The prognostic role of tumor-infiltrating lymphocytes in urothelial carcinoma of bladder: A systematic review and meta-analysis

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Abstract

Background: Tumor-infiltrating lymphocytes (TILs) in the tumor microenvironment are associated with different prognosis in various malignancies. However, their prognostic impact remains controversial in urothelial carcinoma of bladder (UCB). In this systematic review and meta-analysis, we aimed to investigate the prognostic value of TILs in UCB patients.

Methods: A systematic review and meta-analysis was performed using Pubmed, Embase and Cochrane Library. Studies were eligible if they investigated the prognostic value of CD3+, CD4+, CD8+, Foxp3+ lymphocytes or TILs in UCB patients, by time-to-event survival analysis. All studies were appraised for risk of bias using the Quality and Prognosis Studies (QUIPS) criteria. Hazard rations (HRs) with their 95% confidence interval (CIs) from each study were used to generate pooled HRs.

Results: A total of 14 studies assessing the impact of TILs on prognostic outcomes in UCB patients were included in final analysis. The pooled analysis indicated a favorable role of CD3+ TILs (HR 0.74 (95% CI 0.62-0.88) for overall survival) and CD8+ TILs (HR 0.46 (95% CI 0.28-0.74) for OS) in the clinical outcomes of UCB, while Foxp3+ TILs were associated with worse survival (HR 2.21 (95% CI 1.47-3.32) for recurrence-free survival).

Conclusions: This systematic review and meta-analysis confirmed the favorable prognostic impact of CD3+ and CD8+ tumor-infiltrating T cells in UCB patients and found the association between Foxp3+ TILs and worse survival. Future studies using large cohorts and standardized methodology with regard to tumor subsites, stages and treatment modalities are needed to incorporate TILs with clinical practice.

Background

Bladder cancer is the ninth most common malignancy worldwide, with approximately 430,000 new cases and 165,000 deaths each year, which makes it the thirteenth most lethal cancer in terms of mortality(1). Urothelial carcinoma of bladder (UCB) is the most common subtype, which may further subdivides into non-muscle-invasive disease and muscle-invasive disease(2). Despite various advances in diagnostic tools and treatment modalities, the prognosis of UCB still relies mainly on numbers of tumor, prior recurrence rate, T stage, tumor WHO grade, lymph node invasion and remote metastasis (3, 4). Biomarkers indicating therapy responses and time-dependent clinical outcomes are lacking.

In order to find new biomarkers predicting treatment outcomes of UCB, lots of efforts have been taken to reveal the essential properties of cancer cells and their surroundings. Among them, the relationship between tumor cells and immune microenvironment has been an important source of research(5, 6). In fact in recent years, clinical trials targeting this interplay are boosting rapidly and some of them brought favorable outcomes(7, 8). Since the immune microenvironment and immunotherapy becomes increasingly important, understanding the prognostic value of each presence of tumor-infiltrating immune cells might help identify new prognostic biomarkers, predict treatment outcome, and instruct treatment preferences.

Many studies incorporated diverse immune cells including tumor-infiltrating natural killer cells, macrophages, cytotoxic T cells and regulatory T cells, and indicated certain combination of densities could have better

survival(9). However, tumor-infiltrating lymphocytes (TILs) with different subtypes of immune cells have contradicting effects in the tumor microenvironment(10, 11).

Cytotoxic T-lymphocytes comprise the majority of effector T cells which possess antitumor ability by targeting and destroying cancer cells with certain cellular recognition molecules like major histocompatibility complex class I (MHC I)(12, 13). Cytotoxic T-lymphocytes are characterized by the expression of CD8(14). Various studies have explored the association of tumor infiltrated CD8+ lymphocytes with tumor prognosis and found positive outcomes and favorable overall survival (15-17).

The functions of T helper cells, characterized by CD4, are innately complex due to two kinds of cell polarization(18). Type 1 CD4+ lymphocytes are related to cell-mediated immunity by creating a microenvironment and promoting CD8+ T cell-mediated killing of tumor cells, while type 2 CD4+ cells predominantly benefit tumor progression by secreting immunity-compromising cytokines(19, 20).

T regulatory cells (Tregs) are major immunosuppressive subsets of T lymphocytes which maintain immune homeostasis and self-tolerance(21, 22). Tregs are characterized mainly by the transcription factor forkhead box protein P3 (FOXP3)(23). Although Tregs are usually considered to be related to poor survival in cancer patients due to immunosuppressive and tumor-promoting nature, which were confirmed in many studies, several researches suggested otherwise its association with favorable clinical outcomes (24, 25).

In this study, we systematically reviewed the articles about the prognostic values of TIL presence and CD3+, CD4+, CD8+ and FOXP3+ TIL subpopulations in UCB. We aimed to include all the studies that assessed tumor infiltration with CD3+, CD4+, CD8+ and FOXP3+ lymphocytes as well as TIL presence, and evaluate their potential as prognostic biomarkers in urothelial carcinoma of bladder.

Materials And Methods

Search strategy

A computerized search was conducted including the domain (“urothelial carcinoma of bladder”), the determinant (“tumor-infiltrating T-cells”), their synonyms, and a filter for prognostic studies(26). Pubmed/Medline, Embase and The Cochrane Library were searched for publications based on title and abstract from the inception of each database to December 27th, 2019. The search strategy and predetermined inclusion and exclusion criteria were based on a previous study(27). Two researchers (Yang and Hu) independently screened the titles and abstracts according to the criteria. Final selections were made by full-text reading of the selected studies. Discrepancies between the two researchers were discussed and resolved by consensus. T-cell markers assessed in two or more studies were eligible for inclusion.

Inclusion and exclusion criteria

Based on previous studies(27, 28), studies were included where the prognostic value of TILs, CD3+, CD4+, CD8+, and/or FoxP3+ lymphocytes was investigated in patients with urothelial carcinoma of bladder. For TIL marker, studies in which TILs were described as intense and non-intense as well as high and low were included. TILs had to be evaluated immunohistochemically or in hematoxylin- and eosin-stained sections.

Studies concerning intratumoral or tumor stromal lymphocytes were included, and studies that only investigate lymphocytes in other regions were excluded. The prognostic value had to be reported by time-to-event survival analysis with either overall survival (OS), disease-free survival (DFS), relapse/recurrence free survival (RFS), disease-specific survival (DSS), progression-free survival (PFS) or cancer-specific survival (CSS). Animal studies, case reports and commentaries were excluded.

Data extraction

The following data were extracted from included publications: author and title, year of publication, biomarker(s), sample size, tumor subsite, tumor stage, scoring methods, cutoffs and outcome of univariate and multivariate analysis defined by hazard ratio (HR), 95% confidence interval (CI), and p-values. When these parameters were not mentioned in the publication but according Kaplan-Meier curves were available, we digitalized and extracted the data from the Kaplan-Meier curves using the open-source Engauge Digitizer software (http://digitizer.sourceforge.net/), then the univariate HR was estimated. Studies where HRs were not mentioned, Kaplan-Meier curves were not available, or HRs did not match the shown Kaplan-Meier curves were excluded from the meta-analysis.

Assessment of study quality

This study is compliant with the PRISMA checklist(29). All selected publications were appraised for risk of bias using the Quality and Prognosis Studies (QUIPS) criteria, an established and useful tool for systematic reviewers for the critical appraisal of study quality(30). The risk of bias of was assessed as low, moderate or high in six different domains: study participation, study attrition, prognostic factor measurement, outcome measurement, study confounding and statistical analysis, and reporting. For this systematic review, studies that adopted consecutive cohorts and clearly described tumor stage stratification, methods of quantification and treatment modality were valued highest. The risk of bias was assessed by two researchers (Yang and Hu) independently. Discrepancies were resolved by discussion.

Statistical analysis

According to previous studies(27, 28), HRs were used that described the risk of events for high TILs versus low TILs or intense TILs versus non-intense TILs. If the study reported the HRs for low TILs versus high TILs or non-intense TILs versus intense TILs, the reciprocals were taken. The meta-analysis and creation of forest plots was performed in Stata/SE16.0 software.

Results

Study selection and characteristics

The Pubmed/Medline, Embase and The Cochrane Library search identified 2120 records and yielded 1429 hits after removing duplicates (Figure 1). Of them 91 publications remained after these records were screened on titles and abstracts. Then these articles underwent full-text evaluation, of which 18 met our inclusion criteria. Among those being discarded, 12 records were not available; 2 studies were reviews; 18 records were excluded because they were conference abstracts and did not provide sufficient data; 41 records were not

included because their domains, determinants or outcomes mismatched with our criteria. Of those survived full-text assessment, 3 were excluded for not clearly describing staining methods and one study was excluded because there was only one research on CD4+ lymphocytes, which provided limited values. Finally 14 studies were enrolled in this meta-analysis and the study characteristics are shown in Table 1(31-44). All studies investigated TILs by immunohistochemistry or hematoxylin- and eosin (H&E)-stained sections in paraffin-embedded tissue. Most studies included multiple tumor T stages as well as multiple treatment modalities.

According to a previous study, we also paid attention to the definition of different time-to-event variables. OS was defined as the time from the start of the treatment to death or last follow-up. DFS was described as the time from the start of the treatment to the date of first recurrence or last follow-up, while PFS was defined as the time from the start of the treatment to the date of first disease progression or last follow-up. RFS was defined as the time from the start of treatment to the date of clinical recurrence. DSS or CSS were determined as the time from the date of pathological diagnosis to the date of death from bladder cancer or last follow-up.

Quality assessment

The remaining 14 studies were critically appraised for risk of bias using the QUIPS criteria. Consistent with previous studies(27, 28), no studies mentioned study attrition or lost follow-up information. Almost no studies used consecutive cohorts. All studies were clear about their scoring methods, and as there was no consensus on cutoffs, studies used data-dependent cutoffs instead, which was not considered in the prognostic factor measurement domain. Complete quality assessment of these publications included in the meta-analysis is shown in Table 2.

CD3+ TILs as prognostic biomarker

The prognostic value of CD3+ TILs was evaluated in 4 studies. The results of the meta-analysis are displayed in Figure 2. Notably, Yu A et al(34) investigated CD3+ TILs in both tumor core and invasive margin areas independently, thus the data of two cohorts were collected respectively and integrated into this meta-analysis. Overall, the results of pooled meta-analysis showed a favorable advantage of high CD3+ TIL infiltration for OS (pooled HR 0.74 (CI 0.62-0.88)). No other time-to-survival parameters were obtained from these publications.

CD8+ TILs as prognostic biomarker

The prognostic value of CD8+ TILs was assessed in 6 studies, 3 of which presented outcome parameters as both OS and DSS, 2 of which only presented OS and one of which only presented DSS. The results of the meta-analysis are shown in Figure 3. Similar to CD3+ TILs, the study by Yu A et al(34) also investigated CD8+ TILs in both tumor core and invasive margin regions and the results of the two cohorts were independently analyzed. As a result, high CD8+ TILs were associated with a better OS (pooled HR 0.46 (CI 0.28-0.74)). Additionally, high CD8+ TILs showed a trend of improving DSS (pooled HR 0.72 (CI 0.48-1.09)). No else time-to-survival parameters were obtained from these publications.

FoxP3+ TILs as prognostic biomarker

Five studies reported data on the prognostic value of FoxP3+ TILs. Of them, two studies presented outcome parameters as RFS, two presented as OS, two presented as PFS and two presented as CSS. The results of the meta-analysis are shown in Figure 4. An unfavorable disadvantage of high FoxP3+ TILs was observed for RFS (pooled HR 2.21 (CI 1.47-3.32)). The disadvantages were not as significant for OS (pooled HR 1.03 (CI 0.75-1.42)), PFS (pooled HR 1.41 (CI 0.66-3.01)) and CSS (pooled HR 0.99 (CI 0.74-1.31)).

TILs as prognostic biomarker

Five studies demonstrated results on the prognostic value of TILs as a whole. As Wang B et al(31) investigated TILs in discovery cohort and validation cohort, the data of the two independent cohort were analyzed separately. The results of the meta-analysis are displayed in Figure 5. In patients with urothelial carcinoma, the difference of prognostic value of TILs on overall survival was not significant (pooled HR 0.92 (CI 0.52-1.62)). No other time-to-survival parameters were obtained from these studies.

Discussion

Urothelial carcinoma of bladder is one of the most prevailing malignancies across the globe. It is comprised of both benign and malignant subtypes, namely non-muscle invasive bladder cancer (NMIBC) and muscle invasive bladder cancer (MIBC), which present distinct prognosis after diagnosis and treatment(45). Despite recent years' great advances in tumor detection techniques, reliable prognostic biomarkers are still lacking. According to several clinical guidelines, UCB prognosis is mainly dependent on tumor numbers, prior recurrence rate, pathological T stage (depth of invasion), WHO grade, invasion of lymph nodes and remote metastasis to predict disease recurrence and survival(46, 47). UCB is in fact considered to be an "immunogenic" tumor since the famous Bacillus Calmette-Guerin (BCG) therapy, which harnesses immune responses, is well established and widely used in low risk NMIBC context(48-50). Thus the purpose of our study was to investigate the relationship between existing tumor-infiltrated lymphocytes, including the presence of TILs and different subsets, with the prognosis of UCB patients.

Firstly, we investigated the prognostic role of CD3+ T cells in UCB patients. Our meta-analysis showed that high CD3+ TILs were associated with a favorable prognosis in bladder cancer. This result is consistent with other studies which investigated CD3+ TILs in various tumors and ended up with similar outcomes, reflecting that general T cells in the tumor microenvironment are of great importance for clinical outcomes (51-53).

The prognostic value of CD8+ TILs has already been explored widely in most cancer types and many of them concluded that high CD8+ TILs indicated better outcomes(54, 55). Similarly, this meta-analysis suggested that high CD8+ T cell infiltration was significantly correlated with better overall survival. In fact, CD8+ TILs, functionally known as cytotoxic T lymphocytes, take the main adaptive immune responses against tumor by directly contacting and killing tumor cells(56). Thus, it could be easily understood that high tumor-infiltrated CD8+ markers could possibly serve as a favorable prognostic maker.

We failed to conduct a meta-analysis on the relationship between CD4+ TILs and the prognosis of UCB patients due to only one research on CD4+ TILs met our inclusion criteria. Notably, the one study on CD4+ lymphocytes indicated that high infiltration of CD4+ TILs were significantly associated with poor outcome (57). Nevertheless, the importance of CD4+ TILs should be noted. CD4+ T lymphocytes are traditionally

known as T helper cells which assist cytotoxic immune cells to recognize and eliminate tumor cells(58). However, various studies have illuminated their two types of polarizations which exhibiting reciprocal antagonism(18, 20). Type 1 CD4+ T lymphocytes promote CD8+ T cell or NK cell-mediated tumor elimination by secreting cytokines such as IL-12, IFNγ and TNF, while type 2 CD4+ T lymphocytes promote tumor growth by secreting cytokines such as IL-4 and IL-5 (18, 20). This contradictory polarization could possibly explain the conflicting clinical outcomes in other malignancies. Studies of CD4+ TILs with clear discrimination of subpopulation on UCB patients are warranted.

The prognostic value of FoxP3+ TILs was also explored. Of five studies, two considering recurrence-free survival showed that high FoxP3+ TILs were associated with unfavorable clinical outcome. As for overall survival, progression-free survival and cancer-specific survival categories, there were no significant propensions whether high infiltration of FoxP3+ T lymphocytes were correlated with worse prognosis. This could be interpreted with two reasons. On the one hand, the included studies under FoxP3+ category investigated clinical outcomes with different time-to survival events, which resulted in small subgroups and attenuated statistical power. On the other hand, Tregs are also a conflicting subset of T lymphocytes which exhibit internal heterogeneity. In most cancers such as non-small -cell lung cancer and ovarian cancer, Tregs were associated with a poor prognosis, however in colorectal cancer, it can be correlated with a good prognosis(8, 59, 60). This contradiction might due to the combined effects of TIL subsets' interaction in tumor immune microenvironment.

Finally, we conducted a meta-analysis on whole TILs' prognostic role in UCB patients. In the five included studies, TILs were presented as a whole biomarker investigated by staining mononuclear inflammatory cells or combining CD3+ and CD8+ T lymphocytes, which means no subtypes of TILs were divided and analyzed respectively. However, our meta-analysis showed that TILs were not a representative biomarker concerning overall survival of UCB patients. This result might be due to the fact that TILs are consisted of various types of T cell subsets and the internal heterogeneity contributes to the statistical insignificance.

Certain limitations of this review must be noted. The main limitation of our meta-analysis is the heterogeneity regarding to tumor clinical and pathological characteristics among these included studies. The prognostic role of TILs may vary according to different tumor subsites and tumor stages. However, the relatively small sample sizes of included studies prevent us from stratification of different conditions for subgroup analysis. Furthermore, there is also heterogeneity in treatment modalities. UCB patients are generally divided into NMIBC or MIBC subtype according to their histopathologic analysis, which usually require distinct treatment plans. Although these included studies accounted for treatment modality in multivariate analysis, they still didn't combine it to TILs with survival analysis. Notably, it is crucial to determine standardized and validated cut-offs for TIL quantification since clinical practice requires operable parameters. But this meta-analysis couldn't suggest general cutoffs because the methods of quantifying TILs varied among the included studies. Thus in order to incorporate tumor-infiltrated T lymphocyte markers with clinical prognosis, more prospective studies using homogeneous patients, subgrouping tumor subsites, tumor stages and treatment modalities as well as adopting reliable cut-offs for quantification are needed.

Conclusions

In conclusion, our meta-analysis confirmed the prognostic value of CD3+ and CD8+ tumor-infiltrating T lymphocytes in urothelial carcinoma of bladder. High density of CD3+ and/or CD8+ lymphocytes were associated with better overall survival. Moreover, to incorporate different tumor-infiltrating T lymphocyte markers to predict clinical outcomes in real-world practice, large prospective studies using homogeneous cohorts and well-defined cutoffs, regarding to tumor subsites, tumor stage and treatment modalities are needed.

Abbreviations

TILs: tumor-infiltrating lymphocytes; UCB: urothelial carcinoma of bladder; QUIPS: Quality and Prognosis Studies; HR: hazard rations; Tregs: T regulatory cells; FOXP3: forkhead box protein P3; OS: overall survival; DFS: disease-free survival; RFS: relapse/recurrence free survival; DSS: disease-specific survival; PFS: progression-free survival; CSS: cancer-specific survival.

Declarations

Author's contributions

Ping Han: Project development, funding. Zhi-qiang Yang, Yun-jin Bai and Xu Hu: Data collection, analysis, manuscript drafting/editing.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Study characteristics of studies included in meta-analysis.

Study	Sample size	Stage	Treatment	Biomarkers	Survival	Material	Technique
Wang B 2019	226	All	CE, TUR	TILs	OS, DFS	FFPE	IHC/H&E
	417	All	CE, TUR	TILs	OS, DFS	FFPE	IHC/H&E
Rouanne M 2019	147	T1	CE, TUR, BCG	TILs	OS, CSS	FFPE	H&E
Li XD 2019	221	All	CE	TILs	OS	FFPE	IHC/H&E
Yu A 2018	67	All	CE, CT, IT	CD3+, CD8+	OS	FFPE	IHC/H&E
Murai R 2018	115	Ta, T1	TUR	Foxp3+	RFS	FFPE	IHC/H&E
Liu K 2018	102	All	CE, TUR	TILs	OS	FFPE	IHC
Huang HS 2018	259	T4	CE, CT	TILs	OS	FFPE	H&E
Miyake M 2017	154	Ta, T1, Tis	TUR, IT	Foxp3+	RFS, PFS	FFPE	IHC/H&E
Horn T 2016	149	All	CE, CT, TUR	CD3+, CD8+, Foxp3+	OS, CSS	FFPE	IHC
Faraj SF 2015	50	T2, T3, T4	CE, CE, RT	CD8+	OS, DSS	FFPE	IHC/H&E
Wang B 2015	302	Ta, T1	CE, TUR	CD8+, CD103+	OS	FFPE	IHC
Sjodahl G 2014	52	T2, T3, T4	CE	CD3+, CD8+, Foxp3+	DSS	FFPE	IHC
Winerdal ME 2011	37	All	CE	CD3+, Foxp3+	OS, PFS	FFPE	IHC
Sharma P 2007	69	All	CE	CD8+	OS, DFS	FFPE	IHC

Cystectomy (CE), TUR (transurethral resection), BCG (Bacillus Calmette-Guerin), Chemotherapy (CT), intravesical therapy (IT), Radiotherapy (RT), Tumor-infiltrating lymphocytes (TILs), Overall Survival (OS), Disease-free Survival (DFS), Cancer-specific Survival (CSS), Regression-free Survival (RFS), Progression-free Survival (PFS), Disease-specific Survival (DSS), Formalin Fixed, Paraffin Embedded material (FFPE), Immunohistochemistry (IHC), Hematoxylin- and Eosin-staining (H&E)

Table 2. Quality assessment of included studies.

Study	Study Participation	Study Attrition	Prognostic factor	Outcome	Study confounding	Analysis and reporting	Total Risk of Bias
Wang B 2019	i	●		i	i	i	Low
Rouanne M 2019		●	i	i		i	Moderate
Li XD 2019	i	●	i	i		i	Low
Yu A 2018		●		i	i	i	Moderate
Murai R 2018	i	●		i	i	i	Low
Liu K 2018	i	●	i	i	i	i	Low
Huang HS 2018	i	●				i	High
Miyake M 2017	i				i	i	Low
Horn T 2016	i	●		i	i	i	Low
Faraj SF 2015	i				i	i	Low
Wang B 2015	i	●			i	i	Moderate
Sjodahl G 2014	i		i	i	i	i	Low
Winerdal ME 2011	i	●			i	i	Moderate
Sharma P 2007		●	i	i		i	Low

i= low risk of bias, = moderate risk of bias, ● = high risk of bias.

Figures

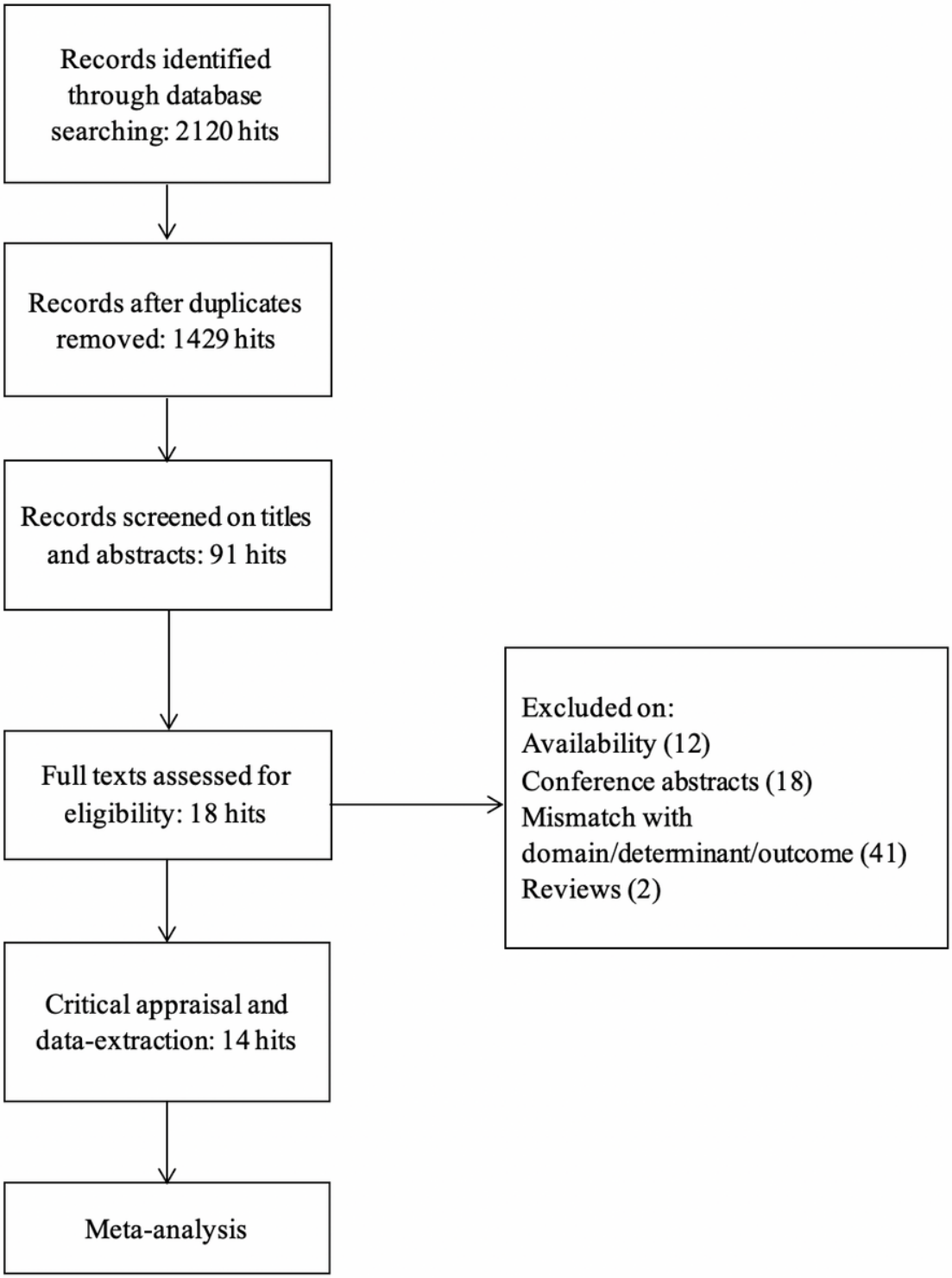

Figure 1

The flow diagram of screening and selecting studies.

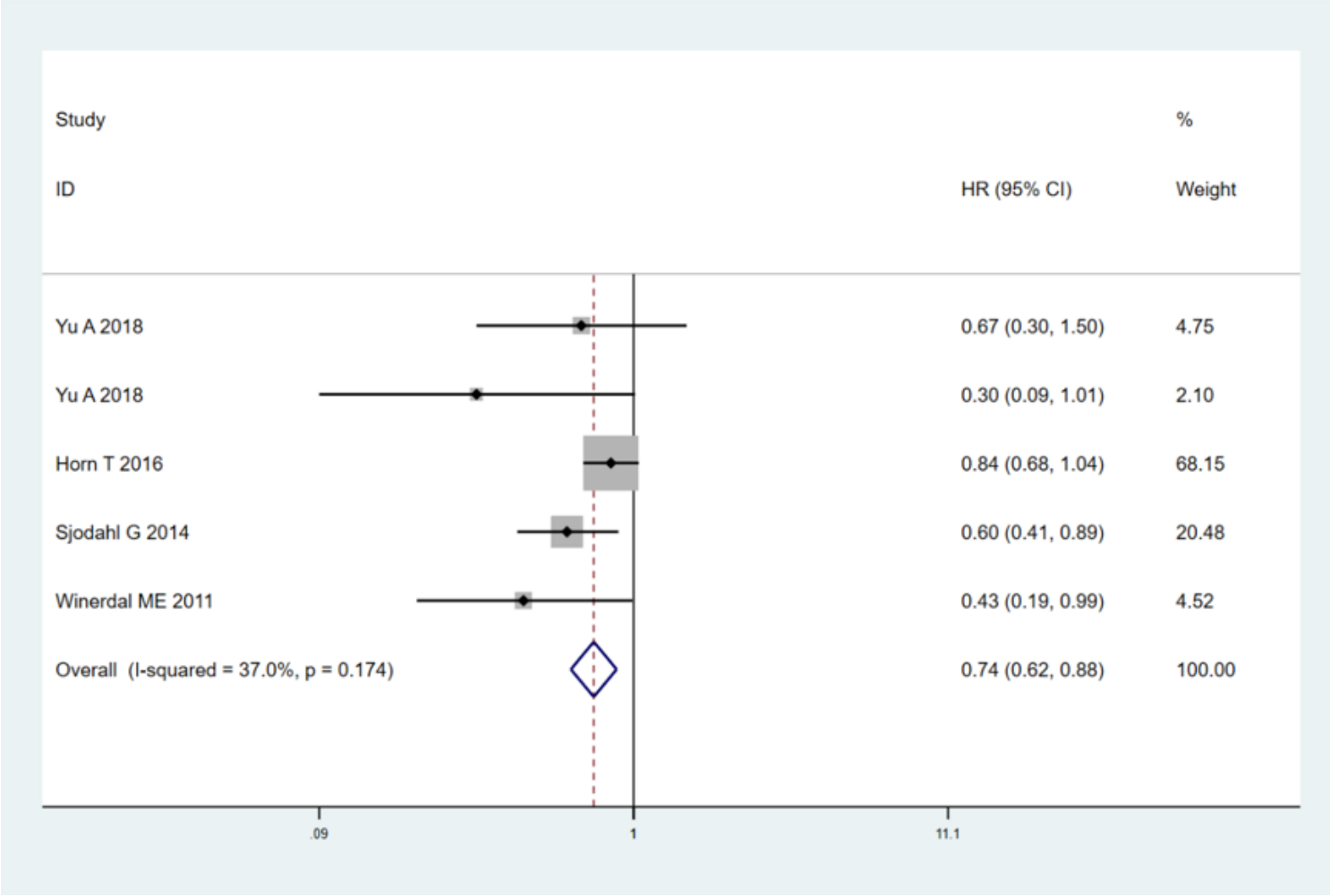

Figure 2

Forest plots of prognostic value of CD3+ TILs on overall survival in patients with UCB.

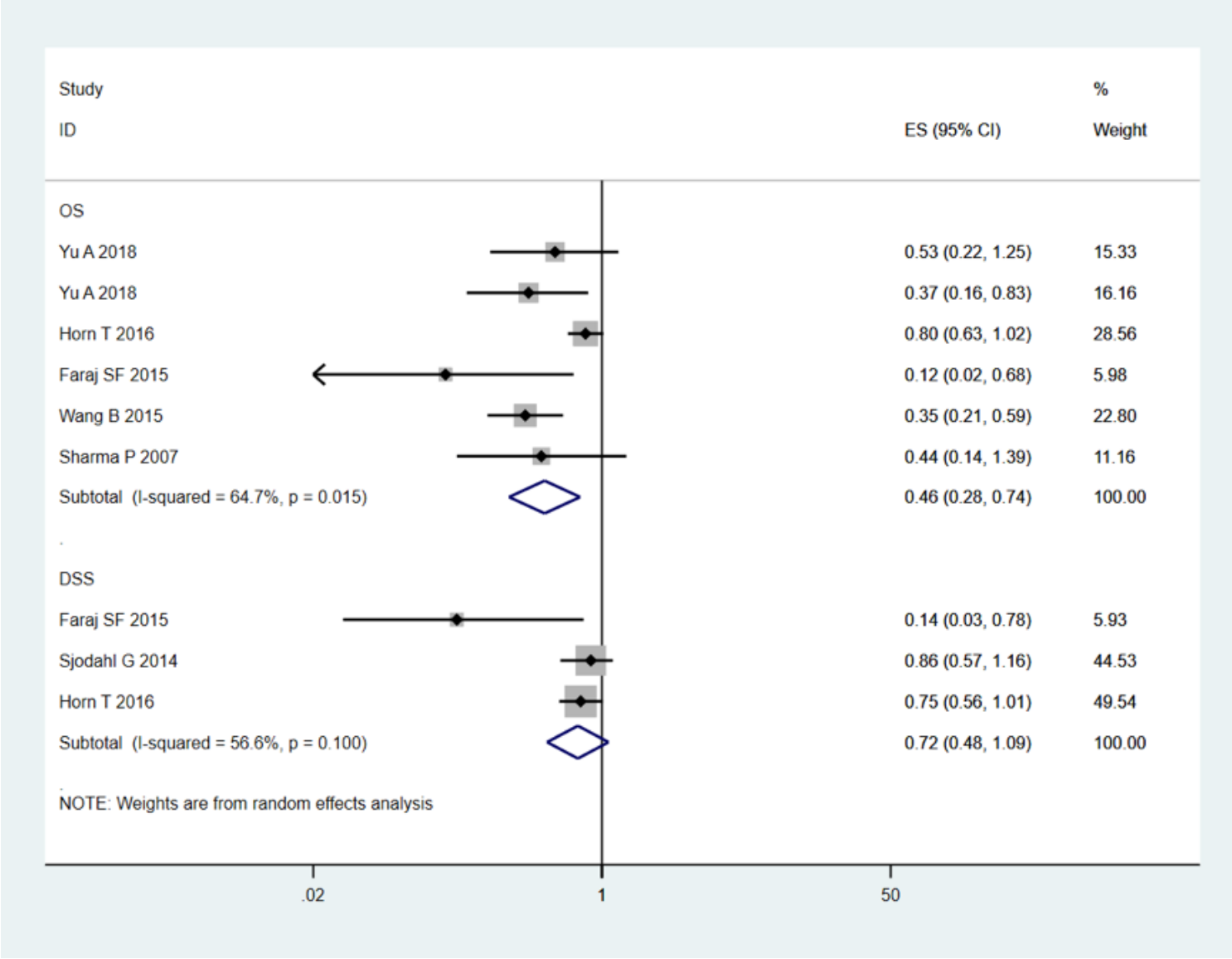

Figure 3

Forest plots of prognostic value of CD8+ TILs on overall survival and disease-specific survival in patients with UCB.

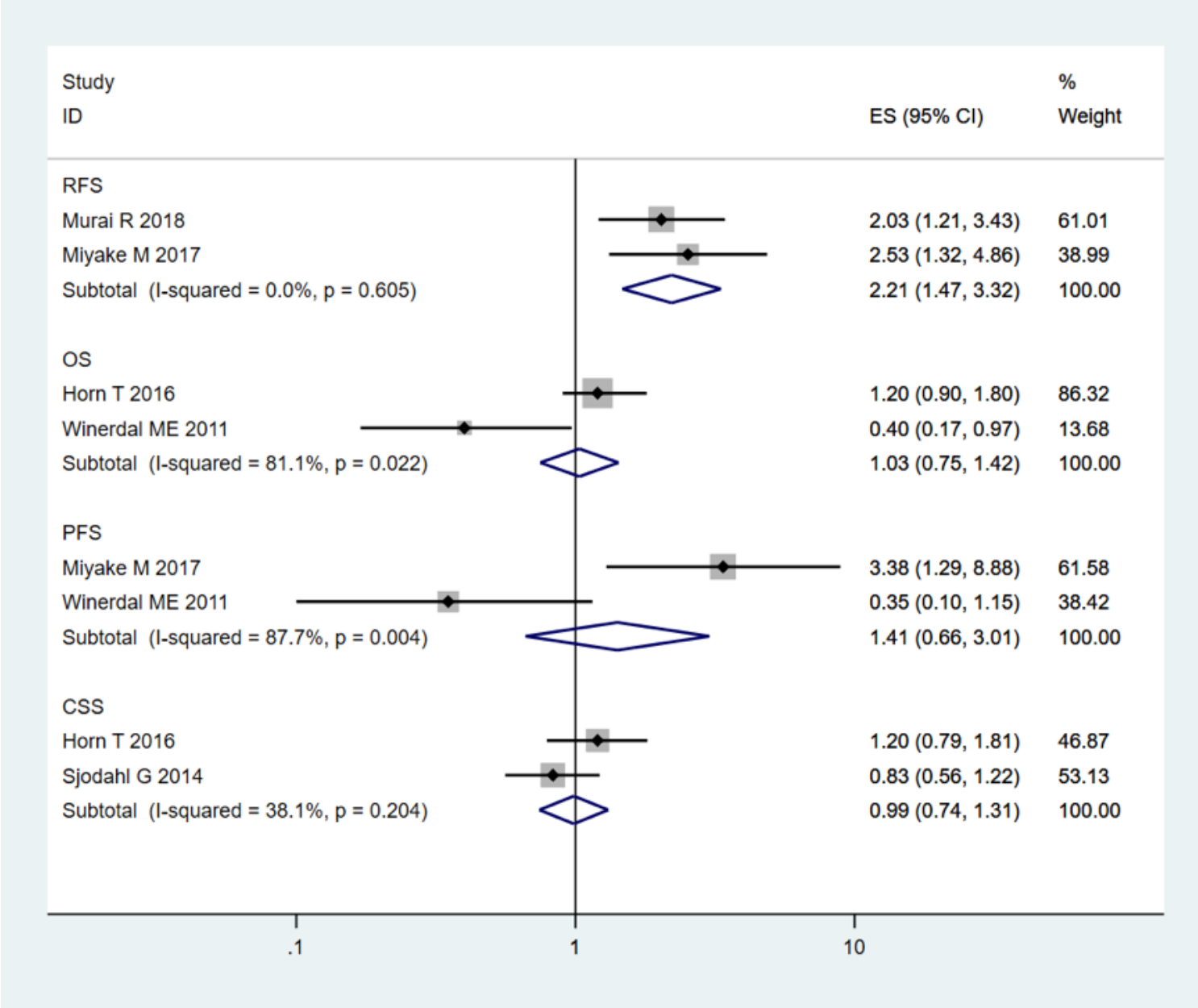

Figure 4

Forest plots of prognostic value of FoxP3+ TILs on overall survival, recurrence-free survival, progression-free survival and cancer-specific survival in patients with UCB.

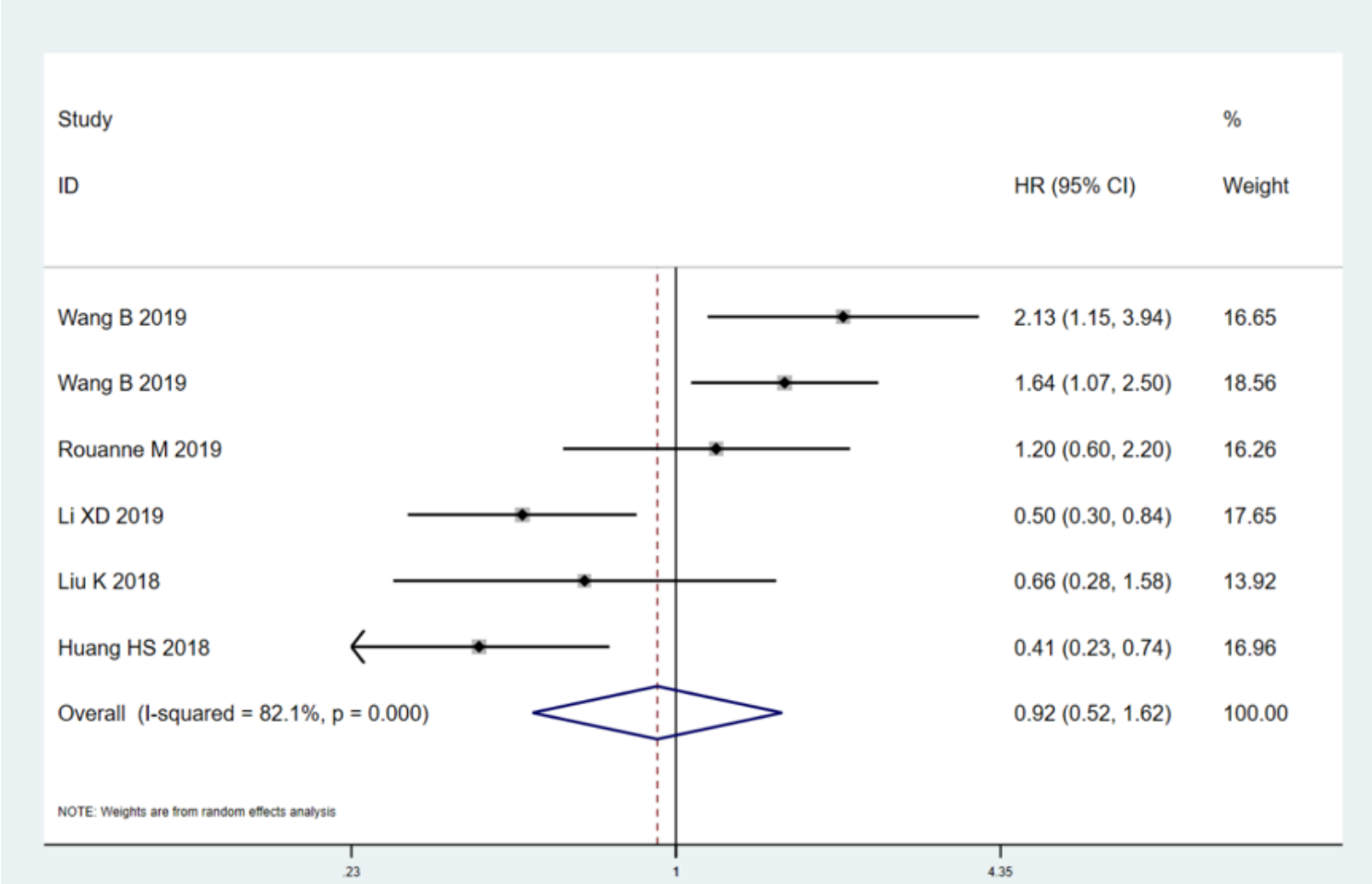

Figure 5

Forest plots of prognostic value of TILs on overall survival in patients with UCB.