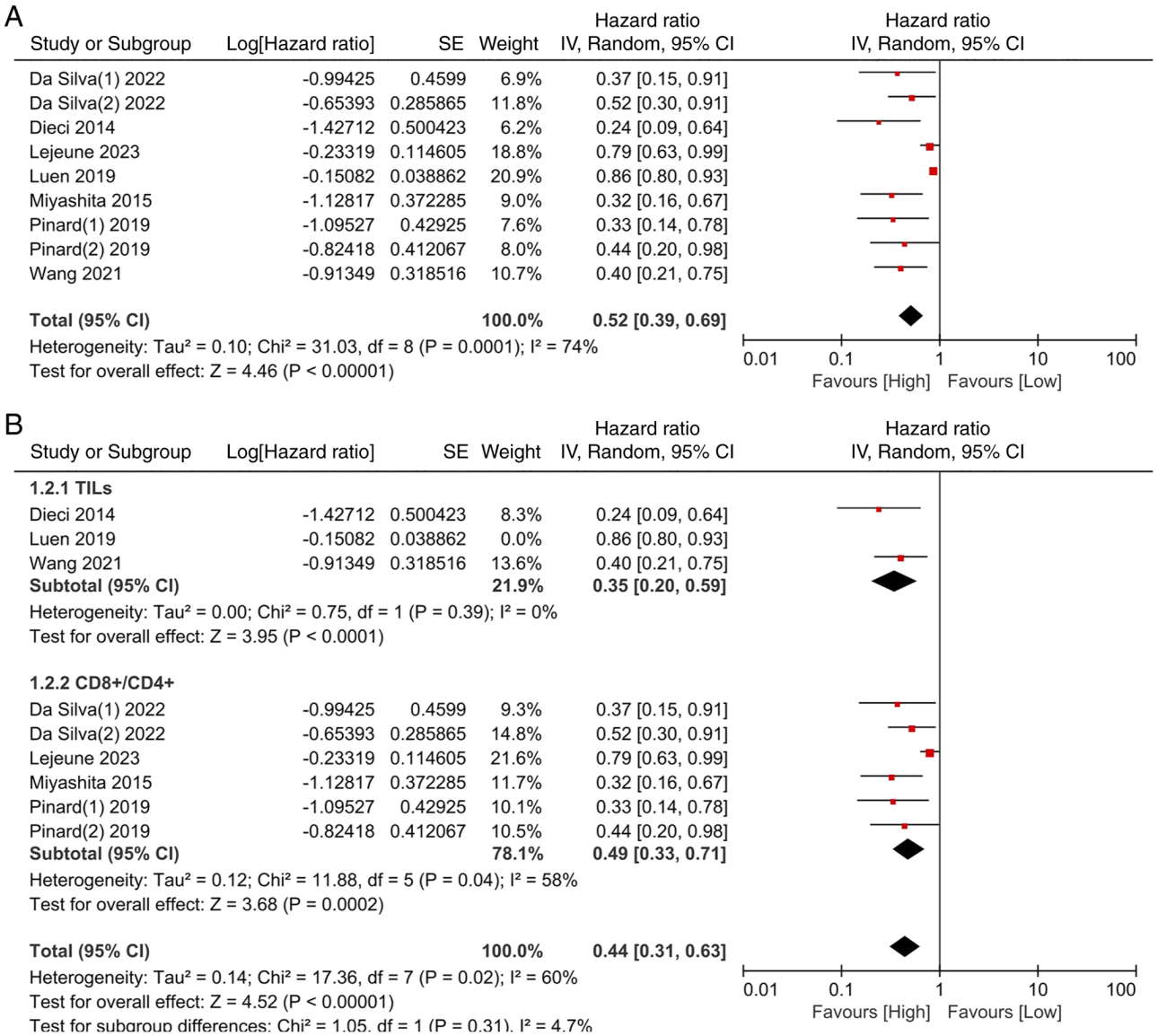

Figure 3. (A) Forest plots representing the association between residual tumor TIL infiltration following neoadjuvant chemotherapy in patients with triple-negative breast cancer and recurrence-free, metastasis-free, event-free and distant recurrence-free interval survival. (B) Subgroup analyses of studies assessing total TILs or TIL subtypes ($CD4^+$ or $CD8^+$). HR, hazard ratio; CI, confidence interval; TIL, tumor-infiltrating lymphocyte.

OS/BCSS were additionally analyzed as long-term outcomes, revealing that those patients with TNBC with higher RT TIL infiltration levels following NAC exhibited a significantly lower risk of death compared with patients with lower levels of TIL infiltration (HR=0.49; 95% CI=0.38-0.65).

Different TIL subtypes play specific roles in the context of antitumor immunity, engaging in cross-regulatory interactions that can shape immune response induction (30). The presence of specific TIL subtypes in RT tissue can contribute to a better or worse patient prognosis, and high levels of infiltration for certain TILs may thus provide a robust means of detecting patients in particular prognostic subgroups (31,32). $CD4^+$ and $CD8^+$ T cells are the primary TIL subtypes (31). $CD4^+$ T cells primarily act as helper T cells that are reliant on antigen-presenting cells to support the activation of other immune cell types following the recognition of soluble tumor-derived antigens, ultimately exerting antitumor roles (33). Through this activity, $CD4^+$ T cells have the potential to increase the numbers of other TILs in the TME or to enhance their functionality (33). Cytolytic $CD8^+$ T cells can directly destroy target tumor cells, making them particularly important mediators of antitumor immunity (34). Greater levels of $CD4^+$ and $CD8^+$ T cell infiltration in the TME have been linked to higher pCR rates and better prognostic outcomes in patients with TNBC (33,35,36). Accordingly, subgroup analyses were conducted in the present study based on the TIL subtypes included in the analyzed studies. With respect to short-term outcomes, high levels of TIL or TIL subtype ($CD4^+$ or $CD8^+$) infiltration were associated with a better prognosis (HR=0.35, 95% CI=0.20-0.59; HR=0.49, 95% CI=0.33-0.71), suggesting a higher risk of metastasis and disease recurrence in individuals with low levels of TIL infiltration. Similar findings were also observed with respect to long-term outcome indicators (HR=0.33, 95% CI=0.19-0.59; HR=0.55, 95% CI, 0.41-0.76).

These meta-analysis results highlight a clear relationship between RT TIL infiltration following prognosis of patients

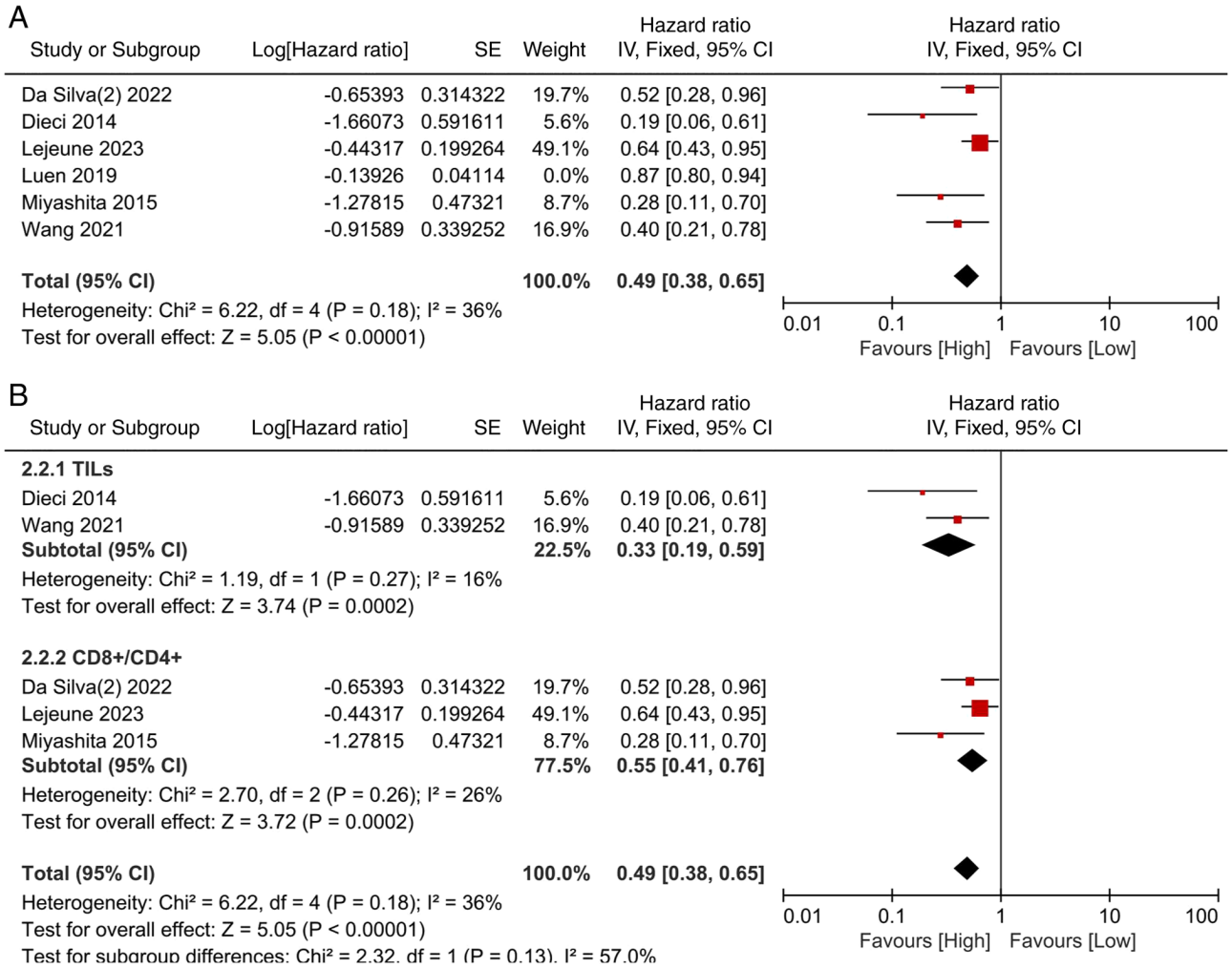

Figure 4. (A) Forest plots representing the association between residual tumor TIL infiltration following neoadjuvant chemotherapy and triple-negative breast cancer patient overall survival and breast cancer-specific survival. (B) Subgroup analyses of studies assessing total TILs or TIL subtypes ($CD4^+$ or $CD8^+$). HR, hazard ratio; CI, confidence interval; TIL, tumor-infiltrating lymphocyte.

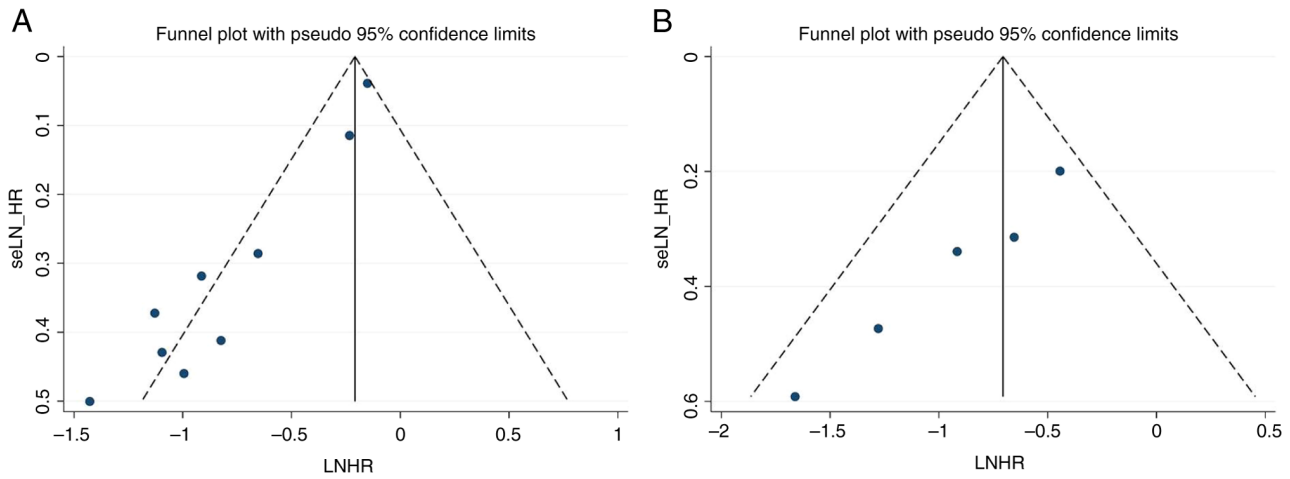

Figure 5. Funnel plots analyzing potential publication bias pertaining to the relationship between TIL infiltration in residual tumor tissue following neoadjuvant chemotherapy in patients with triple-negative breast cancer and their (A) recurrence-free, metastasis-free and event-free survival/distant recurrence-free interval or (B) overall survival and breast cancer-specific survival. LNHR, Logarithm of the Negative Hazard Ratio; seLN_HR, Standard Error of the Logarithm of the Negative Hazard Ratio.

with NAC and TNBC. Residual cancer burden (RCB) has also been used to predict the outcomes of patients with TNBC based on RT levels following NAC (37), suggesting that combining analyses of RCB and RT TIL infiltration profiles may improve

prognostic assessment efforts. Indeed, Luen *et al* (19) observed a significant interactive effect of TIL infiltration and RCB on prognosis, while Asano *et al* (38) determined that the combined assessment of TILs and RCB can predict post-NAC breast cancer recurrence more sensitively compared with TILs alone. Given that TNBC tumors exhibit unique immunological characteristics that can better sensitize these tumors to immunotherapeutic treatment, there is growing interest in the post-NAC immunotherapy-based management of this cancer type (39). As a result, TIL profiles represent increasingly important targets that can be used to stratify patients in post-NAC immunotherapy clinical trials. For example, results from the KEYNOTE-086 study highlighted the fact that sTILs can predict pembrolizumab efficacy when used as a first-line treatment for metastatic TNBC (40). The KEYNOTE-173 study revealed that there was a significant association between high levels of TILs and improved pathological complete response or objective response rate in patients with TNBC who underwent treatment with pembrolizumab (41). These findings were similar to the results of the KEYNOTE-086 study. TIL immune checkpoint receptor expression has been linked to better immunotherapy responses (42,43). Low levels of TIL infiltration may be indicative of the need for chemotherapeutic treatment using cytotoxic drugs and of the potential for the addition of PD-1/PD-L1 therapy. Higher TIL infiltration and lower disease burden, by contrast, may suggest that single-agent immunotherapy represents a viable treatment strategy (44).

The present study is subject to certain limitations. For one, the included studies employed different methods when detecting TILs/TIL subtypes and when selecting the threshold values used to define high levels of TIL infiltration. This, coupled with the inconsistent outcome indicators across studies, is likely to have impacted the overall accuracy of these results. In addition, the pre-NAC TIL infiltration profiles in the patients included in these studies were not evaluated, and the prognostic relevance of dynamic changes in TIL infiltration was thus not assessed, highlighting a promising avenue for future research. Lastly, owing to the limited number of relevant studies published to date, the overall sample size in this meta-analysis was somewhat small, potentially contributing to some degree of bias that may have impacted study results. Additional large-scale studies will be essential to explore the correlation between post-NAC TILs in RT tissue and TNBC patient prognostic outcomes in order to provide a comprehensive and evidence-based approach that can better guide clinical decision-making.

The present meta-analysis results suggest that TIL infiltration in RT tissue following NAC is a valuable prognostic indicator associated with TNBC patient outcomes. Specifically, higher levels of TIL infiltration were found to be associated with a lower risk of disease recurrence, metastasis and death such that patients exhibiting higher levels of RT TIL infiltration tended to exhibit a better prognosis compared with patients with lower levels of RT TIL infiltration. Analyzing levels of total TIL or TIL subtype ($CD4^+$ or $CD8^+$) infiltration in RT tissue following NAC can thus improve the ability of clinicians to predict the efficacy of further adjuvant chemotherapy administration and the prognosis of patients with TNBC.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

BC and ZZ contributed to the conception and design of the study. CW and XL prepared the materials, collected the data and performed the analysis. ZZ drafted the manuscript. BC and ZZ confirm the authenticity of all the raw data. All authors revised the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

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Competing interests

The authors declare that they have no competing interests.

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