

Original Article

BRLF1 transcription activator IgG targeting Epstein-Barr virus hallmarks promising diagnostic efficacy in identification of nasopharyngeal carcinoma: a meta-analysis study

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Abstract: BRLF1 transcription activator IgG (Rta-IgG) targeting Epstein-Barr Virus (EBV) infection has been highlighted as a helpful indicator for nasopharyngeal carcinoma (NPC) diagnosis. Herein, we sought to evaluate the predictive efficacy of Rta-IgG testing for NPC identification. Electronic search was undertaken based on the platforms of online databases. STATA 12.0 and Meta-Disc 1.4 statistical programs were employed for the pooled analyses. Effects of publication bias was assessed utilizing the trim and fill adjustment method. Sixteen cohorts included 17 individual studies with 2658 NPC patients were enrolled. For the discrimination of NPC from non-NPC individuals, single testing of Rta-IgG sustained an estimated AUC (area under ROC curves) value of 0.95 (95% CI: 0.92-0.96), corresponding to a pooled sensitivity of 0.83 (95% CI: 0.78-0.87) and specificity of 0.92 (95% CI: 0.90-0.93). Deek's funnel plot asymmetry test manifested significant publication bias. After filling the estimated missing studies, the adjusted effect of unpublished studies was slightly altered as compared to that of the unadjusted ones. Single measurement of Rta-IgG confers promising diagnostic efficacy to aid in NPC identification. More studies are still warranted to reinforce our preliminary evidence.

Keywords: Epstein-Barr virus, Rta-IgG, nasopharyngeal carcinoma, diagnosis, meta-analysis

Introduction

Nasopharyngeal carcinoma (NPC) is a common malignant tumor with remarkable epidemiological features of unique geographic distribution and racial aggregation [1]. The regions as South China and South East Asia yield the incidence peaks of NPC, with an estimated incidence rate of 10-50/100000/year [2, 3]. Advanced NPC sustains high lethality and remains to be the leading cause for therapeutic failure. In this respect, early and accurate diagnosis continues to be a key approach to obtain an optimal prognosis for the NPC patients.

Epstein-Barr virus (EBV) infection is closely associated with the etiology of NPC, as the presence of high titers of anti-EBV immunoglobulins can be identified in nearly 90% of the NPC patients [2, 4]. The BRLF1 transcription activator (Rta) is encoded by the *BRLF1* gene

and serves as an immediate-early transcription factor linking to the activation of several viral promoters in EBV genome [5]. Additionally, Rta also behaves as a core factor that controls the conversion from the viral latency to the lytic replication [6]. Thus far, evidence does exist to support that elevated titer of sera Rta-IgG is strongly linked to the increased disease risk of incident NPC, and serologic testing of Rta-IgG has therefore been highlighted as a powerful screening predictor in confirming or monitoring NPC [7-22]. Importantly, the examination of such serologic parameter as Rta-IgG is relatively simple and cheap, and thus, can be developed as a routine test for health care [2]. Unfortunately, it seems that different studies may display altered even conflicting results upon Rta-IgG testing in confirming NPC. Apparently, additional medical evidence is expected to testify the diagnosing efficacy of Rta-IgG for NPC. Upon an exhaustive literature

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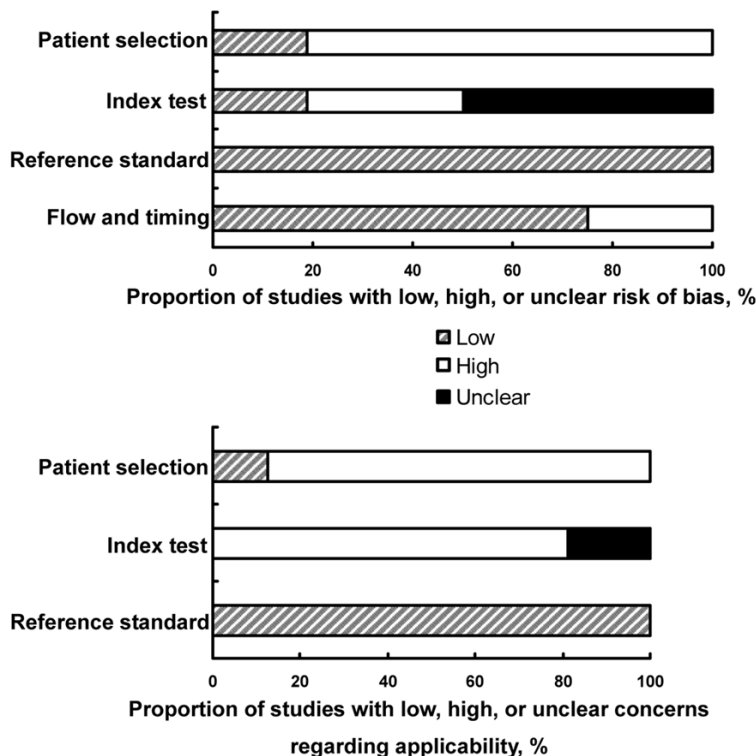
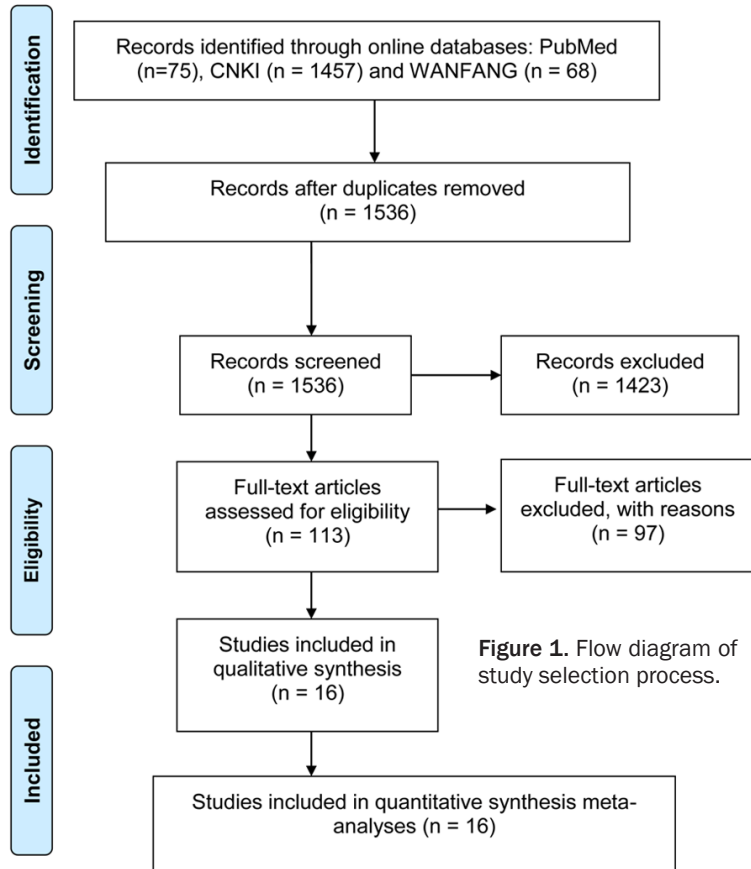


Figure 2. Article quality assessed by the QUADAS II checklist.

review, we conducted a comprehensive meta-analysis and attempted to evaluate the overall diagnostic performance of Rta-IgG testing for NPC identification.

Materials and methods

Publication screening and study selection

The meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [23]. Literature was collected based on the platforms of online PubMed, Chinese National Knowledge Infrastructure (CNKI), and WANFANG databases till June 30th 2016. The following search terms were utilized for identification of available studies without language limitation: “Rta IgG/Rta-IgG/BRLF1 transcription activator IgG”, “Nasopharyngeal Carcinoma/Nasopharyngeal cancer/NPC” and “sensitivity/specificity/diagnosis/ROC/AUC”.

Studies for enrollment should basically meet the inclusion criteria: 1) studies assessed the performance of Rta-IgG for NPC identification; 2) studies with sufficient data to construct the 2×2 table; 3) studies give a clear definition of the control sources; and 4) studies with more than 20 cases. The exclusion criteria were: 1) reported results were insufficient for construction of the 2×2 table; 2) studies failed to clearly interpret the control types; and 3) basic research, review articles, comments, letters, case reports, abstracts in conference, etc.

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Table 1. Main features of the included studies for Rta-IgG in confirming NPC

Author	Year	Area	Case/Control type	Patient/Control size	Test method	Sample type	Cut-off value	TP	FP	FN	TN
Ai et al. [9]	2013	China	NPC vs. Normal	100/60	ELISA	Serial blood	Unclear	77	5	23	55
Cai et al. [7]	2014	China	NPC vs. non-NPC	211/210	ELISA	Serum	Unclear	191	30	20	173
Du et al. [17]	2015	China	NPC vs. Normal	416/688	ELISA	Serum	YD = 0.623	355	45	61	643
Feng et al. [10]	2001	China	NPC vs. Normal	51/47	ELISA	Sera	OD = 0.2	41	4	10	43
								42	5	9	42
Li et al. [21]	2016	China	NPC vs. Normal	56/42	ELISA	Serum	> 30 U/mL	55	4	1	38
Li et al. [13]	2013	China	NPC vs. non-NPC	449/231	ELISA	Serum	S/CO ≥ 1.0	335	17	114	214
Liu et al. [8]	2012	China	NPC vs. Normal	191/197	ELISA	Serum	rOD > 1.0	80	9	111	188
Luo et al. [18]	2013	China	NPC vs. non-NPC	131/200	ELISA	Serum	A > 0.11	102	15	29	185
Qiu et al. [14]	2014	China	NPC vs. non-NPC	272/94	ELISA	Serum	S/CO ≥ 1.0	238	9	34	85
Ren et al. [20]	2006	China	NPC vs. Normal	59/59	ELISA	Serum	rA = 0.22	50	7	9	52
Tang et al. [19]	2014	China	NPC vs. Normal	150/150	ELISA	Serum	rA = 0.22	134	17	16	133
Xia et al. [11]	2015	China	NPC vs. Normal	64/120	ELISA	Serum	> 30 U/mL	48	12	16	108
Xu et al. [15]	2015	China	NPC vs. Normal	75/100	ELISA	Serum	S/CO ≥ 1.0	61	7	14	93
Xu et al. [22]	2016	China	NPC vs. Normal	67/20	ELISA	Serum	Unclear	56	4	11	16
Zhang et al. [16]	2015	China	NPC vs. no-NPC	155/10430	ELISA	Serum	OD > 0.11	127	605	28	9825
Zheng et al. [12]	2009	China	NPC vs. no-NPC	211/413	ELISA	Serum	rA = 0.49	191	41	20	372

NPC: Nasopharyngeal carcinoma, ELISA: Enzyme linked immunosorbent assay, rA: Relative A, YD: Youden index, OD: Optical density, TP: True positive, FP: False positive, FN: False negative, TN: True negative, vs: Versus.

Data extraction and article evaluation

Two reviewers independently evaluated the eligibility of studies and collected the data according to the predesigned extraction forms: the first author, year of publication, country of origin, antibody types, patient size, control size, control sources (healthy people or non-cancer cases), sample types, test method, outcomes of sensitivity and specificity, etc. For the two-stage study contains both training and validation cohorts, each group were considered to be independent. Any disagreement was resolved by group discussion.

Article quality was judged according to the quality assessment for studies of diagnostic accuracy (QUADAS) II checklist [24], in which the concerns for risk of bias and applicability were classified as “low”, “high” or “unclear”, corresponding to an evaluation score of “one”, “zero” and “zero”.

Statistical analysis

All statistical analyses were conducted based on the software of Meta-disc 1.4 (XI Cochrane Colloquium, Barcelona, Spain) and Stata 12.0 (Stata Corporation, College Station, TX, USA). Heterogeneity from the threshold effect was evaluated by the Spearman correlation coefficient,

and that from non-threshold effect was assessed by Cochran’s Q test and inconsistency index (I^2) test (significant level was set at $P < 0.05$ or $I^2 > 50\%$) [25]. In case of the existence of significant consistency, a random effect model will be applied to synthesize the ROC curve, otherwise a fixed effect model will be performed. The bivariate meta-analysis allowed for the estimation of pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR) and diagnostic odds ratio (DOR). Publication bias was examined by the Deek’s funnel plot asymmetry test and the possible effects of publication bias were evaluated by the trim and fill adjustment method [26].

Results

Article search and study quality

According to the predefined criteria, a total of 1536 potentially eligible cohorts in PubMed, CNKI and WANFANG databases were retrieved after excluding the duplicate records. The retrieved records received a detailed review of study title and abstract, and 1423 records were accordingly excluded. Then, the possible eligible studies received full text evaluation were restricted to 113, and 97 of them were not meeting the aim of this study and were eventually discarded. Finally, 16 cohorts included 17

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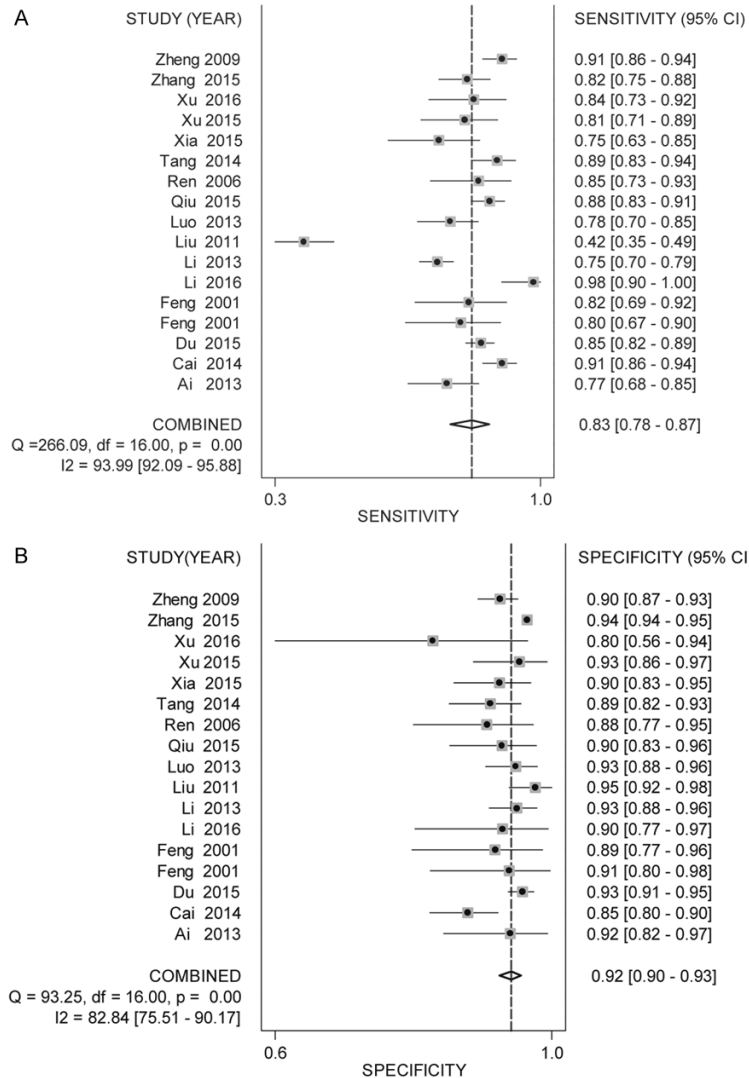


Figure 3. Forest plot of (A) pooled sensitivity and (B) specificity for the included studies.

individual studies were enrolled for this meta-analysis. Flow diagram of study selection process is described in **Figure 1**.

Article quality was judged in terms of the QUADAS II recommendations [24]. Proportion of studies with low, high, or unclear concerns regarding risk of bias and applicability are displayed in **Figure 2**.

Features of retrieved studies

Study population contained 2658 NPC patients and 13061 paired controls. The control cohorts comprise both healthy cases and non-NPC participants with nasopharynx benign diseases. The sample size of NPC and control groups

varying from 20 to 10430, and all NPC patients received a definite diagnosis through the histopathological examination. All studies were conducted in China and the publication date was from 2001 to 2016. All blood samples for testing were coagulation-treated as serum, and the measuring method of Rta-IgG was based on the enzyme linked immunosorbent assay (ELISA). The main features of enrolled studies are summarized in **Table 1**.

Heterogeneity investigation

Exploring of study heterogeneity was based on the evaluation of threshold and non-threshold effects among studies [25]. The results showed that estimated threshold effect appeared in the pooled analysis of Rta-IgG testing, with a spearman correlation coefficient value of 0.504 and *P* value of 0.039, meanwhile, the assessed non-threshold effect seemed to exist in the combined analysis as well ($Q = 34.44$, $P = 0.0047$, $I^2 = 53.5\%$), and therefore, a random effect model was chosen for the statistical analyses.

Diagnostic performance and clinical utility

For the single testing of Rta-IgG directed at EBV, the pooled sensitivity, specificity, PLR, NLR, DOR and AUC of 0.9521 were estimated to be 0.83 (95% CI: 0.78-0.87), 0.92 (95% CI: 0.90-0.93), 10.14 (95% CI: 8.53-12.05), 0.18 (95% CI: 0.14-0.24), 55.15 (95% CI: 41.23-73.77) and 0.95 (95% CI: 0.92-0.96), respectively, corresponding to a diagnostic score of 4.01 (95% CI: 3.72-4.30). Forest plots of pooled sensitivity and specificity as well as the AUC of Rta-IgG testing in confirming NPC are displayed in **Figures 3** and **4A**, respectively.

Fagan's plot manifested apparent improvements of post-test probabilities in pooled study:

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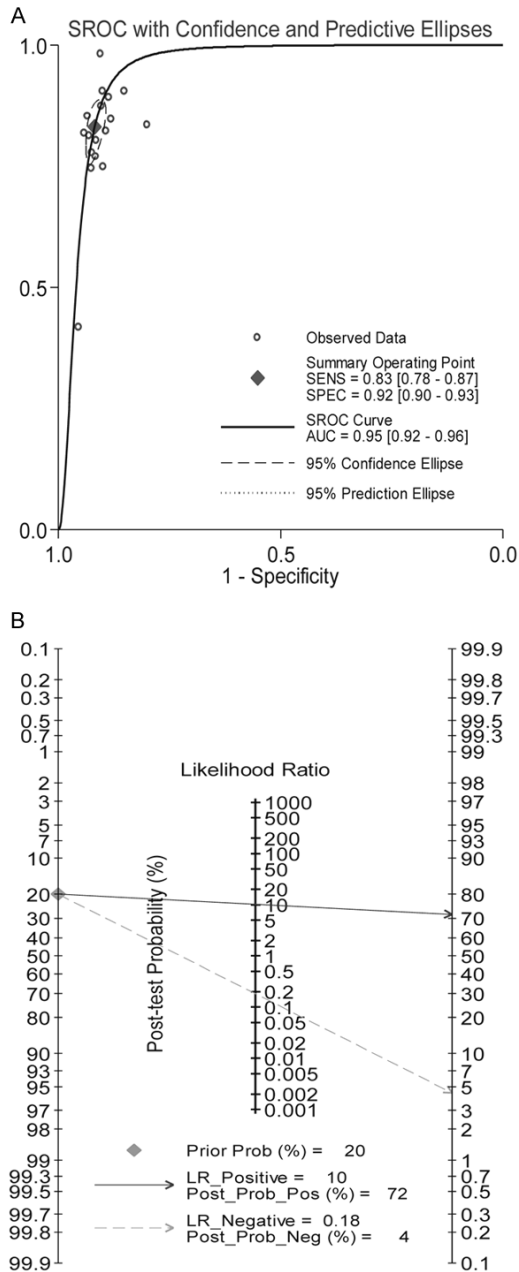


Figure 4. The estimated (A) SROC curve and (B) Fagan's plot of post-test probability.

when the pre-test probability was set to 20%, testing of Rta-IgG could significantly raise the post-test probability of positive result to 72%, and decrease the post-test probability of negative result to 4% (**Figure 4B**).

Influence analysis and meta-regression

Influence analysis was further performed to trace the sources of study heterogeneity. For

the outlier identification analyses, 4 studies for Rta-IgG detection were identified as outliers (**Figure 5**). Accordingly, the pooled diagnostic performance and heterogeneity were altered after an elimination of the outliers (sensitivity: from 0.83 to 0.86, specificity: from 0.92 to 0.90, PLR: from 10.14 to 8.60, NLR: from 0.18 to 0.16, and AUC: from 0.95 to 0.93). Importantly, the *P* value of Spearman correlation coefficient increased from 0.039 to 0.164, suggesting that threshold effect no longer existed after removing the outliers. Meanwhile, the assessed non-threshold effect seemed to be disappeared as well: *I*² dropped from 53.5% to 13.1%, and *P* value altered from 0.0047 to 0.3126.

Meta-regression test was finally conducted according to different covariates, including patient or control size, cut-off value and study quality [25]. As indicated in **Table 2**, the cut-off value likely to be a factor contributed to heterogeneity (*P* = 0.0062), whereas the control types (*P* = 0.8639), NPC size (*P* = 0.3886), control size (*P* = 0.7474), as well as article quality (*P* = 0.7429) showed low likelihood of heterogeneity sources.

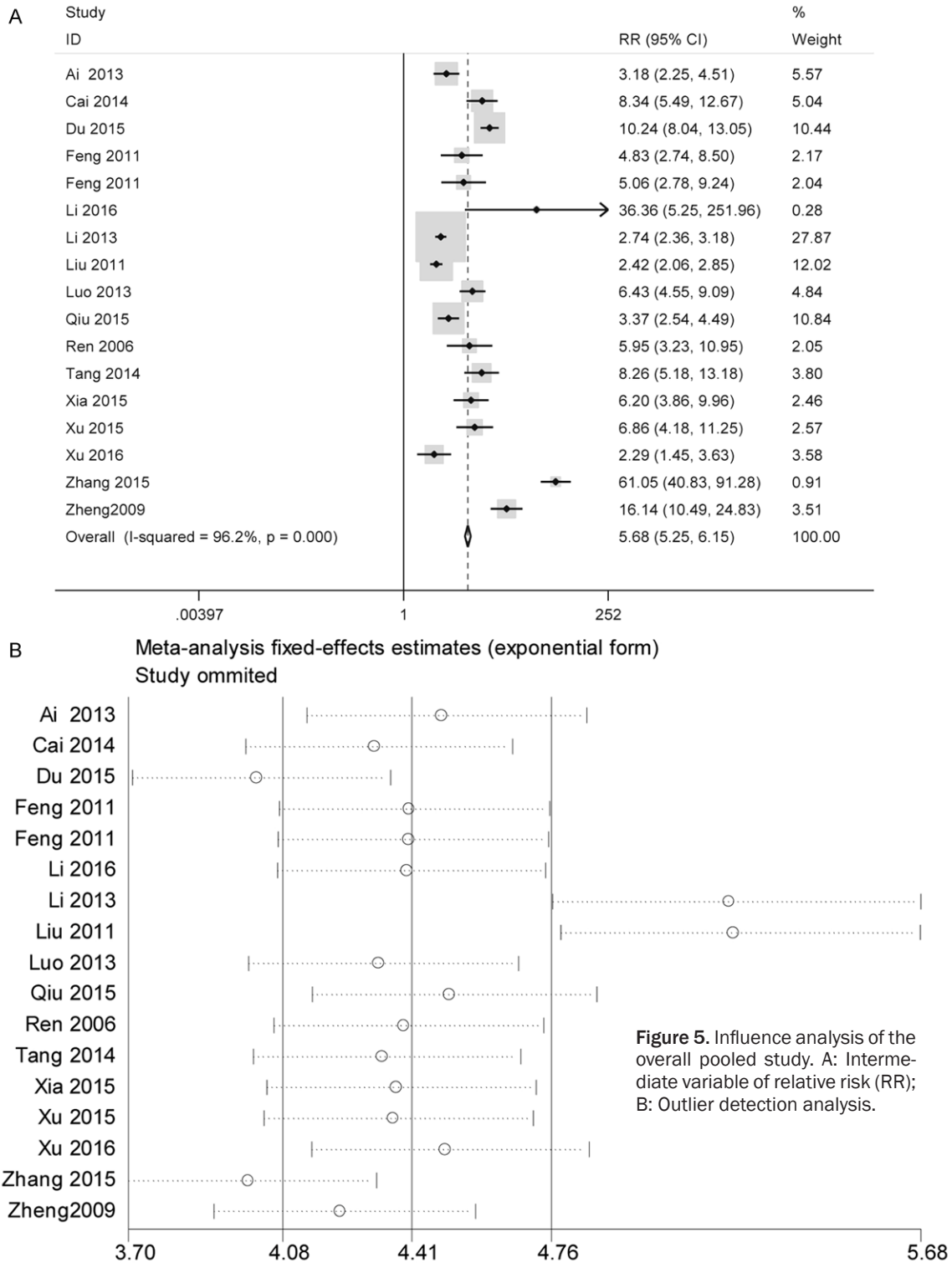
Publication bias

Publication bias was judged by the Deek's funnel plot asymmetry test, and the statistical results revealed a significant publication bias among studies, with a *P* value less than 0.05. As a result, we adjusted the estimated missing studies using the trim and fill method [26]. The linear trimming estimator with a fixed-effects model finally determined 8 missing studies for the pooled analysis of Rta-IgG (**Figure 6**). Nevertheless, the variances of adjusted studies (Variance = 0.645, *P* = 0.000) were slightly attenuated from the unadjusted ones (Variance = 1.201, *P* = 0.000).

Discussion

Epstein-Barr Virus (EBV) infection is a strong factor associated with the occurrence of nasopharyngeal carcinoma (NPC) [2, 4]. The presence of high titer of Rta-IgG against EBV infection has been highlighted as a powerful screening predictor in confirming or monitoring NPC [6-22]. Nonetheless, the diagnostic efficacy of such seromarker revealed a high volatility among studies and as yet remains a problem of

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controversy. We therefore conducted this meta-analysis and made a comprehensive comparison of the predictive value of Rta-IgG testing for NPC identification.

There have been published studies regarding single Rta-IgG detection for NPC diagnosis [6-22]. Most of the studies presented a sensitivity ranged from 0.81 to 0.85, and a specificity

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Table 2. Evaluation of the potential sources of heterogeneity by meta-regression

Study characteristic	P-value	RDOR	95% CI
Control sources (serum from healthy blood, benign disease or non-NPC participants)	0.8639	0.96	(0.59-1.56)
NPC size (NPC < 100 vs. NPC ≥ 100)	0.3886	1.26	(0.71-2.23)
Control size (Control < 100 vs. Control ≥ 100)	0.7474	1.10	(0.60-2.01)
Cut-off value (OD < 1.0 vs. OD ≥ 1.0 vs. other)	0.0062	0.69	(0.54-0.88)
Article quality (QUADAS ≤ 4 vs. QUADAS > 4)	0.7429	1.17	(0.41-3.36)

CI: Confidence interval, RDOR: Relative diagnostic odds ratio, QUADAS: Quality assessment for studies of diagnostic accuracy, NPC: Nasopharyngeal carcinoma, vs: Versus.

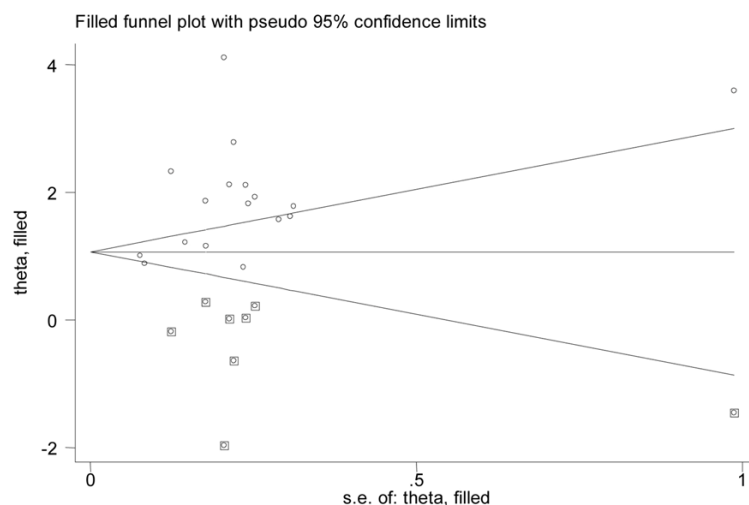


Figure 6. Funnel plot for estimated missing studies using the “trim and fill adjustment method”. Hollow cycle in box indicates the estimated missing studies.

ty ranged from 0.88 to 0.94 for Rta-IgG in confirming NPC. Nevertheless, some study showed that Rta-IgG measurement only yielded a diagnostic sensitivity of 0.77 for NPC screening [18]. Results from this meta-analysis showed that Rta-IgG sustained an overall estimated sensitivity of 0.83, specificity of 0.92 and AUC of 0.95. Notably, the diagnostic odds ratio (termed as DOR [27]) of Rta-IgG measurement was estimated to be 55.15, displaying a powerful discriminatory performance in confirming NPC. Meanwhile, a pooled PLR suggested that Rta-IgG testing retained a ratio of 10.14 between the true positive rate and false positive rate. Besides, an estimated NLR of 0.18 also indicated that laboratorial testing of EA-IgG yielded a false-negative rate of 18%, assigning to Rta-IgG testing a relatively high capability in ruling out NPC. For the clinical utility, testing of Rta-IgG could significantly raise the post-test probability of positive result to 72% and lower the post-test probability of negative result to

4% upon a setting of pre-test probability to 20%. Our analysis demonstrated that single Rta-IgG measurement is a promising and powerful indicator with a relative high efficacy in confirming NPC.

Since heterogeneity is a culprit that causes the pooled results deviated from real, the assessment of study heterogeneity in a meta-analysis is quite essential and necessary [28]. In this meta-analysis, heterogeneity from estimated threshold effect appeared in the pooled analyses of Rta-IgG, which the spearman correlation coefficient value was estimated to be 0.504, with a

P value less than 0.05. The threshold effect is mainly generated by the different cut-off value settings or thresholds used in different studies [25, 27, 28]. Indeed, the cut-off values for Rta-IgG were not uniformed among studies, which may further result in the causes of heterogeneity. On the other hand, the non-threshold effect which could be caused by different ethnicities, testing methods, or sample types [28], seemed to exist in our analyses as well (for the Cochran’s Q test, $P < 0.01$, and I^2 test, $I^2 < 50\%$). Although all studies were conducted in China and all samples were tested in sera and finally examined by ELISA, all NPC participants were not unified for their TNM stages as well as the severity of disease conditions. All these reasons may contribute to the sources of heterogeneity in our study.

We therefore performed influence analysis to trace whether the outlier is a culprit of study heterogeneity, and finally identified 4 outlier

studies. After an elimination of the outliers, the pooled sensitivity altered from 0.83 to 0.86, specificity dropped from 0.92 to 0.90, PLR decreased from 10.14 to 8.60, NLR reduced from 0.18 to 0.16, and AUC lowered from 0.95 to 0.93. The results hinted that the outlier studies act as a key factor contributing to study heterogeneity. On the other hand, an additional evaluation of heterogeneity by meta-regression showed that the diverse cut-off value setting appeared to be a contributor of study heterogeneity, whereas the control types, study size as well as article quality showed low likelihood of heterogeneity sources.

Publication bias is another latent problem which should be seriously interpreted in any meta-analyses [29]. This type of bias occurred in published academic research as articles with positive results are more likely to be published than those with negative results. We identified significant publication bias in our meta-analysis, as a result, we deeply traced its possible effects using the trim and fill adjustment method [26]. Ultimately, after filled the estimated missing studies with a random model, the adjusted effects from unpublished studies were slightly altered as compared to that from the unadjusted ones, hinting that the pooled accuracy is not subject to the bias of publication.

Taken together, our analyses evaluated the predictive value of Rta-IgG targeting EBV, in particular finding that Rta-IgG testing sustains a relatively high diagnostic efficacy for NPC identification. Nevertheless, several limitations such as obvious heterogeneity, population bias as well as complicated control sources presented in our studies. More investigations are still warranted to reinforce this preliminary evidence.

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Disclosure of conflict of interest

None.

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