86466_Auto_Edited.docx

WORD COUNT 4038
TIME SUBMITTED 04-SEP-2023 12:12PM
PAPER ID 102467787

Name of Journal: *World Journal of Gastroenterology*

Manuscript NO: 86466

Manuscript Type: META-ANALYSIS

Diagnostic value of methylated branched chain amino acid transaminase 1/IKAROS family zinc finger 1 in plasma for colorectal cancer

Methylated BCAT1/IKZF1 for CRC diagnosis

Ke Xu, Airu Yu, Shenbin Pan, Jie He

Abstract

BACKGROUND

The diagnostic value of combined methylated branched chain amino acid transaminase 1 (BCAT1)/ IKAROS family zinc finger 1 (IKZF1) in plasma for colorectal cancer (CRC) has been explored since 2015. Recently, several related studies have published their results and showed its diagnostic efficacy.

AIM

To analyze the diagnostic value of methylated BCAT1/IKZF1 in plasma for screening and postoperative follow-up of CRC.

METHODS

The candidate studies were identified by searching the PubMed, Embase, Cochrane Library, CNKI, and Wanfang databases from May 31, 2003 to 1 June, 2023. Sensitivity, specificity, and diagnostic accuracy were calculated by merging ratios or means.

RESULTS

Twelve eligible studies were included in the analysis, involving 6561 participants. The sensitivity of methylated BCAT1/IKZF1 in plasma for CRC diagnosis was 60% (95% confidence interval (CI) 53-67) and specificity was 92% (95%CI 90-94). The positive and negative likelihood ratios were 8.0 (95%CI 5.8-11.0) and 0.43 (95%CI 0.36-0.52), respectively. Diagnostic odds ratio (DOR) was 19 (95%CI 11-30) and area under the curve was 0.88 (95%CI 0.85-0.91). The sensitivity and specificity for CRC screening were 64% (95%CI 59-69) and 92% (95%CI 91-93), respectively. The sensitivity and specificity for recurrence detection during follow-up were 54% (95%CI 42-67) and 93% (95%CI 88-96), respectively.

CONCLUSION

The detection of methylated BCAT1/IKZF1 in plasma, as a non-invasive detection method of circulating tumor DNA, has potential CRC diagnosis, but the clinical application prospect needs to be further explored.

Key Words: BCAT1; IKZF1; methylation; liquid biopsy; colorectal cancer

Xu K, Yu A, Pan S, He J. Diagnostic value of methylated branched chain amino acid transaminase 1/IKAROS family zinc finger 1 in plasma for colorectal cancer. *World J Gastroenterol* 2023; In press

Core Tip: DNA methylation, a commonly used target for detecting ctDNA in plasma, is often explored as diagnostic biomarker of cancer. Here, the present study systematically analyzed 12 studies including 6561 individuals to assess the diagnostic value of methylated branched chain amino acid transaminase 1 (BCAT1)/ IKAROS family zinc finger 1 (IKZF1) in plasma for colorectal cancer (CRC) through meta-analysis. The sensitivity and specificity of methylated BCAT1/IKZF1 in plasma for colorectal cancer diagnosis were 60% (95%CI 53-67) and 92% (95%CI 90-94),

respectively. The detection of methylated BCAT1/IKZF1 has potential in colorectal cancer diagnosis, but the clinical application prospect needs to be explored.

INTRODUCTION

Colorectal cancer (CRC) is the most common malignancy of the digestive system worldwide, with more than 18 million cases each year [1]. During long-term follow-up, 25%-40% of patients with CRC show disease recurrence even if after receiving radical treatment [2, 3]. As is well known, early diagnosis and treatment are crucial for secondary cancer prevention. Both initial diagnosis and diagnosis of relapse after radical treatment have a major impact on the overall survival of patients. Currently, the diagnostic accuracy of CRC has greatly improved through the wide application of CEA testing, colonoscopy, and imaging examination. However, the poor sensitivity of CEA detection, invasiveness of colonoscopy, high cost and radiation problems of imaging examination warrant safer, more convenient, economical, and accurate diagnostic methods in the future.

In recent years, liquid biopsy technology is being increasingly applied to disease diagnosis and treatment, owing to its characteristics such as less trauma, convenience, high speed, and cost efficiency. In the field of oncology, liquid biopsy technology has already been used for the diagnosis, prognosis, and treatment response prediction of diseases. During tumor development, because of the aggressiveness of the tumor and biological phenomena of cell necrosis and apoptosis, circulating tumor DNA (ctDNA) may enter the circulation in the early stages of disease development. Therefore, tumor markers based on ctDNA may play an important role in the early diagnosis of tumors [4, 5]. DNA methylation, mutation, and chromosomal copy number alteration are the most commonly used targets for detecting ctDNA in plasma, and are often explored as biomarkers [6]. The identification of biomarkers can promote the development of liquid biopsy technology, and mining ctDNA-based biomarkers is considered a promising research direction.

In the field of CRC, the accumulation of genetic and epigenetic changes begins more than 10 years before the onset of the disease [7]. Among these changes, genetic mutation and DNA methylation are the most commonly used biomarkers for the diagnosis and clinical prediction of CRC. As a biomarker, DNA methylation is more advantageous than genetic mutation, because DNA methylation is more common and gene mutation is more susceptible to tumor heterogeneity [8-12]. In 2016, the first DNA-methylated tumor diagnostic marker, SEPT9, was approved by the U.S. FDA for CRC screening, which promoted research in this area [13]. To date, several DNA methylation diagnostic markers have been explored for CRC, including APC, BCAT1, IKZF1, ALX4, LINE-1, SDC2, MGMT, RASSF1A, and WIF1 [14-24]. Among them, the diagnostic value of the combination of branched chain amino acid transaminase 1 (BCAT1)/ IKAROS family zinc finger 1 (IKZF1) for CRC has been explored since 2015 [25]. Recently, several clinical studies on the diagnostic accuracy of methylated BCAT1/IKZF1 in plasma for CRC have published their results. Methylated BCAT1/IKZF1 detection in plasma has similar or better diagnostic efficacy. In particular, a study also found that in the follow-up after radical treatment, some patients without imaging evidence have a recent recurrence event after the occurrence of BCAT1/IKZF1 hypermethylation, implying that methylated BCAT1/IKZF1 in plasma may have good timeliness in diagnosis [26]. To fully understand the diagnostic value of BCAT1/IKZF1 hypermethylation in initial diagnosis and postoperative recurrence of CRC, in the present study, we systematically analyzed the sensitivity, specificity, and other diagnostic indicators of methylated BCAT1/IKZF1 detection through meta-analysis, with the aim to provide a theoretical reference for its future clinical application in CRC diagnosis.

MATERIALS AND METHODS

This meta-analysis was performed using the Preferred Reporting Items for Systematic Reviews and Meta-analysis of Diagnostic Test Accuracy Studies (PRISMA-DTA) as the standard. Before performing this meta-analysis, a preliminary plan was developed and registered at https://www.crd.york.ac.uk/PROSPERO/.

Literature search strategy

A Computer search of PubMed, Embase, Cochrane Library, CNKI, and Wanfang databases was performed. Studies on the diagnostic accuracy of methylated BCAT1/IKZF1 in plasma for CRC were collected. The search period was from May 31, 2003, to June 1, 2023. In addition, references in the included literature were traced to supplement and obtain relevant literature. The method of free word retrieval was adopted. The search formula was (BCAT1 or IKZF1) and (methylation or methylated) and ((colorectal or colon or rectal) and (cancer or tumor or carcinoma)).

Inclusion and exclusion criteria

The inclusion criteria were as follows. Patients with CRC confirmed by pathological examination were included in the case group and healthy volunteers without CRC were included in the control group. Methylated BCAT1 and/or IKZF1 in plasma were used as diagnostic markers, and a positive diagnosis was defined as the presence of methylation in either BCAT1 or IKZF1. The exclusion criteria were as follows: reviews, letters, reviews, and conference papers; non-human research; lack of data or incomplete information; and data required for the four-grid statistical table could not be directly or indirectly extracted.

Literature screening and data extraction

Two researchers independently performed literature screening and data extraction, and then cross-checked the data. After removing duplicate references, the titles and abstracts of the remaining articles were screened for inclusion and exclusion criteria to exclude irrelevant studies. Eligible articles were then reviewed in full to filter again and a final inclusion was arrived. The main data extracted included the following. 1. Basic information: first author, publication time, country, design type (prospective or retrospective), detection method, and diagnostic standard. 2. Test subjects: subject selection and number of subjects. 3. Test indicators: number of true positive, false

positive, true negative, and false negative cases. Any differences in the data extraction process were assessed independently by a third researcher.

Quality Assessment

All studies were subjected to quality assessment according to the Cochrane Collaboration's Diagnostic Accuracy Studies Quality Assessment 2 (QUADAS-2). Similarly, two researchers independently assessed the quality of each study. If there was any disagreement, it was discussed with and decided by a third researcher.

Statistical Analysis

Meta-Disc 1.4 and Stata 11.0 software were used for meta-analysis. The spearman correlation coefficient was used to explore whether there was a threshold effect, and the I^2 statistic was used to explore whether there was heterogeneity due to non-threshold effects. If the I^2 was greater than 50%, it indicated obvious heterogeneity among studies, and the random effects model was used for fitting. Otherwise, the fixed effect model was used. The combined statistics were expressed by sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, diagnostic odds ratio, and 95% confidence interval. A summary receiver operating characteristic (SROC) curve was plotted and area under the curve (AUC) was calculated. Subgroup analysis and meta-regression analysis were used to explore the sources of heterogeneity. In addition, publication bias was visually assessed using a funnel plot and quantitatively assessed using Deeks' funnel plot asymmetric linear regression test. In full-text statistics, results with $p < 0.05$ were considered statistically significant.

RESULTS

Characteristics of the included studies

Among the 310 papers obtained through the search formula, 12 papers (11 prospective studies and 1 retrospective study) were included by excluding irrelevant literatures, duplicate studies, basic experimental studies, case reports, and other ineligible

literatures [26-36]. The specific process of inclusion and exclusion is shown in Figure 1, and the detailed information of each included study is shown in Table 1. Next, we assessed the quality of the included studies using the QUADAS-2 tool, and none of the included studies were assessed as having a high-risk bias (Figure 2) (Table S1).

Extraction of literature data

The 12 studies involved 6,561 participants. The number of true positives, false positives, true negatives, and false negatives of BCAT1/IKZF1 methylation in plasma for CRC diagnosis was clearly described by each study. Among them, 5 studies included CRC screening populations, and 7 studies included follow-up populations after radical treatment for CRC. Quantitative PCR was used for assessment in all studies, but the diagnostic criteria for positive results were different among the studies. Six studies set at least one PCR replicate as positive, whereas the other 6 studies defined positive by setting a threshold of methylation rate.

Threshold effect analysis

As different thresholds can lead to different sensitivity, specificity, and diagnostic odds ratio (DOR) of diagnostic tests, resulting in threshold effects, it is necessary to first detect whether the diagnostic method has a threshold effect. Spearman correlation analysis carried out using Meta-Disc 1.4 software showed that the correlation coefficient among the included studies was r = -0.287 (P = 0.366), there was no significant threshold effect and the results were pooled for analysis.

Pooled analysis

The pooled sensitivity and specificity of the 12 studies were 60% (95% confidence interval (CI) 53-67) and 92% (95%CI 90-94), respectively. The positive likelihood ratio and negative likelihood ratio were 8.0 (95%CI 5.8-11.0) and 0.43 (95%CI 0.36-0.52), respectively. The DOR was 19 (95%CI 11-30) and AUC was 0.88 (95%CI 0.85-0.91) (Figure 3). Furthermore, to explore the diagnostic ability of methylated BCAT1/IKZF1

detection for early CRC, we investigated the diagnostic accuracy in patients with different stages of CRC. The diagnostic sensitivity in patients with stage I, II, III, and IV disease was 32% (95%CI 22-43), 66% (95%CI 59-73), 71% (95%CI 63-78), and 91% (95%CI 81-96), respectively (Figure 4).

Subgroup analysis

To further explore the source of heterogeneity, we conducted a subgroup analysis according to the purpose of testing and positive result definition. The results of the subgroup analysis showed that when methylated BCAT1/IKZF1 in plasma was used for CRC screening, the sensitivity was 64% (95%CI 59-69), specificity was 92% (95%CI 91-93), positive likelihood ratio was 8.5 (95%CI 7.1-10.1), negative likelihood ratio was 0.39 (95%CI 0.33-0.45), DOR was 22(95%CI 16-30) and AUC was 0.92 (95%CI 0.89-0.94). When the method was applied to postoperative follow-up and assessment of disease recurrence, the sensitivity was 54% (95%CI 42-67), specificity was 93% (95%CI 88-96), positive likelihood ratio was 7.4(95%CI 3.9-14.2). negative likelihood ratio was 0.49 (95%CI 0.37-0.66), DOR was 15 (95%CI 6-37) and AUC was 0.85 (95%CI 0.81-0.88). The subgroup analysis based on the purpose of testing effectively reduced heterogeneity, especially in the subgroup of CRC screening, where no significant heterogeneity was detected in both sensitivity and specificity analyses (I^2=48.58, I^2=26.75). As the method of evaluating results could directly affect the accuracy of diagnosis, the included studies were divided according to the definition of positive results. The two subgroups were defined as follows: at least one PCR replicate was positive, and exceeding the set threshold was considered positive. When at least one PCR replicate was positive, the sensitivity was 59% (95%CI 50-67), specificity was 91% (95%CI 89-93), and AUC was =0.87(95%CI 0.84-0.90). When result exceeding the set threshold was defined as positive, the sensitivity was 61% (95%CI 49-72), specificity was 94% (95%CI 87-96), and AUC was=0.91(95%CI 0.88-0.93). Combining the principle of real-time PCR and the results of the comprehensive analysis, setting a threshold to define positive result may improve the diagnostic accuracy (Table 2).

Sensitivity and publication bias analysis

To test the robustness of the results, we conducted a sensitivity analysis. The sensitivity analysis showed that after omitting each study one by one, the main results of the meta-analysis did not change significantly, indicating that there was no significant bias in the included studies, and the results were reliable and stable (Figure 5). Deeks' funnel plot was used to assess publication bias, and no significant publication bias was observed (P = 0.604) (Figure 6).

DISCUSSION

Screening and post-treatment follow-up methods for CRC vary worldwide. Currently, the main methods include fecal sample-based testing, colonoscopy, blood sample-based testing, and CT or MRI colonography. The tests based on fecal samples include fecal occult blood tests, fecal immunochemical tests (FIT), gut microbe analysis, and FIT-DNA tests. Among the fecal sample-based tests, Multi-Target Stool DNA Test, approved by the FDA in 2014 for CRC screening is noteworthy. In this test, CRC is diagnosed using the methylation markers NDRG4, BMP3, VIM, and TFP12, combined with mutant KRAS and fecal hemoglobin level. Its sensitivity for CRC screening was 92% and its specificity was 87% [37-39]. Owing to the high diagnostic accuracy of the Multi-Target Stool DNA Test, it is recommended for screening high-risk populations [40]. However, its widespread application still has challenges, such as high cost and commercialization issues [41]. Blood sample assay was vital for non-invasive detection, and the diagnostic markers mainly included DNA mutation, DNA methylation, cfDNA, tumor-derived circulating cells, circular RNA, PIWI-interacting RNA, microRNA, and exosomal microRNA. Researchers have gradually realized the advantages of DNA methylation as a biomarker for tumor diagnosis. Because its changes occur even before the occurrence of tumors, it is theoretically ideal for early screening of tumors [10, 42]. SEPT9 methylation was the first methylated marker approved by the FDA for screening CRC using plasma samples, with a diagnostic sensitivity of 0.679 (95%CI 0.622-0.732)

and specificity of 0.904 (95%CI 0.881-0.923) [43]. However, it is no longer recommended by most guidelines because it is less sensitive than other current tests [44]. Recently, scholars have tried to find other DNA methylation markers with better accuracy for the diagnosis of CRC, such as BCAT1, IKZF1, APC, ALX4, LINE-1, SDC2, MGMT, RASSF1A, and WIF1. They have been used to establish a variety of combinations of diagnostic panels, but there is no sufficient evidence to effectively show their application prospects [14-24]. Owing to the wide application of machine learning in biomedicine, studies on multi-omics and pan-cancer screening products have gradually expanded in the exploration of tumor marker screening based on blood samples. For example, CanerSEEK was a multi-omics tool for detecting ctDNA and proteins for screening various cancers, with a diagnostic sensitivity of up to 84% for CRC [45]. Another new method for pan-cancer screening is GRAIL, which has a diagnostic sensitivity of 82% for CRC, 43.3%, 85.0%, 87.9%, and 95.3% for stage I, II, III, and IV CRC, respectively [46]. It is worth noting that the establishment of most pan-cancer detection methods is still based on DNA methylation as the main diagnostic marker. In the future, more new detection methods will be introduced for the initial diagnosis of CRC and post-treatment follow-up, which will further improve the overall prognosis of CRC.

Recently, multiple studies on the use of methylated BCAT1/IKZF1 in plasma for the diagnosis of CRC have been reported. Overall, its diagnostic accuracy is good, and it may be affected by fewer clinical variables. Based on the results, Clinical Genomics has already completed the commercialization of this detection method, named COLVERA®. At present, it is approved for use in postoperative follow-up detection and assessment of recurrence risk in patients with CRC in the United States and Australia [31]. According to the results of this meta-analysis, methylated BCAT1/IKZF1 in plasma has a sensitivity of 60% (95%CI 53-67) and specificity of 92% (95%CI 91-93) for CRC screening. The results are better than those for CEA detection, and its accuracy is similar to that of SETP9 methylation detection. To further confirm the diagnostic value of methylated BCAT1/IKZF1 detection, a direct cross-over study with methods

such as FIT and Multi-Target Stool DNA Test is needed. As a postoperative follow-up diagnostic method for CRC, the sensitivity of methylated BCAT1/IKZF1 detection 54% (95%CI 42-67), specificity 93% (95%CI 88-96), and AUC was 0.85 (95%CI 0.81-0.88). A study has reported that the sensitivity of this method in the diagnosis of recurrence is as high as 75%, while the sensitivity of the CEA index commonly used in the clinical diagnosis of postoperative recurrence is just 32.1%. It can be seen that it has a major advantage in the postoperative assessment of recurrence. Additionally, some reports suggest that changes in methylated BCAT1/IKZF1 level in plasma occur before imaging changes, which also has the diagnostic timeliness of recurrence [26]. Recently, it has been reported that ctDNA detection using a 15-gene mutation panel can be used to monitor the recurrence of postoperative CRC [47]. Some studies have also found that mesenchymal circulating tumor cell with PRL-3+ is associated with recurrence, which is likely to have a monitoring effect on disease recurrence [48]. However, from the perspective of detection time point, methylated BCAT1/IKZF1 in plasma has major advantages in postoperative follow-up. Earlier, gene mutation indicator detection often required 4-10 wk after surgery, otherwise the sensitivity may decrease, and the method discussed here is not affected by the detection time point [8, 49, 50]. For the stability and reliability of the results, a subgroup analysis was conducted. The Subgroup analysis based on the purpose of detection could effectively reduce the heterogeneity of the results, especially in the CRC screening group. However, there was still some heterogeneity in the postoperative follow-up group, considering this was most likely caused by different time points of monitoring and the different tumor stages. Owing to the limitations of data, further in-depth analysis could not be conducted in this group. From another perspective of grouping, there were differences in the definition of how to determine a positive result. Seven of the included studies defined at least one PCR replicate as positive. In the past two years, it has been found that setting a specific threshold based on this method could substantially improve the specificity of diagnosis without affecting the sensitivity of diagnosis. In this meta-analysis, it was also found that when at least one PCR replicate was positive, the diagnostic sensitivity was 59%

(95%CI 50-67) and specificity was 91% (95%CI 89-93). When thresholds were set, diagnostic sensitivity was 61% (95%CI 49-72), specificity was 94% (95%CI 87-96), and AUC was 0.91(95%CI 0.88-0.93). In the future application of this method, setting a threshold value may further improve the diagnostic accuracy. In terms of diagnostic sensitivity for patients with different stages of CRC, the detection of the methylated BCAT1/IKZF1 in plasma, similar to other detection methods, has a poor diagnostic sensitivity for patients with early-stage CRC, which may limit its clinical application in CRC screening. In the future, the clinical application value of methylated BCAT1/IKZF1 in plasma can be enhanced by combining it with other tests. Overall, in this meta-analysis, we systematically analyzed the diagnostic accuracy of methylated BCAT1/IKZF1 in plasma for CRC. These results can provide a basis for further research and clinical application of BCAT1/IKZF1 methylation in the diagnosis of CRC in the future.

In recent years, researchers have found that methylated BCAT1/IKZF1 in plasma may also be valuable in the prognostic prediction of CRC, suggesting that methylated BCAT1/IKZF1 in plasma is more likely to be found in patients with postoperative incisal margin deficiency, lymph node invasion or distant metastasis [36]. Another study analyzed the 3-year recurrence free survival (RFS) of postoperative patients with CRC and found the RFS of patients with methylation was 56.5%, that of patients without methylation was 83.3% [26].

The multivariate analysis showed that the presence of methylated BCAT1/IKZF1 in plasma was an independent factor for poor RFS in CRC. The value of this method in prognostic prediction may be related to its role in the diagnosis of relapse. The above results suggest that patients with consistently positive BCAT1/IKZF1 methylation in plasma after surgery should be followed up cautiously, and the methylation level should be dynamically monitored. At present, some scholars believe that the cause of positive results after surgery may be related to the existence of minimal residual disease (MRD). However, as there is no gold standard for the diagnosis of MRD in CRC; further research is needed to confirm this hypothesis.

There were some limitations to this meta-analysis. As all included studies were conducted in Australia or the United States, the ability of methylated BCAT1/IKZF1 testing to diagnose CRC in other ethnic groups and regions needs to be further investigated. In addition, not all studies clearly recorded the diagnostic sensitivity for patients with different stages of disease; the diagnostic sensitivity analyzed by stratification needs to be explored. Owing to the limitation of data, the diagnostic efficacy of methylated BCAT1/IKZF1 for patients with different genotypes could not be analyzed separately. Finally, it was not possible to perform a pooled analysis of data on the prognosis, because only two studies recorded prognostic results for this approach.

CONCLUSION

The detection of methylated BCAT1/IKZF1 in plasma, as a non-invasive detection method of circulating tumor DNA, has potential CRC diagnosis, but the clinical application prospect needs to be further explored.

ARTICLE HIGHLIGHTS

Research background

Currently, DNA methylation is one of the most commonly used detection targets for ctDNA in plasma, and is often explored as a diagnostic biomarker for cancer. The diagnostic value of combined methylated branched chain amino acid transaminase 1 (BCAT1)/ IKAROS family zinc finger 1 (IKZF1) in plasma for colorectal cancer (CRC) has been explored since 2015. Recently, several related studies have published their results and showed its diagnostic efficacy.

Research motivation

To fully understand the diagnostic value of methylated BCAT1/IKZF1 in initial diagnosis and postoperative recurrence of CRC.

Research objectives

To evaluate the diagnostic accuracy of methylated BCAT1/IKZF1 in plasma for screening and postoperative follow-up of patients with CRC.

Research methods

We searched the PubMed, Embase, Cochrane Library, CNKI, and Wanfang databases. Studies on the diagnostic accuracy of methylated BCAT1/IKZF1 in plasma for CRC were retrieved. Data extraction, pooled analysis, subgroup analysis, sensitivity analysis, and publication bias analysis were performed.

Research results

The pooled sensitivity and specificity of methylated BCAT1/IKZF1 for CRC diagnosis were 60% (95%CI 53-67) and 92% (95%CI 90-94), respectively. The positive likelihood ratio and negative likelihood ratio were 8.0 (95%CI 5.8-11.0) and 0.43 (95%CI 0.36-0.52), respectively. The diagnostic odds ratio (DOR) and area under the curve (AUC) were 19 (95%CI 11-30) and 0.88 (95%CI 0.85-0.91), respectively.

Research conclusions

The detection of methylated BCAT1/IKZF1 in plasma, as a non-invasive detection method of circulating tumor DNA, has potential in the diagnosis of CRC, but the clinical application value still needs to be explored.

Research perspectives

The detection of methylated BCAT1/IKZF1 in plasma, similar to other detection methods, has poor diagnostic sensitivity for early-stage disease, which may limit its clinical application in CRC screening. In the future, the clinical application of methylated BCAT1/IKZF1 in plasma can be promoted by combining it with other tests.

ACKNOWLEDGEMENTS

We would like to thank Editage (www.editage.com) for English language editing.

86466_Auto_Edited.docx

ORIGINALITY REPORT

15%
SIMILARITY INDEX

PRIMARY SOURCES

1	www.ncbi.nlm.nih.gov Internet	143 words — 3%
2	Yulan Gu, Chuandan Wan, Jiaming Qiu, Yanhong Cui, Tingwang Jiang. "Diagnostic value of circulating tumor DNA as an effective biomarker in cervical cancer: a meta-analysis", Cold Spring Harbor Laboratory, 2019 Crossref Posted Content	51 words — 1%
3	www.spandidos-publications.com Internet	42 words — 1%
4	www.science.gov Internet	38 words — 1%
5	pubmed.ncbi.nlm.nih.gov Internet	35 words — 1%
6	www.labmedica.com Internet	31 words — 1%
7	www.frontiersin.org Internet	27 words — 1%
8	Zhenyu Huo, Feifei Chong, Lingyu Yin, Zongliang Lu, Jie Liu, Hongxia Xu. "Accuracy of the GLIM criteria for diagnosing malnutrition: a systematic review and meta-analysis", Clinical Nutrition, 2022	23 words — 1%

Crossref

9	www.plosone.org Internet	20 words —	< 1%
10	Rosalie V. McDonough, Wu Qiu, Johanna M. Ospel, Bijoy K. Menon, Petra Cimflova, Mayank Goyal. "Multiphase CTA-derived tissue maps aid in detection of medium vessel occlusions", Neuroradiology, 2021 Crossref	18 words —	< 1%
11	Su, Guoming, Zhuqing Fu, Liren Hu, Yueying Wang, Zuguo Zhao, and Weiqing Yang. "16S Ribosomal Ribonucleic Acid Gene Polymerase Chain Reaction in the Diagnosis of Bloodstream Infections: A Systematic Review and Meta-Analysis", PLoS ONE, 2015. Crossref	17 words —	< 1%
12	www.omicsdi.org Internet	17 words —	< 1%
13	Hao Wang, Ying Ba, Qian Xing, Run-Ce Cai. "Differential Diagnostic Value of Bilateral Inferior Petrosal Sinus Sampling (BIPSS) in ACTH-Dependent Cushing Syndrome: a Systematic Review and Meta-Analysis", Research Square Platform LLC, 2020 Crossref Posted Content	16 words —	< 1%
14	journals.plos.org Internet	16 words —	< 1%
15	Qi Li, Ningli Wang, Zhicheng Liu, Lin Li, Zhicheng Liu, Wenjia Wang, Xiaoxue Long, Hongfang Song. "Meta-Analysis of the Relationship between Normal Tension Glaucoma, Open-Angle Glaucoma, and Fractional Pressure Reserve", Molecular & Cellular Biomechanics, 2020 Crossref	15 words —	< 1%

16	journals.lww.com Internet	15 words — < 1%
17	Huiping Li, Yeyuan Zhang, Yujing He, Jianing Huang, Jie Yao, Xieyan Zhuang. "Association between consumption of sweeteners and endometrial cancer risk: a systematic review and meta-analysis of observational studies", British Journal of Nutrition, 2023 Crossref	14 words — < 1%
18	Schreuders, Eline H., Esmée J. Grobbee, Manon C. W. Spaander, and Ernst J. Kuipers. "Advances in Fecal Tests for Colorectal Cancer Screening", Current Treatment Options in Gastroenterology, 2016. Crossref	14 words — < 1%
19	Seval Yılmaz Ergani, Tolgay Tuyan İlhan, Betül Tokgöz Çakır, Burak Bayraktar et al. "Can Triglyceride/Glucose Index (TyG) and Triglyceride/HDL-Cholesterol Ratio (TG/HDL-c) Predict Diabetes in Pregnant Women?", Research Square Platform LLC, 2023 Crossref Posted Content	14 words — < 1%
20	bpsmedicine.biomedcentral.com Internet	14 words — < 1%
21	jamanetwork.com Internet	13 words — < 1%
22	link.springer.com Internet	13 words — < 1%
23	online.reed.es Internet	13 words — < 1%
24	topsecretapiaccess.dovepress.com Internet	13 words — < 1%

25 f6publishing.blob.core.windows.net
Internet

12 words — < 1%

EXCLUDE QUOTES	ON	EXCLUDE SOURCES	< 12 WORDS
EXCLUDE BIBLIOGRAPHY	ON	EXCLUDE MATCHES	< 12 WORDS