Thoracic Cancer

Open Access

Thoracic Cancer ISSN 1759-7706

ORIGINAL ARTICLE

APC gene promoter aberrant methylation in serum as a biomarker for breast cancer diagnosis: A meta-analysis

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Keywords
APC gene; breast cancer; meta-analysis; methylation; promoter.

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Received: 23 October 2017;
Accepted: 22 November 2017.

doi: 10.1111/1759-7714.12580

Thoracic Cancer **9** (2018) 284–290

Abstract

Background: The aim of this study was to evaluate the clinical efficacy of *APC* gene promoter methylation in serum as a biomarker for breast cancer (BC) diagnosis.

Methods: Two reviewers systematically searched online resources to identify the publications relevant to *APC* gene promoter methylation and BC. The data of true positive, false positive, false negative, and true negative were extracted from each included study and pooled for diagnostic sensitivity, specificity, and summary receiver operating characteristic curve.

Results: Twelve studies finally fulfilled the inclusion criteria and were included in this meta-analysis. The diagnostic sensitivity, specificity, positive and negative likelihood ratio, diagnostic odds ratio, and area under the receiver operating characteristic curve were 0.20 (95% confidence interval [CI] 0.17–0.23), 0.96 (95% CI 0.93–0.97), 3.69 (95% CI 1.60–8.50), 0.83 (95% CI 0.75–0.92), 4.58 (95% CI 1.85–11.37) and 0.80, respectively. A Deeks' funnel plot and Egger's line regression test (t = 1.43, P = 0.18) indicated no publication bias was present.

Conclusion: Because of low sensitivity, *APC* gene promoter methylation in serum was not suitable for BC screening. However, as specificity was very high, detection of serum *APC* gene promoter methylation could be used as tool to confirm BC.

Introduction

Breast cancer (BC) is the most diagnosed malignant carcinomas in women worldwide. In China, BC is one of the leading causes of cancer-related death,[1,2] and significantly affects the health and quality of life of women.[3] Generally, the prognosis of advanced BC is poor; however, the prognosis of early stage BC is good, with a high five-year survival rate.[4] Therefore, early detection or screening for BC in high-risk subjects is important to improve the general prognosis of this disease. There is some evidence in the literature that aberrant methylation of cancer-related genes can be detected in the peripheral blood or serum in patients with malignant carcinomas. By contrast, aberrant methylation of cancer-related genes rarely occurs in healthy subjects. This indicates that detecting cancer-related gene aberrant methylation in serum may be a clinically feasible method for cancer diagnosis or screening.

According to previously published studies, aberrant methylation of adenomatous *APC* is usually found in cancer tissue samples of BC patients compared to normal control tissue.[5,6] However, whether the methylation pattern in the serum or blood of BC patients and healthy controls differs is not clear. In the present study, we evaluated the aberrant methylation pattern of the *APC* gene in the serum or blood of BC patients and controls by meta-analysis of published data to determine the clinical applicability of *APC* gene promoter methylation as a biomarker for BC diagnosis.

Methods

Study identification

Two reviewers systematically searched PubMed, Web of Science, the Cochrane Library, Embase, Medline, Chinese Biomedical Literature, and Chinese National Knowledge Infrastructure using the words "breast cancer," "breast neoplasm," "mammary carcinoma," "adenomatous

polyposis coli," "APC," "methylation," and "hypermethylation" for publications related to *APC* gene promoter methylation and BC. The publication search was limited to human studies and the language restricted to English and Chinese.

Inclusion and exclusion criteria

The inclusion criteria were: (i) BC patients with confirmed pathology; (ii) methylation of *APC* gene distribution in the serum of BC patients and control data could be extracted or calculated from the original study; (iii) methylation detection methods were correct; and (iv) English and Chinese language publications. The exclusion criteria were: (i) review or case report studies; (ii) studies without sufficient data, such as the *APC* gene promoter methylation rate could not be extracted or calculated from the original study; (iii) duplicated publications; and (iv) methylation had been detected in cancer tissue instead of in serum or blood. Twelve studies were finally included in this meta-analysis.

Data extraction

Two reviewers independently extracted the main data from each study. In case of disagreement, a third reviewer was consulted for consensus. General information, including study type, first and corresponding author names, year of publication, methylation detection method, patient ethnicity, and *APC* gene methylation frequency in BC and control patients, were extracted from all included studies.

Statistical analysis

MetaDiSc 1.4 (http://www.hrc.es/investigacion/metadisc_en.htm) and Stata/SE 11.0 (StataCorp LP, http://www.stata.com) statistical software were applied for data analysis. Statistical heterogeneity from the 12 studies was assessed by I^2 test.[7] Random-effect (DerSimonian-Laird method) or fixed-effect methods were used to pool the data according to heterogeneity. A Deeks' funnel plot and Egger's line regression test were used to detect publication bias. Diagnostic sensitivity and specificity were calculated using the following equations: sensitivity = true positive/(true positive + false negative); specificity = true negative/(true negative + false positive).

Results

Main study characteristics

Twelve publications relevant to *APC* gene promoter methylation and BC were identified and included in this study.[5,6,8–17]

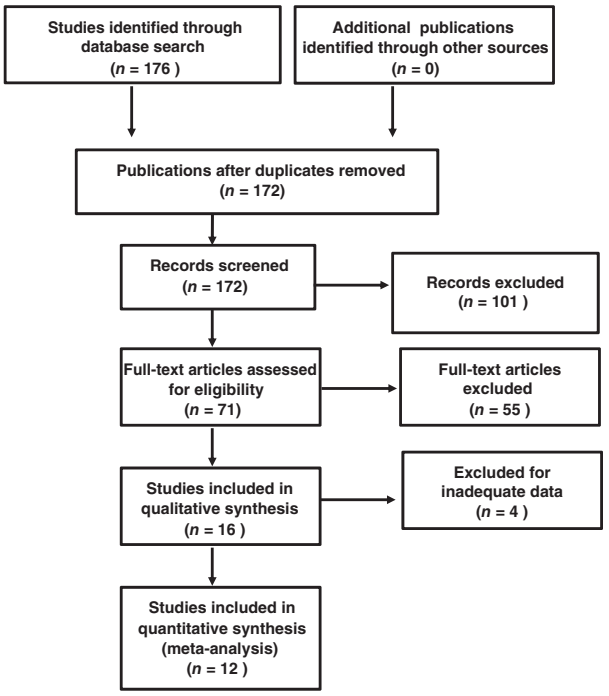

Figure 1 Study screening and inclusion flowchart.

The search process is shown in Figure 1. The ethnicity of the patients in the 12 studies was Caucasian (8), East Asian (3), and African (1). Six studies used methylation-specific PCR (MSP) assay as the *APC* gene promoter methylation detection method, four used quantitative MSP, one used MethyLight, and one used methylation-sensitive high-resolution melting (MS-HRM). The main characteristics of the 12 included studies are shown in Table 1.

Meta-analysis

Pooled sensitivity

Sensitivity was pooled using a random-effect model because of significant statistical heterogeneity (I^2 = 77.1%). The pooled sensitivity was 0.20 (95% confidence interval [CI] 0.17–0.23) for *APC* gene promoter methylation in serum as a biomarker for BC diagnosis (Fig 2).

Pooled specificity

Significant statistical heterogeneity was found regarding the specificity effect size (I^2 = 61.8%). The data was pooled using a random-effect model with the combined specificity of 0.96 (CI 0.93–0.97) for *APC* gene promoter methylation in serum as a biomarker for BC diagnosis (Fig 3).

Pooled positive likelihood ratio

The data was pooled using a fixed-effect model as no statistical heterogeneity existed between the included studies

Table 1 Main characteristics of the included studies

Author	Year	Region	Race	Distribution				Methods
				tp	fp	fn	tn	
Müller *et al.*[8]	2003	Austria	Caucasian	6	0	20	10	MSP
Rykova *et al.*[5]	2004	Russia	Caucasian	4	0	6	6	MSP
Dulaimi *et al.*[6]	2004	US	Caucasian	10	0	24	20	MSP
Taback *et al.*[10]	2006	US	Caucasian	1	0	32	10	qMSP
Hoque *et al.*[9]	2006	West Africa	African	8	0	39	38	qMSP
Zhang *et al.*[11]	2007	China	Asian	28	0	56	10	MSP
Prasad *et al.*[12]	2008	India	Asian	11	0	39	50	MSP
Van der Auwera *et al.*[13]	2009	Belgium	Caucasian	15	1	63	18	qMSP
Brooks *et al.*[15]	2010	US	Caucasian	1	6	49	142	qMSP
Jing *et al.*[14]	2010	China	Asian	14	0	36	50	MSP
Matuschek *et al.*[16]	2010	Germany	Caucasian	25	2	60	20	MethyLight
Wojdacz *et al.*[17]	2011	Denmark	Caucasian	24	13	156	95	MS-HRM

MSP, methylation-specific PCR; qMSP, quantitative methylation specific PCR.

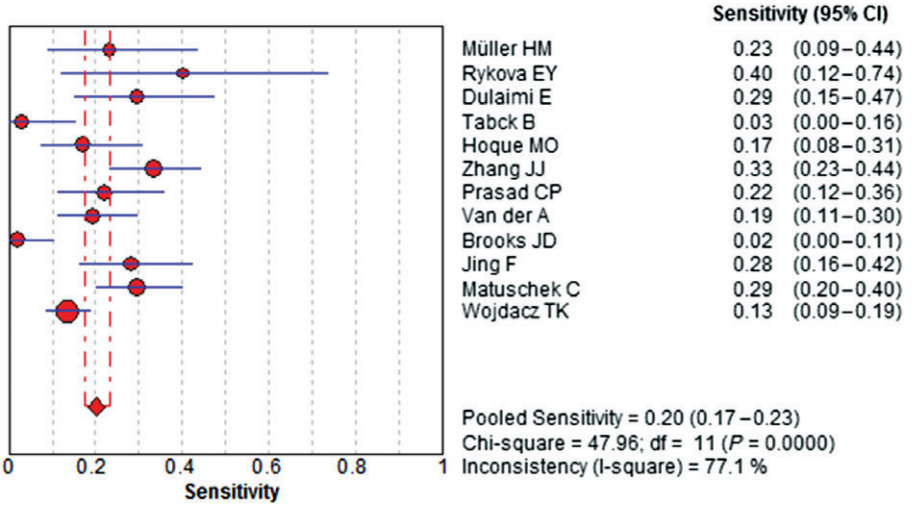

Figure 2 Forest plot of sensitivity for *APC* gene promoter methylation in serum as a biomarker for breast cancer diagnosis. CI, confidence interval.

(I^2 = 47.9%). The pooled positive likelihood ratio (+LR) was 3.69 (95% CI 1.60–8.50) (Fig 4).

Pooled negative likelihood ratio

The negative likelihood ratio (–LR) was pooled by random effect model because of significant statistical heterogeneity across the studies (I^2 = 83.3%). The pooled –LR was 0.83 (95% CI 0.75–0.92) (Fig 5).

Pooled diagnostic odds ratio

The diagnostic odds ratio (Dor) was pooled using a random-effect model for statistical heterogeneity (I^2 = 50%). The pooled Dor was 4.58 (95% CI 1.85–11.37) (Fig 6).

Summary receiver operating characteristic curve

The summary receiver operating characteristic (SROC) curve was synthesized using Stata version 11.0 (StataCorp, College Station, TX, USA). The area under the curve (AUC) of the SROC was 0.80 (Fig 7).

Subgroup analysis

The diagnostic parameters were calculated according to ethnicity and methylation detection method. Subgroup analysis for diagnostic sensitivity, specificity, +LR, –LR, Dor, and AUC are demonstrated in Table 2.

3.4Publication bias analysis

A Deeks' funnel plot and Egger's line regression test (t = 1.43, P = 0.18) were used to evaluate publication bias. No publication bias was found (Fig 8).[18]

Discussion

Breast cancer is one of the leading causes of cancer-related death worldwide. In 2013 in the United States (US),

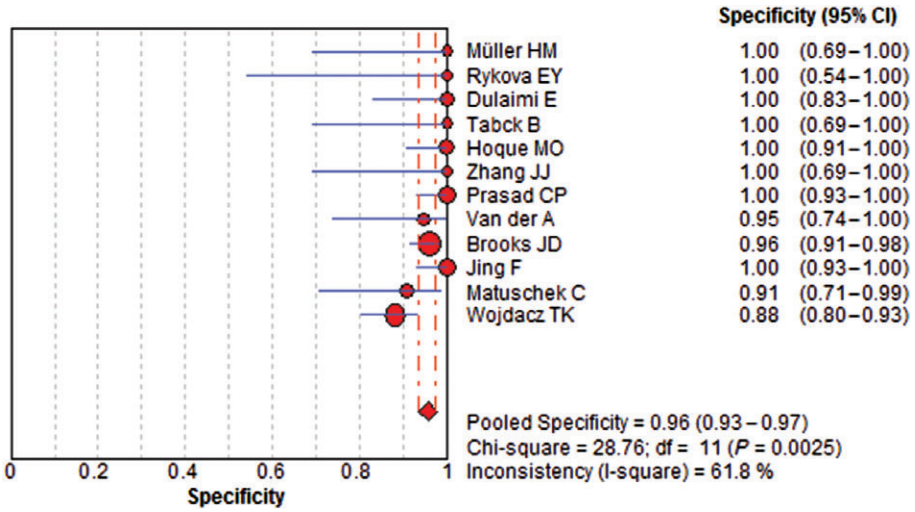

Figure 3 Forest plot of specificity for *APC* gene promoter methylation in serum as a biomarker for breast cancer diagnosis. CI, confidence interval.

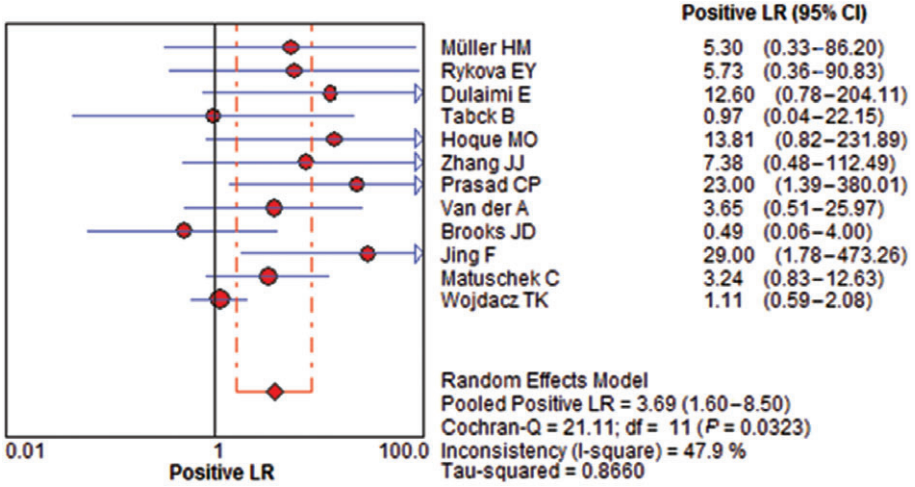

Figure 4 Forest plot of positive likelihood ratio (LR) for *APC* gene promoter methylation in serum as a biomarker for breast cancer diagnosis. CI, confidence interval.

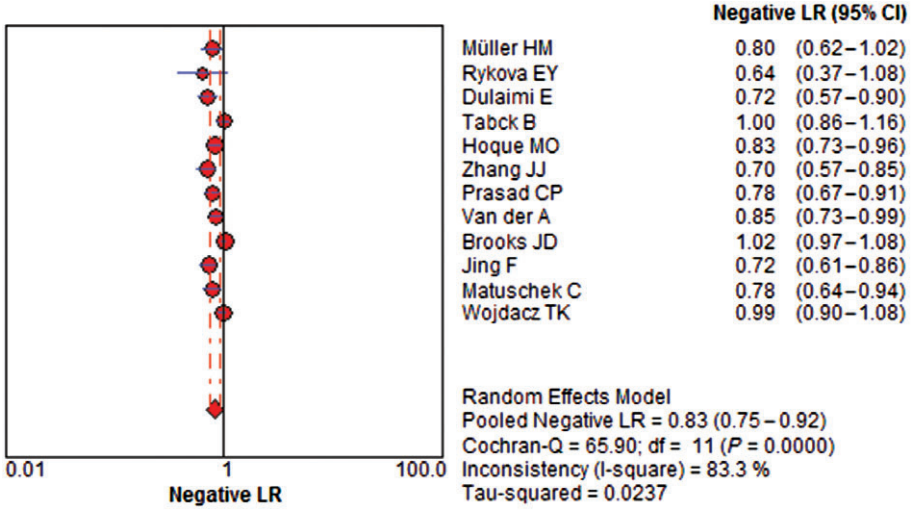

Figure 5 Forest plot of negative likelihood ratio (LR) for *APC* gene promoter methylation in serum as biomarker for breast cancer diagnosis. CI, confidence interval.

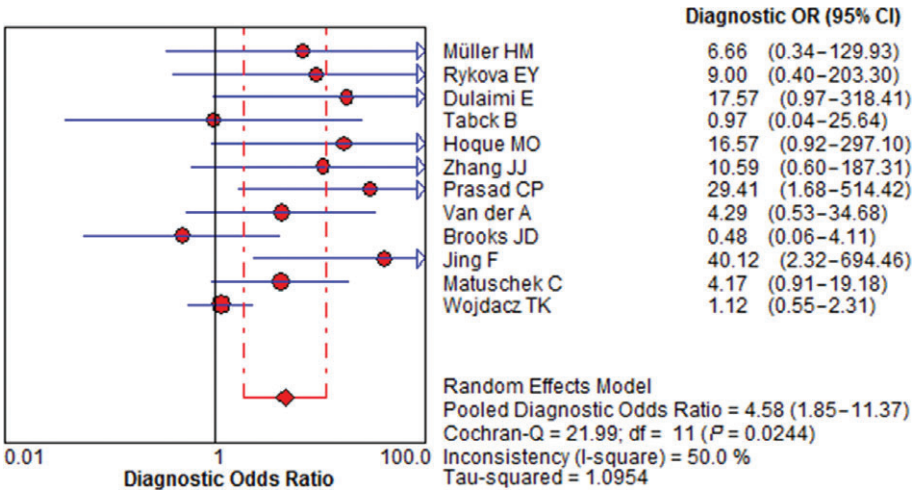

Figure 6 The forest plot of diagnostic odds ratio (Dor) for *APC* gene promoter methylation in serum as biomarker for breast cancer diagnosis. CI, confidence interval.

234 580 new cases were diagnosed and 40 030 patients died as a result of BC.[19] BC is the most commonly diagnosed malignant carcinoma in women and the second highest cause of cancer-related death in the US. Previous publications have demonstrated that BC screening through mammography can significantly improve prognosis by identifying early stage patients.[20,21] However, with relatively high false positive rates, this screening method frequently leads to overdiagnosis. Other biomarkers for BC diagnosis or screening, such as CA15-3 and CA 27-29 levels exhibit the same problem.

Whole genome hypomethylation and tumor suppressor gene promoter hypermethylation is correlated with cancer development and is believed to be a hallmark of many malignant carcinomas.[22] Similar changes are found in blood derived DNA, which suggests the possibility that blood-based DNA methylation markers could serve as new screening or early diagnosis methods.[23,24] Recently, studies have also found that aberrant methylation of cancer-related genes can be detected in the peripheral blood or serum in patients with malignant carcinomas.[15,16] However, in healthy or non-cancerous subjects, aberrant methylation is rarely detected in the serum or blood. This indicates that the detection of aberrant serum methylation may represent a potential biomarker for BC diagnosis or screening.

APC, located on the long arm of chromosome 5 between positions 21 and 22 is a well-characterized typical tumor suppressor gene. The promoter of the *APC* gene is aberrantly methylated in many malignant carcinomas, including BC. Many previously published studies have reported that the aberrant methylation pattern changes in the blood or serum of BC patients and discussed the clinical applicability for screening or diagnosis.[25–28] However, the findings are inconsistent as a result of different inclusion or exclusion criteria, small sample sizes, and different methylation

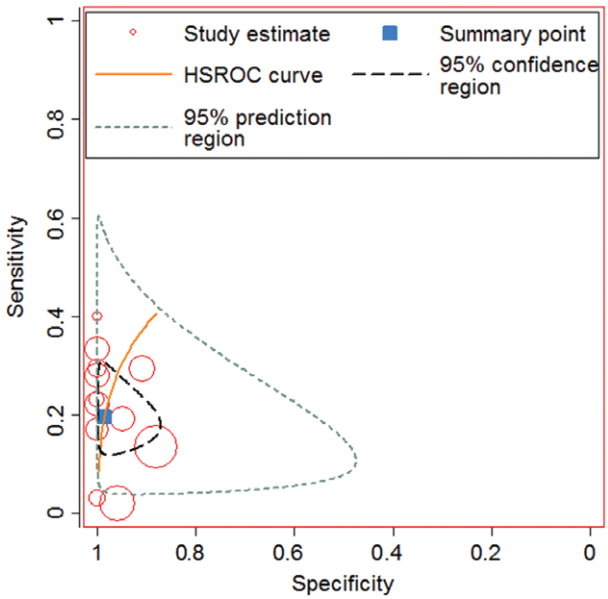

Figure 7 Area under the curve of the summary receiver operating characteristic curve for *APC* gene promoter methylation in serum as a biomarker for breast cancer diagnosis. HSROC, hierarchical summary receiver operating characteristic.

detection methods. Therefore, we screened published studies related to *APC* gene promoter methylation in serum or blood as a biomarker for BC diagnosis and conducted a meta-analysis to further evaluate its clinical usefulness. We found that pooled diagnostic sensitivity, specificity, +LR, –LR, Dor, and area under the ROC curve were 0.20 (95% CI 0.17–0.23), 0.96 (95% CI 0.93–0.97), 3.69 (95% CI 1.60–8.50), 0.83 (95% CI 0.75–0.92), 4.58 (95% CI 1.85–11.37), and 0.80, respectively. The sensitivity was very low at 0.20 (95% CI 0.17–0.23), indicating that the false negative results were high. A high false negative rate will

Table 2 Subgroup analysis according to ethnicity and methylation detection methods (95% CI)

Subgroup	Diagnostic parameters					
	Sensitivity	Specificity	+LR	–LR	Dor	AUC
Total	0.2 (0.17–0.23)	0.96 (0.93–0.97)	3.69 (1.60–8.50)	0.83 (0.75–0.92)	4.58 (1.85–11.37)	0.80
Ethnicity						
East-Asia	0.29 (0.22–0.36)	1.00 (0.97–1.00)	16.76 (3.38–83.10)	0.74 (0.67–0.82)	23.28 (4.46–121.51)	0.55
Caucasus	0.17 (0.14–0.21)	0.94 (0.90–0.96)	1.90 (0.97–3.72)	0.88 (0.79–0.98)	2.28 (1.02–5.09)	0.87
Method						
MSP	0.29 (0.23–0.35)	1.00 (0.98–1.00)	11.03 (3.55–34.28)	0.74 (0.68–0.81)	15.78 (4.78–52.05)	0.54
qMSP	0.12 (0.08–0.17)	0.97 (0.93–0.99)	2.07 (0.49–8.80)	0.93 (0.82–1.06)	2.25 (0.47–10.74)	0.98

+LR, positive likelihood ratio; –LR, negative LR; AUC, area under the curve; CI, confidence interval; Dor, diagnostic odds ratio; MSP, methylation-specific PCR; qMSP, quantitative methylation specific PCR.

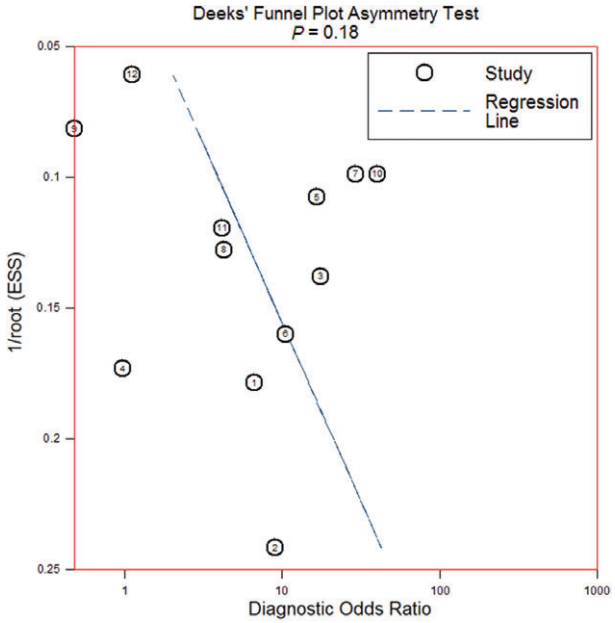

Figure 8 Deeks' funnel plot for evaluation of publication bias. ESS, effective sample size.

lead to a high misdiagnosis rate, thus this method cannot be used as screening biomarker for BC. However, the diagnostic specificity of *APC* gene promoter methylation for BC was very high, which indicated that detection of serum *APC* gene promoter methylation could be used as tool to confirm BC diagnosis.

In conclusion, according to the present evidence, *APC* gene promoter methylation detection has limited applicability for BC screening, but a low false positive rate of APC gene promoter methylation indicates a BC diagnosis and could thus be used as a confirmation assay. However, as the clinical and statistical heterogeneity of the included studies may reduce the reliability of our results, further investigation using well-designed prospective diagnostic studies is required.

Disclosure

No authors report any conflict of interest.

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