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META-ANALYSIS

High expression of anti-apoptotic protein Bcl-2 is a good prognostic factor in colorectal cancer: Result of a meta-analysis

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Abstract

AIM

To systematically evaluate the prognostic-predictive capability of Bcl-2 in colorectal cancer (CRC).

METHODS

A systematic literature search was conducted using PubMed, Web of Science and EMBASE databases. Any eligible study must meet the following criteria: (1) bcl-2 expression was evaluated in human CRC tissues by immunohistochemistry; (2) assessment of the relationships between bcl-2 expression and overall survival (OS), disease free survival (DFS), recurrent free survival (RFS) or clinic-pathological characteristics of CRC was included; (3) sufficient information was provided to estimate the hazard ratio (HR) or odds ratio and their 95% confidence intervals (CIs); and (4) the study was published in English. The impact of Bcl-2 expression on survival of CRC patients were evaluated through this meta-analysis.

RESULTS

A total of 40 eligible articles involving 7658 patients were enrolled in our final analysis. We drew the conclusion that Bcl-2 high expression was significantly correlated with favorable OS (pooled HR = 0.69, 95%CI: 0.55-0.87, P = 0.002) and better DFS/RFS (pooled HR = 0.65, 95%CI: 0.50-0.85, P = 0.001). Additionally, the subgroup analysis suggested that Bcl-2 overexpression was significantly associated with

prognosis (OS) especially in patients came from Europe and America but not Asian and patients who did not receive any adjuvant therapy before surgery. Finally, our present results indicated that expression of bcl-2 protein was associated with high differentiation grade and A/B Ducks' stage.

CONCLUSION

Bcl-2 high expression was significantly correlated with favorable OS and better DFS/RFS. Hence, we propose that Bcl-2 may be a valuable prognostic-predictive marker in CRC.

Key words: Bcl-2; Colorectal cancer; Meta-analysis; Prognostic; Apoptotic

Core tip: No consensus is available in the literature about the prognostic value of Bcl-2 expression in patients with colorectal cancer (CRC). This is the first systematic review and meta-analysis indicating that Bcl-2 is a good prognostic factor in CRC. We investigated the relation in terms of overall survival, disease free survival/recurrent free survival, number of patients, nations, therapy methods, pathological grade.

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INTRODUCTION

Bcl-2 family proteins are key regulators of apoptosis whose dysregulation can cause various pathological consequences including the development of cancer[1]. The anti-apoptotic protein Bcl-2 (B-cell lymphoma-2) is an important member of the Bcl-2 family which controls the release of proapoptotic factors responsible for the activation of caspases by stabilizing the mitochondrial outer membrane[2].

Colorectal cancer (CRC) is one of the most common malignancies worldwide[3,4]. Despite the great progress made in clinical treatment, the morbidity and mortality of CRC remains high. Aberrant expression of Bcl-2 has been implicated in several cancer types including CRC[5]. Nonetheless, data obtained by different researchers were often in disagreement[6-10].

An increasing body of evidence from many studies indicates that Bcl-2 expression may be associated with prognosis in malignancies including CRC[11]. Expression of Bcl-2 has been found to correlate with favorable clinicopathologic parameters and better prognosis by many investigators[7,8,12,13]. In contrast, some groups demonstrated that Bcl-2 was a poor prognostic for cancer patients[9,10,14]. And there are others who found no prognostic significance of Bcl-2 expression in CRC[6,15,16]. Thus, neither the function nor the prognostic value of Bcl-2 expression in patients with CRC is clear to us.

Herein, we carried out this meta-analysis to explore the reason for present contradictory observations and determine the prognostic value of Bcl-2 in patients with CRC.

MATERIALS AND METHODS

Literature search

We identified relevant articles by conducting searches in the PubMed, Web of Science and EMBASE databases using the following terms and all possible combinations: "Bcl-2", "colorectal carcinoma", "CRC", "colon cancer", "rectal cancer". More than this, we examined the references to identify additional eligible studies. The reviews and bibliographies were also retrieved to discern other relevant articles. The most recent search update was October 15th, 2016. After excluding non-related articles through browsing the titles and abstracts of the listed studies, full-text viewing of resting studies was performed. The largest population size study was chosen to avoid duplicate analysis when patients overlap partly or entirely.

Inclusion criteria

The eligible studies included in our meta-analysis must meet the following requirements: (1) bcl-2 expression was evaluated in human CRC tissues by immunohistochemistry (IHC); (2) assessment of the relationships between bcl-2 expression and overall survival (OS), disease free survival (DFS), recurrent free survival (RFS) or clinic-pathological characteristics of CRC was included; (3) sufficient information was provided to estimate the hazard ratio (HR) or odds ratio (OR) and their 95% confidence intervals (CIs); and (4) the study was published in English.

Exclusion criteria

The articles were excluded from our analysis if they have the following characteristics: (1) letters, reviews, case reports, and conference abstracts without original data; (2) lack of necessary data or survival curves to calculate HRs, ORs or the corresponding 95%CIs; and (3) overlapping studies.

Data extraction and assessment of study quality

Data extraction and quality assessment were conducted independently by two primary investigators (Qi H and Shu L) using a standardized form. Discrepancies were arbitrated by a third reviewer. The following characteristics were retrieved: first author's name,

year of publication, country of patients' origin, tumor location, number of patients, age of patients, tumor stage, treatment state before surgery, follow-up time, research technique used, antibody source and dilution, cut-off value, survival data and clinical-pathological data. The quality of each study was tested according to the Newcastle-Ottawa quality assessment scale (NOS).

Statistical analysis

All statistical analysis was performed using the STATA 12.0 software (Stata Corporation, Collage Station, TX, United States). We calculated the pooled HRs and the 95%CIs of all included articles. OS, DFS and RFS were all included in our outcome analysis. We used the raw data directly if HRs and their corresponding 95%CIs were described in the literature. Otherwise, they were extracted from Kaplan-Meier curves published in the article read by Engauge Digitizer version 4.1 (http://digitizer.sourceforge.net/) according to the methods described by Parmar *et al*[17]. At the same time, we also explored the correlation between Bcl-2 expression and clinical-pathological parameters of CRC such as tumor location, tumor grade, Ducks' stage and lymph node metastasis combining the ORs and their 95%CIs. A value of HR > 1 implies a worse prognosis of survival in patients who overexpressed Bcl-2, while a value of OR < 1 indicates an unfavorable parameters in those high Bcl-2 expression patients. The association between Bcl-2 and survival or clinical-pathological factors was considered statistically significant if the 95%CI did not span across 1. The heterogeneity among articles included in this meta-analysis was evaluated by χ^2-based Q statistical test according to Peto's method[18]. The inconsistency index (I^2) ranged from 0% to 100% was used to quantify the proportion of the total variation[19]. A *P*-value for the *Q*-test was presented to assess the heterogeneity among the studies. We chose the random-effects model (the DerSimonian and Laird method) when $P < 0.10$. Otherwise, the fixed-effects model (the Mantel-Haenszel method) was applied[19,20]. Begg's test was used to determine the potential publication bias when $P < 0.05$. Statistical significant was defined as $P < 0.05$.

RESULTS

Literature search and study description

We identified 2274 relevant articles upon screening the keywords from several databases and a total of 40 eligible studies were finally selected to explore the relationship between Bcl-2 expression and CRC patients' survival using the strategy depicted in Figure 1[6-10,12-16,21-50]. The detailed clinical features of each record were shown in Table 1, which enrolled an overall of 7658 CRC patients in this analysis published ranging from 1995 to 2016. Among the 40 studies, 6 studies were conducted in Italy, 5 in Greece, 4 each in China and United Kingdom, 2 each in Netherlands, Sweden, United States, Canada, Finland and Germany, 1 each in Romania, Switzerland, Brazil, South Korea, Ireland, Japan, Australia, India and Austria. As to the prognostic analysis, 34 studies evaluated the correlation between Bcl-2 expression and patients' OS while 13 articles reported the data of Bcl-2 related DFS or RFS.

In OS analysis, 22 of the included articles enrolled more than 100 patients and 12 manuscripts recruited less than 100 patients. Patients from 7 studies received treatment such as radiotherapy, chemotherapy or endocrine therapy before surgery while other 18 studies were not the case, another 9 articles did not provide therapy strategy before surgery.

Methodological quality of selected studies

Each of the 40 eligible studies included in our meta-analysis underwent quality evaluation according to the Newcastle-Ottawa Scale (NOS). NOS scores were judged on eight items of the methodology that categorized into three sections: selection, comparability, exposure and outcome. The quality score of enrolled studies ranged from 5 to 8 with a mean score of 6.5. Eighteen studies scored 7 or more in methodological assessment were defined as high quality (Table 1).

Correlation between Bcl-2 high expression and increased OS or DFS/RFS in CRC

34 studies were included in the analysis to evaluate the association between Bcl-2 high expression and OS. The pooled hazard ratio (HR) for OS was 0.69 (95%CI: 0.55-0.87, $Z = 3.14$, $P = 0.002$). A statistical heterogeneity ($I^2 = 80.0\%$, $P < 0.001$) was observed based on the random-effects model (Figure 2A). A meta-analysis on 13 studies was performed to analyze the correlation between Bcl-2 and DFS/RFS. The pooled HR for DFS/RFS was 0.65 (95%CI: 0.50-0.85, $Z = 3.19$, $P = 0.001$), accompanied with considerable heterogeneity ($I^2 = 59.0\%$, $P = 0.004$) (Figure 2B). These results indicate that high level expression of Bcl-2 is significantly associated with decreased mortality risk in CRC patients and Bcl-2 may be an independent prognostic factor in CRC.

Subgroup analysis and sensitivity analysis of the correlation between Bcl-2 high expression and OS in CRC

To address the heterogeneity in OS, we performed subgroup analysis on the number of patients involved in the study, the origin country of patients, the treatment situation before surgery and the NOS score (Table 2). We found that a significant relationship between high expression of Bcl-2 and OS was exhibited in subgroup with number of patients more than 100 (HR = 0.684, 95%CI: 0.54-0.866, $P = 0.002$) (Figure 3A) and subgroup with origin country of Europe and America (HR = 0.691, 95%CI: 0.553-0.864, $P = 0.001$) (Figure 3B). Additionally, Bcl-2 overexpression showed

Table 1 Main characteristics of the studies included in the meta-analysis

Ref.	Year	Country	Tumor location	Patient(P/N)	Age	Stage	Treatment before surgery	Follow-up time Median(range)	Detection method	Antibody source	Antibody dilution	Cut off value	HR(95%CI) estimation	Quality Score
Cai *et al*[21]	2016	China	Colon and rectum	117(34/83)	52.0 yr (24-87 yr)	Ⅰ-Ⅳ	No	NA	IHC	Thermo Scientific	1:50	> 10%	OS = 0.7 (0.34-1.45) Multivariate	7
Melincovici *et al*[6]	2016	Romania	Colon	31(12/19)	63 ± 11.71 yr	A-D (Ducks)	Yes	NA	TMA/IHC	Dako	1:100	> 5%	OS = 0.211 (0.026-1.718) Univariate	8
Huang *et al*[22]	2015	China	Colon and rectum	190(85/105)	NA	A-D (Ducks)	No	986 d (21-2572d)	IHC	Genetex	NA	Multiply the intensity score by the percentage of labeled cells > 150	OS = 2 (1.21-3.3) Multivariate RFS = 1.32 (0.82-2.13) Multivariate	8
Balzi *et al*[15]	2015	Italy	Colon and rectum	321 (153/168)	< 85 yr	Ⅰ-Ⅲ	No	NA	IHC	Dako	1:50	> 5%	OS = 0.87 (0.51-1.48) Univariate DFS = 0.971 (0.654-1.449) Multivariate	8
Belt *et al*[23]	2014	Netherlands	Colon	160 (81/76)	72.4 yr (34.5-94.0 yr)	T1-4, N1-2, M0	Yes	46.9 mo (3.0-148.6 mo)	TMA/IHC	Dako	1:300	Score ⩾ 1	DFS = 0.409 (0.256-0.653) Univariate	6
Fucini *et al*[9]	2012	Italy	Rectum	66 (27/39)	67 ± 9 yr	Ⅱ-Ⅲ	Yes	105.5 ± 39.6 mo	IHC	Dako	1:50	> 10%	OS = 2.526 (1.146-5.565) Univariate	5
Xu *et al*[10]	2009	China	Colon and rectum	119 (33/86)	57 yr (31-74 yr)	Ⅰ-Ⅳ	No	95 mo (1-203 mo)	IHC	Dako	1:50	> 10%	OS = 3.064 (1.217-7.718) Multivariate	5
Zlobec *et al*[24]	2008	Switzerland	Colon and rectum	1420 (NA)	NA	pT1-4, N0-N2	NA	NA	TMA/IHC	NA	NA	> 30%	OS = 1.15 (0.94-1.39) Multivariate	5
Torsello *et al*[7]	2008	Italy	Colon and rectum	1340 (650/690)	NA	A-D (Ducks)	Yes	5 yr	IHC	Dako	NA	> 30%	OS = 0.221 (0.105-0.464) Univariate	6
Cahlin *et al*[25]	2008	Sweden	Colon	22 (NA)	75 ± 9 yr	A-D (Ducks)	No	68 mo (3.6-127.4 mo)	IHC	Santa Cruz Biotechnology	0.25 μg/mL	NA	OS = 1.43 (1-2.06) Multivariate	5
Tsamandas *et al*[8]	2007	Greece	Rectum	28 (17/11)	64 yr (27-76 yr)	B2 and C (Ducks)	No	47.19 ± 6.2 mo	IHC	Dako	1:40	> 5%	OS = 0.032 (0.007-0.158) Univariate	5
Meleth *et al*[26]	2007	United Kingdom	Colon and rectum	491 (NA)	NA	Ⅰ-Ⅳ	No	5 yr	IHC	NA	NA	Score ⩾ 0.5	OS = 0.67 (0.493-0.92) Multivariate	6
Zavrides *et al*[12]	2006	Greece	Colon and rectum	100 (27/73)	NA	Ⅰ and Ⅲ	No	7 yr (5-9 yr)	IHC	Biogenex	1:10	> 5%	OS:0.273(0.139-0.534) Univariate	7
Georgiou *et al*[27]	2006	Greece	Colon and rectum	170 (64/106)	NA	B and C (Ducks)	NA	46 mo (3-93 mo)	IHC	Dako	1:80	> 10%	OS = 0.556 (0.326-1.031) Univariate	7
Chatla *et al*[28]	2005	United States	Colon and rectum	158 (89/69)	NA	Ⅱ and Ⅲ	No	7.31 yr (< 1-> 20 yr)	IHC	Cambridge Laboratories	1:80	Score ⩾ 0.5	RFS = 0.45 (0.083-2.441) Multivariate	6
Zhao *et al*[29]	2005	China	Colon and rectum	93 (53/40)	51 yr (median)	A-C (Ducks)	NA	60 mo (median)	IHC	NA	NA	Score ⩾ 2	OS = 0.505 (0.317-0.804) Univariate	6
Lustosa *et al*[16]	2005	Brazil	Colon and rectum	116 (58/58)	63.4 yr (30-87 yr)	Ⅰ-Ⅳ	No	28.5 mo (2-96 mo)	IHC	Dako	NA	> 10%	OS = 0.858 (0.433-1.698) Univariate	6
Krajewska *et al*[13]	2005	Sputh Korea	Colon and rectum	106 (NA)	NA	Ⅱ	No	66 mo (median)	TMA/IHC	NA	1:2000	NA	OS = 0.251 (0.111-0.567) Multivariate	6

Rosati *et al*[30]	2004	Italy	Colon and rectum	103 (41/62)	66 yr (29-79 yr)	B and C (Ducks)	Yes	5 yr	IHC	Dako	NA	> 10%	OS = 0.71 (0.37-1.35) Univariate DFS = 1 (0.51-1.96) Univariate	7
Garrity *et al*[31]	2004	Canada	Colon and rectum	366 (97/269)	NA	B2 and C (Ducks)	Yes	8.7 yr (median)	IHC	Dako	1:50	> 10%	OS = 0.99 (0.69-1.429) Multivariate DFS = 0.971 (0.654-1.449) Multivariate	5
Kouraklis *et al*[32]	2003	Greece	Colon	113 (55/58)	70.9 yr (42-94 yr)	B and C (Ducks)	No	NA	IHC	Dako	1:50	> 5%	OS = 0.523 (0.304-0.903) Univariate	8
Scopa *et al*[33]	2003	Greece	Colon and rectum	117 (76/41)	66 yr (25-82 yr)	A-D (Ducks)	No	97 mo (44-142 mo)	IHC	Dako	1:40	Cytoplasmic staining	OS = 1.55 (0.7-3.4) Multivariate	8
Sun *et al*[34]	2003	Sweden	Colon and rectum	138 (82/56)	71 yr (43-94 yr)	A-D (Ducks)	NA	NA	IHC	Nova Castra Laboratories Ltd	1:80	> 5%	OS = 0.504 (0.221-1.146) Univariate	7
Bendardaf *et al*[35]	2003	Finland	Colon and rectum	58 (45/13)	60.3 yr (24.3-78.2 yr)	T2-X,N0-X,M0-1	Yes	NA	IHC	Dako	1:50	Sum the intensity score and expression score ⩾ 1.10	OS = 1.02(0.7-11.5) Univariate	6
Elkablawy *et al*[36]	2001	United Kingdom	Colon and rectum	52 (18/34)	68.8 yr (33-93 yr)	pT2-4, N0-1,M0-1	NA	43.5 mo (2-111 mo)	IHC	Dako	4 μg/mL	Multiply the intensity score and expression score ⩾ 6	OS = 0.552 (0.231-1.319) Univariate	6
Meterissian *et al*[37]	2001	Canada	Colon	76 (62/14)	71.2 yr (40-89 yr)	B (Ducks)	No	59 mo (5-110 mo)	IHC	Dako	1:50	⩾ 30% or stain intensity scale ⩾ 1	OS = 0.35 (0.13-0.94) Univariate DFS = 0.49 (0.19-1.26) Univariate	7
Paradiso *et al*[38]	2001	Italy	Colon and rectum	80 (29/51)	NA	Advanced	No	NA	IHC	Dako	1:40	> 5%	OS = 1.287 (0.76-2.183) Univariate	6
Schwandner *et al*[39]	2000	Germeny	Rectum	160 (47/113)	66.7 yr (31-92 yr)	Ⅰ-Ⅲ	No	38 mo (12-72 mo)	IHC	Dako	1:20	> 10%	DFS = 0.181 (0.056-0.585) Univariate	7
Buglioni *et al*[40]	1999	Italy	Colon and rectum	171 (57/114)	64 yr (56-70 yr)	A-D (Ducks)	No	50 mo (median)	IHC	Dako	NA	A strong homogeneous cytoplasmic immunoreaction	OS = 0.192 (0.0439-0.84) Multivariate DFS = 0.178 (0.0508-0.625) Multivariate	7
Leahy *et al*[41]	1999	Ireland	Colon and rectum	102 (22/80)	69 yr (39.2-85.5 yr)	A-C (Ducks)	No	9.9 yr (9.0-11.2 yr)	IHC	Dako	1:50	> 5%	OS = 0.5 (0.2-1) Multivariate	7
Ishijima *et al*[42]	1999	Japan	Colon and rectum	33 (10/23)	61.6 yr (42-86 yr)	A-D (Ducks)	NA	NA	IHC	Santa Cruz Biotechnology	1:50	> 30%	DFS = 1.051 (0.202-5.464) Univariate	6
Sinicrope *et al*[43]	1999	United States	Colon	137 (71/66)	65.2 yr (26-89 yr)	T2-3, N0,M0	No	105.5 mo (2-281 mo)	IHC	Dako	1:20	> 20%	OS = 0.46 (0.21-1.05) Multivariate RFS = 0.45 (0.21-0.96) Multivariate	7
Hirvikoski *et al*[44]	1999	Finland	Rectum	92 (62/30)	72 yr (52-90 yr)	A-D (Ducks)	Yes	32 mo (0-306 mo)	IHC	Dako	1:200	> 20%	OS = 0.99 (0.55-1.79) Univariate	6
Biden *et al*[45]	1999	Australia	Colon and rectum	66 (49/17)	NA	A-D (Ducks)	NA	NA	IHC	Dako	1:40	> 5%	OS = 0.132 (0.057-0.306) Univariate	5

Ilyas *et al*[46]	1998	United Kingdom	Colon and rectum	66 (40/26)	NA	B (Ducks)	NA	NA	IHC	Dako	1:40	Stain	RFS = 0.77 (0.62-0.96) Univariate	5
Kaklamanis *et al*[47]	1998	United Kingdom	Colon and rectum	224 (73/151)	NA	A-C (Ducks)	NA	36 mo (1-72.5 mo)	IHC	Dako	NA	> 10%	OS = 0.605 (0.375-0.977) Univariate	7
Tollenaar *et al*[48]	1998	Netherlands	Colon and rectum	209 (99/110)	NA	A-C (Ducks)	No	NA	IHC	Boehringer Mannheim	1:200	Score ⩾ 2	OS = 0.978 (0.658-1.453) Univariate	8
Bhatavdekar *et al*[14]	1997	India	Colon and rectum	48 (29/19)	48 yr (25-74 yr)	B and C (Ducks)	NA	29.95 mo (2-36 mo)	IHC	Dako	NA	> 5%	OS = 7.813 (2.375-25.64) Univariate	7
Baretton *et al*[49]	1996	Germeny	Colon and rectum	95 (64/31)	63.8 ± 12.5 yr	pT2-3, N0,M0	NA	Up to 8 yr	IHC	Dianova	1:60	An unequivocally strong cytoplasmic immunoreaction	DFS = 0.504 (0.27-0.943) Univariate	6
Ofner *et al*[50]	1995	Austria	Colon and rectum	104 (47/57)	67.8 yr (35-90 yr)	pT1-4, N0-X,M0-1	NA	NA	IHC	Dako	1:300	Stain	OS = 0.443 (0.252-0.78) Univariate	7

P/N: Positive/Negative; NA: Not assessable.

a favorable OS when the patients adopted no therapy before surgery (HR = 0.696, 95%CI: 0.502-0.964, P = 0.029) (Figure 3C). Our results also indicated that the NOS quality score had no significant effect on the prognostic value of Bcl-2 expression (Figure 3D). Meanwhile, a sensitive analysis was conducted to assess the role of each study on the overall environment. To achieve this, studies were excluded one at a time while the rest were analyzed. HR of Bcl-2 high expression on OS ranged from 0.664 (95%CI: 0.532-0.830) to 0.730 (95%CI: 0.585-0.909) (Figure 4A), and pooled HR of Bcl-2 high expression on DFS/RFS ranged from 0.597 (95%CI: 0.461-0.775) to 0.687 (95%CI: 0.528-0.894) (Figure 4B).

Impact of Bcl-2 high expression on clinicopathological parameters

Twelve studies were selected to assess the association between Bcl-2 high expression and tumor differentiation grade. The pooled OR was 2.475 (95%CI: 1.307-4.685, P = 0.005) with statistical heterogeneity (I^2 = 68.4%, P = 0.000), which indicated that low expression of Bcl-2 was correlated with differentiation of CRC. Correlation between Bcl-2 overexpression and Ducks' stages were also evaluated in twelve studies. The pooled OR was 1.630 (95%CI: 1.009-2.632, P = 0.046) with significant heterogeneity (I^2 = 78.1%, P = 0.000), suggesting that downregulated Bcl-2 expression was associated with the progression of CRC. However, we did not find significant association between Bcl-2 expression and gender or the tumor location, the pooled OR being shown in Table 3.

Publication bias

Begg's test was used to assess the potential publication bias. The funnel plots for the OS (Figure 5A) and DFS/RFS (Figure 5B) indicated that there was no evidence of significant publication bias in our present meta-analysis.

DISCUSSION

It is well documented that defects in the mitochondrial apoptotic pathway are closely related with carcinogenesis. Bcl-2 is a key inhibitor of apoptosis, playing a major role in the maintenance of normal balance between apoptosis and cellular survival.

Currently, effective treatment of CRC remains a big challenge. The majority of patients will experience relapse or distant metastases within 5 years following surgical resection. Abnormal Bcl-2 activation has been implicated during the evolution of CRC. Up to this date, however, the exact role of Bcl-2 in CRC has not been established. The explanation of this inconsistency is not known, perhaps because of the variations with ethnicity and location in the patient population. By the same token, no consistent conclusion about the prognostic value of Bcl-2 expression in CRC patients has been made. So we speculate that the prognostic significance of

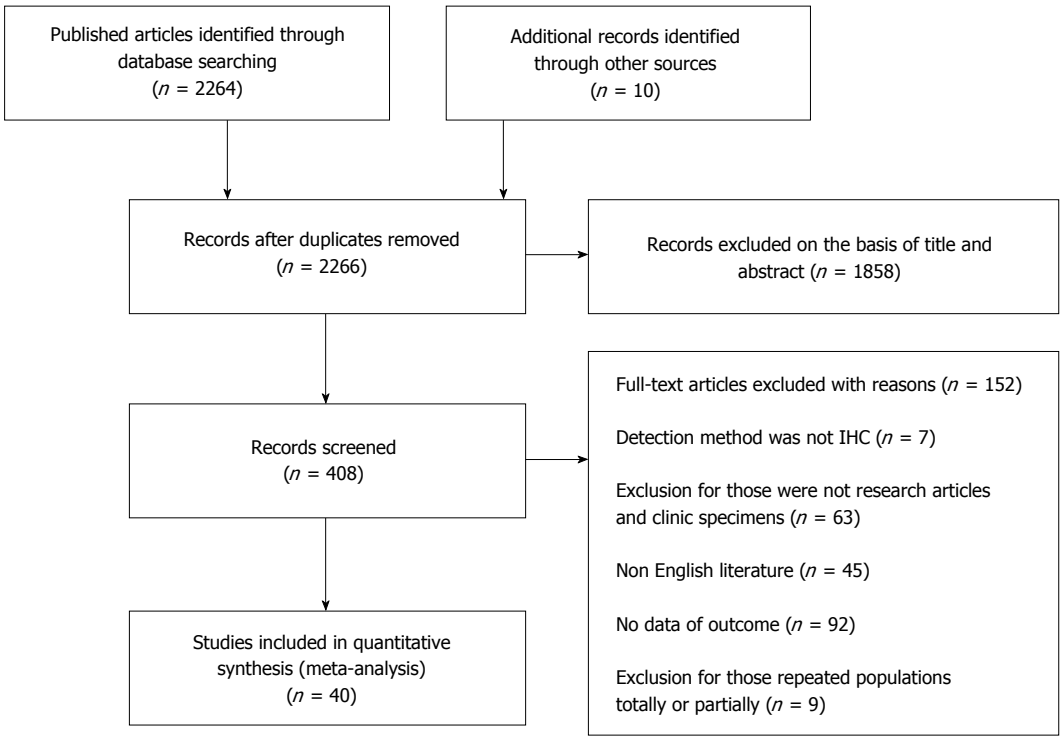


Figure 1 Flow diagram of the selection procedure for the studies.

Table 2 Subgroup analysis of pooled hazard ratios for colorectal cancer patients with overexpressed Bcl-2

Stratified analysis	No. of studies	No. of patients	Pooled HR (95%CI)	*P* value	Heterogeneity	
					I^2 (%)	*P* value
No. of patients						
⩾ 100	22	6274	0.684 (0.54-0.866)	0.002	75.9	0.000
< 100	12	712	0.693 (0.389-1.235)	0.214	85.8	0.000
Study location						
Asia	7	777	1.021 (0.488-2.136)	0.955	88.2	0.000
Europe and America	26	6143	0.691 (0.553-0.864)	0.001	73.9	0.000
Treatment before surgery						
Yes	7	2056	0.772 (0.55-0.947)	0.394	73.8	0.001
No	18	2615	0.696 (0.502-0.964)	0.029	79.9	0.000
Quality score						
⩾ 7	18	2471	0.678 (0.499-0.92)	0.013	71.8	0.000
< 7	16	4515	0.708 (0.503-0.996)	0.047	84.9	0.000

Table 3 Bcl-2 expression and clinicopathological features of colorectal cancer

Clinicopathologial features	No. of studies	No. of patients	Pooled OR (95%CI)	*P* value	Heterogeneity	
					I^2	*P* value
Gender (male *vs* female)	11	1671	1.125 (0.865-1.463)	0.381	30.2%	0.158
Tumor location (colon *vs* rectum)	8	1361	1.168 (0.922-1.480)	0.199	0%	0.628
tumor grade (1 + 2 *vs* 3)	12	1454	2.475 (1.307-4.685)	0.005	68.4%	0.000
Ducks' stage (A + B *vs* C + D)	12	1572	1.630 (1.009-2.632)	0.046	78.1%	0.000

Bcl-2 expression in CRC may be restricted to specific subgroups. To the best of our knowledge, this is the first meta-analysis pertinently investigating the prognostic value of Bcl-2 expression in CRC.

Our meta-analysis incorporated 40 eligible studies with the survival data of OS, DFS and RFS. From our analyses results we found that Bcl-2 high expression is of significant association with increased OS and DFS/RFS in patients with CRC. When the subgroup analyses were conducted, the pooled results demonstrated that high expression Bcl-2 was a favorable prognostic factor in subgroup with number of patients more than 100 and subgroup with origin country of Europe and America. Additionally, Bcl-2 overexpression showed an

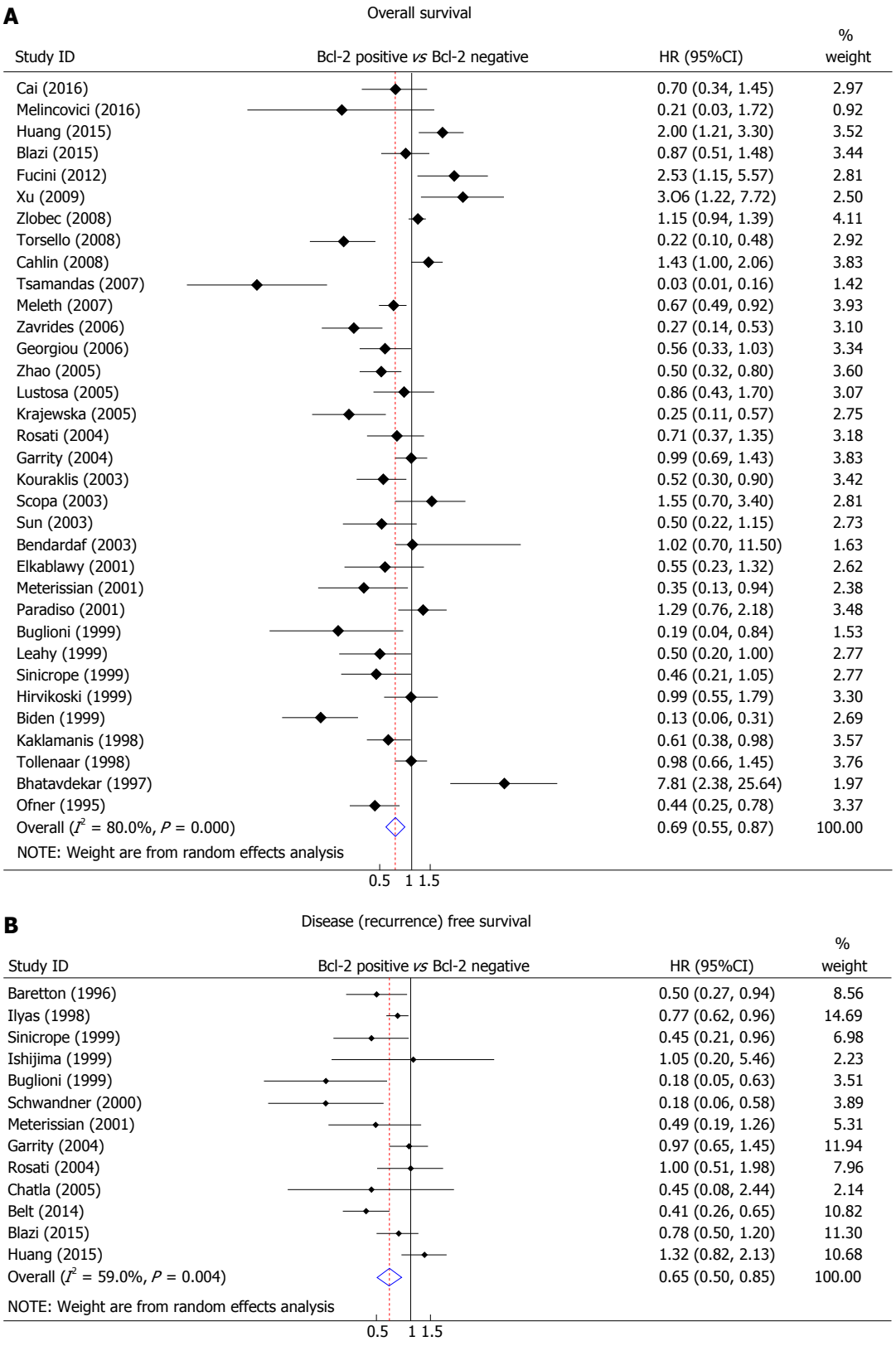
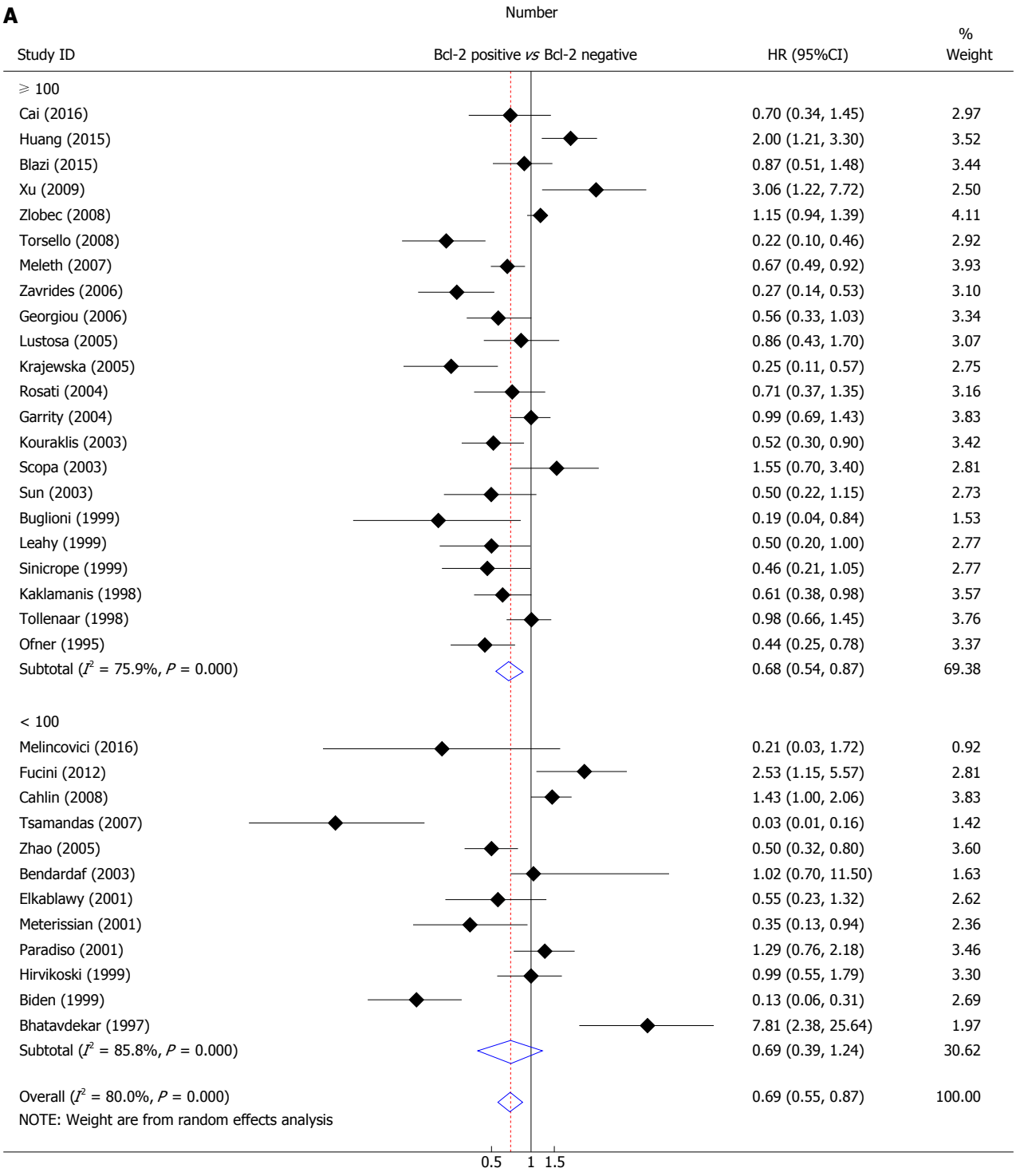


Figure 2 Hazard ratio of Bcl-2 expression associated with (A) overall survival and (B) disease free survival/recurrence free survival. DFS: Disease free survival; RFS: Recurrence frees survival.

increased OS when the patients adopted no therapy before surgery. As to clinicopathological parameters analysis, Bcl-2 was found to express more frequently in tumors with high differentiation grade and A/B Ducks' stage. It should be noted that no publication bias was found in this meta-analysis.

Our study leads to several valuable conclusions. First, expression of Bcl-2 is a favorable factor for


A
Number
Study ID
Bcl-2 positive *vs* Bcl-2 negative
HR (95%CI)
% Weight
⩾ 100
Cai (2016) 0.70 (0.34, 1.45) 2.97
Huang (2015) 2.00 (1.21, 3.30) 3.52
Blazi (2015) 0.87 (0.51, 1.48) 3.44
Xu (2009) 3.06 (1.22, 7.72) 2.50
Zlobec (2008) 1.15 (0.94, 1.39) 4.11
Torsello (2008) 0.22 (0.10, 0.46) 2.92
Meleth (2007) 0.67 (0.49, 0.92) 3.93
Zavrides (2006) 0.27 (0.14, 0.53) 3.10
Georgiou (2006) 0.56 (0.33, 1.03) 3.34
Lustosa (2005) 0.86 (0.43, 1.70) 3.07
Krajewska (2005) 0.25 (0.11, 0.57) 2.75
Rosati (2004) 0.71 (0.37, 1.35) 3.16
Garrity (2004) 0.99 (0.69, 1.43) 3.83
Kouraklis (2003) 0.52 (0.30, 0.90) 3.42
Scopa (2003) 1.55 (0.70, 3.40) 2.81
Sun (2003) 0.50 (0.22, 1.15) 2.73
Buglioni (1999) 0.19 (0.04, 0.84) 1.53
Leahy (1999) 0.50 (0.20, 1.00) 2.77
Sinicrope (1999) 0.46 (0.21, 1.05) 2.77
Kaklamanis (1998) 0.61 (0.38, 0.98) 3.57
Tollenaar (1998) 0.98 (0.66, 1.45) 3.76
Ofner (1995) 0.44 (0.25, 0.78) 3.37
Subtotal (I^2 = 75.9%, P = 0.000) 0.68 (0.54, 0.87) 69.38
< 100
Melincovici (2016) 0.21 (0.03, 1.72) 0.92
Fucini (2012) 2.53 (1.15, 5.57) 2.81
Cahlin (2008) 1.43 (1.00, 2.06) 3.83
Tsamandas (2007) 0.03 (0.01, 0.16) 1.42
Zhao (2005) 0.50 (0.32, 0.80) 3.60
Bendardaf (2003) 1.02 (0.70, 11.50) 1.63
Elkablawy (2001) 0.55 (0.23, 1.32) 2.62
Meterissian (2001) 0.35 (0.13, 0.94) 2.36
Paradiso (2001) 1.29 (0.76, 2.18) 3.46
Hirvikoski (1999) 0.99 (0.55, 1.79) 3.30
Biden (1999) 0.13 (0.06, 0.31) 2.69
Bhatavdekar (1997) 7.81 (2.38, 25.64) 1.97
Subtotal (I^2 = 85.8%, P = 0.000) 0.69 (0.39, 1.24) 30.62
Overall (I^2 = 80.0%, P = 0.000) 0.69 (0.55, 0.87) 100.00
NOTE: Weight are from random effects analysis
0.5
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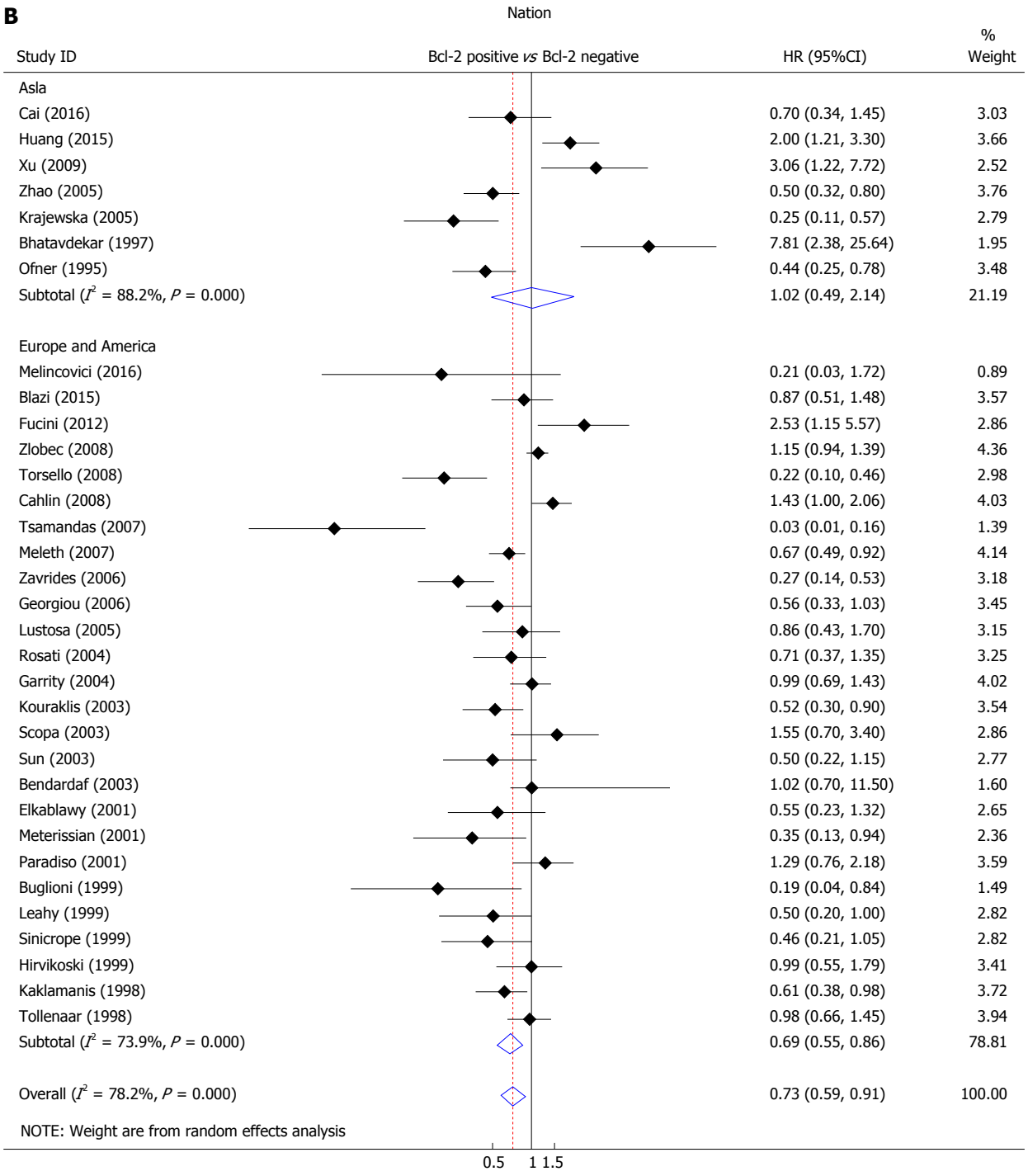
1.5


B
Nation
Study ID
Bcl-2 positive vs Bcl-2 negative
HR (95%CI)
% Weight
Asla
Cai (2016) 0.70 (0.34, 1.45) 3.03
Huang (2015) 2.00 (1.21, 3.30) 3.66
Xu (2009) 3.06 (1.22, 7.72) 2.52
Zhao (2005) 0.50 (0.32, 0.80) 3.76
Krajewska (2005) 0.25 (0.11, 0.57) 2.79
Bhatavdekar (1997) 7.81 (2.38, 25.64) 1.95
Ofner (1995) 0.44 (0.25, 0.78) 3.48
Subtotal (I^2 = 88.2%, P = 0.000) 1.02 (0.49, 2.14) 21.19
Europe and America
Melincovici (2016) 0.21 (0.03, 1.72) 0.89
Blazi (2015) 0.87 (0.51, 1.48) 3.57
Fucini (2012) 2.53 (1.15 5.57) 2.86
Zlobec (2008) 1.15 (0.94, 1.39) 4.36
Torsello (2008) 0.22 (0.10, 0.46) 2.98
Cahlin (2008) 1.43 (1.00, 2.06) 4.03
Tsamandas (2007) 0.03 (0.01, 0.16) 1.39
Meleth (2007) 0.67 (0.49, 0.92) 4.14
Zavrides (2006) 0.27 (0.14, 0.53) 3.18
Georgiou (2006) 0.56 (0.33, 1.03) 3.45
Lustosa (2005) 0.86 (0.43, 1.70) 3.15
Rosati (2004) 0.71 (0.37, 1.35) 3.25
Garrity (2004) 0.99 (0.69, 1.43) 4.02
Kouraklis (2003) 0.52 (0.30, 0.90) 3.54
Scopa (2003) 1.55 (0.70, 3.40) 2.86
Sun (2003) 0.50 (0.22, 1.15) 2.77
Bendardaf (2003) 1.02 (0.70, 11.50) 1.60
Elkablawy (2001) 0.55 (0.23, 1.32) 2.65
Meterissian (2001) 0.35 (0.13, 0.94) 2.36
Paradiso (2001) 1.29 (0.76, 2.18) 3.59
Buglioni (1999) 0.19 (0.04, 0.84) 1.49
Leahy (1999) 0.50 (0.20, 1.00) 2.82
Sinicrope (1999) 0.46 (0.21, 1.05) 2.82
Hirvikoski (1999) 0.99 (0.55, 1.79) 3.41
Kaklamanis (1998) 0.61 (0.38, 0.98) 3.72
Tollenaar (1998) 0.98 (0.66, 1.45) 3.94
Subtotal (I^2 = 73.9%, P = 0.000) 0.69 (0.55, 0.86) 78.81
Overall (I^2 = 78.2%, P = 0.000) 0.73 (0.59, 0.91) 100.00
NOTE: Weight are from random effects analysis
0.5
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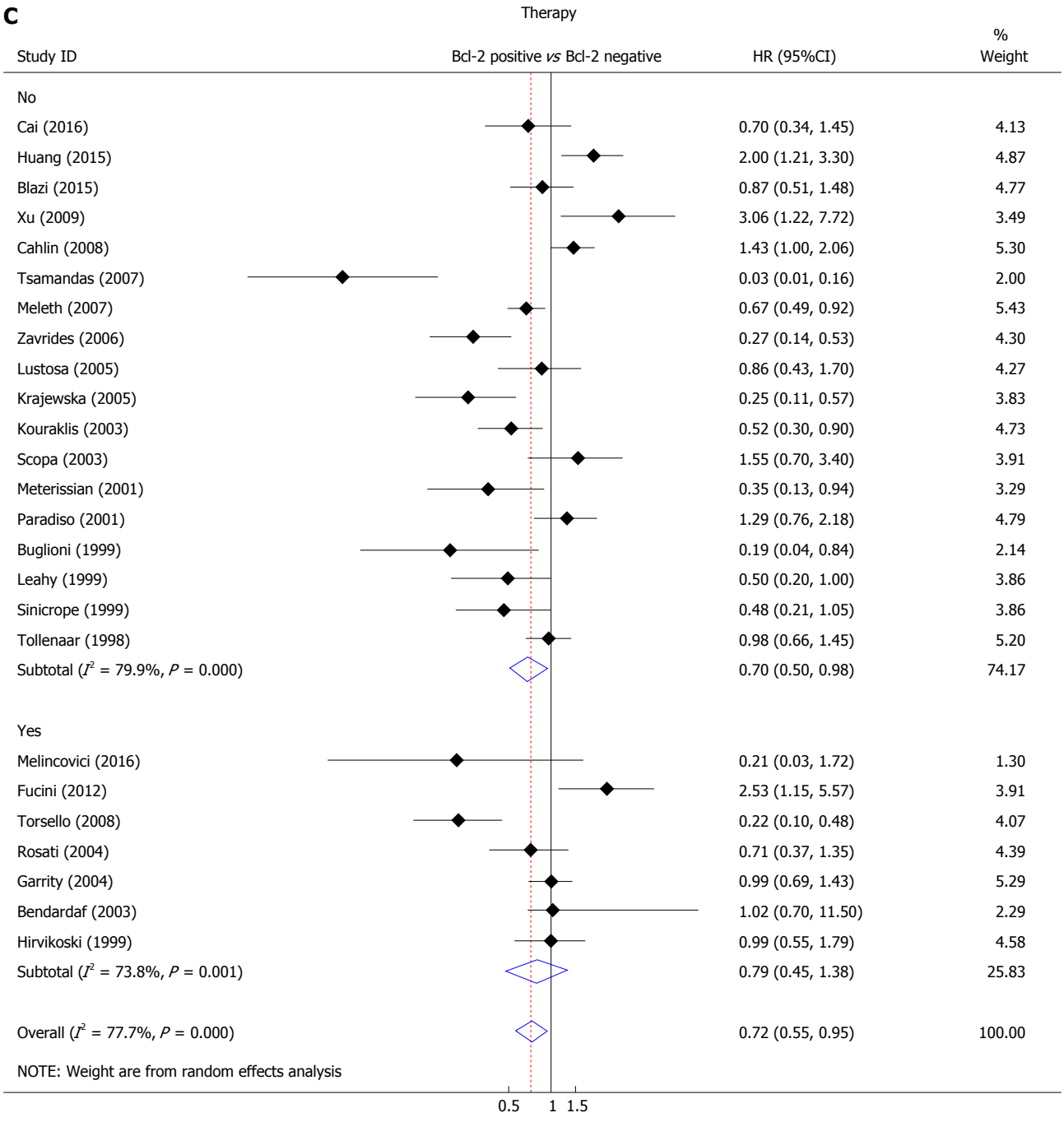
C


Therapy
Study ID
Bcl-2 positive vs Bcl-2 negative
HR (95%CI)
% Weight
No
Cai (2016) 0.70 (0.34, 1.45) 4.13
Huang (2015) 2.00 (1.21, 3.30) 4.87
Blazi (2015) 0.87 (0.51, 1.48) 4.77
Xu (2009) 3.06 (1.22, 7.72) 3.49
Cahlin (2008) 1.43 (1.00, 2.06) 5.30
Tsamandas (2007) 0.03 (0.01, 0.16) 2.00
Meleth (2007) 0.67 (0.49, 0.92) 5.43
Zavrides (2006) 0.27 (0.14, 0.53) 4.30
Lustosa (2005) 0.86 (0.43, 1.70) 4.27
Krajewska (2005) 0.25 (0.11, 0.57) 3.83
Kouraklis (2003) 0.52 (0.30, 0.90) 4.73
Scopa (2003) 1.55 (0.70, 3.40) 3.91
Meterissian (2001) 0.35 (0.13, 0.94) 3.29
Paradiso (2001) 1.29 (0.76, 2.18) 4.79
Buglioni (1999) 0.19 (0.04, 0.84) 2.14
Leahy (1999) 0.50 (0.20, 1.00) 3.86
Sinicrope (1999) 0.48 (0.21, 1.05) 3.86
Tollenaar (1998) 0.98 (0.66, 1.45) 5.20
Subtotal (I^2 = 79.9%, P = 0.000) 0.70 (0.50, 0.98) 74.17
Yes
Melincovici (2016) 0.21 (0.03, 1.72) 1.30
Fucini (2012) 2.53 (1.15, 5.57) 3.91
Torsello (2008) 0.22 (0.10, 0.48) 4.07
Rosati (2004) 0.71 (0.37, 1.35) 4.39
Garrity (2004) 0.99 (0.69, 1.43) 5.29
Bendardaf (2003) 1.02 (0.70, 11.50) 2.29
Hirvikoski (1999) 0.99 (0.55, 1.79) 4.58
Subtotal (I^2 = 73.8%, P = 0.001) 0.79 (0.45, 1.38) 25.83
Overall (I^2 = 77.7%, P = 0.000) 0.72 (0.55, 0.95) 100.00
NOTE: Weight are from random effects analysis
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1.5

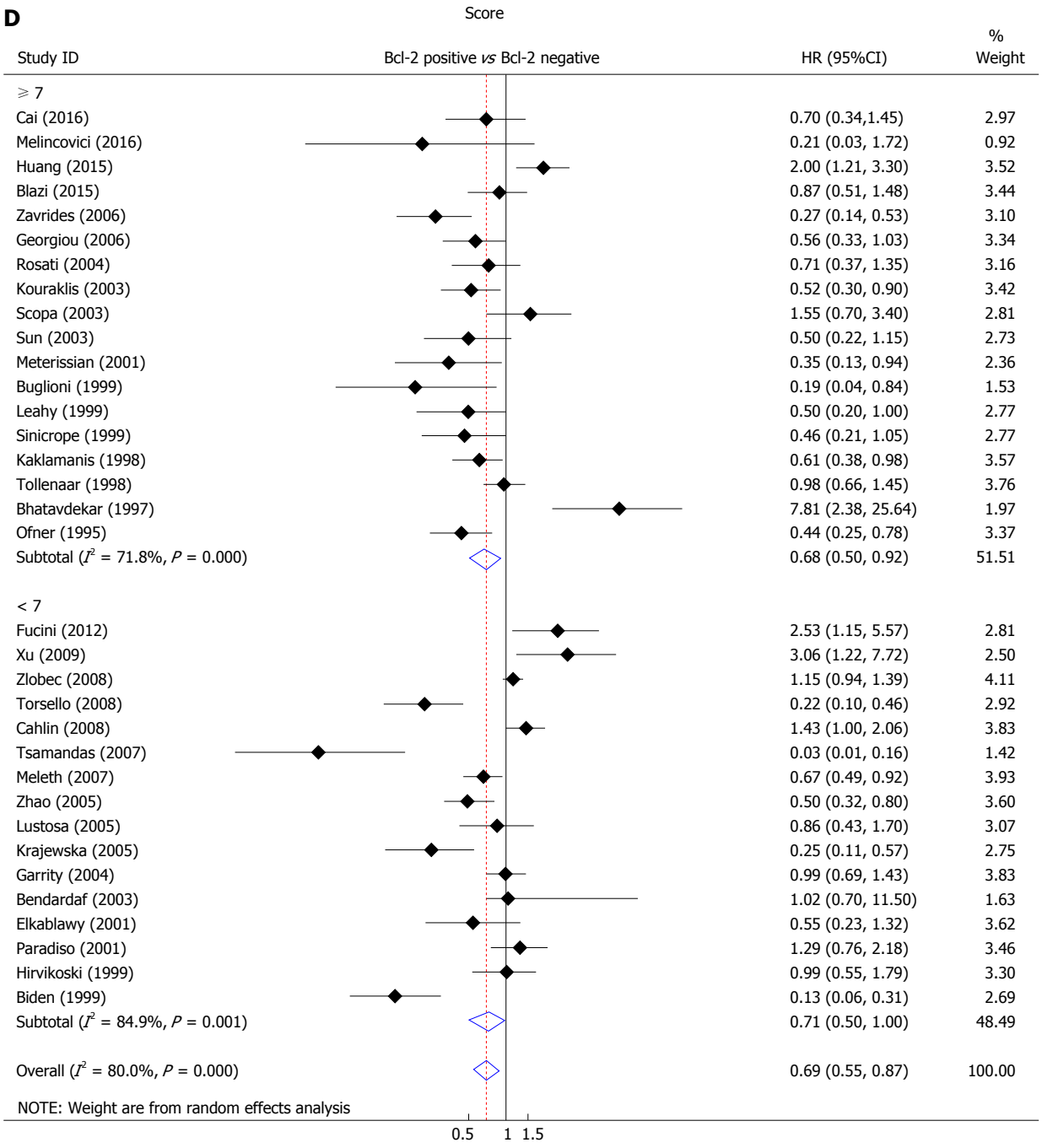

Figure 3 Hazard ratio of Bcl-2 expression associated with overall survival in the subgroup. A: Patients' number; B: Patients' country of origin; C: The condition of treatment before surgery; D: Quality score of study.

CRC. The relationship between Bcl-2 expression and transformation from normal epithelium to invasive cancer is not entirely clear. However, there is evidence to suggest that during the evolution of CRC, the role of Bcl-2 oncoprotein is believed to be in the early stages of carcinogens[51,52]. Moreover, lack of Bcl-2 expression has been proved to be correlated with invasion, metastasis and recurrence of CRC. Our meta-analysis revealed that the upregulation of Bcl-2 was related to favorable prognosis in both OS and DFS/RFS. This is contradictory to the anti-apoptotic function of Bcl-2, which may be due to the interactions of various proteins involved in apoptotic pathways such as p53, Fas and so on. Second, our present results indicated that expression of Bcl-2 protein was associated with pathological grade and clinical stage, consistent with what Zavrides *et al*[12] reported. The survival of CRC patients largely depends on disease stage at the time of diagnosis and differs greatly between stages. It was reported earlier that Bcl-2 expression correlated with improved survival, a significantly higher MFS for the subgroup of patients with Dukes' B[53]. It is logical to assume that the primary role of Bcl-2 during carcinogenesis and progression of CRC may depend

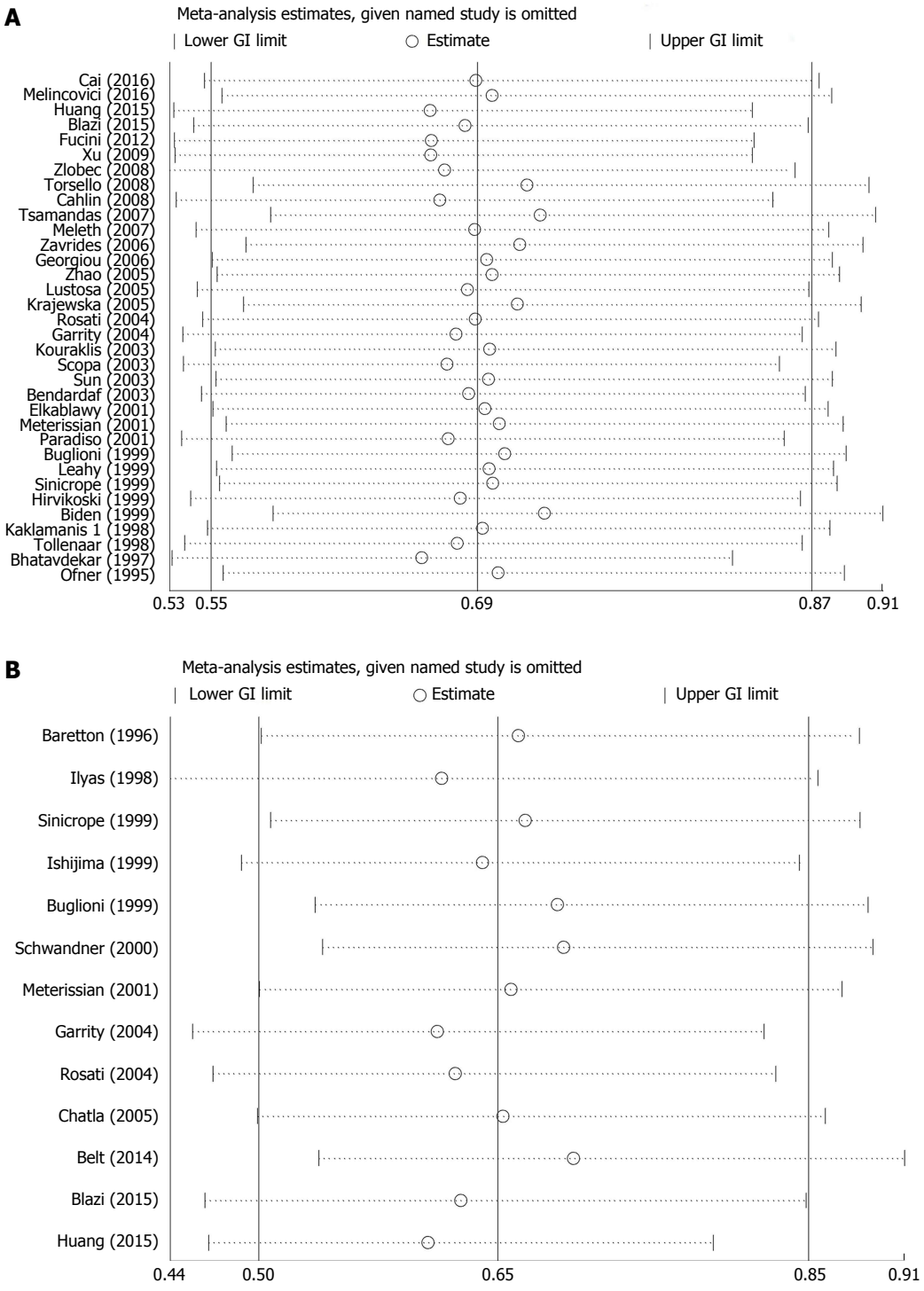

Figure 4 Sensitivity analysis for the meta-analysis of (A) OS and (B) disease free survival/recurrence free survival in all patients.

on disease stage. However, further study on large sample is needed to confirm this speculation. Third, the prognostic role of Bcl-2 expression on CRC patients is evident in Caucasian populations but not yet the case in Asians. Genomic polymorphisms among various ethnic groups may be the explanation. Thus, the clinical value of Bcl-2 should be studied separately based upon different population structures and aggregates in the future research. Additionally, the favorable effect of Bcl-2 expression on CRC patients' overall survival is insignificant in subgroup receiving preoperative treatment. Some studies have suggested that preoperative chemoradiation can influence cancer cell's apoptosis and treatment effect by changing Bcl-2 expression. Thus, it seems easy to explain such observation. However, Long-term prospective studies are needed to verify this.

Recently, targeting proteins involved in apoptotic pathways appeared as an attractive strategy to assist anticancer therapy. A particular concern has been

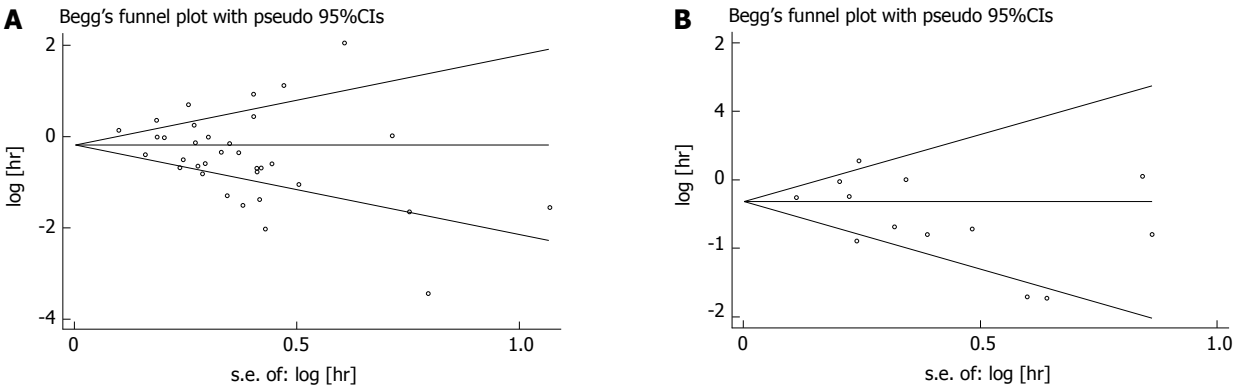

Figure 5 Begg's publication bias plot. It showed no publication bias for the studies regarding the association of Bcl-2 expression with (A) OS or (B) disease free survival/recurrence free survival in the meta-analysis. Each point represents a separate study for the indicated association.

focused on the development of agents capable of inhibiting the activity of Bcl-2 family members that are overexpressed in various malignancies[54,55]. In this regard, it seems that we need to reassess the of small-molecule drugs targeting Bcl-2. There is a serious need for more *in vivo* experiments to explicate the detailed mechanism. Finally, using Bcl-2 alone to evaluate prognostic information of CRC patients with different stages is probably limited. On the other hand, integration of multiple biomarkers may provid sufficient support for clinical application[56-59]. We suggest focusing on the combination of key markers within the prominent pathways that occupy an important role in clinical prognosis, which better reflects the overall molecular environment in CRC. A systematic study on the prognostic value of multi-marker proteins in CRC patients can also be performed in the future.

There exists some limitations that should be noted in our meta-analysis. We only recruited articles published in English, thus a language bias might exist. Some HRs and their corresponding 95%CIs were extracted from the survival curves. However, these data might be less reliable than those directly obtained from survival data. We use random effects model to deal with heterogeneity, however, the inter-study heterogeneity resulted from different populations, different antibody source and varying cutoff values was inevitable.

In summary, our meta-analysis suggests that expression of the Bcl-2 protein is associated with favorable prognosis in patients with CRC. Subgroup analysis showed that Bcl-2 overexpression may become a good prognosis factor in CRC where patients come from Europe and America but not Asian and patients not receive any adjuvant therapy before surgery. These significant associations were more remarkable in CRCs with high grade of differentiation and A/B Ducks' stage. Our analysis also found those significant associations only be find in populations more than 100. This told us that further prospective studies with larger sample sizes are required to validate the prognostic value of Bcl-2 expression in CRC.

COMMENTS

Background

An increasing body of evidence from many studies indicates that Bcl-2 expression may be associated with prognosis in malignancies including colorectal cancer (CRC). However, neither the function nor the prognostic value of Bcl-2 expression in patients with CRC is clear to us.

Research frontiers

Currently, effective treatment of CRC remains a big challenge. Abnormal Bcl-2 activation has been implicated during the evolution of CRC and speculated playing a major role in the prognosis of CRC. Up to this date, however, the exact role of Bcl-2 in CRC has not been established.

Innovations and breakthroughs

In the present study, the authors explored the reason for present contradictory observations and determine the prognostic value of Bcl-2 in patients with CRC. This is the first meta-analysis pertinently investigating the prognostic value of Bcl-2 expression in CRC.

Applications

The present study allows understanding the prognostic-predictive capability of Bcl-2 in CRC.

Peer-review

This systematic review and meta-analysis of retrospective studies adds useful information for practice and research, and probably for policy.

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