Prognostic value of tumor-infiltrating lymphocytes in gliomas: A Systematic Review

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Research article

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Abstract

Background: Glioma is the most common primary brain tumor with poor prognosis. Some studies have learned the prognostic role of tumor infiltrating lymphocytes (TILs) in gliomas. But conflict conclusions were drawn by these studies. In order to reach an agreement, we systematically performed a meta-analysis.

Method: A systematic literature research was conducted on the Web of Science, EMBASE, PubMed, Cochrane Library and China National Knowledge Infrastructure. The eligible articles which met the inclusion criteria were included in our study. The clinical outcomes of included patients were defined as progression-free survival (PFS) and overall survival (OS). The basic characteristics and relevant data were extracted. Hazard ratios (HRs) with 95% confidence intervals (CIs) were pooled to evaluate the prognostic role of TILs in gliomas. Newcastle Ottawa Scale (NOS) was used to assess the quality of included studies.

Results: Eight articles published from 2008 to 2019 were finally included in our study. And 25 studies were conducted in these articles. We assessed the prognostic role of TILs in gliomas by subgroup analysis according to the subtypes of TILs. The pooled HRs for OS revealed that high density of CD3+ and CD4+ TILs were related to the poor prognosis of gliomas (HR for CD3+ TILs=1.266; HR for CD4+ TILs= 2.128). The pooled HR for PFS indicated that only high density of FOXP3+ TILs were related to poor prognosis (HR=2.785; 95%CI=1.848, 4.197).

Conclusion: High density of CD3+ and CD4+ TILs may be a potential candidate for predicting poor OS of gliomas while high density of FOXP3+ TILs may serve as a good biomarker for predicting poor PFS of gliomas.

Background

Glioma is the most common primary brain tumor with poor prognosis[1]. Glioblastoma, which accounts for 16% brain tumors in adults, has a median overall survival of less than 12 months[2]. Medulloblastoma, which is another type of glioma, commonly occurs in children. Over 80% medulloblastoma patients have achieved a five-year survival with the improvement of treatment[3]. But the quality of survivors is still a major issue because of the substantial neurologic and cognitive sequelae. In addition, ependymoma and neuroblastoma, commonly occurring in children, also have a poor prognosis[3,4]. So it is necessary to identify valid biomarkers for predicting the prognosis of these patients.

Tumor infiltration lymphocytes (TILs), part of complex microenvironments, playan important role in tumor development and growth[5]. Until now, they have been identified as good biomarkers in ovarian cancer, breast cancer and non-small cell lung cancer[6–8]. Recently, TILs have also gained attentions in the prognosis of gliomas. However, some studies indicated that TILs were good biomarker for gliomas[9–14] while some studies revealed that no significant correlation was observed between TILs and gliomas[9,10,13,15]. In order to reach an agreement, we systematically reviewed the studies focused on glioma and TILs. And in this study, we assessed the prognostic role of TILs in gliomas by subgroup analysis according to the subtypes of TILs.

Method

Search strategy

A systematic literature search was conducted in Web of Science, EMBASE, PubMed, MEDLINE, Cochrane Library and China National Knowledge Infrastructure before May 2020. The key words including tumor-infiltrating lymphocytes, prognosis, glioma and all possible combinations were used to search the study.

Inclusion criteria

The studies were included if they met the following criteria: (1) published as original articles with high quality; (2) tumor tissues were isolated from humans; (3) the prognostic role of TILs in gliomas were studied; (4) containing the essential information to estimate the effects of TILs.

Data extraction

The data was independently extracted by Y.L.S. and J.Z.. The general information, including country, sample number, median age, sex (M/F), stage, diagnosis, TIL subtypes, detective methods, cut-off value, follow-up time, outcomes, HR, 95%CIs, and p-values were extracted from these studies. In addition, the included studies were quantified with Newcastle Ottawa Scale (NOS) which was used for the quality assessment in meta-analysis[16]. When HR and 95%CIs were not mentioned in these articles but Kaplan-Meier curves were shown in these articles, the data was extracted and digitized according to Tierney's report[17]. If the results of multivariate analyses and univariate analyses were both shown in the article, the multivariate analyses results were chosen to calculate the pooled HR.

Quality assessment of included studies

The Newcastle Ottawa Scale (NOS) was used to assess the quality of included studies. In our study, we assessed the quality of each study included in these article respectively. And the studies with 7 points or more than 7 points were defined as high quality studies[18].

Statistical analyses

The pooled HRs were calculated by STATA 11.0 (*StataCorp, College Station, TX, USA*). And the pooled HR was considered to be significant if the p value was less than 0.05 and 95% CI did not overlap 1[19]. The heterogeneity between studies was evaluated by chi-squared test and I^2 statistic, and substantial

heterogeneity was defined as p≤0.05 or I^2≥50%[20]. The random-effects model was used to calculate the pooled HR whether these included studies had a fine homogeneity because of the differences in treatment strategies and patients characteristics[21]. If the heterogeneity was significant, sensitivity analysis was performed to assess the contribution of each study in heterogeneity.

Results

Study selection

Our study was designed, conducted and reported based on the PRISMA statement. The article was developed by the order of guidelines of system reviews. The process of study selection was shown in **Figure 1**. Briefly, a total of 323 articles were included for initial evaluation after duplicate remove. And 20 articles were selected by screening the title and abstract. Non-related articles, cases, and reviews were excluded. Eight articles were finally identified as eligible studies for further assessment[9-15,22].

Basic characteristics of selected studies

Eight articles published from 2008 to 2019 were included. And 25 studies were conducted in these articles. The basic characteristics of each study were shown in **Table 1**. Among these studies, 20 studies were performed in East Asian (8 studies in China, 4 studies in Japan, 3 studies in India, and 5 studies in Korea), 4 studies were performed in USA, and 1 study was performed in France. A total of 928 samples were included in these studies. All the studies were considered to have high quality according to the NOS assessment. The included studies explored the prognosis role of CD4, CD3, FOXP3, and CD8 positive TILs in gliomas. The tumor samples were all detected by immunohistochemistry staining. And OS and PFS were both assessed in these studies. The follow-up time, HR and p values were also shown in our study.

Pooled analysis for CD4+ TILs in gliomas

As shown in **Figure 2**, 3 studies were pooled for the analysis of the prognostic role of CD4+ TILs in gliomas. It revealed that high density of CD4+ TILs was related to short OS (HR=2.128; 95% CI=1.373-3.297; P=0.001). And no significant heterogeneity was observed in these studies (I^2=48.6%; P=0.143)

Pooled analysis for CD3+ TILs in gliomas

Among these studies, 6 studies focused on the relationship between CD3+ TILs and the prognosis of gliomas. The pooled analysis indicated that high density of CD3+ TILs were associated with shorter OS (HR=1.266; 95% CI=1.055-1.518; P=0.011). And no significant heterogeneity was observed in these studies (I^2=0%; P=0.427). However, no significant correlation was observed between CD3+ TILs and PFS of gliomas (HR=0.521; 95% CI=0.161-1.690; P=0.277). The sensitivity analysis of the studies revealed that the study of “Murata 2018” was the major source of heterogeneity. After excluding this study, I^2 changed to 0%. A significant correlation between high density of CD3+ TILs and PFS was observed (HR=0.290; 95% CI=0.109-0.775; P=0.014).

Pooled analysis for FOXP3+ TILs in gliomas

Four studies explored the relationship between FOXP3+ TILs and OS of gliomas, and another four studies explored the relationship between FOXP3+ TILs and PFS of gliomas. The pooled HRs were analyzed respectively and shown in **Figure 2 and Figure 3**. Different with CD3+ TILs, high density FOXP3+ TILs were associated with short PFS (HR=2.785; 95% CI=1.848-4.197; P≤0.001) but was not significantly related with OS (HR=1.537; 95% CI=0.961-2.457; P=0.073). No significant heterogeneity was observed in the studies which analyzed the relationship between FOXP3+ TILs and PFS of gliomas (I^2=0%; P=0.861). A significant heterogeneity was observed in the studies which explored the relationship between CD3+ TILs and OS (I^2=78.6%; P=0.003). Different conclusions were draw by sensitivity analysis. And a significant heterogeneity still existed no matter which study was excluded.

Pooled analysis for CD8+ TILs in gliomas

Eight studies assessed the prognostic value of CD8+ TILs in gliomas. Among these studies, 5 studies analyzed the relationship between CD8+ TILs and OS and 3 studies analyzed the relationship between CD8+ TILs and PFS. The pooled HRs revealed that no significant relationship was observed between CD8+ TILs and prognosis of gliomas (HR for OS= 1.813; 95% CI=0.780-4.214; P=0.786 HR for PFS=1.117; 95% CI=0.501-2.494; P=0.786). And a significant heterogeneity were observed in these studies (I^2= 86.0%; P≤0.001). So we performed sensitivity analysis to figure out the origin of heterogeneity. The significant heterogeneity still existed after excluding each study, and different conclusions were drawn by the analysis.

Discussion

Glioma is the most common primary brain tumor with poor prognosis[1]. Finding a good biomarker for predicting the prognosis of glioma has always been a hotspot. Recently, more and more attention has been focused on the TILs which were part of complex microenvironments[5,23]. But conflict conclusions were draw by these studies. In order to reach an agreement, we performed this meta-analysis.

Considering the heterogeneity of TILs, we pooled HRs according to the subtypes of TILs. As shown in Fig. 2 and Fig. 3, high density of CD3 + and CD4 + TILs were significantly related to poor OS while high density of FOXP3 TILs was related to poor PFS. But CD8 + TILs were not a good predictor for glioma. CD3 which was a marker of most T cells were proved to improve the prognosis in many kinds of tumors[24–26]. But in our study, high density of CD3 + TILs was identified to be related to poor OS. On the contrary, high density of CD3 + TILs was related to better PFS after excluding the study which contributed to the high inner heterogeneity. The similar conclusion was also drawn by Ding et al. in hepatocellular carcinoma[27]. And the mechanisms were not exactly explained in previous study, which needs to be answered in the future.

CD4 + TILs were considered to be a double-edge sword for tumor development. On the one hand, they helped the activation of cytotoxic T lymphocytes. On the other hand, they dampened the anti-tumor immunity and promoted tumor development[28]. In gliomas, CD4 + FOXP3 + lymphocytes were identified to act as a dominant immune escape mechanism[29]. CD4 + FOXP3 + lymphocytes obviously suppressed the activation of naïve T cells. After depleting CD4 + FOXP3 + lymphocytes, the CD8 + anti-tumor cells were detected. In addition, CD4 + FOXP3 + lymphocytes in gliomas were reported to be correlated with angiogenesis which promoted the tumor progression[30]. In agreement with these studies, our study elucidated that high density of CD4 + TILs predicted the poor OS of gliomas. Generally, FOXP3 + TILs were considered to predict poor prognosis because of the immune escape role[29]. Recently, FOXP3 was also verified to be expressed in glioma cells by quantitative RT-PCR, immunohistochemistry and FACS analysis[31]. And these might explain the prognostic role of FOXP3 + TILs in gliomas.

Intriguingly, CD8 + TILs were frequently considered to be the pivotal effector of anti-tumor immune. And they were verified to improve the OS of several tumors, like breast cancer, colorectal cancer, gastric cancer, and melanoma[20,24–26]. But in this study, no correlation was observed between CD8 + TILs and gliomas. This might be related to blood-brain barriers and the specificity of the tumor environment in brain[32,33]. In addition, the inner heterogeneity of the studies which assessed the role of CD8 + TILs in gliomas was significant. So more studies that focused on the role of CD8 + TILs in gliomas are needed in the future.

Although our study figures out three potential markers for predicting the prognosis of gliomas and attracts more attentions on the targets in glioma patients, a significant heterogeneity was observed in our study, which might be due to the heterogeneity of gliomas. Previous studies proved that the prognosis of the different gliomas was very different. Limited numbers of studies were included in our study, as well as the subgroup analysis of different subtypes of gliomas. So more studies should be focused on the evaluation of different TILs in different gliomas, which will made the conclusion more convincing.

Conclusions

In conclusion, our study provided evidence for the prognostic role of TILs in gliomas and identified CD4 and CD3 for OS of gliomas and FOXP3 for PFS of gliomas. However, due to the heterogeneity among studies, more well-designed studies are warranted in the future.

Abbreviations

TILs, tumor infiltrating lymphocytes; FACS, fluorescence activated cell sorting; HR, hazard ratio; CI, confidence interval; OS, overall survival; PFS, progression free survival; Newcastle Ottawa Scale (NOS)

Declarations

-Ethics approval and consent to participate

Not applicable.

-Consent for publication

Not applicable.

-Availability of data and materials

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

-Competing interests

The authors declare that they have no competing interests.

-Funding

None.

-Authors' contributions

YS and JZ worked together on the conception, data analysis and interpretation, and drafting of the manuscript. They contributed equally to this work. LY contributed to the study design, and manuscript editing. XM supervised development of work, helped to evaluate the manuscript and acted as corresponding author. All authors read and approved the final manuscript.

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Yanlin Song and Jing Zhang contributed equally to this work.

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Tables

Table 1 Characteristics of the included articles

Study(author, year)	Country	Sample No	Age (median)	Sex (M/F)	Stage	Diagnosis	TILs (Subtypes)	Detective methods	Cut-off value	Follow-up (months)	Outcome	HR
Wu 2019	TCGA	152	-	-	□	Glioblastoma	CD4	IHC	median	90	OS	1.5
Wu 2019	China	30	46	15/15	□-□	Gliomas	CD4	IHC	median	60	OS	2.6
Orrego 2018	USA	43	47	22/21	□	Glioblastoma	CD4	IHC	median	36	OS	2.9
Murata 2018	Japan	16	13.5	11/5	-	Medulloblastoma	CD3	IHC	median	264	OS	2.3
Jha 2019	India	126	-	-	-	Diffuse midline gliomas	CD3	IHC	-	137	OS	2
Jha 2019	India	126	-	-	-	Diffuse midline gliomas	CD3	IHC	-	137	OS	1.2
Nam 2019	Korea	178	-	-	□/□	Ependymoma	CD3	IHC	median	241	PFS	0.3
Nam 2019	Korea	178	-	-	□/□	Ependymoma	CD3	IHC	median	241	PFS	(0.0
Murata 2018	Japan	16	13.5	11/5	-	Medulloblastoma	CD3	IHC	median	264	PFS	1.8
Yue 2014	China	62	-	43/19	□	Glioblastoma	FOXP3	IHC	-	48	OS	2.8
Yue 2014	China	62	-	43/19	□	Glioblastoma	FOXP3	IHC	-	48	OS	2.3
Heimberger 2008	USA	135	44	-	□-□	Gliomas	FOXP3	IHC	-	-	OS	1.2
Heimberger 2008	USA	135	44	-	□-□	Gliomas	FOXP3	IHC	-	-	OS	1.0
Nam 2019	Korea	178	-	-	□/□	Ependymoma	FOXP3	IHC	median	241	PFS	2.3
Nam 2019	Korea	178	-	-	□/□	Ependymoma	FOXP3	IHC	median	241	PFS	3.9
Yue 2014	China	62	-	43/19	□	Glioblastoma	FOXP3	IHC	-	48	PFS	(6.
Yue 2014	China	62	-	43/19	□	Glioblastoma	FOXP3	IHC	-	48	PFS	2.4
Madkouri 2017	France	186	64	105/81	□	Glioblastoma	CD8	IHC	median	65.8	OS	0.5
Jha 2019	India	126	-	-	-	Diffuse midline gliomas	CD8	IHC	-	137	OS	5.8
Orrego 2018	USA	43	47	22/21	□	Glioblastoma	CD8	IHC	median	36	OS	2.9
Murata 2018	Japan	16	13.5	11/5	-	Medulloblastoma	CD8	IHC	median	264	OS	2.3
Yue 2014	China	62	-	43/19	□	Glioblastoma	CD8	IHC	-	48	OS	1.1
Murata 2018	Japan	16	13.5	11/5	-	Medulloblastoma	CD8	IHC	median	264	PFS	2.8
Yue 2014	China	62	-	43/19	□	Glioblastoma	CD8	IHC	-	48	PFS	1.1
Nam 2019	Korea	178	-	-	□/□	Ependymoma	CD8	IHC	median	241	PFS	0.4

Figures

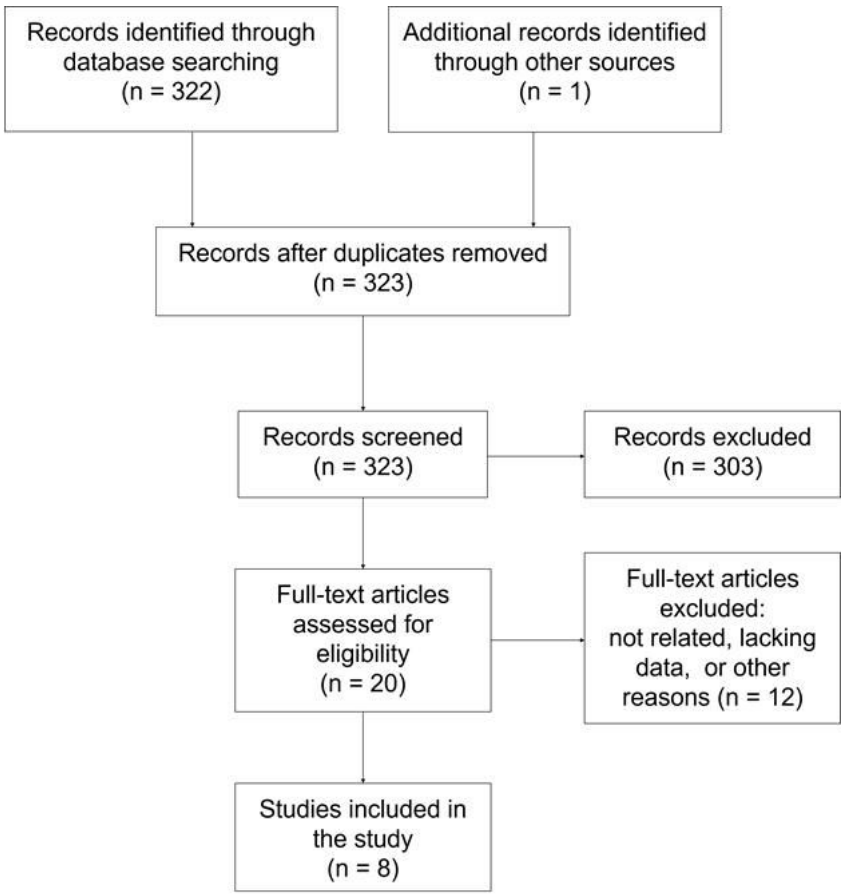

Figure 1

Selection process of studies

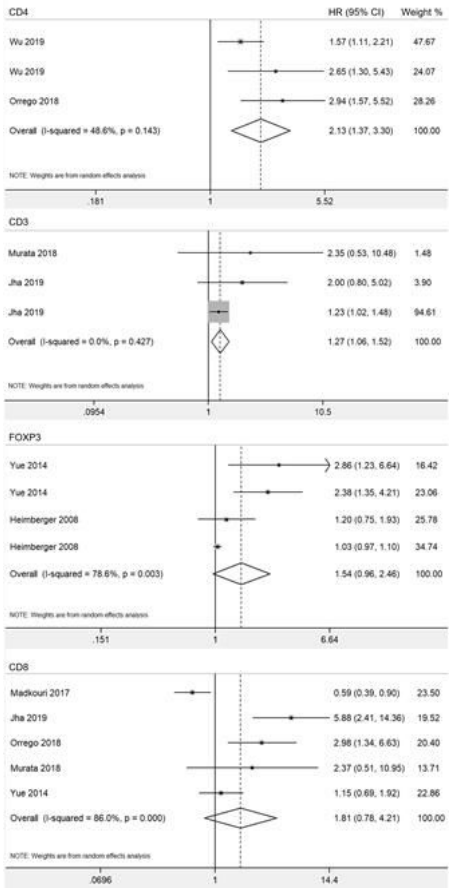

Figure 2

Pooled hazard ratios of higher density of TILs for overall survival in patients with gliomas.

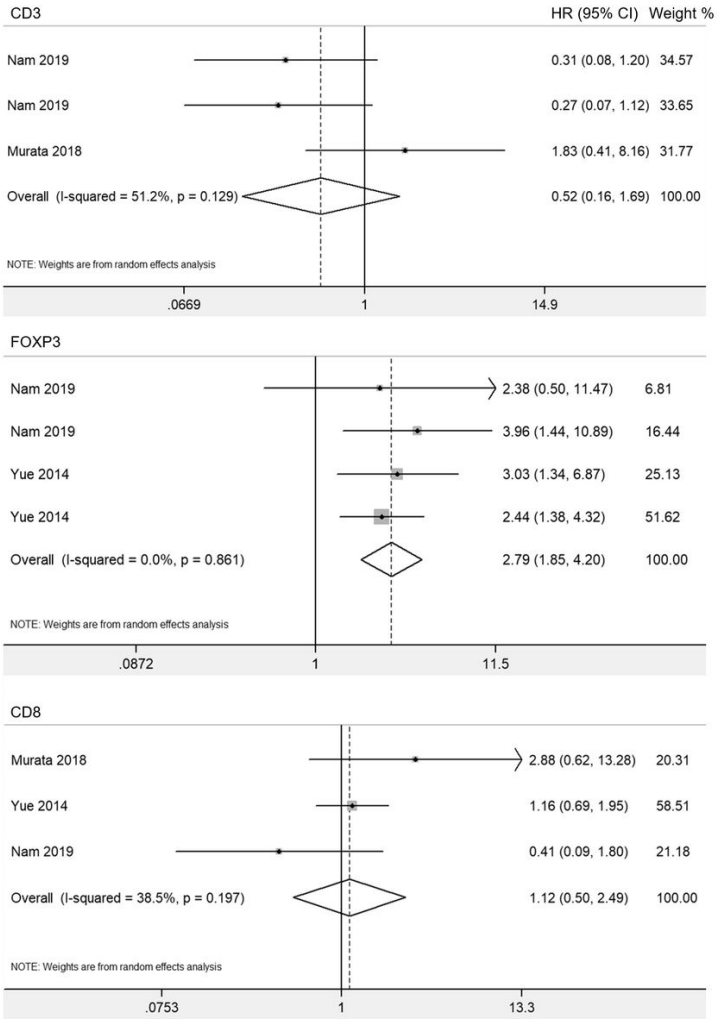

Figure 3

Pooled hazard ratios of higher density of TILs for progression free survival in patients with gliomas.

Supplementary Files

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- PRISMA2009ChecklistMSWord.doc