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Review Article

Diagnostic value of serum P16 gene promoter methylation in gastric cancer: a meta-analysis

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Abstract: A meta-analysis was carried out to explore the clinical diagnostic value of P16 gene promoter methylation in gastric cancer using serum samples. Information was retrieved up to June 21st, 2016 from PubMed, EMBASE, Ovid (Medline), Google Scholar and CNKI. The pooled sensitivity, specificity, positive likelihood ratio (+LR) and negative likelihood ratio (-LR) were calculated by Meta-DiSc1.4, 15 articles containing 1291 cases were retrieved from databases. The median methylation rate for gastric cancer group was 30.43% (range: 10%-84.62%), the healthy control group's methylation range was 0.0% (range: 0.0%-18.18%). The methylation rate in gastric cancer was statistically higher than in the healthy control ($P < 0.05$). The sensitivity of P16 MSP in diagnosing gastric cancer was 0.487 (95% CI: 0.446-0.528), specificity was 0.989 (95% CI: 0.976-0.996), the +LR was 15.417 (95% CI: 6.303-37.709), the -LR was 0.537 (95% CI: 0.423-0.68), and the Area under the SROC Curve (AUC) was 0.9163.

Keywords: Gastric cancer, P16, promoter methylation, meta-analysis, sensitivity and specificity

Introduction

Gastric cancer (GC) is one of the most common malignanttumors in the world. Approximately 40-65% of GC patients will experience a recurrenceand die from the disease [1]. One of its main survival limits is the late detection of primary and recurrent tumors. Serum tumor markers such as CEA and CA19-9 are reliable diagnostic indicators, but they lack sensitivity and specificity [2, 3]. In contrast, serum DNA-based techniques make for a promising alternative for detecting early tumors in patients. Free DNA fragments are released from tumors into the circulation by necrosis and/or apoptosis, such fragments increase in the serum from cancer patients [4, 5]. Tumor-specific DNA alterations should be detectable in patient serum. The methylation-specific polymerase chain reaction (MSP), which is able to detect the methylation status in tumor peripheral blood is an important method for cancer detection. P16 hypermethylation has been recently reported to reach about 40% in gastric cancer [6, 7], suggesting that the detection of P16 in serum might be one of the reliable diagnostic and prognostic markers for GC.

P16, also known as CDKN2A, is a tumor suppressor protein that is capable of regulating the cell cycle negatively through the inhibition of cyclin D-CDKN4 activity [8, 9]. The inactivation of P16 breaks down the regulatory mechanism of the cell cycle and is speculated to correlate strongly with carcinogenesis [10]. Several studies have suggested that promoter DNA methylation in tumor suppressor gene P16 might be involved in the development and progression of GC [11] and might serve as a promising prognostic marker [12, 13]. A recent case-control study shows that the mutations of the P16 gene might contribute to the development of poorly differentiated gastric-esophageal adenocarcinoma [14]. However, published trials fail to provide a consistent diagnostic advantage for P16 MSP approach in GC [15].

As far as we know, there is no searchable meta-analysis that focuses on the serum level P16 MSP with GC. As a result, an overall analysis is conducted here to further evaluate the exact diagnostic value of the serum P16 promoter MSP in GC.

Materials and methods

Search strategy

A literature search via PubMed, EMBASE, Ovid (Medline), Google Scholar and CNKI databases

Table 1. Summary of the characteristics of included studies

Author	Year	QUADAS score#	TP	FP	TN	FN	Sensitivity	Specificity
Li-yue Z, et al	2014	5	11	2	57	21	34.38%	96.61%
Hiroshi K, et al	2005	9	17	0	10	46	26.98%	100.00%
Daisuke I, et al	2004	7	36	0	10	73	33.03%	100.00%
WK Leung, et al	2005	6	6	4	18	45	11.76%	81.82%
Wang Y, et al	2010	5	21	0	16	48	30.43%	100.00%
Chong Q, et al	2007	7	44	0	20	8	84.62%	100.00%
Abbaszadegan MR, et al	2008	7	14	0	50	38	26.92%	100.00%
Lee TL, et al	2002	6	28	0	30	26	51.85%	100.00%
Yasuaki K, et al	2003	8	6	0	16	54	10.00%	100.00%
Tani N, et al	2006	5	3	0	10	23	11.54%	100.00%
Sing-Huang T, et al	2007	4	2	0	10	2	50.00%	100.00%
Yi-Chen Wu, et al	2014	9	63	0	10	29	68.48%	100.00%
Koike H, et al	2004	9	9	0	10	32	21.95%	100.00%
Shi-jun L, et al	2005	5	15	0	20	29	34.09%	100.00%
Liu YH, et al	2005	6	12	0	15	72	30.43%	100.00%

#Study Quality Score; TP = True Positive; FP = False Positive; TN = True Negative; FN = False Negative.

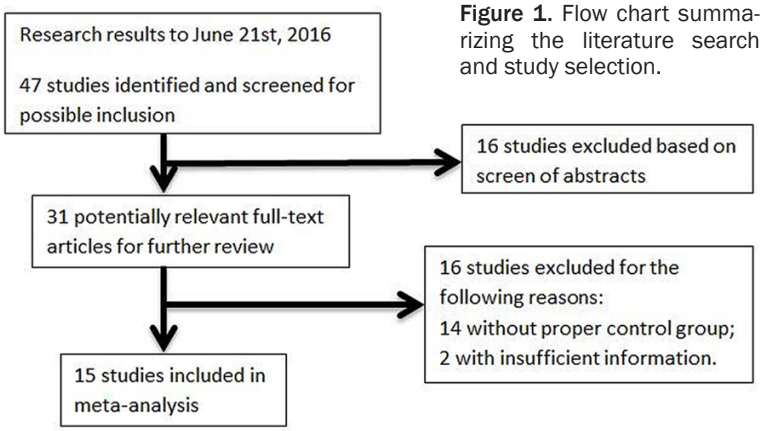

Figure 1. Flow chart summarizing the literature search and study selection.

was conducted on June 21, 2016. Articles that assess the role of serum P16 promoter methylation status in gastric cancer were retrieved. The following keywords were used for searching: (1) Gastric or stomach, and (2) Cancer or carcinoma or tumor or neoplasm or carcinogenesis, and (3) Methylation or hypermethylated or MSP, and (4) Serum or plasma or blood, and (5) P16 or CDKN2A.

The studies were random, controlled clinical trials, or prospective studies. The identified articles were supposed to provide information on the measurement of P16 methylation in the serum of gastric cancer patients and normal controls to be included in this analysis. The patients involved were supposed to be definitely diagnosed with gastric cancer without other complications, previous gastric cancer, coexisting cancer or any adjuvant therapy before serum acquisition.

Literature screening and data extraction

Two reviewers screened the titles and abstracts of the identified studies. The relevant articles were further searched for the full text and the obtained literature was reviewed based on the above-mentioned inclusion and exclusion criteria. At last, the reference lists of acquired studies were hand searched to identify the other relevant studies.

For data extraction, articles were reviewed by two independent investigators. Extracted data included the first author's name, year of publication, the number of cancer patients and healthy controls, research methodology, P16 methylation count, the number of true positive (TP), false positive (FP), false negative (FN) and true negative (TN). Standard deviations (SD) were estimated based on a formula when only a range was reported [16]: Estimate SD = Range/4 ($15 < n < 70$); Range/6 ($n > 70$). Cross-checking was made after the data extraction. The completed databases were compared and discussed by both investigators to find if the results were the same.

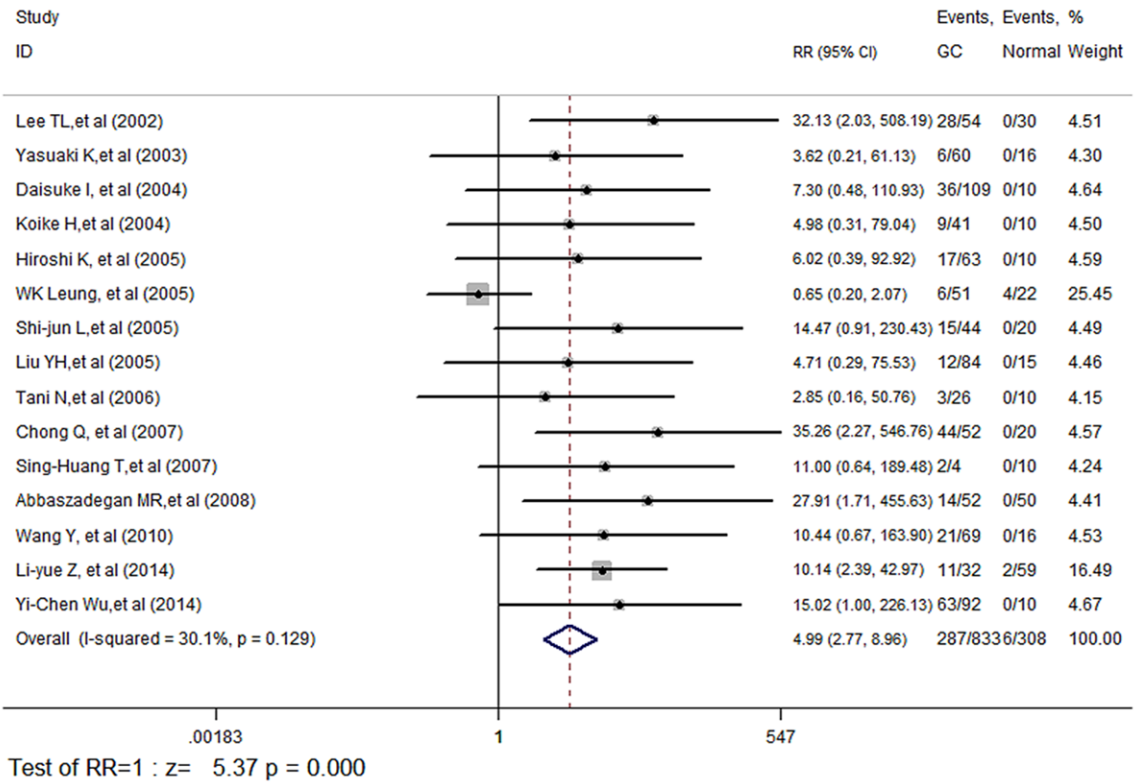

Figure 2. Meta-analysis of methylation rate in gastric cancer patients and healthy controls. The methylation rate in gastric cancer was statistically higher than in the healthy control (P < 0.001).

Study quality assessment

Quality was assessed based on the QUADAS (quality assessment of diagnostic accuracy studies) [17] criteria. Studies were awarded stars on the basis of the validity of the study design, clarity of the inclusion standard of the patients, propriety of the statistical method, and the discussion of the possible bias. The range of possible scores was 0 to 12 stars. And the result was presented in **Table 1**.

Statistical analysis

Meta-DiSc1.4 was used to do the statistical analysis. The diagnosis index of sensitivity, specificity, positive likelihood ratio (+LR) negative likelihood ratio (-LR), and area under the receiver operating characteristic (ROC) were calculated according to a random effect model or fixed effect model. The heterogeneity test and meta-analysis were conducted by Stata 12.

For categorical variables, statistical analysis was performed by using RR and 95% CI for the efficacy analysis statistics. For continuous variables, weighted mean difference (WMD) was used. The results were considered statistically significant when $P < 0.05$ and when the 95% confidence interval (CI) did not include the value 1 (for categorical variables) or 0 (for continuous variables).

Statistical heterogeneity was assessed by I^2. It was regarded as significant if $I^2 > 50\%$. If this occurred, reanalyzing the data with random effects models, then a meta-regression would be made to explore the source of heterogeneity. The publication bias was accessed by funnel plot, Egger's or Begg's test. Subgroup analyses would also be used to further assess the possible reason for heterogeneity.

Results

Selected studies

According to our defined criteria, the electronic database search retrieved 47 articles. By carefully screening the title, study type and abstracts, 16 articles were excluded for several

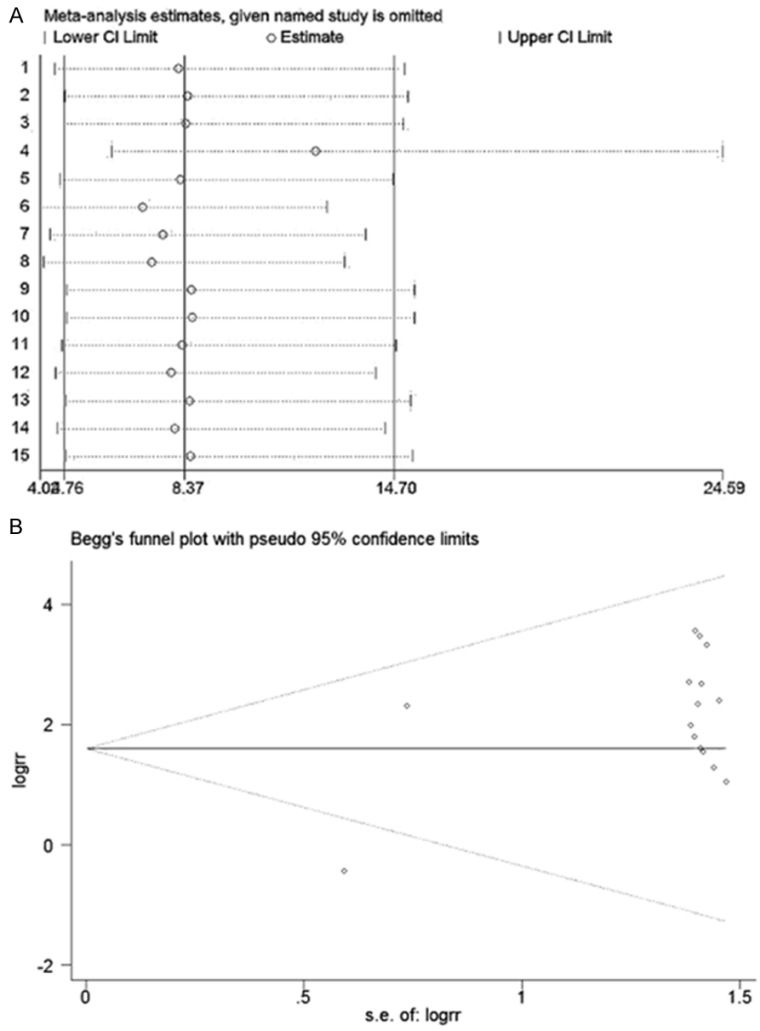

Figure 3. Sensitivity analysis (A) and funnel plots (B) of the included studies. Nopublication bias was observed in these studies.

reasons (e.g. The studied objects did not exactly match selection criteria). And after reading the full-text papers of the existing 31 articles, we finally enrolled 15 eligible studies which were published between 2002 and 2014 into our meta-analysis [18-34], Search processes were presented in **Figure 1**.

Study quality

A total of 1291 patients were enrolled in the 15 studies, in which 6 studies involved patients from mainland China, 6 from Japan, 2 from Hong Kong, and the other 2 from Singapore and Iran respectively. There were no eligible studies from America or Europe which might be due to the low incidence of gastric cancer in western countries; and that few studies focused on the relationship of serum P16 promoter methylation condition and patients with GC in America or Europe.

In the extracted database, median methylation rate for GC group was 30.43% with a range of 10%-84.62%. The median methylation rate for the healthy control group was 0.0% with a range of 0.0-18.18%. As was shown in **Figure 2**, the methylation rate in gastric cancer was statistically higher than in the healthy control ($P < 0.001$). Since there was no statistical heterogeneity between the studies ($I^2 = 30.1\%$ $P = 0.129$), the random effects model was adopted.

The results of sensitivity analysis suggested that no single study could influence the overall pooled estimates (**Figure 3A**). No obvious publication bias was observed in these studies, as we found no evidence of obvious asymmetry in the Begger's funnel plots (**Figure 3B**). Egger's test also didn't display strong statistical evidence for publication bias ($P = 0.260$).

However, with the involved studies, heterogeneity could be observed in following analysis: analysis of sensitivity ($I^2 = 90.5\%$, $P = 0.000$) and specificity ($I^2 = 43.8\%$, $P = 0.036$). Thus, random effects analysis was adopted for the above-mentioned analysis. The pooled sensitivity and specificity were 0.487 (95% CI: 0.446-0.528) and 0.989 (95% CI: 0.976-0.996) respectively (**Figure 4**).

Since heterogeneity also existed in +LR and -LR studies ($I^2 = 50.6\%$, $P = 0.013$ for +LR, and $I^2 = 90.0\%$, $P = 0.000$ for -LR), we pooled the +LR and -LR by randomized effects model as well. The pooled diagnosis +LR and -LR were 15.417 (95% CI: 6.303-37.709), and 0.537 (95% CI: 0.423-0.680) respectively (**Figure 5**).

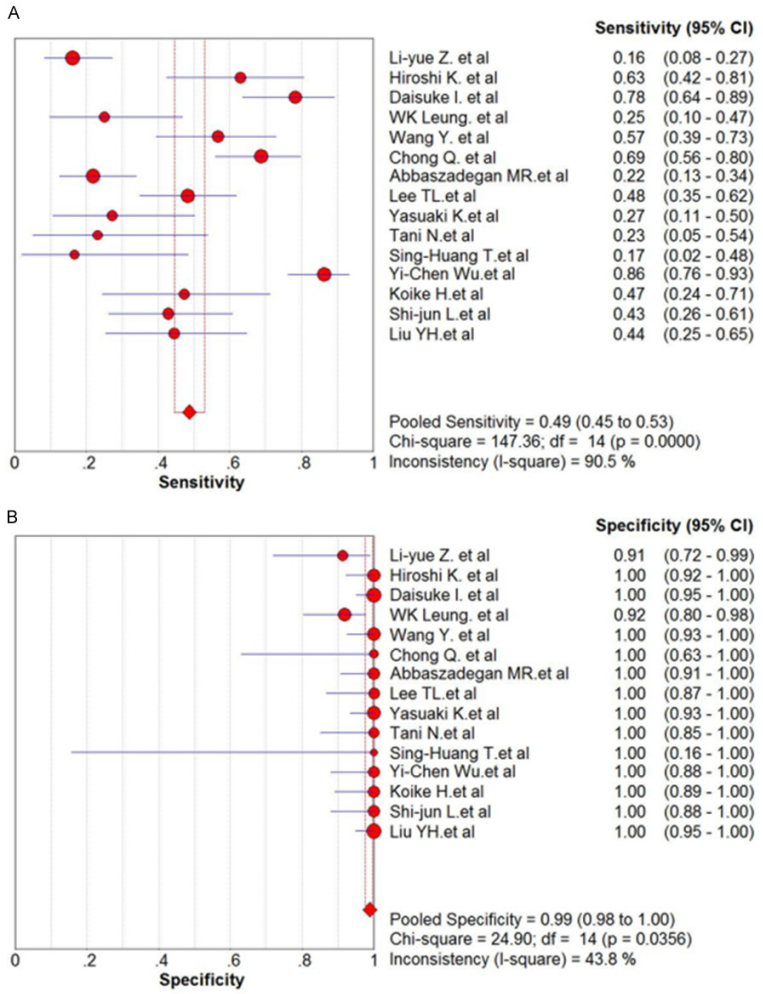

Figure 4. Forest plot of the pooled sensitivity (A) and specificity (B). The pooled sensitivity was 0.487 (95% CI: 0.446-0.528) and pooled specificity was 0.989 (95% CI: 0.976-0.996).

The diagnostic odds ratio was pooled by random effect model for its significant heterogeneity. The pooled diagnostic odds ratio was 31.665 (95% CI: 11.168-89.783) (**Figure 6**). The area under the ROC curve was 0.9163 with Q* = 0.8491 (0.0341); indicating a relatively high level of diagnostic accuracy for serum P16 gene promoter methylation.

When we used the Spearman model to study the correlation between the heterogeneity and threshold effect, we found that the correlation coefficient of P16 methylation in the diagnosis of GC was -0.472 (P = 0.075). This indicated that the heterogeneity of the study was not caused by the threshold effect.

Meta-regression & subgroup analysis

To further investigate the sources of the heterogeneity, we respectively added the publication year, region and QUADAS score to the Meta-Regression analysis. As the results showed, the heterogeneity between the studies might largely be related to the QUADAS score [RDOR = 7.92 (95% CI: 1.13-55.68)].

However, when a sub-group analysis was made on diagnostic odds ratio between the studies that QUADAS score > 6 and ≤ 6, no statistical change had occurred (Supplementary Figure 1). Other indexes showed the similar results.

It was assumed in the discussion that the testing standard and testing method (equipment) was various among institutions, which might lead to the heterogeneity.

Discussion

P16 promoter methylation is an early event in the carcinogenesis of gastric cancer [35, 36]. Promoter DNA methylation of P16 may lead to the alteration of P16 function, which may result in the uncontrolled proliferation of tumor cells and aggravate the progression of GC [37].

Our meta-analysis results revealed that the frequencies of P16 promoter methylation in the GC patients' serum were higher than those of normal controls, which suggested that promoter methylation of P16 gene might play a key role in the development of GC.

The sensitivity and specificity of serum P16 promoter methylation in the diagnosis of gastric cancer were 0.487 (95% CI: 0.446-0.528) and 0.989 (95% CI: 0.976-0.996), indicating that this method was effective enough to rule out negative cases with a diagnostic ability as well. In contrast, the sensitivity and specificity of traditional serum GC diagnosis symbols (CA50 and CEA and CA19-9) were around 40% and 74%, and even lower in early GC diagnosis.

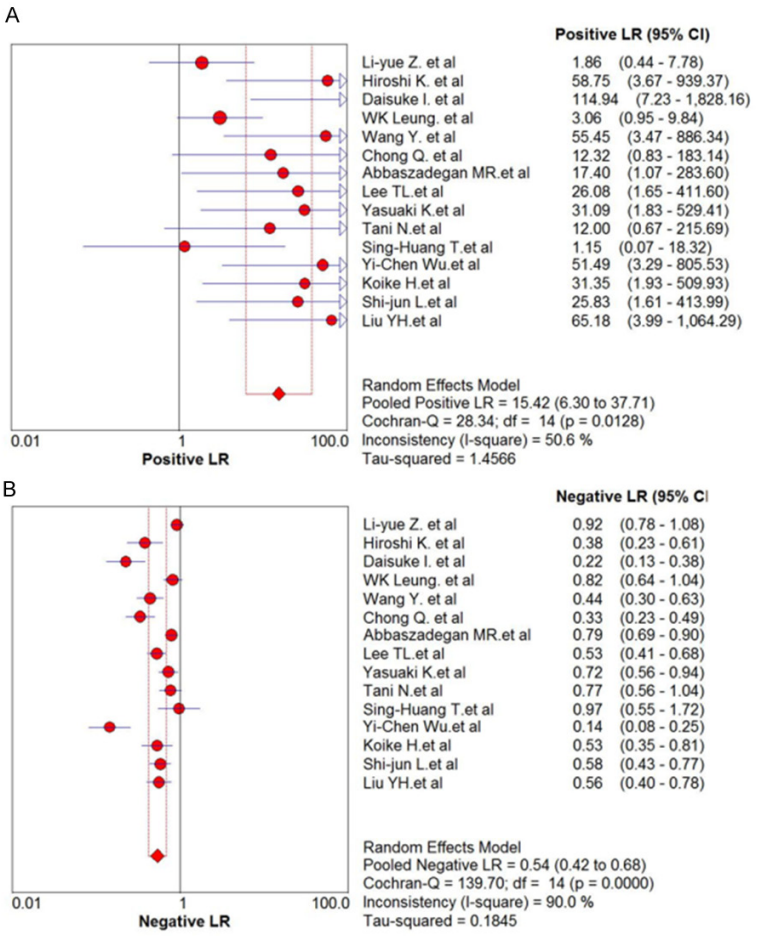

Figure 5. The forest plot of the pooled positive likelihood ratio (A) and negative likelihood ratio (B). The pooled diagnosis +LR was 15.417 (95% CI: 6.303-37.709), and -LR was 0.537 (95% CI: 0.423-0.680).

Diagnostic Odds Ratio (DOR) is a comprehensive index of sensitivity and specificity. It could reveal the accuracy of the diagnostic method independently [38]. The greater the value is, the stronger the ability to identify the method. The DOR was 31.67 (95% CI: 11.17-89.78) in this meta-analysis, indicating that the serum P16 promoter methylation can be used as a better indicator for GC diagnosis.

The positive likelihood ratio and negative likelihood ratio were 15.417 (95% CI: 6.303-37.709), and 0.537 (95% CI: 0.423-0.68). This meta-analysis showed the probability of serum P16 methylation above the threshold was 15.417 times higher in GC patients than the control group and the probability that patients appear serum P16 methylation below the threshold was 0.573 times of the control group. Although they could not indicate the possibility of the increase of gastric cancer when the serum P16 methylation was above the threshold, serum P16 methylation could still be used as a method for large-scale screening and early diagnosis when current GC diagnosis is not ideal.

The area under the SROC curve (AUC), Q (SE) and DOR value are important parameters for diagnosis [39-41]. According to the literature, the AUC that is greater than 0.97 can be an ideal method; It would be very good when between 0.96~0.93; and good when between 0.92 and 0.75; if it is less than 0.75, the method is still acceptable [40, 41] in a certain degree. In this meta-analysis, the AUC of comprehensive diagnosis was 0.9163; Q (SE) values were 0.8491 (0.0341) and DOR values were 31.67. These results showed that serum P16 methylation was of good diagnostic value for gastric cancer.

The current literature often suggests the diagnostic value be improved with combined diagnosis [42]. Thus, if we combined serum P16 methylation screen with clinical symptoms, endoscopy, X-ray barium meal, et al, it would add sensitivity and become one of the most effective methods for the diagnosis of early gastric cancer, and large-scale screening.

Our study still had some limitations. Firstly, the definition of "methylation amplified results, varies in different studies, and a review bias might exist. Secondly, meta-analysis is a retrospective study that may lead to subject selection bias, affecting the reliability of our results. Thirdly, our meta-analysis failed to fully explain the heterogeneity causes, which may limit further evaluation. At last, the inclusion criteria for cases and controls were not well defined in all included studies, which might also influence our results.

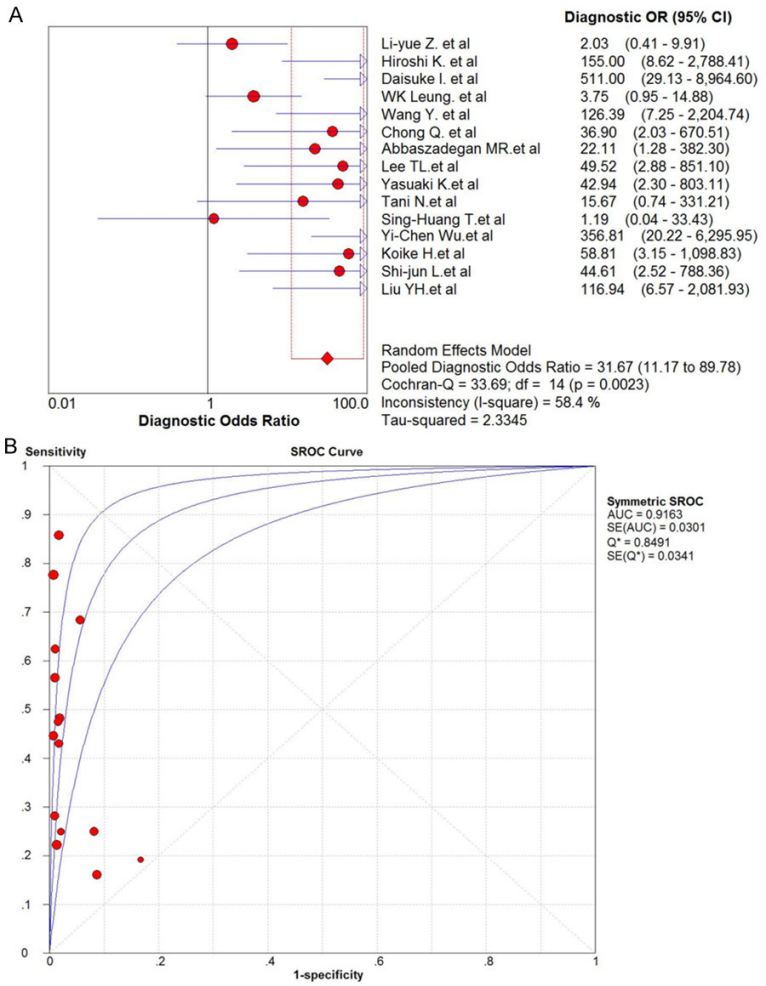

Figure 6. Diagnostic value of P16 MSP in serum. A. The forest plot of thepooled diagnostic odds ratiowas 31.665 (95% CI: 11.168-89.783). B. The area under the ROC curve was 0.9163 with Q* = 0.8491 (0.0341).

All in all, our findings were consistent with previous studies, which indicated that serum P16 promoter methylation could be a useful and promising biomarker for diagnosis of GC.

Disclosure of conflict of interest

None.

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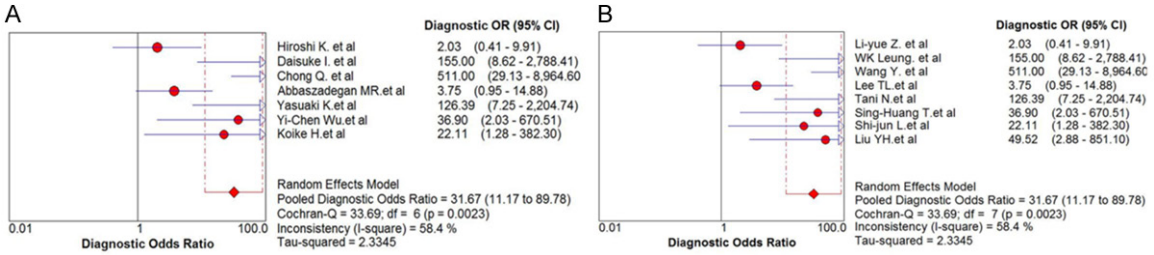

Supplementary Figure 1. The sub-group analysis of the diagnostic odds ratio. (A) QUADAS score > 6 and (B) QUADAS score ≤ 6.

Analysis of diagnostic threshold

Spearman correlation coefficient: -0.472 *p*-value = 0.075
(Logit (TPR) vs Logit (FPR)
Moses' model (D = a + bS)

Weighted regression (inverse variance)

Var	Coeff.	Std. Error	T	*p*-value
a	3.236	2.178	1.486	0.1611
b (1)	-0.051	0.523	0.098	0.9238

Tau-squared estimate = 2.1089 (Convergence is achieved after 9 iterations)
Restricted Maximum Likelihood estimation (REML)
No. studies = 15
Filter OFF
Add 1/2 to all cells of the studies with zero

Meta-regression (inverse variance weights)

Var	Coeff.	Std. Err.	*p*-value	RDOR	[95% CI]
Cte.	1.752	2.1339	0.4307	----	----
S	-0.712	0.5888	0.2543	----	----
Area	-1.770	1.1799	0.1644	0.17	(0.01; 2.36)
Score	3.309	1.2233	0.0221	27.35	(1.79; 417.55)
Year	-0.296	1.1622	0.8042	0.74	(0.06; 9.91)

Tau-squared estimate = 1.1205 (Convergence is achieved after 7 iterations)
Restricted Maximum Likelihood estimation (REML)
No. studies = 15
Filter OFF
Add 1/2 to all cells of the studies with zero

Meta-regression (inverse variance weights)

Var	Coeff.	Std. Err.	*p*-value	RDOR	[95% CI]
Cte.	1.675	2.2072	0.4639	----	----
S	-0.187	0.4892	0.7097	----	----
Score	2.080	0.9544	0.0519	8.01	(0.98; 65.42)
Year	0.282	1.1487	0.8106	1.33	(0.11; 16.62)

Tau-squared estimate = 1.3215 (Convergence is achieved after 6 iterations)
Restricted Maximum Likelihood estimation (REML)
No. studies = 15

Filter OFF
Add 1/2 to all cells of the studies with zero

Meta-regression (inverse variance weights)

Var	Coeff.	Std. Err.	*p*-value	RDOR	[95% CI]
Cte.	1.824	1.9553	0.3693	----	----
S	-0.162	0.4553	0.7276	----	----
Score	2.069	0.8953	0.0394	7.92	(1.13; 55.68)

Tau-squared estimate = 1.0691 (Convergence is achieved after 6 iterations)
Restricted Maximum Likelihood estimation (REML)
No. studies = 15
Filter OFF
Add 1/2 to all cells of the studies with zero

Meta-regression (inverse variance weights)

Var	Coeff.	Std. Err.	*p*-value	RDOR	[95% CI]
Cte.	3.221	2.5614	0.2347	----	----
S	0.000	0.6272	0.9997	----	----
Area	0.164	1.0870	0.8827	1.18	(0.11; 12.89)
Year	0.018	1.4719	0.9906	1.02	(0.04; 25.98)

Tau-squared estimate = 2.6245 (Convergence is achieved after 6 iterations)
Restricted Maximum Likelihood estimation (REML)
No. studies = 15
Filter ON (score ≤ 10)
Add 1/2 to all cells of the studies with zero

Summary sensitivity

	Study	Sen	[95% Conf. Iterval.]	TP/ (TP + FN)	TN/ (TN + FP)
Li-yue Z, et al	0.162	0.084	- 0.271	11/68	21/23
Hiroshi K, et al	0.630	0.424	-0.806	17/27	46/46
Daisuke I, et al	0.783	0.636	-0.891	36/46	73/73
WK Leung, et al	0.250	0.098	-0.467	6/24	45/49
Wang Y, et al	0.568	0.395	-0.729	21/37	48/48
Chong Q, et al	0.688	0.559	-0.798	44/64	8/8
Abbaszadegan MR, et al	0.219	0.125	-0.340	14/64	38/38
Lee TL, et al	0.483	0.350	-0.618	28/58	26/26
Yasuaki K, et al	0.273	0.107	-0.502	6/22	54/54
Tani N, et al	0.231	0.050	-0.538	3/13	23/23
Sing-Huang T, et al	0.167	0.021	-0.484	2/12	2/2
Yi-Chen Wu, et al	0.863	0.762	-0.932	63/73	29/29
Koike H, et al	0.474	0.244	-0.711	9/19	32/32
Shi-jun L, et al	0.429	0.263	-0.606	15/35	29/29
Liu YH, et al	0.444	0.255	-0.647	12/27	72/72
Pooled Sen	0.487	0.446	-0.528		

Heterogeneity chi-squared = 147.36 (d.f. = 14) P = 0.000
Inconsistency (I-square) = 90.5%
No. studies = 15
Filter OFF
Add 1/2 to all cells of the studies with zero

Summary specificity

	Study	Spe	[95% Conf. Iterval.]	TP/ (TP + FN)	TN/ (TN + FP)
Li-yue Z, et al	0.913	0.720	-0.989	11/68	21/23
Hiroshi K, et al	1.000	0.923	-1.000	17/27	46/46
Daisuke I, et al	1.000	0.951	-1.000	36/46	73/73
WK Leung, et al	0.918	0.804	-0.977	6/24	45/49
Wang Y, et al	1.000	0.926	-1.000	21/37	48/48
Chong Q, et al	1.000	0.631	-1.000	44/64	8/8
Abbaszadegan MR, et al	1.000	0.907	-1.000	14/64	38/38
Lee TL, et al	1.000	0.868	-1.000	28/58	26/26
Yasuaki K, et al	1.000	0.934	-1.000	6/22	54/54
Tani N, et al	1.000	0.852	-1.000	3/13	23/23
Sing-Huang T, et al	1.000	0.158	-1.000	2/12	2/2
Yi-Chen Wu, et al	1.000	0.881	-1.000	63/73	29/29
Koike H, et al	1.000	0.891	-1.000	9/19	32/32
Shi-jun L, et al	1.000	0.881	-1.000	15/35	29/29
Liu YH, et al	1.000	0.950	-1.000	12/27	72/72
Pooled Spe	0.989	0.976	-0.996		

Heterogeneity chi-squared = 24.90 (d.f. = 14) P = 0.036
Inconsistency (I-square) = 43.8%
No. studies = 15
Filter OFF
Add 1/2 to all cells of the studies with zero

Summary positive likelihood ratio (random effects model)

	Study	LR+	[95% Conf. Iterval.]	% Weight
Li-yue Z, et al	1.860	0.445	-7.778	10.47
Hiroshi K, et al	58.750	3.674	-939.37	6.02
Daisuke I, et al	114.94	7.226	-1828.2	6.04
WK Leung, et al	3.063	0.953	-9.839	11.50
Wang Y, et al	55.447	3.469	-886.34	6.03
Chong Q, et al	12.323	0.829	-183.14	6.21
Abbaszadegan MR, et al	17.400	1.068	-283.60	5.98
Lee TL, et al	26.085	1.653	-411.60	6.06
Yasuaki K, et al	31.087	1.825	-529.41	5.87
Tani N, et al	12.000	0.668	-215.69	5.74
Sing-Huang T, et al	1.154	0.073	-18.316	6.04
Yi-Chen Wu, et al	51.486	3.291	-805.53	6.08
Koike H, et al	31.350	1.927	-509.93	5.98
Shi-jun L, et al	25.833	1.612	-413.99	6.02
Liu YH, et al	65.179	3.992	-1064.3	5.97
(REM) pooled LR+	15.417	6.303	-37.709	

Heterogeneity chi-squared = 28.34 (d.f. = 14) P = 0.013
Inconsistency (I-square) = 50.6%
Estimate of between-study variance (Tau-squared) = 1.4566
No. studies = 15
Filter OFF
Add 1/2 to all cells of the studies with zero

Summary negative likelihood ratio (random effects model)

	Study	LR-	[95% Conf. Iterval.]	% Weight
Li-yue Z, et al	0.918	0.779	-1.081	7.62
Hiroshi K, et al	0.379	0.235	-0.612	5.97
Daisuke I, et al	0.225	0.132	-0.383	5.64
WK Leung, et al	0.817	0.639	-1.044	7.29
Wang Y, et al	0.439	0.305	-0.631	6.66
Chong Q, et al	0.334	0.226	-0.494	6.50
Abbaszadegan MR, et al	0.787	0.688	-0.901	7.71
Lee TL, et al	0.527	0.409	-0.678	7.26
Yasuaki K, et al	0.724	0.559	-0.937	7.23
Tani N, et al	0.766	0.563	-1.042	6.97
Sing-Huang T, et al	0.969	0.547	-1.716	5.41
Yi-Chen Wu, et al	0.144	0.082	-0.253	5.47
Koike H, et al	0.533	0.351	-0.811	6.34
Shi-jun L, et al	0.579	0.434	-0.772	7.08
Liu YH, et al	0.557	0.399	-0.778	6.84
(REM) pooled LR-	0.537	0.423	-0.680	

Heterogeneity chi-squared = 139.70 (d.f. = 14) P = 0.000
Inconsistency (I-square) = 90.0%
Estimate of between-study variance (Tau-squared) = 0.1845
No. studies = 15
Filter OFF
Add 1/2 to all cells of the studies with zero

Summary diagnostic odds ratio (random effects model)

	Study	DOR	[95% Conf. Iterval.]	% Weight
Li-yue Z, et al	2.026	0.414	-9.912	9.45
Hiroshi K, et al	155.00	8.161	-2788.4	6.27
Daisuke I, et al	511.00	29.128	-8964.6	6.32
WK Leung, et al	3.750	0.945	-14.879	9.99
Wang Y, et al	126.39	7.246	-2204.7	6.34
Chong Q, et al	36.902	2.031	-670.51	6.25
Abbaszadegan MR, et al	22.109	1.279	-382.30	6.35
Lee TL, et al	49.525	2.882	-851.10	6.37
Yasuaki K, et al	42.939	2.296	-803.11	6.19
Tani N, et al	15.667	0.741	-331.21	5.94
Sing-Huang T, et al	1.190	0.042	-33.426	5.41
Yi-Chen Wu, et al	356.81	20.221	-6295.9	6.31
Koike H, et al	58.810	3.148	-1098.8	6.19
Shi-jun L, et al	44.610	2.524	-788.36	6.31
Liu YH, et al	116.94	6.568	-2081.9	6.29
(REM) pooled DOR	31.665	11.168	-89.783	

Heterogeneity chi-squared = 33.69 (d.f. = 14) P = 0.002
Inconsistency (I-square) = 58.4%
Estimate of between-study variance (Tau-squared) = 2.3345
No. studies = 15
Filter ON (score ≤ 10)
Add 1/2 to all cells of the studies with zero

Meta-analysis

	Study	RR	[95% Conf. Interval]	% Weight
1	10.141	2.393	42.967	16.49
2	6.016	0.389	92.919	4.59
3	7.300	0.480	110.931	4.64
4	0.647	0.202	2.069	25.45
5	10.443	0.665	163.896	4.53
6	35.264	2.274	546.757	4.57
7	27.906	1.709	455.632	4.41
8	32.127	2.031	508.189	4.51
9	3.623	0.215	61.133	4.30
10	2.852	0.160	50.756	4.15
11	11.000	0.639	189.482	4.24
12	15.022	0.998	226.128	4.67
13	4.976	0.313	79.037	4.50
14	14.467	0.908	230.433	4.49
15	4.706	0.293	75.535	4.46
I-V pooled RR	4.986		8.961	100.00

Heterogeneity chi-squared = 20.02 (d.f. = 14) P = 0.129
I-squared (variation in RR attributable to heterogeneity) = 30.1%
Test of RR = 1: z = 5.37 P = 0.000

Begg's test

adj. Kendall's Score (P-Q) = -19
Std. Dev. of Score = 20.21
Number of Studies = 15
z = -0.94
Pr > |z| = 0.347
z = 0.89 (continuity corrected)
Pr > |z| = 0.373 (continuity corrected)

Egger's test

	Std_Eff	Coef.	Std. Err.	t	P > |t|	[95% Conf. Interval]
Slope	-.9817515	.8344077	-1.18	0.260	-2.78438	.8208768
Bias	2.365757	.720236	3.28	0.06	.8097822	3.9211733