

RESEARCH ARTICLE

Ki-67 is a Valuable Prognostic Factor in Gliomas: Evidence from a Systematic Review and Meta-analysisWen-Jie Chen¹, De-Shen He², Rui-Xue Tang¹, Fang-Hui Ren¹, Gang Chen^{1*}**Abstract**

Ki-67 has been widely used as an indicator of cell proliferation in gliomas. However, the role of Ki-67 as a prognostic marker is still undefined. Thus, we conducted a meta-analysis of the published literatures in order to clarify the impact of Ki-67 on survival in glioma cases. Eligible studies were identified in PubMed, EMBASE, ISI Web of Science, Cochrane Central Register of Controlled Trials, Science Direct and Wiley Online Library with the last search updated on August 31, 2014. The clinical characteristics, overall survival (OS) and progression-free survival (PFS) together with Ki-67 expression at different time points were extracted. A total of 51 studies, covering 4,307 patients, were included in the current meta-analysis. The results showed that overexpression of Ki-67 can predict poor OS (HR=1.66, 95% CI: 1.53-1.80; Z=11.87; $p=0.000$) and poor PFS (HR=1.67, 95% CI: 1.47-1.91; Z=7.67; $p=0.000$) in gliomas. Moreover, subgroup analyses also indicated that high level of Ki-67 expression was related to poor OS and PFS in glioma patients regardless of region, pathology type, cut-off value and statistical method. In conclusion, the current meta-analysis revealed that Ki-67 expression might be a predicative factor for poor prognosis of glioma patients, emphasizing its importance as a predictor.

Keywords: Ki-67 - glioma - meta-analysis - immunohistochemistry - prognosis - survival

Asian Pac J Cancer Prev, 16 (2), 411-420

Introduction

Glioma is the most common intracranial neoplasia in adults, which arises from the brain or spinal cord tissues (Hernandez-Pedro et al., 2013). Glioma accounts for approximately 80% of all primary malignant brain tumors (Kohler et al., 2011). Malignant glioma is the most frequent type of primary brain tumors, with an annual incidence of 5/100,000 individuals (Wen and Kesari, 2008). The highly invasive nature of this tumor prevents complete tumor resection and causes significant neurologic morbidity and mortality (Kouri et al., 2012; Zhao et al., 2013). Despite advances in diagnostic and therapeutic techniques, the prognosis for most glioma patients remains dismal (Nazarenko et al., 2012; Westermarck, 2012).

Ki-67, a non-histone protein, is a DNA-binding nuclear protein expressed throughout the cell cycle in proliferating cells, but not in quiescent (G0) cells (Tadbir et al., 2012; Fakhrjou et al., 2013). Ki-67 has been used to distinguish growing cells from non-growing cells (Haroon et al., 2013; Dang et al., 2014; Huang and Chen, 2014). Furthermore, Ki-67 is a reliable indicator of tumor cell proliferative activity that has been associated with the histological grade for glioma (Hu et al., 2013). In spite of a large number of studies performed in glioma patients, the prognostic value of Ki-67 for survival remains controversial. Therefore,

we performed a systematic review of the literatures with meta-analysis to assess the prognostic value of Ki-67 for prognosis.

Materials and Methods*Literature search*

A literature search via PubMed, EMBASE, ISI Web of Science, Cochrane Central Register of Controlled Trials, Science Direct and Wiley Online Library was conducted to search articles that evaluated Ki-67 in glioma (Last search was updated on August 31, 2014). The keywords and text words were used as follows: (1) ("glioma" OR "astrocytoma" OR "glioblastoma" OR "oligodendroglioma" OR "oligoastrocytoma" OR "Ependymomas" OR "Gliomatosis Cerebri" OR "brain cancer" OR "brain neoplasm" OR "brain tumor" OR "GBM" OR "AA" OR "AO" OR "DIPG"); (2) ("Ki-67" OR "Ki67" OR "MIB-1" OR "proliferative index" OR "proliferative activity" OR "mitotic index" OR "labeling index" OR "mitotic count" OR "proliferative marker"); (3) ("prognos*" OR "surviv*" OR "follow-up" OR "mortality" OR "predict" OR "outcome").

Selection Criteria

All English and Chinese studies were included and

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all eligible articles that examined the association between the expression of Ki-67 and overall survival (OS) or progression free survival (PFS) were gathered. However, the papers which only have abstracts were excluded because of insufficient data for meta-analysis. Hence, we first checked the titles of the publications and the abstracts to find exactly those articles that examined the relationship between Ki-67 and OS or PFS in glioma patients. After the abstracts met these conditions, the full texts were analyzed and included into our meta-analysis according to the following criteria: (i) studies were written as full paper; (ii) expression levels of Ki-67 were compared to OS or PFS; (iii) expression of the protein was evaluated in tumor tissues by immunohistochemistry (IHC), western blot or mRNA by reverse transcription and polymerase chain reaction (RT-PCR) analysis; (iv) Hazard ratios (HR) and 95%CI for OS or PFS were provided or could be calculated from the sufficient data.

Data extraction

Two reviewers independently reviewed all articles and extracted data in separate databases. The following information was collected from each study: name of first author, year of publication, pathology type, WHO grade, number of patients involved, Ki-67 assay, cut-off value, HR with 95%CI. Disagreements were resolved through discussion among the authors.

Statistical analysis

A study was considered as significant if the P-value was 0.05 for the statistical test comparing the survival distributions between the groups of low and high Ki-67 expression. A study was classified as 'positive' when Ki-67 expression was identified as a good prognosis factor for survival in univariate analysis. While a study was regarded as 'negative' if the Ki-67 overexpression was associated with a significant detrimental effect on survival. Finally, a study was considered as 'not significant' if no difference between groups expressing or not Ki-67 was detected. The intensity of relationship between the expression levels of Ki-67 and OS or PFS was described as HRs. Positive expression of Ki-67 indicated poor prognosis in patients with glioma if $HR > 1$ with the 95%CI not overlapping 1. From some published researches, HR and 95%CI could be directly obtained by using univariate or multivariate survival analysis. Otherwise, HR and 95%CI were calculated by Kaplan-Meier survival curves using the software Engauge Digitizer Version 4.1 (<http://digitizer.sourceforge.net/>) and the method (Parmar et al., 1998). HR and 95%CI were also calculated from the sufficient data by SPSS 20.0. The pooled HR corresponding to the 95%CI was used to assess the prognostic value of Ki-67 in patients. Statistical heterogeneity was tested by Cochrane's Q test (Chi-squared test; Chi^2) and inconsistency (I^2) (Lau et al., 1997; Higgins and Thompson, 2002). If there was no obvious heterogeneity, the fixed-effects model (Mantel-Haenszel method) was used to estimate the pooled HR. Otherwise the random-effects model (DerSimonian and Laird method) was applied. Funnel plot and Begg's rank correlation method were designed for assessing risk of publication bias. STATA 11.0 (STATA Corp., College,

TX) was used to perform statistical analysis.

Results

Studies selection and characteristics

The abstracts and titles of primary 924 studies were identified by using the search strategies. Following deduplication, two reviewers independently screened the identified titles and abstracts. They subsequently agreed that 141 articles should be retrieved for detailed review; for these manuscripts, full texts were obtained. On cautious review of study methodologies, 71 of the 141 studies were excluded for showing no information related to Ki-67/MIB1 and survival. Of the candidate studies, 13 articles failed to completely describe the available survival data. Finally, 51 published studies in total were included in the analysis after eliminating articles unsatisfying the selection criteria and duplicates (Jaros et al., 1992; Torp et al., 1992; Montine et al., 1994; Ellison et al., 1995; Heegaard et al., 1995; Kros et al., 1996; Coons et al., 1997; Pollack et al., 1997; Dehghani et al., 1998; McKeever et al., 1998; Ritter et al., 1998; Figarella-Branger et al., 2000; Rodriguez-Pereira et al., 2000; Ho et al., 2001; Reavey-Cantwell et al., 2001; Zhong et al., 2001; Bredel et al., 2002; Pollack et al., 2002; Bowers, 2003; Chiang et al., 2003; Neder et al., 2003; Wessels et al., 2003; Zamecnik et al., 2003; Preusser et al., 2005; Uematsu et al., 2005; Kleinschmidt-DeMasters et al., 2006; Donato et al., 2007; Kanamori et al., 2008; Kuo et al., 2009; Laks et al., 2009; Li et al., 2009; Armstrong et al., 2010; Nabika et al., 2010; Watanabe et al., 2010; Yoshida et al., 2010; Habberstad et al., 2011; Margraf et al., 2011; Qiang et al., 2011; Shen et al., 2011; Zawrocki et al., 2011; Okita et al., 2012; Park et al., 2012; Phi et al., 2012; Preusser et al., 2012; Tove et al., 2012; Abd El Atti et al., 2013; Huang et al., 2013; Liu et al., 2013; Yang et al., 2013; Tian et al., 2014; Yue et al., 2014). A flow diagram of the study selection process was presented in Figure 1.

The main information of the 51 articles was summarized in Table 1. Considering the selected studies, 17 were carried out in Europe, 15 in America, 18 in Asia and 1 in Africa. Immunohistochemistry (IHC) was the only technique performed to detect the expression of Ki-67 protein. Out of 51 studies published between the years 1992 and 2014, all had the sufficient information for HR extraction, including 46 studies evaluable for OS and 16 for PFS. In the studies with OS, 16 studies provided the multivariate HRs, 9 articles also reported the univariate HRs, 15 showed survival curves available to calculate the HRs, while 7 studies provided the sufficient data to be calculated by SPSS 20.0 and 3 studies showed HRs without mentioning the methods used. In the PFS analysis group, 8 studies reported HRs with 95%CI in the multivariate models, 8 studies showed the HRs according to univariate analysis, 5 studies showed survival curves that were available to calculate the HRs and 1 study provided the data that could be calculated by SPSS 20.0. Subsequently, the perspective of individual glioma pathology type was taken into account. In the OS analysis group, astrocytoma was studied most with twenty-nine studies being included, while 4 studies included oligodendrogial and

Table 1. Characteristics of Studies Included in the Meta-analysis

Referenc	year	Re-gion	Cancer type(N)	Grade	N (M/F)	cutoff	OS	PFS	HR
Torp	1992	Nor-way	GBM20	IV	20 (11/9)	Ki-67> 1.5%	p=0.12,HR=2.27 (0.82,6.38)		Estimated
Jaros	1992	UK	GBM8, AA32	III-IV	40 (27/13)	Ki-67> 5%	p<0.00,HR=7.36 (2.84,19.09)		Estimated
Montine	1994	US	A11, AA12, GBM13	II-IV	36 (17/19)	Ki-67≥ 7.5%:	p<0.00, HR=1.84 (0.48,7.05)		Survival curve
Ellison	1995	UK	FA24, AA31, GBM68	III-IV	123 (77/46)	Ki-67> 2%,	p=0.04, HR=1.04 (1.01,1.06)		ND
						Ki-67 2-5%	p<0.00, HR=3.13 (1.81,5.44)		HR (Un-adjusted)
						Ki-67 2-5%	p=0.40, HR=1.43 (0.76,2.66)		HR (Ad-justed)
						Ki-67 >5%	p<0.00, HR=3.21 (1.95,5.28)		HR (Un-adjusted)
						Ki-67 >5%	p=0.40,HR=1.04 (0.55,1.95)		HR (Ad-justed)
Hee-gaard	1995	Den-mark	ODG28	II	28	Ki-67> 3%	p<0.00, HR=2.97 (1.04,8.50)		Survival curve
Kros	1996	Neth-er-lands	ODG 108	II	108	MIB-1>10%	p=0.001, HR=1.59 (1.06,2.40)		Survival curve
						MIB-1≥20%	p=0.01, HR=1.73 (1.13,2.65)		Survival curve
Pollack	1997	US	GBM16, AA10	III-IV	26 (12/14)	MIB-1>12%	p=0.04,HR=3.88 (1.09,13.8)	p=0.04, HR=3.19 (1.03,9.87)	Estimated
Coons	1997	US	ODG55, OA26	II	81 (51/30)	Ki-67> 5%	p=0.04,HR=5.86 (2.65,12.95)		Survival curve
Ritter	1998	US	E34	II	34 (19/15)	MIB-1>20%	p=0.00, HR=5.7 (2.00,16.4)		ND
McKeever	1998	US	A50	II	50 (35/15)	MIB-1>2%	p=0.00,HR=1.301 (1.16,1.46)		HR (Mul-tivariate)
Deh-ghani	1998	Ger-many	ODG82	II	82	Ki-67> 2%	p<0.00, HR=7.60 (2.97,19.4)		Survival curve
						Ki-67> 5%	p=0.03, HR=1.88 (0.94,3.74)		Survival curve
Fiarella-Branger	2000	US	E37	II	37 (21/16)	Ki-67 >1%	p=0.01, HR=3.18 (1.40,7.22)	p=0.00, HR=3.18 (1.40,7.22)	HR (Mul-tivariate)
Rodri-guez-Pereira	2000	Spain	A42, AA25, GBM25, ODG62, AODG, OA3	II-IV	137 (85/52)	MIB-1 in 1%	p<0.00, HR=1.03 (1.02,1.07)		HR (Mul-tivariate)
Reavey-Cantwell	2001	US	GBM32	IV	32 (22/10)	Ki-67> 20%	p=0.16, HR=1.8 (0.8,4.2)		HR(Uni-variate)
							p=0.09, HR=2.15 (0.89,5.17)		HR (Mul-tivariate)
Zhong	2001	China	A94	ND	94	Ki-67>8.5%	p=0.00,HR=6.88 (2.75,10.2)		Survival curve
Ho	2001	Tai-wan	E48, AE24, ME9	II-III	81	MIB-1≥6%	p=0.01, HR=16.5 (2.1,127.2)	p<0.00, HR=23.5 (5.4,101.8)	HR (Uni-variate)
Bredel	2002	Aus-tria	GBM 111	IV	111	MIB-1≥27%	p=0.04, HR=1.49 (1.03,2.14)		Survival curve

Pollack	2002	US	AA43	III	98	MIB-1>18%		$p=0.02$, HR=2.49 (1.17, 5.32)	Survival curve	
						MIB-1>36%		$p=0.02$, HR=5.86 (2.71,12.7)	Survival curve	
			GBM 42	IV		MIB-1>18%		$p=0.04$, HR=1.69 (1.01,2.85)	Survival curve	
						MIB-1>36%		$p=0.04$, HR=2.33 (1.23,4.41)	Survival curve	
			Other eligible13	ND		MIB-1>18%		$p<0.01$, HR=1.71 (1.05,2.79)	Survival curve	
						MIB-1>36%		$p<0.01$, HR=2.09 (1.32,3.31)	Survival curve	
Wessels	2003	UK	A47	II	47 (25/22)	MIB-1>1%		$p=0.02$, HR=3.63 (1.22, 10.8)	HR (Univariate)	
								$p=0.01$, HR=4.69 (1.56,14.2)	HR (Multivariate)	
Bowers	2003	US	PA141	I	141 (78/63)	MIB-1 \geq 2%		$p=0.02$, HR=1.20 (1.03,1.40)	HR (Univariate)	
Zamecnik	2003	Czech Republic	E31	II11, III 20	31 (19/12)	MIB-1 LI>7%		$p<0.00$, HR=27.0 (3.5,210.3)	HR (Univariate)	
								$p=0.03$, HR=8.8 (1.9,48.6)	$p=0.00$, HR=10.9 (2.3,52.2)	HR (Multivariate)
Chiang	2003	Taiwan	GBM 68	IV	68 (41/27)	MIB-1>35%		$p<0.01$, HR=2.42 (1.45,4.02)	Survival curve	
Nede	2004	Brazil	A11, AA5, GBM31		40 (27/13)	MIB-1>3%		$p<0.00$, HR=10.241 (2.37,44.26)	Estimated	
Uematsu	2005	Japan	ND	II9, III12, IV8	29 (17/12)	Ki-67 \geq 10%		$p=0.03$, HR=4.01 (1.19,13.5)	Estimated	
Preusser	2005	Austria	E58	II	58	Ki-67>20.5%		$p=0.02$, HR=2.42 (1.09,5.40)	Survival curve	
Klein-schmidt-DeMasters	2006	US	GBM28	IV	28 (22/6)	MIB-1>29.3%		$p=0.10$, HR=1.01 (0.37,2.75)	Estimated	
Donato	2007	Italy	GBM43	IV	39	MIB-1>20%		$p=0.00$, HR=4.24 (1.68,10.71)	Survival curve	
Li	2009	China	primary GBM 95 secondary GBM21	IV	116 (76/40)	ND		$p=0.009$, HR=2.44 (1.25,4.77)	$p=0.00$, HR=2.95 (1.47,5.91)	HR (Multivariate)
Kanamori	2009	Japan	ODG13, OA5, AODG26, AOA12	II-III	56 (32/24)	Ki-67 \geq 25%			$p=0.00$, HR=3.8 (1.4,9.5)	HR (Multivariate)
Laks	2009	US	ND	ND	30	ND		$p=0.04$, HR=17.0 (1.0,286)	$p=0.03$, HR=4.71 (1.12,19.7)	HR (Multivariate)
Kuo	2009	Taiwan	ODG40, OA9	III	49 (27/22)	Ki-67 \geq 5%		$p=0.05$, HR=2.92 (0.99,8.55)	$p=0.01$, HR=3.55 (1.36,9.26)	HR (Univariate)
								$p=0.00$, HR=10 (2.42,41.67)		HR (Multivariate)
Yoshida	2009	Japan	GBM38	IV	38 (23/15)	MIB-1>20%		$p=0.02$, HR=2.35 (1.04,5.3)	Survival curve	
Watanabe	2010	US	DA12, AA9, GBM44	II-IV	65 (40/25)	MIB-1>25%		$p=0.04$, HR=2.49 (1.03,6.06)	HR (Multivariate)	
Armstrong	2010	US	E63	II	63	MIB-1 \geq 10%		$p=0.28$, HR=3.50 (1.15,10.64);	$p=0.00$, HR=4.69 (1.56,14.2)	HR (Univariate)

Nabika	2010	Japan	AA24, GBM35	III-IV	59 (35/ 24)	MIB-1> 17.3%	$p=0.31$, HR=1.02 (0.98,1.04)		HR (Mul- tivariate)
Zawrocki	2011	Pol- land	E39	II	39 (22/17)	Ki-67 >3%	$p=0.00$, HR=3.79 (1.23,4.03)		HR (Uni- variate)
Jin	2011	China	GBM	IV	156	Ki-67 >25%	$p=0.00$, HR=1.49 (1.06,2.11)	$p=0.01$, HR=1.16 (1.13,2.18)	Survival curve
Habber- stad	2011	Nor- way	AA	III	27 (17/10)	MIB- 1>10%	$p=0.03$, HR=2.85 (1.03,7.88)		Estimated
Shen	2011	China	LGA27, AA20, GBM24	II-IV	71 (39/32)	Ki-67 $\geq 10\%$	$p=0.02$, HR=6.45 (2.05,16.7)		HR (Mul- tivariate)
Margraf	2011	US	PA80	II	80 (40/40)	MIB-1 \geq 2%	$p=0.03$, HR=1.92 (1.04,3.53)		Survival curve
Tove	2012	Nor- way	A104	ND	104	Ki-67> 4.45%	$p=0.26$, HR=1.35 (0.70,2.62)		Survival curve
Okita	2012	Japan	GBM 189	IV	189 (121 /68)	MIB-1> 30%	$p=0.9$, HR=1.07 (0.40,2.93)		HR (Mul- tivariate)
						MIB-1> 10%	$p=0.00$, HR= 5.25 (1.67,20.6)		HR (Mul- tivariate)
Yang	2012	China	A,OA	II	341	Ki-67 $\geq 10\%$	$p=0.00$, HR=3.40 (1.64,7.02)	$p=0.09$, HR=1.61 (0.93,2.77)	HR (Mul- tivariate)
			AG	I	122	ki-67 $\geq 10\%$	$p=0.72$, HR=0.91 (0.53,1.55)	$p=0.74$, HR=0.93 (0.62,1.41)	HR (Mul- tivariate)
			GBM	IV	202	ki-67 $\geq 10\%$	$p=0.49$, HR=1.09 (0.85,1.39)	$p=0.04$, HR=1.25 (1.01,1.54)	HR (Mul- tivariate)
Phi	2012	Korea	E	II	33 (18/15)	Ki-67> 8.8%	$p=0.43$, HR=1.04 (0.95,1.13)	$p=0.00$, HR=1.06 (1.02,1.11)	HR (Uni- variate)
Preusser	2012	Aus- tria	AOA	III	76	Ki-67 \geq 22.7%	$p=0.00$, HR=1.92 (1.07,3.46)	$p=0.00$, HR=2.03 (1.26,3.29)	Survival curve
Park	2012	Korea	LGA (Biopsy)	II	33 (20/13)	MIB- 1>6%		$p=0.11$, HR=114 (0.97,1.35)	HR (Uni- variate)
								$p=0.11$, HR=1.26 (0.95,1.66)	HR (Mul- tivariate)
			LGA (Resec- tion)		24 (12/12)			$p=0.00$, HR=1.17 (1.06,1.29);	HR (Uni- variate)
								$p=0.01$, HR=1.25 (1.04,1.50)	HR (Mul- tivariate)
Liu	2013	China	AA	III	48 (31/17)	ND	$p<0.01$, HR=4.14 (1.83,9.41)	$p<0.01$, HR=3.34 (1.53,7.29)	ND
Abd El Atti	2013	Egypt	A111	ND	111 (74/37)	MIB-1> 10.1%	$p=0.08$, HR=2.66 (0.90,7.87)		HR (Mul- tivariate)
Huang	2013	China	41DA, 30AA, 31 GBM	II41, III30, IV31	102 (55/47)	Ki-67 $\geq 10\%$	$p=0.02$, HR=7.75 (2.63,18.4)		HR (Mul- tivariate)
Tian	2014	China	ND	I-II117	312 (182/ 130)	ND	$p=0.03$, HR=1.23 (0.83,1.61)		ND
				III- IV195					
Yue	2014	China	GBM	IV	62 (43/19)	Ki-67> 20%	$p=0.08$, HR=0.62 (0.36,1.06)	$p=0.38$, HR=1.26 (0.75,2.17)	HR (Uni- variate)
							$p=0.09$, HR=1.70 (0.92,3.13)		HR (Mul- tivariate)
							$p=0.02$, HR=1.92 (1.10,3.33)		HR (Mul- tivariate)

*A for astrocytoma;AA for anaplastic astrocytoma;AE for anaplastic ependynoma;AOA for anaplastic oligoastrocytoma;AODG for anaplastic oligodendrogloma;DA for diffuse astrocytoma;E for ependynoma;FA for fibrillary astrocytoma; GBM for glioblastoma ;LGA for low grade astrocytoma;ME for myxopapillary ependynoma;OA fo oligoastrocytoma;ODG for oligodendrogloma;PA for pilocytic asrocytoma

Table 2. Summarized HRs of Overall and Subgroup Analyses for OS

Stratified analysis	Study (N)	HR	p	Heterogeneity	
				I ²	p
Region					
Europe	17	1.68 (1.49,1.90)	0	85.30%	0
America	12	2.79 (1.80,4.33)	0	72.10%	0
Asia	16	1.73 (1.44,2.07)	0	85.40%	0
Africa	1	2.66 (0.90,7.87)	0.08	-	-
Cutoff					
L(<10%)	18	2.42 (1.98,2.95)	0	88.70%	0
H(≥10%)	24	1.49 (1.34,1.67)	0	79.30%	0
Tumor type					
A	29	1.73 (1.55,1.94)	0	84.80%	0
OT	4	2.28 (1.53,3.40)	0	52.20%	0.06
E	8	4.07 (1.97,8.42)	0	87.60%	0
Method					
Estimated	7	3.39 (1.91,6.01)	0	46.20%	0.08
Survival curve	14	2.34 (1.82,3.02)	0	63.20%	0
HR(univariate)	9	2.50 (1.38,4.53)	0	84.70%	0
HR(multivariate)	15	1.34 (1.20,1.52)	0	80.90%	0

Table 3. Summarized HRs of Overall and Subgroup Analyses for PFS

Stratified analysis	Study (N)	HR	p	Heterogeneity	
				I ²	p
Region					
Europe	2	6.81 (1.30,35.7)	0.02	78.5%	0.01
America	10	2.30 (1.68,3.15)	0.00	71.3%	0.00
Asia	16	1.34 (1.18,1.53)	0.00	76.8%	0.00
Cutoff					
L(<10%)	18	1.71 (1.40,2.09)	0.00	83.3%	0.00
H(≥10%)	9	1.46 (1.22,1.75)	0.00	67.0%	0.00
Tumor type					
A	11	1.47 (1.29,1.66)	0.00	65.8%	0.00
OT	2	4.42 (2.40,8.15)	0.00	0.00%	0.46
E	5	5.83 (1.84,18.48)	0.00	89.7%	0.00
Method					
Estimated	2	3.53 (1.70,7.33)	0.00	0.00%	0.82
Survival curve	14	1.98 (1.52,2.59)	0.00	55.7%	0.02
HR(univariate)	9	1.29 (1.09,1.52)	0.00	81.9%	0.00
HR(multivariate)	15	1.78 (1.35,2.33)	0.00	72.8%	0.00

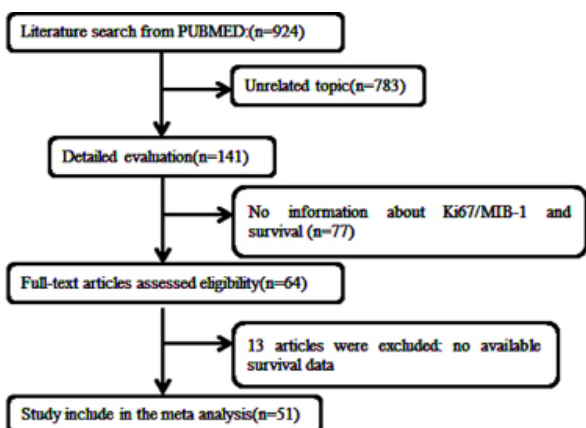


Figure 1. Flow Diagram of Study Selection

oligoastrocytic tumors, 8 studies included ependymomas, 2 studies included several glioma types and 3 studies showed no information about tumor type. Moreover, there were 11 studies in the astrocytoma group, 5 studies in the ependymoma group, 2 studies with oligodendroglial and oligoastrocytic tumors, 1 study including several glioma

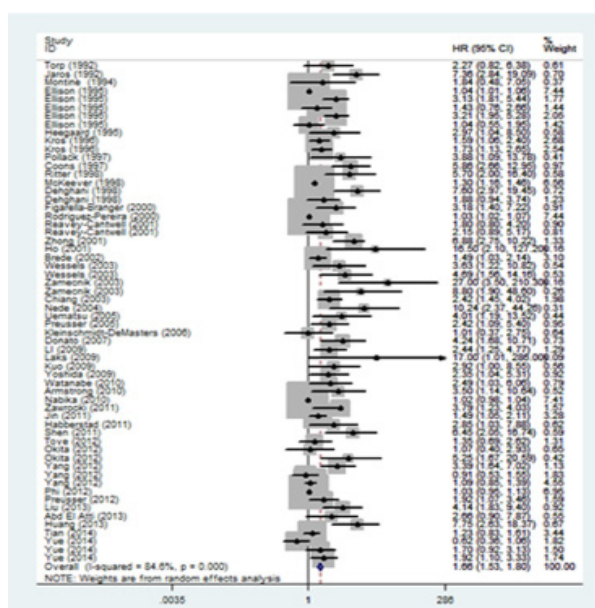


Figure 2. Forest Plot Showing the Combined Relative HR from Random-effect for OS

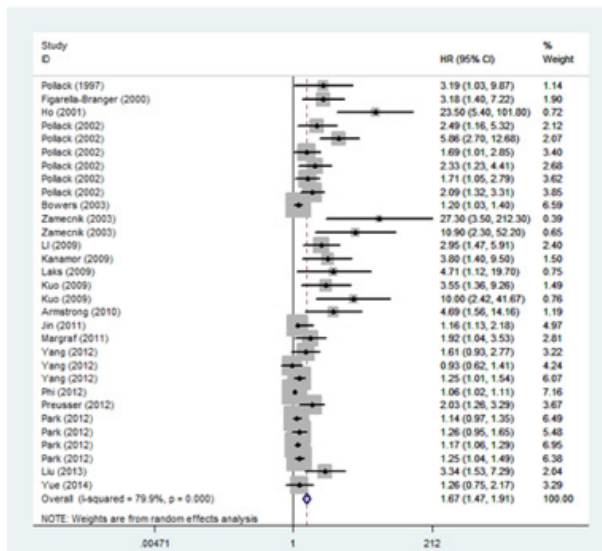


Figure 3. Forest Plot Showing the Combined Relative HR from Random-effect PFS

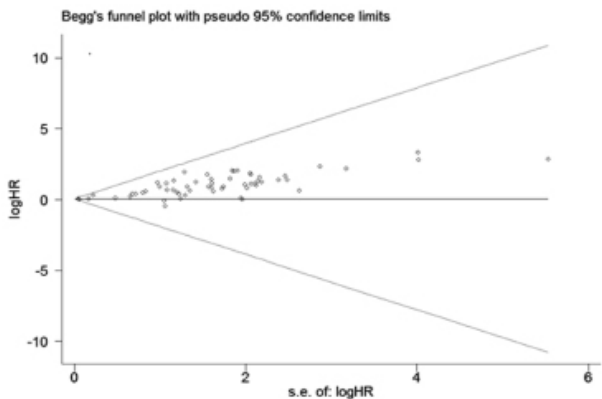


Figure 4. Funnel Blot was Designed to Visualize a Potential Publication Bias for OS Studies

types and 1 study with no information about glioma type in PFS analysis group. The cut-offs of antibodies used to define immunohistochemical positivity also varied between reports, 42 OS analysis group selected the percentage of positive staining as the cut-off point, including 24 studies $\geq 10\%$, 18 studies $< 10\%$.

Meta-analysis

In this meta-analysis, we evaluated 46 studies dealing with Ki-67 expression and OS. The pooled HR was 1.66 (95%CI:1.53-1.80; $Z=11.87$; $p=0.000$, Figure2) with Heterogeneity ($I^2=84.6\%$ $p=0.000$). It suggested that overexpression of Ki-67 was significantly related with the worse prognosis of glioma and high Ki-67 expression was a valuable prognostic factor for overall survival in glioma patients. Moreover, we also performed subgroup analysis by region, glioma pathology type, cut-off value and statistical method. The result showed that significant relationships between high Ki-67 expression and OS were exhibited especially in the subgroup of oligodendroglial and oligoastrocytic tumors (HR=1.99, 95%CI, 1.56, 2.55; $Z=5.48$, $p=0.000$) and HR estimated by original data (HR=3.28, 95% 2.16, 4.97; $Z=5.60$, $p=0.000$, Table2). In the subgroup analysis according to the study region, the combined HR was 2.79 (95%CI: 1.80-4.33; $Z=4.60$;

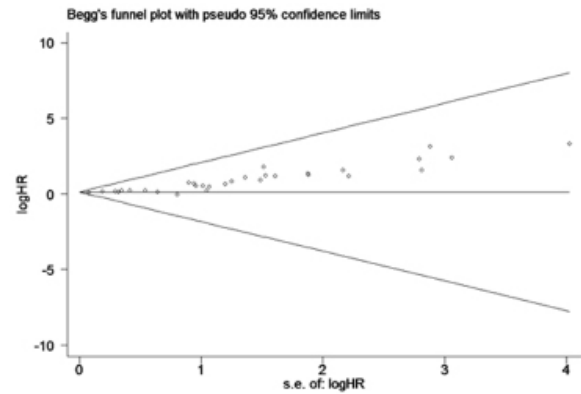


Figure 5. Funnel Blot was Designed to Visualize a Potential Publication Bias for PFS Studies

$p=0.000$) for America countries. When stratified according to the cut-off value, the combined HRs of the subgroup with cut-off $< 10\%$ showed an inverse effects on survival (HR=2.42; 95%CI 1.98-2.95; $Z=8.72$, $p=0.000$, Table2).

The nineteen studies in PFS analysis group were also statistically significant with a pooled estimate of risk of 1.67 (95%CI:1.47-1.91; $Z=7.67$; $p=0.000$, Figure3) with Heterogeneity ($I^2=79.9\%$ $p=0.000$). Statistically significant differences were also identified for all survival subgroup analyses (Table3).

Heterogeneity analysis results

Obvious heterogeneity of subjects was found in 3 of the 4 analysis groups (OS for all, $p=0.00$, $I^2=84.6\%$; OS for astrocytoma, $p=0.000$ and $I^2=84.8\%$; OS for oligodendroglial and oligoastrocytic tumors, $p=0.063$, $I^2=52.2\%$; OS for ependymoma, $p=0.000$, $I^2=87.6\%$). Meanwhile, we conducted a meta regression to evaluate the potential factors responsible for the obvious heterogeneity (Table2). In the subgroup that HR estimated by original data did not show obvious heterogeneity ($p=0.084$, $I^2=46.2\%$). In the PFS analysis group, obvious heterogeneity of subjects was also found (PFS for all, $p=0.000$, $I^2=79.9\%$). However, PFS for oligodendroglial and oligoastrocytic tumors ($p=0.458$, $I^2=0.0\%$) and HR estimated by original data ($p=0.861$, $I^2=0.0\%$) reached low heterogeneity (Table3).

Publication Bias

Figure4 and Figure5 showed that the funnel plot was unsymmetrical, that indicated there existed publication bias. The Begg rank correction test also detected evidence for publication bias among studies of Ki-67 and OS ($p=0.04$) and PFS ($p<0.001$) in glioma patients. The funnel plot revealed an apparent asymmetry that suggested the presence of a potential publication bias, a language bias, inflated estimates by a flawed methodologic design in smaller studies, and/or a lack of publication of small trials with opposite results.

Discussion

This meta-analysis showed that Ki-67 overexpression was associated with worse OS and PFS in glioma patients. Recently, a few studies have suggested that Ki-67 is an essential prognostic factor in human glioma (Yoshida et

al., 2010; Preusser et al., 2012). In our systematic review with meta-analysis, patients with Ki-67 positive tumors had significantly worse survival than those with Ki-67 negative ones. The mechanism underlying the effect of Ki-67 expression on tumor progression and prognosis remains essentially uncertain. However, it has to be considered that positivity for the Ki-67 antigen may reflect the ability of a cell to continue to proliferate after the time of tumor resection. This nonhistone nuclear protein is expressed throughout the active parts of the cell cycle (G1, S, G2, and mitosis). The Ki-67 protein can be detected during all active phases of the cell cycle but not in resting cells and can be used as a tool to estimate the growth fraction of any human cell population. Besides, Ki-67 immunostaining can easily be performed on various types of cytological and histological preparations, such as smear, squash, cytocentrifuge preparations, and histological sections. Thus, the importance of the Ki-67/MIB-1 labeling index (LI) as a prognostic and predictive factor in human malignancies has been debated for many years.

In this meta-analysis, we had dealt with highly significant heterogeneity among OS analysis and PFS analysis group. Although we used random effects models to analyze the data, heterogeneity was still a potential problem to affect the results of meta-analysis. The information of the included studies revealed that the heterogeneity could be attributed to the differences in the publication years, the pathology types and stages of the tumors, the cut-off values of Ki-67, study region and the risk evaluation methods.

Our meta-analysis was carried out using literature published results, and we therefore acknowledge some limitations of our approach which is, however, much less expensive than a meta-analysis using individual patients data. The language selection can favour positive studies, following the assumption that they are more often published in English, whereas the negative ones tend to be published more often in local journals.

One of the possible sources of confusion is the usage of the same cohort of patients in different publications. It might be difficult to avoid inclusion of some patients more than once in the meta-analysis, although publications clearly based on the analyses of the same patients cohorts were excluded. We have assumed that authors have been honest and have not reported the results from the same cohort of patients without mentioning in their publications.

Another potential source of bias is related to the method for extrapolating the HR. If the authors did not report the individual HR together with its variance, we calculated it from the survival comparison statistic and its variance whenever possible. If not, we extrapolated it from the survival curves using several time points during follow-up for reading the corresponding survival rates, assuming that censored observations were uniformly distributed. Reading the survival rates on the graphical representation of the survival curves was performed independently by three of the authors, but this strategy can not eliminate complete inaccuracy in the extracted survival rates. Consequently, the estimated HR might be less reliable than when obtained from published statistics.

Additionally, some studies have used 10% as the cut-

off (arbitrary value), whereas others have chosen mean, median, the optimal cut-off value or arbitrary values. These differences might be responsible for the difficulty in determining a standard threshold in daily practice. In the context of this meta-analysis, we may assume that increased Ki-67 leads to an increased risk of relapse and/or death and that a relative increase is estimated although the baseline risk (the risk in the group considered Ki-67 negative) is not the same in all the studies.

In conclusion, this meta-analysis has yielded significant association between Ki-67 overexpression and worse OS and PFS in patients with glioma. Ki-67 can be a potential prognostic indicator for glioma patients. In addition, it is rather necessary that better designed studies need to be enrolled into such kind of analysis in the future, to provide a better conclusion about the relationship between Ki-67 expression and the outcome of patients with glioma. The value of Ki-67 for molecular staging of glioma also needs to be confirmed in controlled trials involving larger number of patients with longer follow-up, before any definitive conclusions can be made.

Acknowledgements

The study was supported by the fund of Guangxi Zhuang Autonomous Region University Student Innovative Plan (No. 201410598026), China. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the paper.

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