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Diagnostic and Prognostic Value of Serum Interleukin-6 in Colorectal Cancer

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Abstract: The application of serum interleukin-6 (IL-6) in the diagnosis and prognosis of colorectal cancer (CRC) has been evaluated in many studies, whereas the results were contradictive.

The aim of this study was to systematically evaluate this issue.

An original study was conducted to explore the diagnostic value of serum IL-6 in CRC. Pubmed, Embase, and Cochrane library databases were searched for eligible studies.

For diagnostic meta-analysis, aggregate data (AD) and individual participant data (IPD) meta-analyses were both adopted. The sensitivity and specificity were pooled and a summary receiver-operating characteristic (ROC) curve was constructed. For prognostic meta-analysis, study-specific hazard ratios (HRs) of IL-6 for survival were summarized. Secondary analysis of survival data was performed to synthesize the Kaplan–Meier curves.

Total 17 studies (including our study) were included in this meta-analysis. The pooled sensitivity, specificity, and area under curve (AUC) of serum IL-6 were 0.72 (95% CI: 0.46–0.88), 0.74 (95% CI: 0.56–0.86), and 0.79 (95% CI: 0.75–0.82) in CRC diagnosis, respectively. Further, IPD meta-analysis strengthened the diagnostic value of serum IL-6 (the AUC, sensitivity, and specificity were 0.794, 0.606, and 0.839, respectively). For prognostic analysis, the high serum level of IL-6 was inversely associated with overall survival (OS) (pooled HR = 1.76, 95% CI: 1.42–2.19, $P < 0.001$) and disease-free survival (DFS) (pooled HR = 2.97, 95% CI: 1.76–5.01, $P < 0.001$). The synthesized Kaplan–Meier curves indicated that CRC patients with higher serum IL-6 level had a worse OS ($P = 0.0027$) and DFS ($P < 0.001$), which further support the prognostic value of serum IL-6 in CRC patients.

The present study confirmed that serum IL-6 may be a potential biomarker for CRC diagnosis, and the high serum IL-6 level was associated with poor prognosis for both CRC overall survival and disease-free survival.

The study has been registered in an international registry of systematic reviews PROSPERO (CRD42013006485).

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Abbreviations: AD = aggregate data, AUC = area under curve, CRC = colorectal cancer, DFS = disease-free survival, FN = false-negative, FP = false-positive, HR = hazard ratio, IL-6 = interleukin-6, IPD = individual participant data, LRs = likelihood ratios, OD = optical density, OS = overall survival, QUADAS = quality assessment of diagnostic accuracy studies, ROC = summary receiver-operating characteristic, RR = risk ratio, sROC = summarize in receiver-operating characteristic curves, TN = true-negative, TP = true-positive.

INTRODUCTION

Colorectal cancer (CRC) is one of the most common cancers worldwide and also an important source of cancer-related death.[1,2] The present clinical examinations, such as colonoscopy and fecal occult blood test, have been widely used for CRC screening.[3,4] However, these tests have certain limitations. For example, colonoscopy is invasive, and fecal occult blood test screening suffers for its low sensitivity for polyps, especially smaller ones.[5] Therefore, development of noninvasive diagnostic and prognostic biomarkers is critical for CRC early detection and curative treatment interventions, which can significantly reduce its morbidity and mortality.

It has been reported that cancer-associated inflammation is a key determinant of disease progression and survival in CRC,[6] which can contribute to tumor angiogenesis, invasion, and metastatic spread.[7,8] As an inflammatory cytokine, IL-6 can be involved in immune regulation, hematopoiesis, and carcinogenesis.[9] It can act as a tumor promoter in colorectal neoplasms by activating the downstream oncogenic transcription factors in epithelial cells, such as NF-κB and STAT3.[10–12] Several meta-analyses and systematic reviews[13–16] have assessed the association between serum IL-6 and risk of CRC, whereas these pooled results were insignificant. Of note, Kakourou et al[13]

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reported a significantly positive association between the serum IL-6 level and risk of colon cancer, but the results were opposite for rectal cancer.

There are also many independent studies assessed the diagnostic[17–22] and prognostic[23–32] value of serum IL-6 level for CRC patients, whereas the results were inconsistent. Therefore, we conducted a quantitative database-based analysis to comprehensively explore the potential diagnostic and prognostic value of serum IL-6 in CRC.

METHODS

Original Study

We conducted an original study to explore the diagnostic value of IL-6 in CRC. Serum samples were collected in EDTA tubes from 72 healthy controls in Hangzhou First People's Hospital and from 72 CRC patients before surgical operation in Zhejiang Cancer Hospital. All samples were immediately frozen in liquid nitrogen and kept at −80 °C until analysis. No patients had received neo-adjuvant treatment including radiotherapy or chemotherapy before surgery and diagnosis, and all patients were enrolled with written informed consent under institutional review board-approved protocols of Zhejiang University. Serum IL-6 levels were measured using Human IL-6 Platinum ELISA kit (eBioscience, Inc, Vienna, Austria). The optical density (OD) value was detected at a wavelength of 450 nm according to the provided protocol and a standard linear regression curve was established with good fitting degree ($R^2 = 0.984$). According to the instruction manual of the ELISA kit, the limit of detection of human serum IL-6 was 0.92 pg/mL. The calculated overall intra-assay and inter-assay coefficient of variation was 3.4% and 5.2%, respectively. In addition, the instruction manual also illustrated the freeze-thaw and storage stability of the ELISA kit. The data from this original study were included in the meta-analysis process.

To determine the diagnostic performance of IL-6 level in CRC, receiver operating characteristic (ROC) analysis was performed and the area under the curve (AUC) value was calculated. The optimal cutoff threshold was determined at the point on the ROC curve at which (sensitivity + specifi-specificity-100%) was maximal. Sensitivity and specificity were calculated with this cutoff value.

Meta-analysis

This meta-analysis was designed, conducted, and reported according to PRISMA and MOOSE statements.[33,34] The review has been registered in an international registry of systematic reviews PROSPERO (CRD42013006485).

Literature Search and Study Selection

Systematic literature searches were conducted in Pubmed, Embase, and Cochrane library database (to October 2015) to identify eligible studies. The following terms were applied: ''interleukin-6'' OR ''interleukin6'' OR ''interleukin 6'' OR ''IL-6'' OR ''IL6'' OR ''IL 6''; ''colon'' OR ''rectal'' OR ''colorectal''; ''cancer'' OR ''tumor'' OR ''carcinoma'' OR ''neoplasm''. References of relevant articles and reviews were also scanned for potentially missing studies. Titles and abstracts were first scanned, and then full articles of potential eligible studies were reviewed. Articles as full papers in English and Chinese were evaluated for eligibility. The retrieved studies were carefully examined to exclude potential duplicates or overlapping data.

Articles were included if they met all the following criteria: (1) studies evaluated the diagnostic or prognostic value of serum IL-6 level in CRC patients; (2) for diagnostic studies, histologic assessment should be applied as reference standard for colorectal cancer; (3) for prognostic studies, the endpoint of follow-up should be overall survival (OS) and/or disease-free survival (DFS); (4) for studies analyzed the diagnostic value of IL-6, absolute numbers of true-positive (TP), false-positive (FP), true-negative (TN), and false-negative (FN) results were provided or could be calculated; for prognostic studies, hazard ratio (HR) or risk ratio (RR) values with 95%CI were provided or could be calculated. In addition, studies assessed the tissue level of IL-6, mRNA level of IL-6, and colon adenomas were excluded. Reviews, meeting abstracts, letters, comments, editorials, and case reports were also excluded because of the limited data.

Data Extraction and Quality Assessment

Two reviewers (Xu JM and Ye Y) independently collected data using standardized forms and discrepancies were resolved by a third investigator. We extracted the following information from each study: first author, year of publication, origin of the study population, patient characteristics (age, sex, cancer type, and stage), source of samples, number of participants, IL-6 assay method, follow-up time and variables adjusted for in the analysis. For diagnostic studies, number of TP, FP, TN, and FN was extracted. An individual participant data (IPD) meta-analysis approach was conducted to assess the diagnostic performance of serum IL-6 level in CRC patients. The principal investigators of relevant studies were contacted and asked to provide the raw data. For prognostic studies, HR or RR estimates with 95% CIs for overall survival (OS) and disease-free survival (DFS) were extracted. If the HRs and their 95% CIs were not provided, the numbers of deaths or recurrences and total samples in each study were extracted to calculate these numbers.[35] The quality of the diagnostic and prognostic studies was assessed using the quality assessment of diagnostic accuracy studies (QUADAS).[36]

Statistical Analysis

To assess the diagnostic performance of IL-6 for CRC, the analyses of sensitivity, specificity, likelihood ratios (LRs), and diagnostic odds ratio were performed and data were finally summarized in receiver-operating characteristic curves (sROC). The sensitivity and specificity were calculated according to the numbers of TP, FP, TN and FN. The mathematical methods for the positive and negative LR were sensitivity/(1 – specificity) and (1 – sensitivity)/specificity, respectively. If the positive LR≥5.0 and the negative LR≤0.2, the test was defined clinically effective.[37] The extent of heterogeneity across studies was checked by the chi-square test and I^2 test[38] and the publication bias was tested by Deek's funnel plot method.[39] Source of heterogeneity was explored by subgroup and sensitivity analysis. For the IPD meta-analysis, the serum IL-6 level of each CRC patient and control were obtained from the raw data, which provided by the principal investigators of relevant studies. ROC analysis was performed and the AUC value was calculated. The optimal cutoff threshold was determined at the point on the ROC curve at which (sensitivity + specificity – 100%) was maximal. Sensitivity and specificity were calculated with this cutoff value.

To assess the prognostic performance of IL-6 in CRC, either a fixed- or random-effect model was adopted to pool the study-specific HRs (ORs), according to the extent of

heterogeneity. The significance of the pooled HR was determined by Z test ($P < 0.05$ was considered to be significant). Heterogeneity across studies were checked by the chi-square test and I^2 test (I^2 test quantifies the proportion of total variation across studies due to heterogeneity rather than chance). $P \leq 0.10$ and/or $I^2 > 50\%$ indicates significant heterogeneity, and the random-effect model was used. Otherwise, a fixed-effect model was applied. Begg's funnel plots and Egger's linear regression test were used to assess publication bias.

We performed a secondary analysis of prognostic survival data according to Guyot's algorithm,[40] including extracting data from the Kaplan–Meier curves of published studies and reconstructing the pooled survival curve. Digitization of the Kaplan–Meier curves was achieved by Engauge software, which provided the coordinates of the curves. The detailed information on numbers at risk were extracted to estimate the number of censored individuals.[40] Individual patient data was reproduced imitatively using the R software and the survival data were aggregated to form combined survival curves. The accuracy of this method has been evaluated and several other studies have adopted the method as well.[41,42]

All analyses were conducted using the Stata software (version 11.0; StataCorp, College Station, TX), Meta-Disc (version 1.4, Unit of clinical biostatics, the Ramoy Cajal Hospital, Madrid, Spain), IBM SPSS Statistics (version 18.0, SPSS Inc., Chicago, IL), Engauge Digitizer (version 4.1, by Mark Mitchell, free software with absolutely no warranty), and R (version 3.2.1, The R Foundation for Statistical Computing). All *P* values presented were 2-sided. The association was considered significant if the *P* value was ≤ 0.05.

RESULTS

Study Selection and Characteristics

We searched Pubmed, Embase, and Cochrane library database and 1990 articles were included. A total of 658 duplicate articles were removed and 1229 papers were excluded by reviewing titles and abstracts. And 103 articles were left for further evaluation, and then we excluded 87 papers which did not meet the inclusion criteria. Not on the right topic or targeted population (n = 34), insufficient data (n = 46), language was neither English nor Chinese (n = 2), review article, meeting abstract, letters or comments (n = 5).

Finally, 17 studies were included in this meta-analysis (including our study). The selection process was shown in Figure 1 and the characteristics of the included studies were shown in Tables 1 and 3. Among the included studies, 7 articles[17–22] reported the diagnostic value of serum IL-6 (including our study) for CRC patients, whereas 10 articles[23–32] examined the prognostic value of serum IL-6 in CRC.

Diagnostic Value of Serum IL-6 for CRC Patients

An original study was first conducted to explore the potential value of IL-6 in CRC diagnosis, in which 72 CRC patients and 72 normal controls were enrolled. The ROC analysis revealed that serum IL-6 might be a potential biomarker in discriminating patients with CRC from controls. The area under the AUC value was 0.818 (95% CI: 0.751–0.885; Figure 2A). The sensitivity and specificity were 72.22% and 75.00%, respectively, at a cutoff value of 2.14 pg/mL.

Finally, 7 studies with 654 patients assessed the diagnostic value of IL-6 level for CRC (including our study). The included studies were conducted in Europe (n = 4), China (n = 2), and

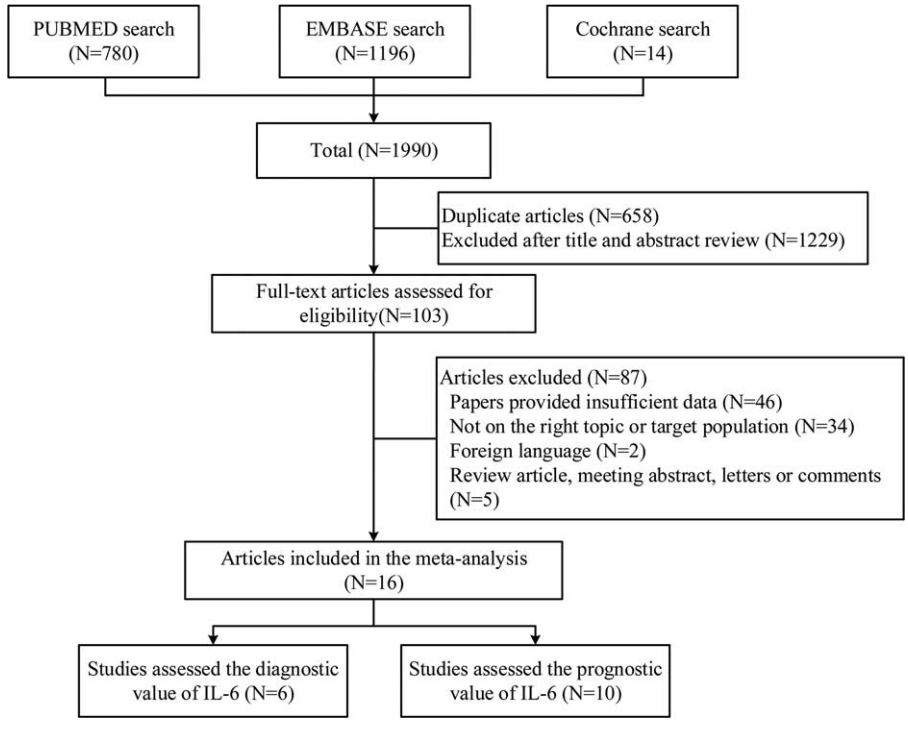

FIGURE 1. Flow diagram of study selection process.

TABLE 1. Characteristics of the Diagnostic Studies

Study	Country	Design	Gender	Age (years)	No. of Participants	Cut-Off Point (pg/mL)	IL-6 Measure	Sensitivity	Specificity	TP	FP	FN	TN	QUADAS Score
Pengjun et al, 2013[17]	China	Case-control	F/M	CRC:58,control:57	149CRC,69conrtols	N/A	Immunoassay kit*	0.869	0.550	149	56	23	69	12
Kantola et al, 2012[18]	Finland	Case-control	F/M	CRC:67.9,conrtol:67.3	148CRC,86controls	4.24	Cytokine Panel†	0.826	0.738	115	30	24	84	11
Eldesoky et al, 2011[19]	Egypt	Case-control	F/M	Mean:49	35CRC,30controls	6.70	ELISA kit‡	0.680	0.590	35	21	17	30	12
Bunger et al, 2011[20]	Germany	Case-control	F/M	CRC:71.2,control:65.5	50CRC,50controls	N/A	Biochip array§	0.080	0.960	50	2	575	50	12
Groblewska et al, 2008[22]	Poland	Case-control	F/M	CRC:65.3,control:43	76CRC,35controls	3.06	ELISA kit‖	0.895	0.857	76	6	9	35	12
Kaminska et al, 2005[21]	Poland	Case-control	F/M	CRC:62,control:38.9	157CRC,50controls	N/A	ELISA kit#	0.861	0.460	157	59	25	50	11
Wu et al, 2013	China	Case-control	F/M	Mean:55.4	72CRC,72controls	2.14	ELISA kit**	0.722	0.750	72	24	28	72	12

CRC = colorectal cancer; FN = false negative; FP = false positive; N/A = not available; QUADAS = quality assessment of diagnostic accuracy studies; TN = true negative; TP = true positive.
* Multiplex bead-based sandwich immunoassay kit, HCYTOMAG-60K; Millipore, MA, USA.
† Bio-Plex Pro Human premanufactured 27-Plex Cytokine Panel, Bio-Rad, Hercules, CA, USA.
‡ Quantikine quantitative human IL-6 sandwich ELISA kits, R and D system, Minneapolis Minnesota.
§ Multiplex biochip array in combination with the evidence investigator readout equipment, Randox laboratories Ltd, Crumlin, UK.
‖ R&D Systems, Abingdon, UK.
R&D Systems, Minneapolis.
** Human IL-6 Platinum ELISA, eBioscience, Inc, Vienna, Austria.

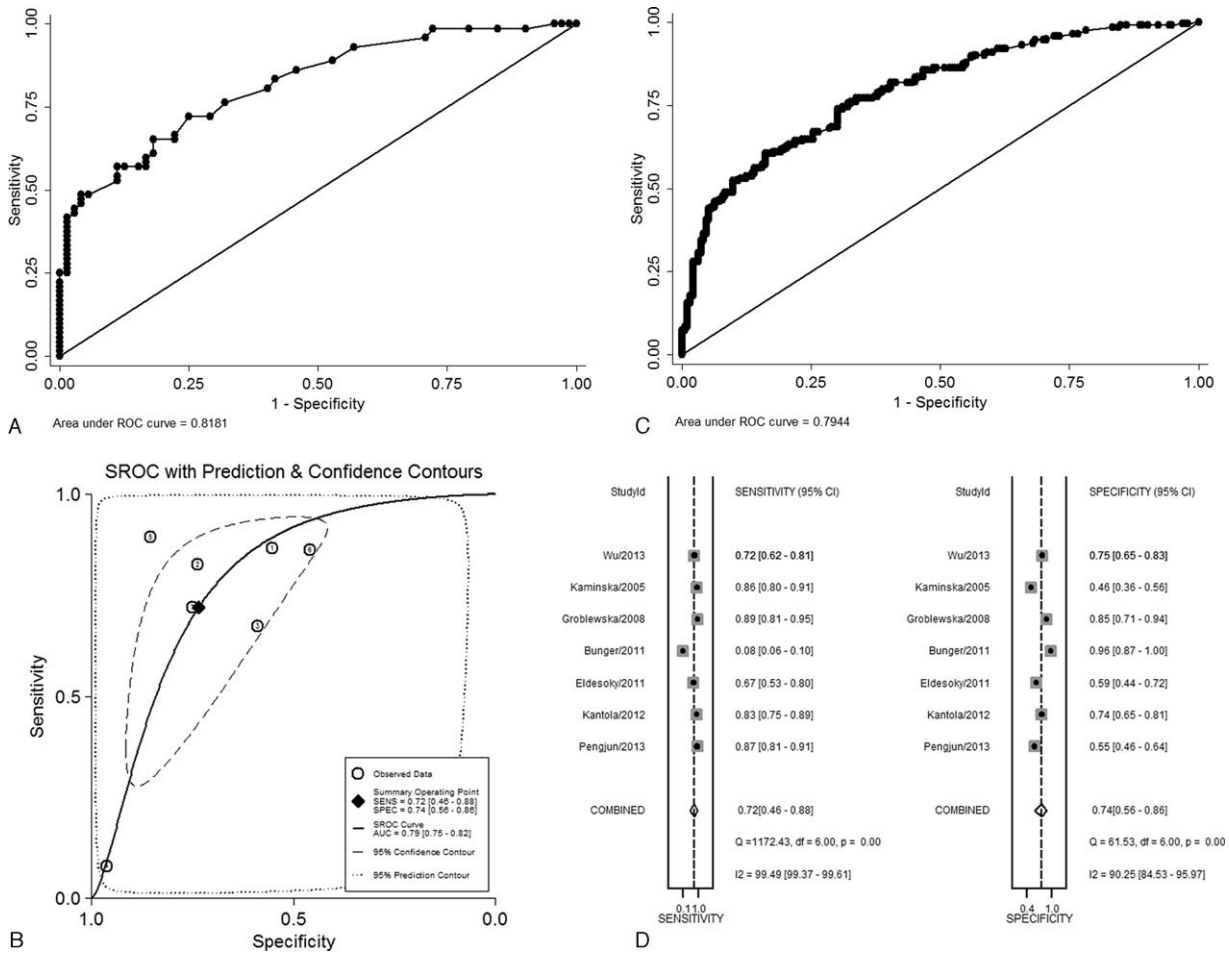

FIGURE 2. The ROC and sROC curve for the diagnostic analysis. (A) The AUC value of IL-6 in CRC from the original study. (B) Summary receiver operating characteristic curves for IL-6 in the diagnosis of CRC. (C) The AUC value of IL-6 in CRC from IPD meta-analysis. (D) Forest plots of sensitivities and specificities of serum IL-6 in the diagnosis of CRC. AUC = area under curve, CRC = colorectal cancer, IPD = individual participant data, ROC = summary receiver-operating characteristic, sROC = summarize in receiver-operating characteristic curves.

Africa (n = 1). Sample size of each study ranged from 65 to 234. The studies adopted different methods to measure the level of IL-6, such as Elisa (n = 4), multiplex bead-based sandwich immune assay kit (n = 1), Bio-Plex Pro Human pre-manufactured 27-Plex Cytokine Panel (n = 1), and cytokine and growth factor multiplex biochip array (n = 1). The quality assessments were shown in Supplementary Table 1, http://links.lww.com/MD/A617.

Together, the area under the sROC curve was 0.79 (95% CI: 0.75–0.82) (Figure 2B) and the pooled sensitivity and specificity were 0.72 (95% CI: 0.46–0.88) and 0.74 (95% CI: 0.56–0.86), respectively (Figure 2D), indicating that IL-6 has a relatively moderate diagnostic performance in CRC. Significant heterogeneity was found in both sensitivity ($Q = 1172.43$; $df = 6.00$; $P = 0.00$; $I^2 = 99.49\%$) and specificity ($Q = 61.53$; $df = 6.00$; $P = 0.00$; $I^2 = 90.25\%$). The pooled positive LR, negative LR and diagnostic OR were 2.33 (95% CI: 1.74–3.14), 0.33 (95% CI: 0.09–1.25), and 7.69 (95% CI: 4.40–13.43), respectively. Through metaregression and subgroup analysis, as shown in Table 2, the heterogeneity across studies might come from country of region, IL-6 measure methods, and sample size. When stratifying the studies according to sample size, the pooled sensitivity changed from 0.72 (95% CI: 0.46–0.88) to 0.85 (95% CI: 0.82–0.88) in large sample size subgroup, with a significant decrease in heterogeneity from $Q = 1172.43$; $df = 6.00$; $P = 0.00$; $I^2 = 99.49\%$ to $Q = 1.08$; $df = 2$; $P = 0.58$; $I^2 = 0.00\%$. In addition, the heterogeneity decreased to in Asia subgroup ($I^2 = 88.4\%$) and Elisa subgroup ($I^2 = 83.6\%$) when stratifying the studies according to country of region and IL-6 measure methods, respectively.

Sensitivity analysis was also conducted to explore the potential impact of within-study heterogeneity. After removing the single study in which sensitivity is the lowest (sensitivity = 8%),[20] the pooled sensitivity changed from 0.72 (95% CI: 0.46–0.88) to 0.83 (95% CI: 0.80–0.85), with a significant decrease in heterogeneity from $Q = 1172.43$; $df = 6.00$; $P = 0.00$; $I^2 = 99.49\%$ to $Q = 20.95$; $df = 5.00$; $P = 0.00$; $I^2 = 76.10\%$.

To further explore the potential value of serum IL-6 in CRC diagnosis and illustrate the significant heterogeneity across the studies, a meta-analysis of IPD was applied. Three studies (including our study) with 264 CRC patients and 193 controls presented the IPD data.[18,22] In the multivariable analysis which included IL-6, age, gender, weight, height, side of the tumor, TNM stage and postoperative adjuvant therapy,

TABLE 2. Subgroup Analysis of Diagnostic Studies

Factor	SEN (95% CI)	SEN Heterogeneity (Chi-Square/df/P/I^2)	SPE (95% CI)	SPE Heterogeneity (Chi-Square/df/P/I^2)	AUC (95% CI)
Country of region					
Asia	0.81(0.76–0.86)	8.6/1/0.0034/88.4%	0.64(0.57–0.70)	9.41/1/0.0022/89.4%	–
Europe	0.39(0.36–0.42)	695.78/3/0.0000/99.6%	0.69(0.64–0.74)	56.86/3/0.0000/94.7%	0.8321
Africa	0.68	–	0.59	–	–
IL-6 measure methods					
Elisa	0.81(0.77–0.85)	18.28/3/0.0004/83.6%	0.63(0.57–0.68)	29.96/3/0.0000/90.0%	0.8278
Others	0.34(0.31–0.37)	582.61/2/0.0000/99.7%	0.70(0.64–0.75)	36.41/2/0.0000/94.5%	0.8028
Sample size*					
Large	0.85(0.82–0.88)	1.08/2/0.5828/ 0.0%	0.58(0.53–0.64)	19.02/2/0.0001/89.5%	0.8830
Small	0.27(0.24–0.30)	415.85/3/0.0000/99.3%	0.78(0.72–0.83)	25.26/3/0.0000/88.1%	0.8820

– = could not be calculated; AUC = area under the curve; CI = confidence interval; ELISA = enzyme linked immunosorbent assay; IL-6 = interleukin-6; SEN = sensitivity; SPE = specificity.

* Large sample size means the colorectal cancer patients number≥100, whereas the small sample size means the CRC patients number <100.

the serum IL-6 level served as an independent diagnostic marker for CRC. The AUC value was 0.794 (95% CI: 0.754–0.835; Figure 2C). The sensitivity and specificity were 60.61% and 83.94%, respectively, at a cutoff value of 4.2 pg/mL.

Prognostic Value of Serum IL-6 for CRC Patients

A total of 10 studies with 860 CRC patients were included in the prognostic analysis. The included studies were conducted in Europe (n = 4) and East Asia (n = 6). Eight studies adopted Elisa method to measure the serum level of IL-6, whereas 1 study applied the QuantiGlo Human IL-6 Immunoassay method, and another applied xMAP technology developed by Luminex (Riverside, CA). The quality assessments were shown in Supplementary Table 2, http://links.lww.com/MD/A617. Six studies reported the impact of IL-6 on CRC OS and 3 on CRC DFS, and 1 study reported both OS and DFS. For overall survival, the pooled HR was 1.76 (95% CI: 1.42–2.19, $P < 0.001$), indicating that higher serum IL-6 level predicate poor OS for CRC patients (n = 679) (Figure 3A). There was no significant heterogeneity across the studies ($I^2 = 0.0\%$, $P = 0.443$). For DFS, the pooled HR was 2.97 (95% CI: 1.76–5.01, $P < 0.001$), indicating that higher serum IL-6 level predicate poor DFS for CRC patients (n = 313) (Figure 3B). There was also no significant heterogeneity across the studies ($I^2 = 10.4\%$, $P = 0.341$).

Among the included studies, 3 studies[24,28,32] on CRC OS and 3 studies[26,30,31] on CRC DFS provided detailed data of the Kaplan–Meier survival curves. A secondary analysis based on these studies was conducted and the pooled Kaplan–Meier curves was reconstructed, as shown in Figure 3C and D. A total of 294 and 181 CRC patients were included for the pooled OS and DFS curves, respectively. The results indicated that higher serum IL-6 level was significantly associated with worse OS ($P = 0.0027$) and DFS ($P < 0.001$) in CRC patients, which provided more reliable evidence for the prognostic efficacy of IL-6.

Publication Bias

Begg's funnel plot and Egger-weighted regression indicated that there was no significant publication bias.

DISCUSSION

As a pleiotropic cytokine, several epidemiological studies have investigated the association between serum IL-6 level and the risk of CRC and the application of IL-6 as a biomarker for CRC diagnosis and prognosis has gained much attention in recent years. In the present study, we summarized the results of published observational studies, including 6 studies assessed serum IL-6 for CRC diagnosis and 10 studies assessed serum IL-6 for CRC prognosis. Finally, 17 studies (including our study) were included to perform the meta-analysis on this issue.

For the diagnostic value of IL-6, the results of this meta-analysis indicated that serum IL-6 may be a potential noninvasive biomarker for CRC diagnosis. The pooled sensitivity, specificity, and AUC were 0.72, 0.74, and 0.79, respectively. The diagnostic odds ratio (DOR) combines the strengths of both sensitivity and specificity, and is reported to be a useful indicator for the evaluation of diagnostic method.[43] The DOR value of IL-6 from this study was 7.69, indicating a moderate diagnostic accuracy. However, the positive LR (2.33) and negative LR (0.33) suggested that IL-6 may be not adequate to discriminate CRC patients. Subgroup analysis indicated that country of region, the IL-6 measure method and sample size might contribute to the heterogeneity across studies. The laboratory performance of various analytical tests used to measure IL-6 could partially explain the significant heterogeneity. We divided the studies into ''ELISA'' group and ''other methods'' group, and the SEN heterogeneity was more significant in ''other methods'' group (chi-square = 582.61) than ''ELISA'' group (chi-square = 18.28). Furthermore, the differences and partial data deficiency of cutoff values could also contribute to the heterogeneity. In order to validate the diagnostic value and further explain the heterogeneity, we adopted the sROC model to pool the diagnostic evaluation of IL-6 from different studies, and the IPD (including raw data of 457 participants) method recalculated the cutoff value of IL-6 and made it more accurate (pooled cutoff value 4.20 pg/mL). In addition, we found significant heterogeneity decrease during the sensitivity analysis. The heterogeneity decreased significantly after removing the single study[20] in which sensitivity is the lowest. Although an IPD meta-analysis method was adopted and the results were consistent between IPD-analysis and our original study, considering the significant heterogeneity,

TABLE 3. Characteristics of the Prognostic Studies

Study	Country	Sex (M/F)	Age (Years)	No. of Participants	End Point	Follow-Up Time	Cut-Off Point (pg/mL)	IL-6 Measure	Study Quality	Adjustment for Covariates
Reitter et al, 2014[23]	Austria	382/344 (total 726)	Median 62	98CRC	OS	Median 705 days	N/A	xMAP technology*	13	Age, gender, cytokine levels
Hazama et al, 2014[24]	Japan	N/A	N/A	17 CRC	OS	About 48 months †	2	ELISA kit	12	N/A
Lee et al, 2013[26]	Korea	43/34	43–79(median 64.4)	77CRC	DFS	Median 19.7 months (range 6.1–59.1)	9	ELISA kit	13	Secondary analysis of survival data‡
Shimazaki et al, 2013[25]	Japan	29/17	43–86(mean 70.3)	46CRC	OS	About 3000 days†	2.41	Quanti Glo Human IL-6 Immunoassay	13	Age, gender, tumor location, TNM stage
Yeh et al, 2010[27]	China	56/43	30–82(mean 63.8)	99CRC	OS	7.8 years (range 0.5–11.5)	10	ELISA kit	11	Age, gender, BMI, tumor location, TNM stage, histological grade and CEA, WBC, BUN, albumin, hemoglobin, creatinine, AST, alkaline phosphatase level
Li et al, 2010[28]	China	47/32	23–84(median 65)	79CRC	OS	7 years	13.2	ELISA kit	11	Secondary analysis of survival data‡
Kwon et al, 2010[29]	Korea	79/53	26–83(mean 62)	132CRC	OS and DFS	Median 18.53 months (range 0.73–43.17)	11.68	ELISA kit	11	Age, gender, tumor size, lymph node ratio, differentiation, TNM stage, CEA level
Nikiteas et al, 2005[30]	Greek	39/35	33–86(mean 66.83)	74CRC	DFS	Median 18.57 months (range 1–32)	8	ELISA kit	11	Secondary analysis of survival data‡
Galizia et al, 2002[31]	Italy	34/16 (total 50)	30–83(mean 65.4)	30CRC	DFS	Mean 22.2 ± 6.6 months (range 5.2–26.1)	8.4	ELISA kit	11	Secondary analysis of survival data‡
Belluco et al, 2000[32]	Italy	119/91	27–92(mean 64)	208CRC	OS	Median 46 months	10	ELISA kit	13	Age, gender, tumor location, grade, TNM stage, CEA level

CRC = colorectal cancer; DFS = disease-free survival; ELISA = enzyme-linked immunosorbent assay; IL-6 = interleukin 6; N/A = not available; OS = overall survival.

* xMAP technology developed by Luminex (Riverside, CA).

† The precise follow-up time was not given; data extracted from figure in the origin article.

‡ Secondary analysis of survival data was performed according to Guyot's algorithm, the method of which derived from the published Kaplan–Meier survival curves a close approximation to the original individual patient time-to-event data.

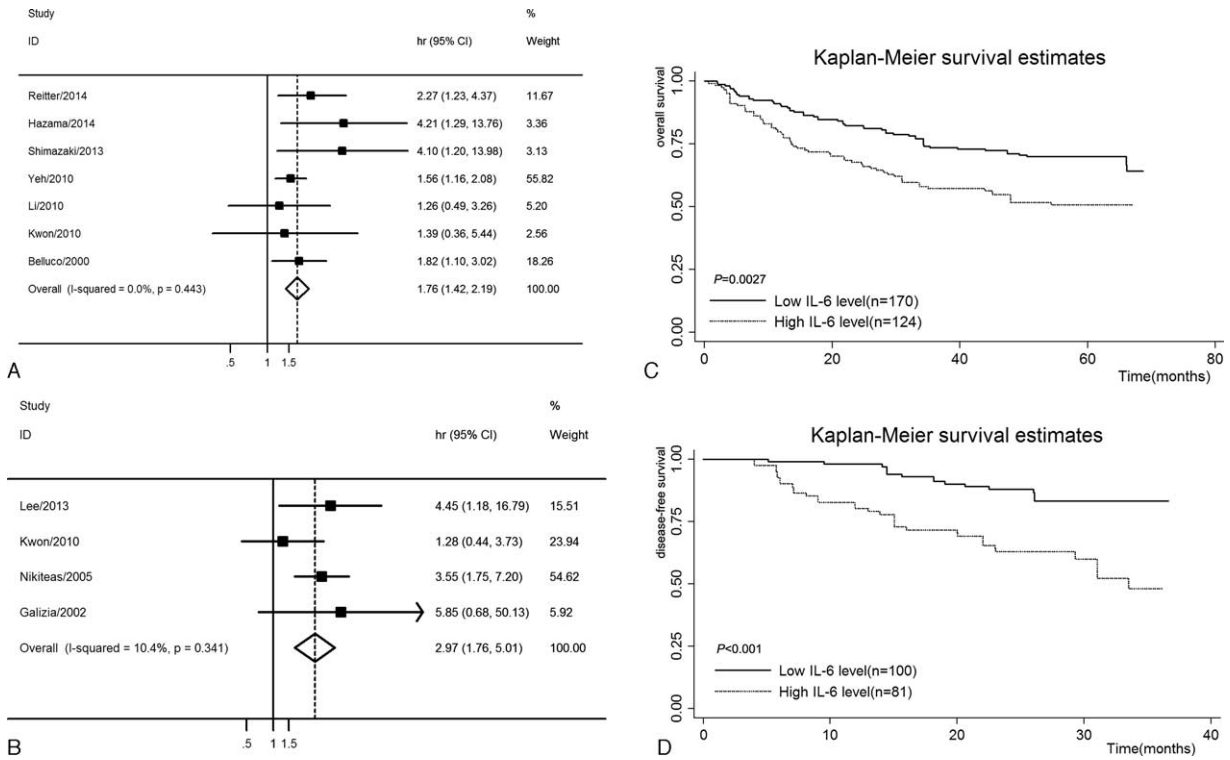

FIGURE 3. Results of prognostic analysis for serum IL-6 in CRC. (A) Forest plot of studies evaluating serum IL-6 level for CRC OS. (B) Forest plot of studies evaluating serum IL-6 level for CRC DFS. (C) Reconstructed Kaplan–Meier survival estimates of OS by secondary analysis of survival data. (D) Reconstructed Kaplan–Meier survival estimates of DFS by secondary analysis of survival data. CRC = colorectal cancer, DFS = disease-free survival, OS = overall survival.

different cutoff values, different IL-6 measure methods, and the lack of validation cohorts, the conclusion should be taken more cautiously and more cohort studies are warranted to further strengthen it.

For the prognostic value of IL-6, the results of the meta-analyses indicated that the serum IL-6 level was a promising biomarker to predict OS and in CRC patients. Compared to patients with low serum IL-6 level, patients with increased level of IL-6 had a 1.76-fold higher risk of poor OS, and 2.97-fold higher risk of poor DFS. The synthesized survival Kaplan–Meier curves indicated that CRC patients with higher serum IL-6 level had a worse OS ($P = 0.0027$) and DFS ($P < 0.001$), which further strengthened the prognostic value of serum IL-6 in CRC patients. In addition, there was no significant heterogeneity for the prognostic analyses, which indicated the prognostic efficacy of IL-6 was valid and reliable.

As a pro-inflammatory cytokines, IL-6 seems to be at the center stage in human cancer development by regulating cancer cell growth and thereby contribute to tumor promotion and progression.[44] Secreted IL-6 binds to its membrane receptor (IL-6R), composed of the ligand-binding (gp 80) and signal-transducing subunits (gp 130), results in the activation of certain functions involved in carcinogenesis.[45] Signal transduction through gp130 is mediated by 2 pathways: the JAK-STAT3 pathway and the Ras-MAPK-pathway.[9] It has been reported that STAT3 activation is an important step for the promotion and progression of cancer through the induction of various target genes.[46] These target genes are involved in tumor cell survival (eg Bcl-2, Survivin, Mcl-1), proliferation (eg c-Myc, Cyclin D1, Cyclin B), angiogenesis (eg HIF1alpha, VEGF), metastasis (eg MMP2, MMP9), cell adhesion (eg ICAM-1, TWIST1), inflammation (eg IL-6, IL-17, IL-23, Cox2), and so on.[46,47] Besides, as a critical NF-κB-dependent pro-tumorigenic cytokine produced by lamina propria myeloid cells, IL-6 activates STAT3 in both inflammatory cells and the epithelial cells from where the tumors arose. Specifically, activation of STAT3 induced the upregulation of key genes involved in cell proliferation and survival, and increased the nuclear localization of β-catenin, which contributes to colorectal carcinogenesis.[48] Thus, all these findings support a vital role for IL-6 in tumorigenesis, whereas IL-6 expression can be associated with tumor stage, size, metastasis and survival of patients with CRC,[49] and therefore, could explain the diagnostic and predictive value of IL-6 in CRC.

Our study had several important strengths. First, we conducted a relatively thorough systematic search and applied a comprehensive analytical approach to evaluate the diagnostic and prognostic value of IL-6 in CRC patients. Second, an original study was also conducted to explore the diagnostic potential of serum IL-6 in CRC, and an IPD meta-analysis was then used, which further supported the conclusions of the study. Third, a full secondary analysis of survival data contributed to the reliability of the prognostic value of serum IL-6. Finally, most of the included studies were of high methodological quality for analysis, and the methods of this study were rigorous and followed the guidelines for conducting and reporting systematic reviews.

This study also has some limitations. First, most of the diagnostic studies enrolled healthy people as controls and were not blind designed. This may affect the diagnostic performance and more cohort studies are warranted to further strengthen it. Second, significant heterogeneity was found among diagnostic studies. Although subgroup and IPD analyses were applied, the results could not fully explain the observed heterogeneity.

CONCLUSION

The present study confirmed that serum IL-6 may be a potential biomarker for CRC diagnosis, and the high serum IL-6 level was associated with poor prognosis for both CRC overall survival and disease-free survival.

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