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Review Article

Clinical significance of neutrophil gelatinase-associated lipocalin (NGAL) in colorectal cancer: a meta-analysis renewal

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Abstract: Background and objectives: More observational studies from different parts of the world have been executed to demonstrate the molecular biological relationship between NGAL and colorectal cancer. Our previous study in 2014 has extracted data from 5 studies with literature screening until early 2013. This meta-analysis aimed to recruit more recent papers and explore the overall accuracy of NGAL detection on diagnosis. Materials and methods: We divided this meta-analysis into two layers: 1. distinguishing adenocarcinoma from other abnormalities and normal tissue; 2. distinguishing adenocarcinoma and abnormalities with high possibility of carcinogenesis from benign hyperplasia and normal tissue Results: For Layer 1, the pooled sensitivity and specificity of all studies were 0.81 (95% CI, 0.78-0.84) and 0.56 (95% CI, 0.52-0.60). The pooled positive likelihood ratio and negative likelihood ratio were 2.29 (95% CI, 1.40-3.74) and 0.34 (95% CI, 0.18-0.63). The pooled diagnostic odds ratios was 11.29 (95% CI, 3.36-38.01). The area under the summary receiver operating characteristic curve for the diagnosis of colorectal cancer was 0.8476. For Layer 2, the pooled sensitivity and specificity of all studies were 0.76 (95% CI, 0.73-0.79) and 0.63 (95% CI, 0.58-0.67). The pooled positive likelihood ratio and negative likelihood ratio were 7.11 (95% CI, 1.97-25.63) and 0.35 (95% CI, 0.23-0.54). The pooled diagnostic odds ratios was 23.10 (95% CI, 6.55-81.44). The area under the summary receiver operating characteristic curve for the diagnosis of colorectal cancer was 0.8900. Conclusion: NGAL is more suitable for screening adenocarcinoma and abnormalities with high possibility of carcinogenesis from benign hyperplasia and normal tissue.

Keywords: NGAL, colorectal cancer, diagnosis, accuracy, meta-analysis

Introduction

Colorectal cancer (CRC) is one of the most common gastrointestinal cancers in the world [1]. With the modern therapies, the 5-year survival rate is still not ideal; therefore early diagnosis is the easiest way to reduce its mortality. The majority of colorectal cancer patients can be cured successfully, for there is a strong correlation between the tumor stage of diagnosis and the 5-year survival rate [2]. Aside from traditional screening techniques including fecal occult blood test and colonoscopy, finding an effective biomarker which can be useful for early diagnosis, especially for abnormalities with high risks of carcinogenesis for colorectal cancer, including familial multiple intestinal polyps, precancerous changes of local epithelia or mucosa, or atypical hyperplasia, will be desirable. Our previous meta-analysis involving 5 studies was published in 2014 to evaluate the diagnostic precision of NGAL [3]. For the recent 2 years, a few of related studies have been published and some of them have contributed contradictory results with previous papers which made the overall picture of NGAL in diagnosis of colorectal cancer ambiguous, thus we carried out this meta-analysis to further elucidate the possibility of NGAL testing in colorectal cancer.

The observational studies involving diagnosis of cancers by a variety of biomarkers will usually depend on immunohistochemical slices. However, in this analysis, we recruited one paper with ELISA results, and tried to make its

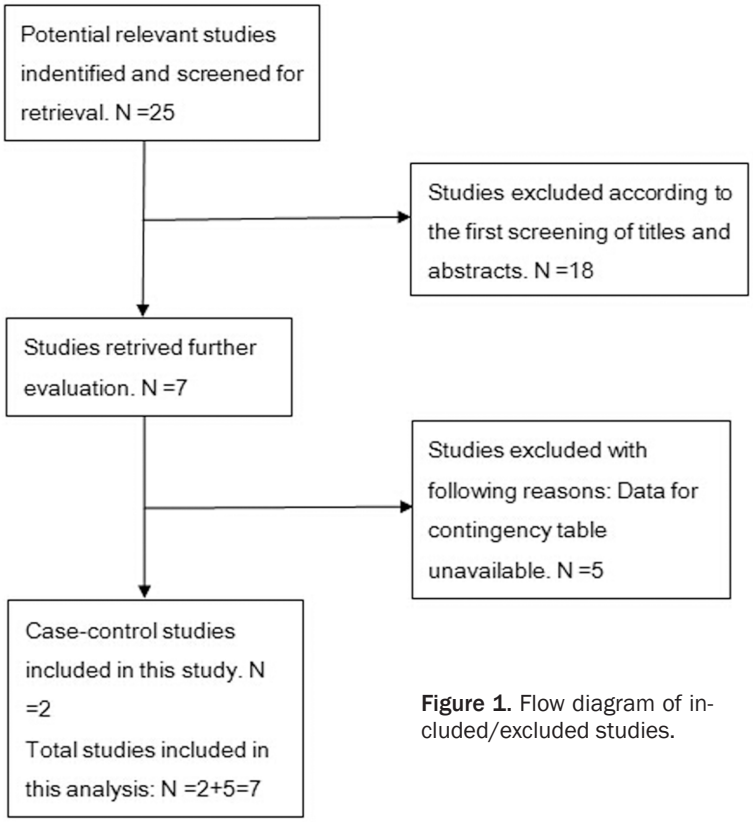

Figure 1. Flow diagram of included/excluded studies.

results comparable to immunohistochemical results.

Material and method

Search strategy and publication selection

We followed the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines for the conduct of meta-analyses of observational cohort studies. We conducted a literature search on Pubmed, Ovid and China National Knowledge Infrastructure (CNKI) databases, including all newly published papers from March 2011 until May 2015 with a combination of the following terms: neutrophil gelatinase-associated lipocalin; NGAL; lipocalin 2; colorectal cancer; colon cancer; and rectal cancer. There were no language restrictions.

We reviewed potentially associated publications by checking their titles and abstracts, and then procured the most relevant papers for a further examination. Moreover, the reference lists of the selected papers were also screened for any potential information. The criteria used for the literature selection were listed as follows: 1. papers clearly describing studies about the association of NGAL with colorectal cancer; 2. colorectal cancer pathological diagnoses and sources of cases and controls should be stated; 3. test methods and completeness of data, or any information that may help infer the results should also be offered. Accordingly, the following exclusion criteria were also used: 1. design and definition of the experiments were obviously different from those of the selected papers; 2. source of cases and controls and other essential information could not be obtained; and 3. reviews and repeated literature.

Data extraction and study quality assessment

The quality of each included study was assessed using the diagnostic accuracy tool QUADAS (*ie.* Quality assessment for studies of diagnostic accuracy [maximum score 14]). Data including author, publication year, region, study population, the measurement method of NGAL, and completeness of data (the numbers of true-positive, false-positive, true-negative and false-negative results to allow reconstruction of the diagnostic 2-by-2 table) were extracted.

Statistical analysis

The studies were analyzed using the chi-square-based Q-statistic test to assess heterogeneity and I^2 to estimate the degree of heterogeneity. Statistically significant heterogeneity was considered when the *P* value was less than 0.05 and the I^2 value was more than 50%. If there was significant heterogeneity, we used the random-effect model (DerSimonian and Laird). Otherwise, we used the fixed-effect model (Mantel-Haenszel).

The bivariate model was applied for diagnostic meta-analysis to perform the pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR). All the pooled estimates with the corresponding 95% CI were initially calculated using the appropriate statistical analysis

Table 1. Basic information of the 7 studies for this analysis

Auther	Year	Method	Type of cancer patients	Patients of other types and control	Country	QUADAS
Zhang et al	2009	IHC	32 colon cancer 51 rectum cancer	81 adjacent normal tissue	China	11
Chen and Wu	2007	IHC	23 colon cancer	20 normal colonic mucosa 28 Peutz-Jeghers polyps	China	12
Sun et al	2011	IHC	287 colorectal cancer	94 normal mucosa 145 adenoma	China	12
Nielsen et al	1996	IHC	14 colorectal cancer	14 adjacent normal tissue	Denmark	13
Barresi et al	2011	IHC	48 colon cancer	48 adjacent normal tissue	Italy	12
Odabasi et al	2014	IHC	15 colon cancer	14 hyperplasia polyps 36 dysplasia	Turkey	13
Duvillard et al	2014	ELISA	219 colorectal cancer	250 benign illnesses in abdomen (eg. inguinal hernia, diverticulitis)	France	13

model. We constructed summary receiver operator characteristic (sROC) curves. The area under the curve (AUC) value with Q value was also calculated to present an overall summary of test performance to differentiate between a diseased and a non-diseased participant. The Spearman correlation coefficient of sensitivity and 1-specificity was calculated to estimate the threshold effect. The publication bias of included studies was assessed using the effective sample-size funnel plot and Egger's test.

Statistical analysis was implemented by Meta-Disc 1.4, and Stata 11.0 softwares.

Results

Search results and study characteristics

The systematic literature search generated a total of 25 references based on the search strategy. We excluded 18 studies after screening the titles and abstracts, because they were duplication in these databases, or not relevant to our interests. After a careful review, in the remaining 7 studies, 5 studies were discarded, because of lack of sufficient data for constructing the 2-by-2 contingency tables [4-8]. Finally, 2 new studies [9, 10] were chosen together with the 5 studies [11-15] in our previous meta-analysis to evaluate the diagnosis value of NGAL. A flow chart showing the study selection procedure is given in **Figure 1**.

We established a database according to the extracted information from these 7 studies. In order to accomplish a deeper clarification of the relationship of NGAL expression with colorectal adenocarcinoma, we additionally studied NGAL's distinguishing ability for normal and a variety of abnormal status, therefore we stratified our database into 2 layers: layer 1 aiming to distinguish adenocarcinoma from the rest of the abnormalities and normal tissue; Layer 2 aiming to distinguish adenocarcinoma and abnormalities with high possibility of carcinogenesis from benign hyperplasia and normal tissue.

The overall information was listed in **Table 1**. All the selected 7 studies were single-center trials from different part of the world and included nearly 1391 colorectal cancer patients, patients of other type of colorectal abnormalities and health controls. NGAL was measured by IHC in paraffin section in most of the studies and by ELISA in one of them. The quality of each study was appraised according to QUADAS.

Diagnostic accuracy analyses

For Layer 1 analysis, the forest plot of sensitivity, specificity, PLR, NLR and DOR for NGAL test in the diagnosis of colorectal cancer was shown in **Figures 2** and **3A**. The overall pooled sensitivity and specificity of all studies were 0.81 (95% CI, 0.78-0.84) and 0.56 (95% CI, 0.52-0.60), respectively. The overall pooled PLR and NLR were 2.29 (95% CI, 1.40-3.74) and 0.34 (95% CI, 0.18-0.63). The pooled DOR was 11.29 (95% CI, 3.36-38.01).

For Layer 2 analysis, the forest plot of sensitivity, specificity, PLR, NLR and DOR for NGAL test in the diagnosis of colorectal precancerous abnormalities was shown in **Figures 3B** and **4**. The overall pooled sensitivity and specificity of all

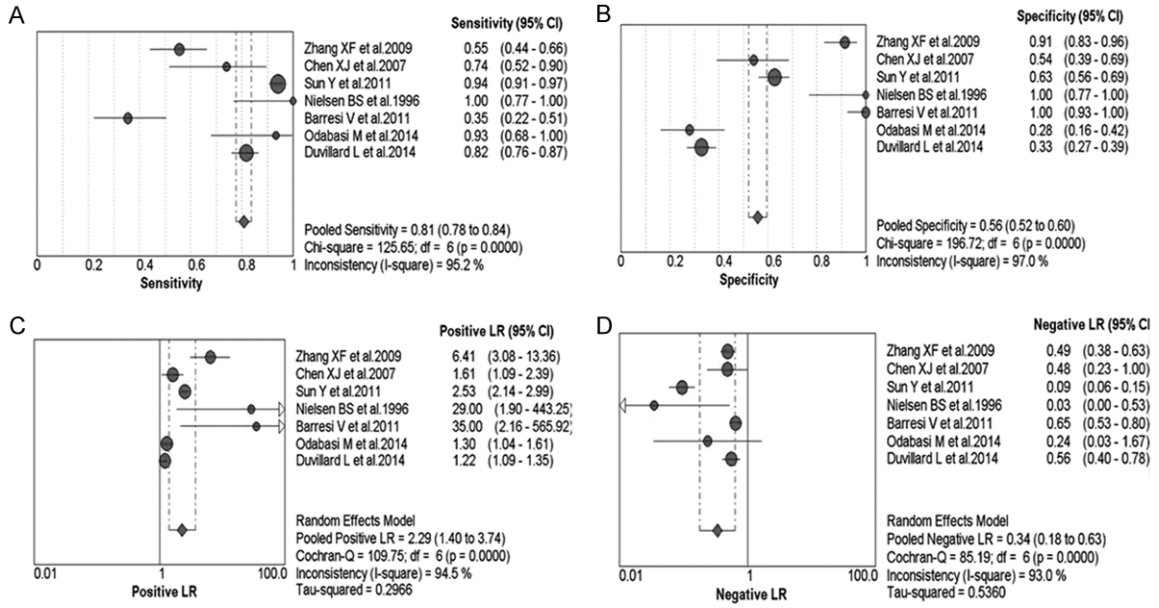

Figure 2. Forest plots of the pooled sensitivity (A), specificity (B), positive LR (C), and negative LR (D) of NGAL for the diagnosis of breast cancer. The solid circles represent each individual study and the diamond represents the pooled diagnostic odds ratio. The size of the circle is proportional to the size of the study included. Error bars are 95% confidence intervals. LR = likelihood ratio. Results are for Layer 1 analysis.

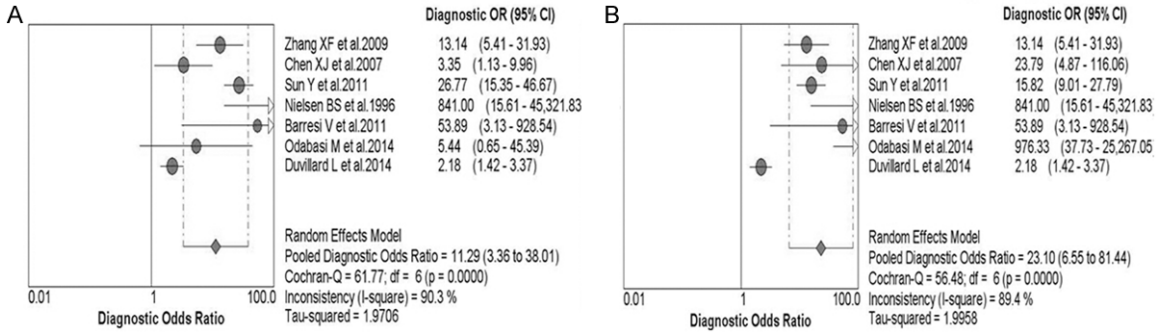

Figure 3. Forest plots of the pooled diagnostic odds ratio (DOR) of NGAL for the diagnosis of breast cancer/pre-cancerous abnormalities. The solid circles represent each individual study and the diamond represents the pooled DOR. The size of the circle is proportional to the size of the study included. Error bars are 95% confidence intervals. A and B. Results are for Layer 1 and Layer 2 analysis, respectively.

studies were 0.76 (95% CI, 0.73-0.79) and 0.63 (95% CI, 0.58-0.67), respectively. The overall pooled PLR and NLR were 7.11 (95% CI, 1.97-25.63) and 0.35 (95% CI, 0.23-0.54). The pooled DOR was 23.10 (95% CI, 6.55-81.44).

Summary receiver-operating characteristics

The sROC curve for NGAL expression showing true-positive rates against false-positive rates from each study displays the trade-off between sensitivity and specificity. For Layer 1 analysis, 7 studies were included to construct the sROC curve in **Figure 5A**. The AUC for the diagnosis of colorectal cancer was 0.8476 and the Q* value was 0.7789. For Layer 2 analysis, sROC curve was shown in **Figure 5B**. The AUC for the diagnosis of colorectal cancer was 0.8900 and the Q* value was 0.8207.

Test of heterogeneity

We performed a threshold analysis to explore the threshold effect, which was evaluated with the spearman correlation coefficient, by using Moses' model weighted by inverse variance.

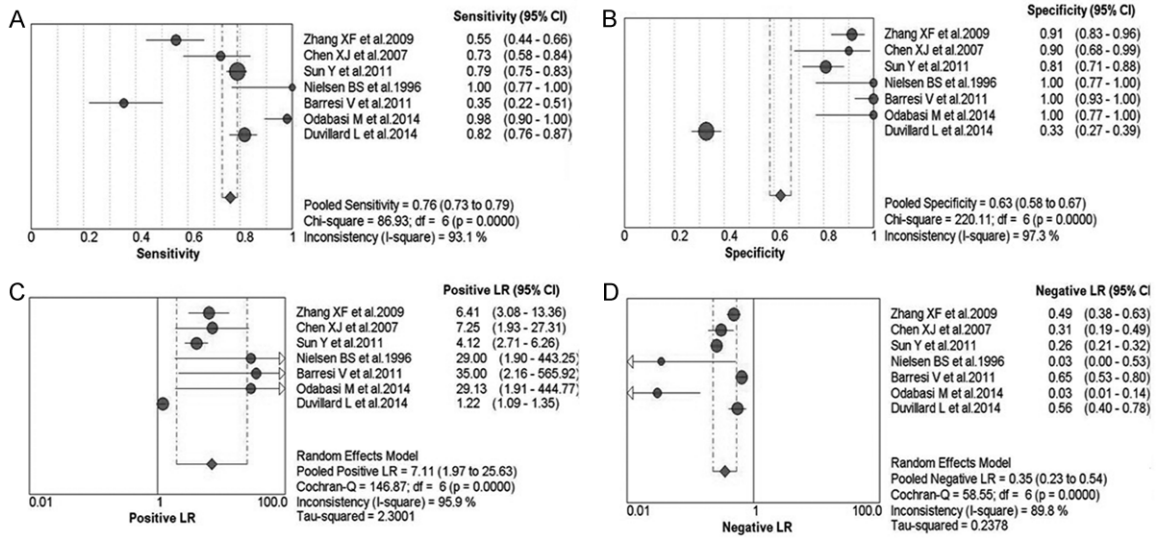

Figure 4. Forest plots of the pooled sensitivity (A), specificity (B), positive LR (C), and negative LR (D) of NGAL for the diagnosis of breast pre-cancerous abnormalities. The solid circles represent each individual study and the diamond represents the pooled diagnostic odds ratio. The size of the circle is proportional to the size of the study included. Error bars are 95% confidence intervals. LR = likelihood ratio. Results are for Layer 2 analysis.

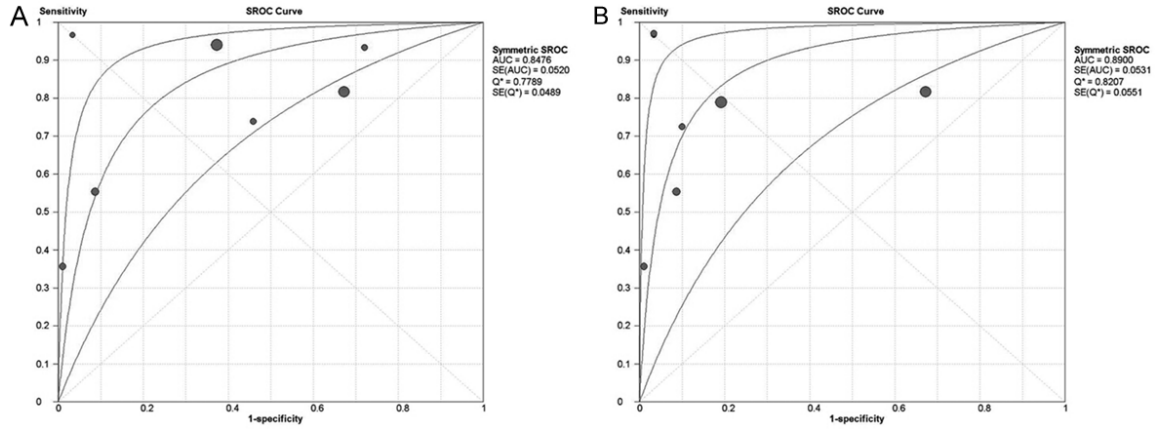

Figure 5. sROC curve of all included studies in the diagnosis of breast cancer/pre-cancerous abnormalities. The solid circles represent each individual study in the meta-analysis. The size of the circle is proportional to the size of the included study. A and B. Results are for Layer 1 and Layer 2 analysis, respectively.

We did not find a statistically significant difference neither (spearman correlation coefficient = 0.250, P = 0.589) for Layer 1, nor (spearman correlation coefficient = 0.126, P = 0.788) for Layer 2. The Spearman's correlation coefficient indicated that the heterogeneity of the 7 included studies was not related to threshold effect.

The Cochran Q test and the I^2 statistic were used to evaluate the presence of statistical heterogeneity occurred in the studies of Layer 1 shown in **Figures 2** and **3A**. We found the pooled sensitivty (chi-square = 125.65, I^2 = 95.2%, P < 0.001), specificity (chi-square = 196.72, I^2 = 97.0%, P < 0.001), PLR (chi-square = 109.75, I^2 = 94.5%, P < 0.001), NLR (chi-square = 85.19, I^2 = 93.0%, P < 0.001), and DOR (chi-square = 61.77, I^2 = 90.3%, P < 0.001).

Statistical heterogeneity occurred in the studies of Layer 2 was shown in **Figures 3B** and **4**. Here are the pooled sensitivty (chi-square = 86.93, I^2 = 93.1%, P < 0.001), specificity (chi-square = 220.11, I^2 = 97.3%, P < 0.001), PLR (chi-square = 146.87, I^2 = 95.9%, P < 0.001),

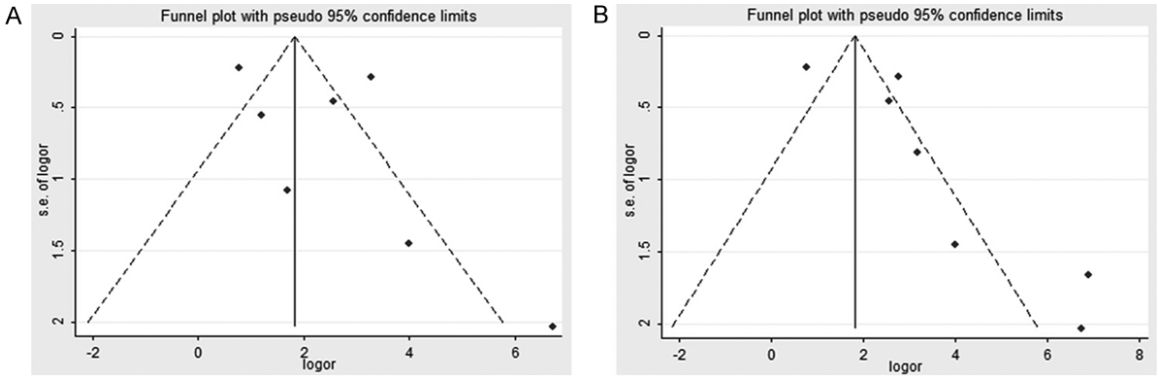

Figure 6. The funnel plot for the assessment of publication bias. Symbols represent each study in the meta-analysis. A and B. Results are for Layer 1 and Layer 2 analysis, respectively.

NLR (chi-square = 58.55, I^2 = 89.8%, P < 0.001), and DOR (chi-square = 56.48, I^2 = 89.4%, P < 0.001).

Publication bias

Funnel plot and Egger's test were performed to access the publication bias of the 7 studies stratified into two layers. The shape of funnel plots showed symmetry in **Figure 6**. The *P* value of Egger's test was 0.39 and 0.06, respectively. The result did not suggest any evidence of publication bias.

Discussion

Some research papers published after 2012, expressed their contradictory opinions on the diagnostic accuracy of NGAL in colorectal cancer, which differed from the 5 studies we have meta-analyzed. The sensitivity of lipocalin2 was found to be 24% and insufficient for use in primary disease detection [5]. NGAL overexpression could not distinguish dysplasia from adenocarcinoma [9]. Despite a significant increase in serum NGAL in CRC patients, NGAL may not be a suitable biomarker for diagnosis, especially early detection [10]. Therefore, we conducted this renewal to re-evaluate the efficacy of NGAL in CRC diagnosis. Other recent published papers with approval opinions on NGAL diagnosis, but failed to provide enough data to construct 2-by-2 table [6-8], thus they were excluded from this renewal.

In the present meta-renewal, for the first time, we brought into 1 research paper using serum ELISA assay for NGAL detection [10] and we tried to make reasonable use of its quantitative data as described below. The original quantified data from it were tri-segmented by NGAL concentration points 75 ng/ml and 104 ng/ml, and ELISA kit were purchased from R&D Systems. In two other studies using ELISA kit from the same source, the median NGAL concentration was 105.9 ng/ml in CRC patient group and 86.4 ng/ml in control group [5], and 67.96 ng/ml in CRC patients and 23.33 ng/ml in controls [7]. In another study using ELISA kit from Gentofte, Denmark, the average NGAL concentration was 102.3 ng/ml in CRC patient group and 0.6 ng/ml in control group, which data was incomparable to the previous. Therefore, in order to generate a 2-by-2 table, we deducted the tri-segmented data by choosing 75 ng/ml as proposed "cut point", which correspondingly consistent to the dataset of the previous two researches.

In this study, we have explored the relationship between NGAL expression and colorectal cancer and its precancerous abnormalities by peeling this analysis into two layers. Layer 1 analysis was dedicated to distinguish adenocarcinoma from the rest of the abnormalities and normal tissue; Layer 2 analysis was dedicated to distinguish adenocarcinoma and abnormalities with high possibility of carcinogenesis from benign hyperplasia and normal tissue. Median or mean values of serum NGAL concentration were significantly higher in CRC patients than in controls [5, 7, 10], and serum NGAL of colorectal adenoma patients was in between of the two parties [7]. This shift of "distinguishing ability" will cause a downregulation in diagnostic threshold for the relationship between colorectal abnormality series and NGAL expression.

When PLR > 10 or NLR < 0.1, the possibility of approving or negating a diagnostic ability of a disease significantly increased [3]. In this analysis, the pooled PLR and NLR were 2.29 and 0.34, 7.11 and 0.35, in Layer 1 and Layer 2 analyses, respectively, and PLRs were of big difference between layers yet NLRs were of little difference. The pooled PLR in Layer 2 indicated that the possibility of NGAL test making a correct diagnosis for positive result is 7.11 times higher than making a wrong diagnosis for positive result, and this possibility even is 3 times higher in Layer 2 than in Layer 1.

Using the bivariate model, we found that the AUC for the diagnosis of colorectal cancer was 0.8476 for Layer 1 analysis and 0.8900 for Layer 2 analysis, which increased more than 5%. The pooled DOR of NGAL was 11.29 and 23.10, respectively. The DOR is a single indicator to evaluate the diagnostic value of proposed test. The pooled DOR of NGAL of Layer 2 was 23.10, which indicated that the ratio of the odds of positivity in colorectal cancerous abnormalities to that in normal and benign subjects. Furthermore, the pooled DOR of NGAL was 2 times higher for Layer 2 than for Layer 1, which indicated the ratio of the odds of positivity in colorectal cancer and other abnormality patients to that in the normal and benign subjects was 2 times higher than the ratio of the odds of positivity in colorectal cancer patients to that in the non-cancer subjects.

The overall results of this study, mainly PLR, AUC and DOR demonstrated that NGAL detection may have a greater power in distinguishing adenocarcinoma and precancerous abnormalities from benign hyperplasia and normal tissue, than in distinguishing adenocarcinoma from the other abnormalities and normal tissue. Therefore, NGAL detection may be more effective in screening adenocarcinoma and precancerous abnormalities than colorectal cancer diagnosis. The observational studies involving diagnosis of cancers by biomarkers, basically need comparing immunohistochemical results with pathological "gold standard" before drawing a conclusion. As the consuming of time and complexity in manipulate immunohistochemical slices and ambiguousity in trans-interpretation of light microscopy results, recent observational studies have resorted to immunosorbent assay which was prepared by commercial kits and can be easily operated by scientific personnel without long-term training, and more convenient in unification results and more accessible to clinical application. More in-depth studies were needed to reveal the relationship between serum NGAL and colorectal cancer, which may have a higher value in clinical pre-cancerous screening than tissue slices for its non-invasive feature.

Some limitations of this present study must be considered. Studies included in this meta-analysis were fairly few. Particularly, only one study using ELISA assay met the inclusion criteria because more studies were excluded for shortage in essential information. In order to make a step closer to clinical application, studies involving immunoassay results should be taken into higher consideration. As studies using ELISA kits from different sources are difficult to compare with one another, more diagnostic studies applying ELISA kits with quantified and comparable data should be encouraged in the future, as the "cut point" and "core distinguishing ability" (in distinguishing cancer from non-cancer, or abnormality from normal) will only show up upon the quantified data accumulating to a certain extent.

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Disclosure of conflict of interest

None.

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