Research Article

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CK20 mRNA expression in serum as a biomarker for colorectal cancer diagnosis: A meta-analysis

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Abstract: The aim of this study was to evaluate the diagnostic value of serumCK20 mRNA as a biomarker for colorectal cancer diagnosis by meta-analysis.

Clinical studies related to serum CK20 mRNA expression for colorectal cancer diagnosis were searched in the databases of Pubmed, Cochrane Library, Embase, ISI Web of Knowledge, CNKI and Wanfang. The number of true positive (tp), false positive (fp), false negative (fn) and true negative (tn) of the original included publications were extracted by two reviewers independently. The diagnostic sensitivity, specificity, positive likely hood ratio (+LR), negative likelyhood ratio (-LR), diagnostic odds ratio (DOR) and area under the symmetric ROC curve (AUC) were pooled by random or fixed effect method according to the statistical heterogeneity among the studies.

After screening the databases, nineteen publications met the inclusion criteria and were finally included in this meta-analysis. The diagnostic sensitivity and specificity were pooled by random effect model(I2>50%). The pooled diagnostic sensitivity and specificity of CK20 mRNA in serum as biomarker for colorectal cancer were 0.49 (95% CI:0.46 to 0.51) and 0.94 (95%CI:0.92-0.96) respectively. The pooled +LR and −LR were 10.90 (95%CI:5.78 to 20.55) and 0.51 (95%CI:0.45 to 0.57) respectively by random-effect method. The pooled DOR was 22.31 with the 95% CI of 11.65 to 42.71. The pooled area under the ROC curve (AUC) was 0.72for CK20 mRNA in serum as a biomarker for colorectal cancer diagnosis. Conclusion Serum CK20 mRNA expression was significantly elevated in colorectal cancer patients which could be a promising serum biomarker for colorectal cancer diagnosis with high specificity.

Keywords: Colorectal cancer; CK20; Biomarker; Diagnosis; Meta-analysis.

1 Introduction

Colorectal cancer accounting for 1,000,000 new cases per-year is one of the most diagnosed malignant carcinoma world-wide [1]. Its incidence ranks the third just behind lung cancer and breast cancer. In China, colorectal cancer is one of the leading causes of cancer related death with an incidence rate of 13.6/100,000 for males and 9.2/100,000 for females which is still on the rise [2]. The prognosis of colorectal cancer is relatively good for early stage patients. However, the long-term survival rate is very low for advanced metastatic disease. Therefore, early diagnosis is important for improving the survival of patients with colorectal cancer. Serum biomarkers for colorectal cancer screening or diagnosis is generally used clinically such as CEA, CA199 and *et al* [3, 4]. Cytokertation 20 (CK20) protein is widely used as a serum biomarker for cancer screening or diagnosis. However, the efficacy of using the serum level of CK20mRNA as a biomarker for colorectal cancer diagnosis is not clear according to the previously published studies with small sample sizes. Therefore, we

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performed this meta-analysis to further evaluate the diagnostic value of CK20mRNA level in serum for colorectal cancer.

2 Material and methods

2.1 Studies selection

Clinical studies related to serum CK20 mRNA for colorectal cancer diagnosis were searched in the databases of Pubmed, Cochrane Library, Embase, ISI Web of Knowledge, CNKI, and Wanfang. The "colorectal cancer/CRC'', "colon cancer", "rectal cancer", "colorectal carcinoma", "colorectal neoplasm", "colorectal tumor", "colon carcinoma", "rectal carcinoma", "colon neoplasm", " rectal neoplasm", "colon tumor", "rectal tumor", "CK20", "cytokertation 20" were used as the MeSH and free text words when searching the databases. The inclusion criteria were: colorectal cancer patients with histology or cytology conformation; the CK20 mRNA expression in serum was detected by real-time quantitative PCR (RT-qPCR) or other confirmed methods; the patients distribution of true positive(tp), false positive(fp), false negative(fn) and true negative(tn) in colorectal patients and controls could be drawn from the original included publications. All potential relevant studies were assessed in details, and all citations of the included articles were further evaluated in order to identify additional suitable studies. The publication searching was done by two reviewers independently and cross checked.

2.2 Data extraction from the original studies

The data and main information of each include publications were extracted by two authors independently and checked by the third reviewer. The sample size, first and corresponding authors, number of subjects distributed to tp, fp, fn and tn were carefully extracted from the included publications.

2.3 Statistical analysis

The statistical analysis was done by MetaDiSc1.4 (http://www.biomedsearch.com) software. Chi-square and I^2 test were used to calculate the statistical heterogeneity among the included studies. Fixed or random effect method was used to pool the sensitivity, specificity, +LR, -LR, and DOR according to the statistical heterogeneity results. The publication bias was evaluated by funnel plot and Egger's line regression test.

3 Results

3.1 General character of the included 19 publications

After searching the related electronic databases, 19 studies [5-23] met the inclusion criteria and were finally included in this meta-analysis. The publication searching procedure is demonstrated in Figure 1. The publication year ranges from 1999 to 2012. Serum CK20 mRNA was examined by RT-qPCR and FQ-PCR assay in 15 and 4 studies respectively. The general information of the included 19 publications is demonstrated in Table 1.

3.2 Pooled sensitivity

Due to significant statistical heterogeneity across the studies, the diagnostic sensitivity was pooled by random effect model (I^2=85.6%). The pooled diagnostic sensitivity of CK20 mRNA in serum as a biomarker for colorectal cancer was 0.49 with the 95% CI of 0.46 to 0.51 through random effect method, Figure 2.

3.3 Pooled specificity

Statistical significance was also found in diagnostic specificity (I^2=82.2%). The data was pooled by random effect method. The pooled specificity was 0.94 with the 95%CI of 0.92-0.96, Figure 3.

3.4 Pooled +LR and –LR

Significant heterogeneity was found in +LR and –LR (I^2=74.9% and 81.9%). The pooled +LR and –LR were 10.90 (95%CI:5.78 to 20.55) and 0.51 (95%CI:0.45 to 0.57) respectively for serum CK20 mRNA in the diagnosis of colorectal cancer, Figure 4.

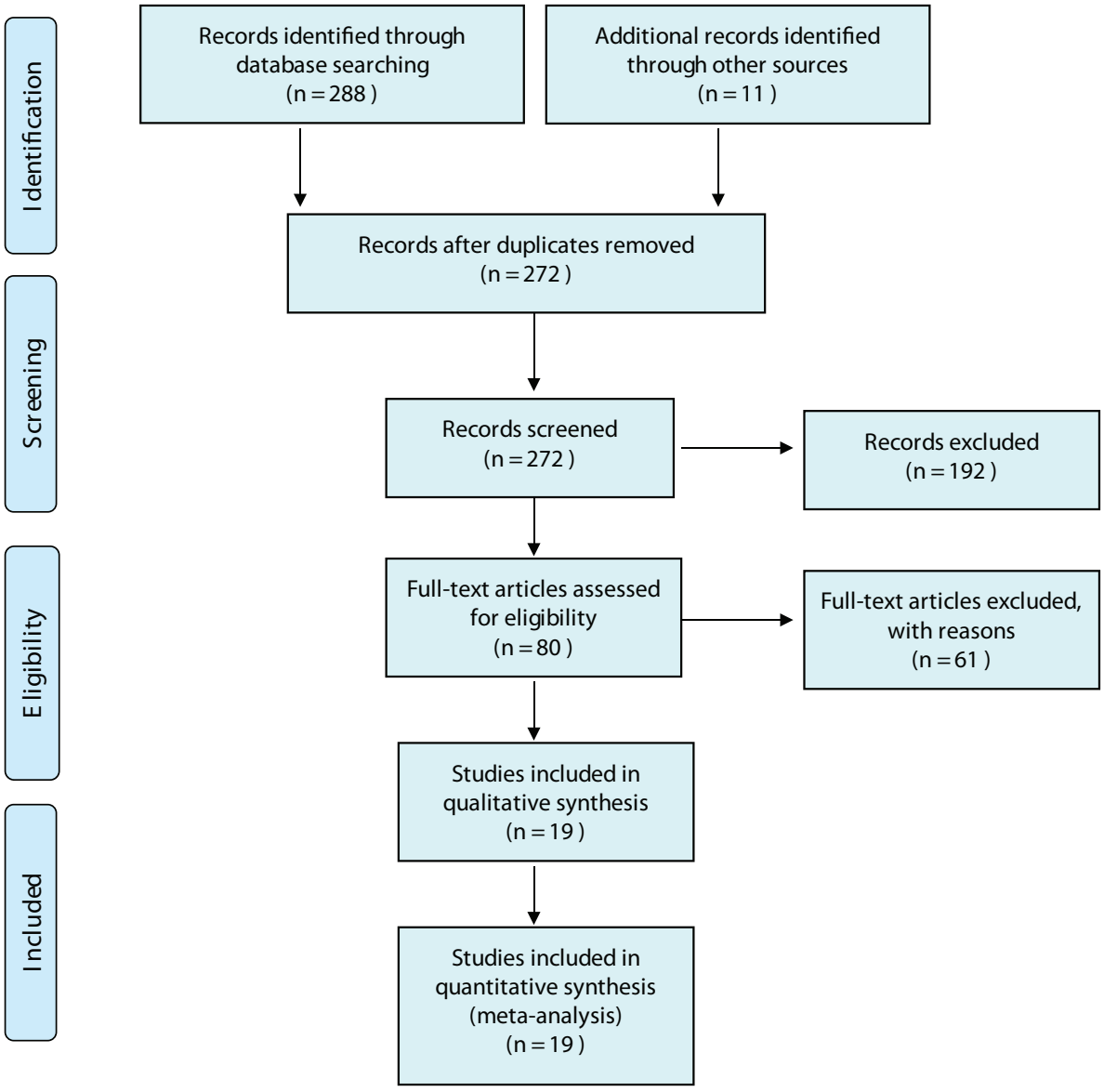

Figure 1: The publication searching flow chart

3.5 Pooled DOR

With statistical heterogeneity, the DOR was calculated by random effect method (I^2=64.2%). The pooled DOR was 22.31 with the 95% CI of 11.65 to 42.71, Figure 5.

3.6 Pooled ROC

The pooled area under the ROC curve was 0.72 for CK20 mRNA in serum as a biomarker for colorectal cancer, Figure 6.

3.7 Subgroup analysis

We performed subgroup analysis according to control type and CK20 mRNA detection methods. The pooled sensitivity, specificity, +LR, -LR, DOR and AUC of the population based control group, hospital based control group, RT-qPCR group and FQ-PCR group are shown in Table 2. The diagnostic value changed a little for different subgroup analysis.

Table 1: The general characters of the included 19 publications

Author	Year	Sample size	Distribution				Control type	Country	Methods
			TP	FP	FN	TN			
Richard Q[5]	1999	170	35	1	65	69	Population based	Britain	RT-qPCR
Chausovsky G[6]	1999	57	15	0	20	22	Population based	Israel	RT-qPCR
Lin GL[7]	2002	67	23	0	24	20	Mixed	China	RT-qPCR
Sun JW[8]	2004	62	19	0	23	20	Population based	China	RT-qPCR
Cui M[9]	2004	122	62	0	40	20	Population based	China	RT-qPCR
Zeng QG[10]	2004	89	41	6	12	30	Hospital based	China	RT-qPCR
Yin HZ[11]	2005	57	34	0	13	10	Population based	China	FQ-PCR
Yin HZ[11]	2005	57	34	0	13	10	Hospital based	China	FQ-PCR
Dandachi N[17]	2005	134	46	17	36	35	Mixed	Austria	RT-qPCR
Guo J[18]	2005	50	31	0	9	10	Hospital based	China	FQ-PCR
Xu D[19]	2006	198	46	2	122	28	Population based	China	RT-qPCR
Katsumata K[20]	2006	54	18	0	22	14	Population based	Japan	RT-qPCR
Wang ZC[12]	2007	211	35	0	101	73	Mixed	China	FQ-PCR
Shen C[21]	2008	281	74	21	82	104	Mixed	China	RT-qPCR
Lagoudianakis EE[22]	2009	58	28	0	14	14	Population based	Greece	RT-qPCR
Wong SC[23]	2009	342	62	3	70	207	Mixed	China	FQ-PCR
Wu F[13]	2009	142	44	0	48	50	Population based	China	RT-qPCR
Chen P[14]	2009	56	18	0	18	20	Population based	China	RT-qPCR
Chen P[14]	2009	57	18	1	18	10	Hospital based	China	RT-qPCR
He Q[15]	2010	151	49	0	52	50	Population based	China	RT-qPCR
Li LH[16]	2012	110	48	3	32	27	Hospital based	China	RT-qPCR
Li LH[16]	2012	110	48	0	32	30	Population based	China	RT-qPCR

3.8 Publication bias

The Deeks' funnel plot showed no significant publication bias in the aspect of serum CK20 mRNA as a biomarker for colorectal cancer diagnosis (t=0.56, P=0.58), Figure 7.

4 Discussion

Colorectal cancer, also known as bowel cancer and colon cancer, is the development of cancer from the colon or rectum. It is one of the leading causes of cancer related death world-wide [24, 25]. Population based colorectal cancer screening for high risk patients is a key method for identifying early stage patients and improving the prognosis. The National Comprehensive Cancer Network (NCCN) colorectal cancer guideline recommends fecal occult blood tests plus colonoscopy for colorectal cancer screening [26, 27]. Colonoscopy examination for colorectal cancer diagnosis has high specificity and sensitivity. However, colonoscopy examination is mildly invasive, expensive, and a complex operation procedure, which

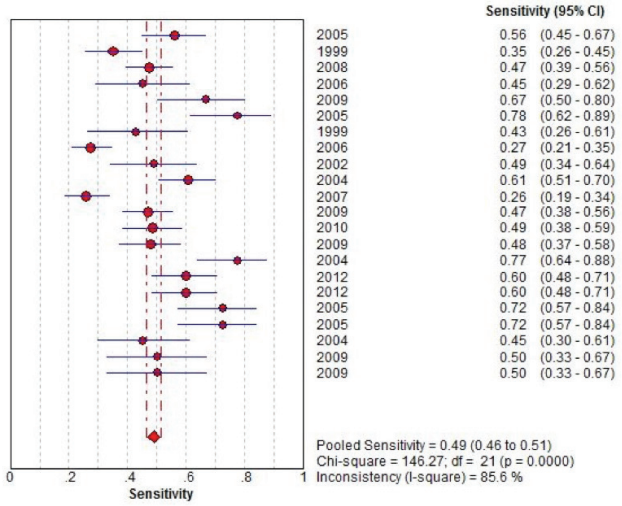

Figure 2: The forest plot of diagnostic sensitivity for serum CK20 mRNA in diagnosis of colorectal cancer

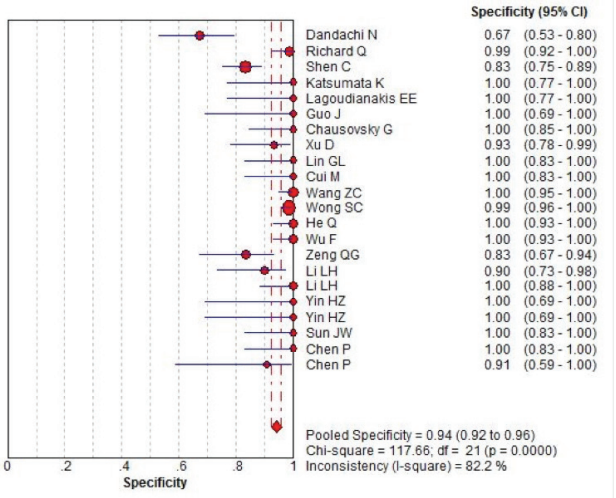

Figure 3: The forest plot of diagnostic specificity for serum CK20 mRNA in diagnosis of colorectal cancer

is not suitable for population based screening. It was believed that the ideal colorectal cancer screening or early diagnosis method should be of high sensitivity, high specificity, non-invasive, and easy to perform. Serum biomarkers are easy to access, non-invasive, less expensive and easy to test, which makes them suitable for colorectal cancer screening or diagnosis [28].

CK20 is a member of the intermediate filament protein family and a prominent component of the intestinal epithelium. CK20 expression is confined to astrointestinal epithelium, urothelium, and Merkel cells of the epidermis, as well as malignancies that originate from the aforementioned sites [29, 30]. CK20 mRNA expression in serum detected by RT-qPCR was widely investigated for colorectal cancer screening. For most studies published in the previ-

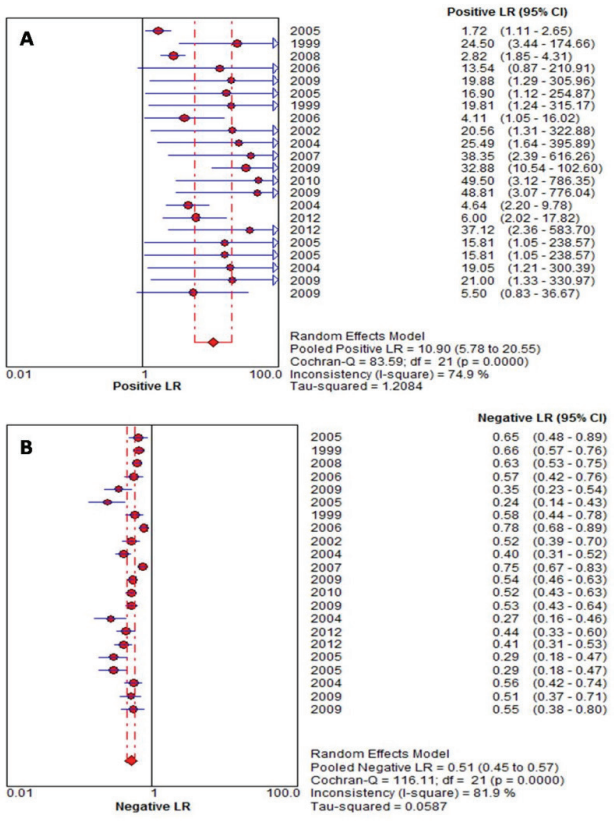

Figure 4: The forest plot of diagnostic +LR and -LR for serum CK20 mRNA in diagnosis of colorectal cancer

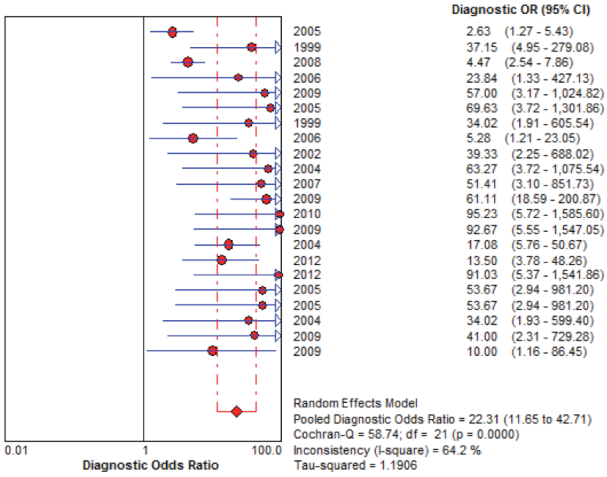

Figure 5: The forest plot of DOR for serum CK20 mRNA in diagnosis of colorectal cancer.

ous years, CK20 mRNA was elevated in colorectal cancer patients compared to healthy controls or benign colorectal disease patients. However, its clinical value as a biomarker for colorectal cancer diagnosis is not clear for different study design. And, because of small sample size for

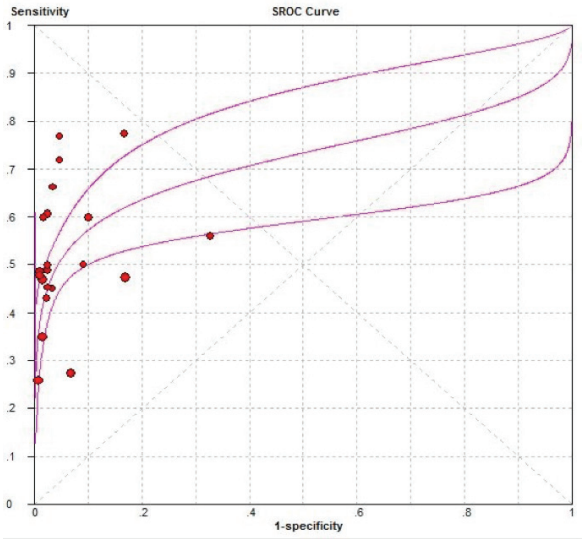

Figure 6: ROC cure of CK20 mRNA in serum as biomarker for colorectal cancer diagnosis.

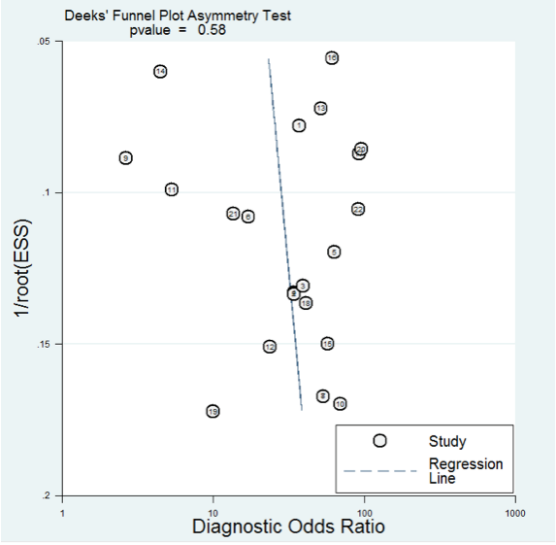

Figure 7: Publication bias evaluated by Deeks' funnel plot

Table 2: Subgroup analysis according to the control type and CK20 mRNA detection methods

Factors	Sen(95%CI)	Spe(95%CI)	+LR(95%CI)	-LR(95%CI)	DOR(95%CI)	AUC
Control type						
Population based	0.47(0.44-0.50)	0.99(0.98-1.00)	15.51(7.81-30.83)	0.51(0.44-0.60)	29.37(14.30-60.34)	0.86
Hospital based	0.67(0.61-0.73)	0.90(0.82-0.95)	5.61(3.21-9.82)	0.36(0.26-0.49)	17.44(8.46-35.94)	0.88
Methods						
RT-qPCR	0.49(0.46-0.52)	0.92(0.89-0.94)	8.30(4.37-15.60)	0.53(0.47-0.60)	16.60(8.42-32.75)	0.71
FQ-PCR	0.49(0.44-0.54)	0.99(0.77-1.00)	26.77(11.17-64.15)	0.41(0.27-0.61)	59.21(23.64-148.29)	0.95

each individual study, the results were inconclusive and clinical value was limited. To further assess the diagnostic value of serum CK20 mRNA as a biomarker for colorectal cancer diagnosis or screening, we searched the open published studies related to CK20 mRNA and colorectal cancer diagnosis and performed this meta-analysis. We found that CK20 mRNA almost cannot be detected in the serum of healthy people or benign colorectal disease patients. This made the false positive rate very low. However, the positive rate of serum CK20 mRNA in colorectal patients was also relative low. This made the serum CK20 mRNA a high specificity and low sensitivity biomarker for colorectal cancer diagnosis. Due to the low sensitivity (0.49, 95%CI:0.46-0.51), serum CK20 mRNA is not suitable for colorectal screening especially for the population based trials. However, a very high specificity (0.94, 95%CI: of 0.92-0.96), means that serum CK20 mRNA can be used as a confirmation test for colorectal cancer diagnosis.

Conflicts of interest: The authors declare no conflicts of interest regarding the publication of this manuscript.

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