Comparative Diagnostic Accuracy of Fecal Protein Biomarkers for Colorectal Advanced Neoplasms: A Systematic Review and Meta-Analysis

Atefeh Nasir Kansestani
Zhejiang University
Mohammad Erfan Zare
Zhejiang University
Qingchao Tong
Zhejiang University
Jun Zhang (jameszhang2000@zju.edu.cn)
Zhejiang University

Research Article

Keywords: colorectal cancer, fecal test, diagnostic accuracy, meta-analysis.

Posted Date: July 14th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-684598/v1

Abstract

Early diagnosis of colorectal advanced neoplasms (AN), including colorectal cancer (CRC) and advanced adenoma (AA), has positive effect on survival rate. As the first attempt, the aim of this meta-analysis was to compare the diagnostic accuracy of fecal protein biomarkers for detection of colorectal neoplasms with consideration of wide range covariates.

Until Jun 10, 2021, a systematic literature search was performed on Web of Sciences, Scopus and PubMed. The diagnostic accuracies were calculated using the bivariate/hierarchical random effect model. Biomarkers were determined clinically applicable (CA) if they had area under the curves> 0.70, positive and negative likelihood ratio >2 and <0.5, respectively.

A total of 47059 test results were extracted from 16 Immunochemical fecal occult blood test (iFOBT), 26 Pyruvate Kinase-M2 (PK-M2) and 23 Fecal Calprotectin (FC) studies.

Only iFOBT, PK-M2 and FC for CRC plus iFOBT and PK-M2 for AN were CA. iFOBT had significantly superior accuracy (P= 0.02 versus PK-M2 and P< 0.01 versus FC for CRC; P< 0.01 versus PK-M2 for AN). Regarding covariates, lateral flow method of PK-M2 measurement increased its accuracy for CRC detection compared to enzyme-linked immunosorbent assay (P< 0.01). Briefly, iFOBT is the most accurate fecal biomarker for diagnosis of CRC and AN.

Introduction

Colorectal cancer (CRC) is currently the third most prevalent malignancies and the second reason of death among cancerous patients [1]. Despite the fulfillment of major efforts like screening programs, increasing trend of new cases in recent years indicates that better strategies require for early diagnosis of CRC and advanced adenoma (AA) as its precursor [2].

Early diagnosis of colorectal advanced neoplasms (AN), including CRC and AA, has a positive correlation with a high survival rate owing to implementation of proper treatments. Today, colonoscopy is considered as the gold standard for AN diagnosis and screening [2,3]. However, colonoscopy is an expensive, invasive and operator's skill-dependent technique. In addition, it needs unpleasant bowel preparation and occasionally causes serious complications. Therefore, implementing non-invasive biomarkers for diagnosis of AN seems to necessary [4].

Among different introduced biomarkers for diagnosis and screening of AN, fecal protein biomarkers have special importance due to their low cost, non-invasive and simple sampling procedure attributes [5]. The first introduced fecal biomarker for AN was guaiac-based fecal occult blood test (gFOBT) and since its introduction, it could save many human lives, despite its low sensitivity. After a while, this method is replaced by Immunochemical fecal occult blood test (iFOBT) which has much higher sensitivity [2,5]. In the recent decades, some promising fecal protein biomarkers are introduced for diagnosis and screening of CRC and other ANs. Nonetheless, there is not precise information regarding comparison of their accuracies for finding the most accurate biomarker. Given the above information, as the first attempt, the aim of this evidence based meta-analysis was to compare the diagnostic accuracy of fecal protein biomarkers for detection of CRC, AA and AN with consideration of wide range covariates.

Methods

Search strategy

The search strategy of the present systematic review was carried out based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. We performed systematically searches on the electronic databases containing Web of Sciences, Scopus and MEDLINE/PubMed until Jun 10, 2021, without any language restriction. Also, the Chinese National Knowledge Infrastructure (CNKI) data base for Chinese full text articles and the Scientific Information Database (SID) data base for Persian full text articles were searched.

The following search keywords were used: ("Colorectal cancer" OR "CRC" OR "Colorectal malignancy" OR "Colorectal tumor" OR "Adenoma" OR "Colorectal neoplasm") AND ("Fecal biomarker" OR "Laboratory tests" "Diagnostic biomarker", "Screening Biomarker"). Finally, the reference lists of each selected paper and related systematic and narrative reviews on this topic were assessed to identify missed studies. To exclude the duplicate papers, we imported records into the EndNote software (Version X9, Thomson Reuters).

Study selection and data extraction

Two reviewers (A.N.K and M.E.Z) independently screened the title and abstract of all obtained records for eligibility and inclusion. Included criteria were: 1) patients for whom a fecal protein biomarker was used to detect CRC, AA or AN; 2) CRC and AA should be confirmed by colonoscopy and pathology reference standards; 3) specific diagnostic information was sufficient to construct a 2×2 contingency table; 4) For each fecal biomarker, at least 4 studies should be found. Exclusion criteria were set as following: 1) duplicated studies, review articles, editorials, case reports, and clinical guidelines; 2) insufficient data reporting to construct the 2×2 contingency table; CRC and colorectal AA were not verified by aforementioned reference standards.

A custom-made form was utilized for data extraction. The results of iFOBT were extracted in those studies which be accomplished it along with other assessed biomarkers. To achieve more reliable results in case-control designed studies, we construct 2×2 contingency tables by comparing the specific characteristic versus not only healthy controls but also other patients which had not that specific characteristic. In order to homogenization of different units, we transformed mg/L (=µg/mL) to µg/g by multiplying each value by a factor of 5.

In CRC patients, the percentage of distal and late stage tumors were extracted. Proximal tumors were defined as those located from cecum to transverse colon and distal tumors located from splenic flexure to rectum. Besides, late stage tumors were defined as CRC stages III+IV or Dukes' stages C+D against 0+I+II or Dukes' stages A+B which were categorized as early stage tumors [6]. Colorectal adenomas were defined as AA when the following features existed: 1) high-grade dysplasia; 2) tubulovillous or villous components; 3) multiple adenomas or individual lump with ≥ 1 cm in size. AN contained CRC and/or AA.

Quality Assessment and publication bias

The methodological quality of each included study was assessed utilizing the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool. QUADAS-2 evaluates four key domains made up of "patient selection", "index test", "reference standard", and "flow and timing" in two categories, "risk of bias" for all four domains and "applicability" for the first three domains in the diagnostic accuracy studies. Each category was finally scored as low, high or unclear according to the assessment criteria. All disagreements were resolved by consensus after discussion. Further, to evaluation of potential publication bias, the linear regression method was utilized for assessing the asymmetry of Deeks' funnel plot. P <0.1 for the slope coefficient reveals the presence of publication bias.

Statistical analysis

To construct 2×2 contingency table, we calculated true positive, false positive, true negative and false negative for each included study. Standard bivariate method was employed to calculate the summary points including pooled-sensitivity, pooled-specificity, pooled-positive Likelihood Ratio (PLR+), pooled-negative Likelihood Ratio (PLR-) and pooled-diagnostic Odds Ratio (PDOR). Using hierarchical model, we plotted summary receiver operating characteristic (HSROC) to achieve the area under the curve (AUC) as a global measure of test performance. Overall diagnostic accuracy of each biomarker was interpreted according to AUC, PLR+ and PLR-. The relationship between AUC value and diagnostic accuracy is described as following: 0.5 to 0.70 is interpreted as not acceptable, 0.71 to 0.79 acceptable, 0.80-0.89 good and 0.90-1 excellent. Also, based on PLR+ and PLR-, diagnostic accuracy of each biomarker is divided into four categories. The PLR- values <0.1, 0.1–0.2, 0.2–0.5 and >0.5 respectively represents substantial, moderate, small and not meaningful evidences to rule out the disease existence. The PLR+ values >10, 5–10, 2–5 and <2 respectively consider as substantial, moderate, small and not meaningful evidences to rule in the disease existence. The results of LRs were summarized by a scattergram.

In this study we considered clinically applicable biomarkers if they had AUC> 0.70, PLR+ > 2, and PLR-< 0.5. To compare diagnostic accuracy of different clinically applicable biomarkers, relative-DORs (RDOR) and their P-values were computed.

Between study heterogeneity were evaluated using Higgins' inconsistency index (I^2). I^2 > 50% implied substantial heterogeneity, which was then assessed to find potentially sources of heterogeneity by subgroup analysis, meta-regression, and threshold analysis.

In the present study, calculations were conducted and summarized for reporting with considering 95% confidence interval (95% CI) and reports were defined as statistically significance when P< 0.05 (Except publication bias). All statistical analyses were performed by "midas" commands in Stata software (Stata Corporation, College Station, TX, USA, version 12.0) and *RevMan* 5.3 was employed to draw comparative HSROC plots.

Results:

Study selection

Among 2581 initial records, 840 studies were excluded owing to duplication and 1670 were excluded after screening of title and abstract. In this stage, the most exclusion reasons were: 1) review articles, editorials, case reports, and clinical guidelines; 2) laboratory biomarkers were evaluated on non-fecal samples (Serum and tissue), and 3) non-protein biomarkers such as molecular biomarkers, microbiome mass, and others. Finally, 71 studies were undergone full text assessment. From this number, 22 studies were excluded due to following reasons: 1) lack of verification by reference standard (Colonoscopy and pathology) (n= 13), and 2) insufficient data to construct the 2×2 contingency table (n=9). Eventually, 49 studies with 47059 test results were included in the present study (**Fig. 1A**).

Pursuant to inclusion criteria, Pyruvate Kinase-M2 (PK-M2) and Fecal Calprotectin (FC) were found as eligible biomarkers for further assessment.

Sixteen from 49 included studies reported the data of iFOBT besides the other assessed biomarkers with 13769 test results [7–22]. All 16 studies had the results of iFOBT for diagnosis of CRC (5610 test results), 10 studies had results for AA (4008 test results) and 11 studies had results for AN (4151 test results). One study evaluated iFOBT with two different commercial kits, so we constructed two separated 2×2 contingency tables from this article [13].

From 26 PK-M2 included studies with 12213 test results [10–19,23–38], 25 studies reported the results of CRC detection (5706 test results), 10 studies for AA (3781 test results) and 10 studies for AN (2726 test results). One study assessed PK-M2 by two different methods, therefore we constructed two 2×2 contingency tables from this article [10].

We found 23 studies with 21077 test results for FC [7–9,16,17,20–22,27,39–52] which all of them had the information of CRC diagnosis with 9747 test results. The results of FC for detection of AA and AN were extracted from 9 articles with 5665 test results for each condition, respectively. There were two studies which evaluated FC by two different methods, so we constructed two separately 2×2 contingency tables for each article [22,41].

There were 2 studies that evaluated all three biomarkers [16,17] and one study evaluated PK-M2 and FC at the same time for CRC diagnosis [27]. **Table 1** summarized the main characteristics of included studies in this review.

Quality assessment and publication bias

The quality of included studies were assessed using QUADAS-2 tool and their results were illustratively summarized for each biomarker (**Fig. 1B-D**). The quality assessment result of included studies in iFOBT group revealed the major risk of bias in the “flow and timing” and “patient selection” categories mainly due to all patients were not included in analysis and case-control study design, respectively (**Fig. 1B**). Regarding PK-M2 included studies, the major risk of bias occurred in “patient selection” category because of case-control study design. Also, there were 3 studies with high and 9 studies with unclear risk of bias in “index test” category as a result of not pre-specified the thresholds and unclear situation of index test interpretation without knowledge of reference standard result, in orderly (**Fig. 1C**). Concerning FC included studies, the greatest risk of bias referred to “flow and timing” and “index test” owing to the same aforementioned reasons (**Fig. 1D**). Included studies for all biomarkers had no concerns regarding applicability.

Table 2 includes the publication bias analyses of each group. As regards the CRC diagnosis, the Deeks’ funnel plot asymmetry test indicated there were not significant publication bias in datasets of iFOBT, PK-M2 and FC biomarkers. (**Supplemental Fig. 1A-C)**. In relation to AA detection, significant publication bias in iFOBT dataset and absence of publication bias in PK-M2 and FC datasets were found (**Supplemental Fig. 2A-C)**. Concerning AN diagnosis, analyses represented not significant publication bias in iFOBT and PK-M2 while indicated significant publication bias in FC datasets (**Supplemental Fig. 3A-C**).

Diagnostic accuracy of fecal biomarkers

Table 2 presents the diagnostic accuracy of different fecal biomarkers for detection of CRC, AA and AN. In order to CRC diagnosis, all 3 assessed biomarkers were applicable according to their PLR+, PLR- and AUC (>2, <0.5, and >0.70, respectively) (**Table 2** and **Fig. 2A-D**). **Fig. 2E** shows the LR scattergram of CRC clinically applicable biomarkers.

Our results showed there was not any applicable biomarker for diagnosis of AA, individually. Moreover, the analyses showed iFOBT and PK-M2 were clinically applicable for detection of AN, whereas FC was not applicable (**Table 2** and **Fig. 3A-C**). **Fig. 3D** presents the LR scattergram of CRC clinically applicable biomarkers.

Comparison of fecal biomarkers diagnostic accuracies

The most useful parameter for comparison of test accuracies between different biomarker groups or subgroups is DOR. Thus, we used individual DORs and their relatives to compare diagnostic accuracies of clinically applicable biomarkers.

Among CRC clinically applicable biomarkers, the accuracy of iFOBT was significantly higher than PK-M2 and FC. The accuracies of PK-M2 and FC had not significant difference (**Table 2**). In addition, the AUC of iFOBT was the highest among other biomarkers and based on LR scattergram, only iFOBT had upper moderate power of accuracy to both rule in and rule out of CRC existence (**Fig. 2E**).

Among AN clinically applicable biomarkers, the accuracy of iFOBT was significantly higher than PK-M2. Further, in compare to PK-M2, the AUC of iFOBT was higher (**Table 2**). In line with LR scattergram, iFOBT had upper moderate power of accuracy to confirm but not exclude AN existence whereas PK-M2 had lower moderate power of accuracy to confirm and exclude AN existence (**Fig. 3D**).

So as to determine the effect of biomarkers’ combination on diagnostic accuracy, we extracted the results of double combination including iFOBT+PK-M2, iFOBT+FC, PK-M2+FC as well as triple combination, namely iFOBT+PK-M2+FC, from primary studies, if they had these data. We considered the final result as positive if at least one of the biomarkers was positive and the negative results were determined if all double or triple combined biomarkers were negative. The data of iFOBT+PK-M2 could be extracted from three studies [15,17,18], following iFOBT+FC from three [17,20,22], PK-M2+FC from one [17] and iFOBT+PK-M2+FC from two [16,17] studies. Our analysis could not find any combined biomarker that significantly increases the diagnostic accuracy compared to individual biomarkers (**Supplemental Table 1**). Moreover, PK-M2+FC and iFOBT+PK-M2+FC had significantly lower accuracy for diagnosis of AN when they compared to individual iFOBT.

Subgroup analysis

Our results demonstrated substantial heterogeneity among studies in different groups when calculating the pooled sensitivity and specificity (**Table 2**). Thus, to find the potential sources of heterogeneity we performed subgroup analyses.

We separated each group of studies into 7 subgroups on the basis of method of measurements (*Latex agglutination immunoturbidimetry (LAIT) for iFOBT as well as* enzyme-linked immunosorbent assay (ELISA) for PK-M2 and FC versus lateral flow), cutoff values (≥ 20 μg/g versus <20 μg/g for *iFOBT*, >4 U/mL versus 4 U/mL for PK-M2 and >50 μg/g versus 50 μg/g for FC), study type (Cohort versus case-control) in addition 4 domains of QUADAS-2 "risk of bias" category (Low risk versus high or unclear "risk of bias") (**Table 3**). Significant difference in a subgroup indicates it could be considered as a source of heterogeneity.

For diagnosis CRC, lateral flow method of PK-M2 measurement led to significantly increase in the overall accuracy **(Fig. 4)**. Moreover, in the FC group, case-control study design and high or unclear "risk of bias" in "patient selection" domain led to significantly increase in overall accuracy.

Regarding detection of AA and AN, there was not any subgroup to change the overall accuracy. Due to similar subset of each covariate, subgroup analyses of study type and "patient selection" domain in iFOBT and cutoff value in PK-M2 and FC groups were not feasible for AA diagnosis. Also, study type and "patient selection" domain in iFOBT and cutoff value in PK-M2 and FC groups were not executable in AN group.

Threshold effect and meta-regression analysis

In addition to subgroup analysis, we performed threshold effect and univariate meta-regression analysis to evaluate further causes of heterogeneity.

In diagnostic accuracy studies, one of the most important source of heterogeneity is the threshold effect. Our analysis showed the diagnostic threshold effect was not significant as a source of heterogeneity for iFOBT and FC to CRC, AA, and AN diagnosis. Regarding PK-M2, despite not significant threshold effect in CRC and AA groups, it significantly makes heterogeneity in AN detection ($P < 0.01$) (**Table 2**).

So as to univariate meta-regression analysis, we considered some covariates including the mean age of patients, % male as gender frequency, % distal tumors as CRC tumor site, and % late as CRC tumor stage. Our results demonstrated none of the aforementioned covariates made sensitivity and specificity heterogeneity. It should be noted due to lack of FC biomarker data, analysis of aforesaid covariates in AA group as well as the impact of CRC tumor stage on heterogeneity were not feasible (**Table 4**).

Discussion

For the first time, our present systematic review and meta-analysis summarized and compared the diagnostic performances of all available fecal protein biomarkers, namely iFOBT, PK-M2 and FC for screening of CRC, AA, and AN. Also, uniquely, we assessed the impact of tumor site, tumor stage, method of measurement and different cutoff values on the performance of these biomarkers.

The overall quality of the included studies for each biomarker was relatively high according to QUADAS-2 tool. In summary, the range of low risk studies in "risk of bias" category for all four domains were 50-95.6%, reflects moderate to very low risk of bias and all included studies had no concern regarding "applicability" in all three domains. In order to evaluation of the impact of QUADAS-2 domains on the overall accuracy, we conducted subgroup analysis based on low versus high or unclear risk from "risk of bias" category. The results showed, despite the impact of different domains on sensitivities and specificities, only "patient selection" domain in FC group for CRC detection could significantly affect the overall accuracy that we have discussed about its reasons in the fourth following paragraph (Table 3).

The first important aim of our study was to determine the most accurate fecal protein biomarker. Our analyses showed iFOBT, PK-M2 and FC biomarkers were clinically applicable for CRC, as well as iFOBT and PK-M2 for AN, and there was not anyone for AA according to their AUC, positive and negative LRs. Plus, combination of biomarkers could not increase the accuracy for detection of each condition. The overall accuracy of iFOBT was significantly higher than PK-M2 and FC for CRC detection ($P = 0.02$ and < 0.01, respectively) and significantly higher than PK-M2 for AN diagnosis ($P < 0.01$). Pursuant to our search results, before ours, there was not any meta-analysis to compare the accuracy of various fecal biomarkers for diagnosis of different intestinal neoplasms. Nonetheless, Li *et al.* [53] using 4 research papers, conducted a direct comparison between iFOBT and PK-M2 for CRC screening. Despite the small number of studies to achieve convincing results, in confirm to our findings, they indicated iFOBT had significantly higher accuracy in compare to PK-M2. Further, all of our included articles which were contained comparison data had higher iFOBT accuracy than PK-M2 and/or FC for diagnosis of both CRC and AN, except the results of Kim *et al.* [10]. Kim *et al.* assessed the accuracy of two different methods of PK-M2 measurement and compared with iFOBT in CRC and adenoma patients. Their results showed regardless of measurement method, PK-M2 accuracy was superior to iFOBT for the diagnosis of CRC and adenoma. The most suspected reason for this contradiction is a technical mistake related to measurement equipment which a systematic error gave rise to decrease the accuracy of iFOBT in Kim's study. To clarify this issue, we tested the iFOBT accuracy of Kim versus the other studies. The results indicated the performance of iFOBT in Kim's study was significantly lower than others (RDOR = 0.19 (95% CI, 0.04–0.10); $P = 0.04$) that indicates systematic error is possible.

Today, the most widely used biomarker for detection of colorectal neoplasms is FOBT. Two commonly used FOBTs are gFOBT and iFOBT which has been proven that iFOBT has superior diagnostic performances [2,54]. Our results showed iFOBT is clinically applicable for CRC diagnosis with upper moderate overall accuracy in line with its positive and negative LR results. Also, it is clinically applicable for AN with upper moderate accuracy only for

confirmation, not for exclusion. Overall accuracy of iFOBT in our present study is similar to previous published meta-analyses [55–57]. However, we evaluated more covariates in our research to shed light on the different strengths and limitations of iFOBT implementation. The first unique covariate was the measurement method. Today, there are two common methods for measurement of iFOBT, qualitative rapid lateral flow and quantitative latex agglutination immunoturbidimetry, whereas before the present study there was no data about their overall accuracy differences. According to our findings, there is not any difference between these two methods of measurement with different commercial brands for diagnosis of all three conditions (Table 3). Another covariate was cutoff to find the optimal iFOBT value. In previous published meta-analysis, Lee *et al* [55], proposed lower 20 μg/g cutoff may increase sensitivity of iFOBT for the detection of CRC compared to upper 20 μg/g values. So, we analyzed the difference of accuracies between lower 20 μg/g versus upper 20 μg/g values not only for CRC detection, but also for AA and AN. Our results indicated that there were not significant differences among different cutoff value for detection of CRC, AA and AN (Table 3). Meanwhile, the results of univariate meta-regression analysis showed age, gender, CRC tumor site and stage could not affect the sensitivity and specificity of iFOBT for diagnosis of all three conditions (Table 4). The results of the most recently published meta-analysis confirmed our findings in term of the impact of tumor site on iFOBT performance [57]. However, concerning CRC tumor site, the results of Hirai *et al.* meta-analysis [56] are not completely in consistent with ours. They concluded the overall accuracy of iFOBT for proximal colon was significantly lower than distal colon, but it's not too convincing conclusion given that largely overlapping confidence intervals in the site-specific sensitivities.

PK-M2 is a non-organ specific promising tumor biomarker which has been shown its concentration elevates in various types of tumors [53]. For the first time in 2004, Hardt *et al* [38] demonstrated PK-M2 concentration was elevated in the feces of CRC patients and it could be used as a biomarker. So far, several studies have been conducted on fecal PK-M2 in CRC patients and results showed contradictory accuracies. As to final conclusion that whether fecal PKM2 could be used as a biomarker for diagnosis of colorectal neoplasms or not, diagnostic accuracy meta-analysis must be carried out. Following two earlier versions [53,58], the latest diagnostic accuracy meta-analysis of PK-M2 for CRC detection was published in 2015, which included 8 researches [59]. Nonetheless, all aforementioned studies included only CRC patients, without evaluation the impact of different covariates on PK-M2 performance. In this study, plus update the body of evidences using 26 included research articles, we uniquely assessed the diagnostic accuracy of PK-M2 for detection of AA and AN in addition to CRC. Further, we evaluated the impact of different covariates on the performance of PK-M2. Our findings indicated PK-M2 was clinically applicable for diagnosis of CRC and AN, and not for AA, with lower moderate accuracy for both disease confirmation and exclusion given that its LR results. These results are compatible with previous meta-analyses regarding the accuracy of PK-M2 for diagnosis of CRC [53,58,59]. To provide new insights on elucidating the PK-M2 performance, we assessed different covariates on its accuracy. One of our important findings was the impact of PK-M2 measurement method on its performance. Subgroup analysis in CRC group demonstrated rapid lateral flow could significantly increase the accuracy of PK-M2 in compare to ELISA method (RDOR = 0.14 (95% CI, 0.04–0.48); P < 0.01) **(**Fig. 4**)**. These findings were similar to the study results of Kim *et al.* [10]. Moreover, we re-analyzed the difference of iFOBT and lateral flow PK-M2 measurement accuracies. The results revealed when lateral flow PK-M2 measurement was implemented, it eliminates the initial significant difference with iFOBT accuracy for CRC detection (RDOR = 1.79 (95% CI, 0.38–8.46); P = 0.43), whereas accuracy of iFOBT for AN was still significantly superior to lateral flow PK-M2 (RDOR = 0.28 (95% CI, 0.10–0.81); P = 0.02). Lower accuracy of ELISA method could be derived from the bio-stability of tumor PK-M2 in stool sample. There are some evidences have been shown tumor PK-M2 in stool sample could be dramatically affected by sample storage time [60]. By nature, ELISA is a time consuming method, whereas lateral flow is a rapid technique which commonly utilized in point of care tests (POCTs). Also, our results have been implied age, gender, cutoff value, CRC tumor site and stage could not affect the PK-M2 accuracy (Table 3 **and** Table 4).

FC is released in feces following mucosal neutrophil degradation as a result of intestinal inflammation. The level of FC increases in a wide range of intestinal diseases that are along with inflammation, including inflammatory bowel disease, CRC and AA [52]. The results of numerous studies indicated a broad range of FC sensitivity for detection of CRC, from 33–100% (Fig. 2C). The latest meta-analysis with 20 included articles regarding the performance of FC for CRC and adenoma diagnosis was performed in 2018 [61]. However, this prior paper evaluated all adenomas, not advanced type which is clinically important as the precursors of CRC. Meanwhile, there were no data concerning the impact of measurement technique, type of included studies, and CRC site-specific on FC accuracy. In the present research, besides update the data using 23 included research articles regarding CRC, we assessed the diagnostic accuracy of FC in order to AA and AN detection for the first time as well as the impact of various covariates on FC performance. Our results in consistent with the previous meta-analysis [61] indicated FC has lower moderate accuracy for diagnosis of CRC based on its LR values. Also, we could determine it is not applicable to detection of AA and AN. Evaluated covariates including age, gender, method of measurement and CRC tumor site had not significant effect on FC accuracy (Table 4). Nonetheless, case-control study design and "patient selection' domain from QUADAS-2 "risk of bias" category had a significant impact on FC performance for diagnosis of CRC (Table 3). These two covariates are relatively similar, because high risk point is given to case-control studies in "patient selection' domain. As aforementioned, FC has low specificity for intestinal disorders, therefore its overall accuracy is declined in cohort study designs which include patients with different intestinal disorders.

One of the most important strengths of this study was the adoption of rigorous inclusion and exclusion criteria in three widely used medical databases without language restriction. Diagnostic accuracy comparison of multiple biomarkers and subgroup analysis by different method of measurement and cutoff values are another unique strength. Besides, we analysis the impact of the site and the stage of tumors on the biomarkers' performances in CRC group which has not been conducted in previous meta-analyses. Despite the strengths, there are some limitations that should be taken into consideration for interpreting our findings. First, the accuracy for AN detection may be under- or overestimated because it is strongly influenced by the proportion of CRC and AA cases in the study population. Second, the data of AA were not available to determine site-specific accuracy.

Conclusion

In summary, our results determined iFOBT is still the most accurate fecal biomarker for diagnosis of CRC and AN among clinically applicable types. Besides, lateral flow method of PK-M2 measurement should be implemented instead of ELISA due to its higher efficacy on PK-M2 performance. There is not any clinically applicable fecal biomarker for AA diagnosis as an important precursor of CRC. So, further researches is recommended to find new comprehensive biomarkers.

Declarations

Acknowledgments

This research received no grant from any financial organizations or funding agency in the public, commercial, or not for profit sectors.

Author contributions

A.N.K, M.E.Z and J.Z conceived and designed the project. A.N.K and M.E.Z reviewed literatures, A.N.K, M.E.Z and Q.T extracted the data. A.N.K and M.E.Z performed statistical analysis and wrote the manuscript. J.Z reviewed the manuscript and provided suggestions for further development. All performances were conducted under supervision of J.Z.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information

References

1. Sung, H. *et al.* Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA. Cancer J. Clin.* ahead of print (2021) doi:10.3322/caac.21660.

2. Loktionov, A. *et al.* Biomarker measurement in non-invasively sampled colorectal mucus as a novel approach to colorectal cancer detection: screening and triage implications. *Br. J. Cancer***123**, 252–260 (2020).

3. Ogunwobi, O. O., Mahmood, F. & Akingboye, A. Biomarkers in colorectal cancer: Current research and future prospects. *Int. J. Mol. Sci.***21**, 1–20 (2020).

4. Mármol, I., Sánchez-de-Diego, C., Dieste, A. P., Cerrada, E. & Yoldi, M. J. R. Colorectal carcinoma: A general overview and future perspectives in colorectal cancer. *Int. J. Mol. Sci.***18**, (2017).

5. Toma, S. C., Ungureanu, B. S., Patrascu, S., Surlin, V. & Georgescu, I. Colorectal Cancer Biomarkers - A New Trend in Early Diagnosis. *Curr. Heal. Sci. J.***44**, 140–146 (2018).

6. Yau, T. O., Tang, C. M., Harriss, E. K., Dickins, B. & Polytarchou, C. Faecal microRNAs as a non-invasive tool in the diagnosis of colonic adenomas and colorectal cancer: A meta-analysis. *Sci. Rep.***9**, 1–13 (2019).

7. Turvill, J. *et al.* Diagnostic accuracy of one or two faecal haemoglobin and calprotectin measurements in patients with suspected colorectal cancer. *Scand. J. Gastroenterol.***53**, 1526–1534 (2018).

8. Högberg, C., Karling, P., Rutegård, J. & Lilja, M. Diagnosing colorectal cancer and inflammatory bowel disease in primary care: The usefulness of tests for faecal haemoglobin, faecal calprotectin, anaemia and iron deficiency. A prospective study. *Scand. J. Gastroenterol.***52**, 69–75 (2017).

9. Karl, J. *et al.* Improved Diagnosis of Colorectal Cancer Using a Combination of Fecal Occult Blood and Novel Fecal Protein Markers. *Clin. Gastroenterol. Hepatol.***6**, 1122–1128 (2008).

10. Kim, Y. C. *et al.* The usefulness of a novel screening kit for colorectal cancer using the immunochromatographic fecal tumor M2 pyruvate kinase test. *Gut Liver***9**, 641–648 (2015).

11. Guan-Fu, W. J., Yi-Hua, L. P. & Xin-Min, S. Evaluation of fecal tumor m2 pyruvate kinase combined with fecal occult blood test as a detecting tool for colorectal cancer. *J Sun Yat Sen Univ (Med Sci)***27**, 350-353 (2006).

12. Shastri, Y. M. *et al.* Comparison of an established simple office-based immunological FOBT with fecal tumor pyruvate kinase type M2 (M2-PK) for colorectal cancer screening: Prospective multicenter study. *Am. J. Gastroenterol.***103**, 1496–1504 (2008).

13. Mulder, S. A. *et al.* Tumor pyruvate kinase isoenzyme type M2 and immunochemical fecal occult blood test: Performance in screening for colorectal cancer. *Eur. J. Gastroenterol. Hepatol.***19**, 878–882 (2007).

14. Vogel, T. *et al.* Vergleich verschiedener stuhltests zur detektion von neoplasien des kolon. *Dtsch. Medizinische Wochenschrift***130**, 872–877 (2005).

15. Zaccaro, C. *et al.* Power of screening tests for colorectal cancer enhanced by high levels of M2-PK in addition to FOBT. *Intern. Emerg. Med.***12**, 333–339 (2017).

16. Rutka, M. *et al.* Diagnostic accuracy of five different fecal markers for the detection of precancerous and cancerous lesions of the colorectum. *Mediators Inflamm.***2016**, 2492081 (2016).

17. Parente, F. *et al.* A combination of faecal tests for the detection of colon cancer: A new strategy for an appropriate selection of referrals to colonoscopy? A prospective multicentre Italian study. *Eur. J. Gastroenterol. Hepatol.***24**, 1145–1152 (2012).

18. Caviglia, G. P., Cabianca, L., Fagoonee, S. & Gili, F. M. Colorectal cancer detection in an asymptomatic population: Fecal immunochemical test for hemoglobin vs. Fecal M2-type pyruvate kinase. *Biochem. Medica***26**, 114–120 (2016).

19. Cho Chi Hyun *et al.* Evaluation of the Performance of a Fecal Tumor M2-PK Rapid Kit Using Stool Specimens for Detection of Colorectal Tumors. *Ann. Clin. Lab. Sci.* 154–160 (2016).

20. Widlak, M. M. *et al.* Diagnostic accuracy of faecal biomarkers in detecting colorectal cancer and adenoma in symptomatic patients. *Aliment. Pharmacol. Ther.***45**, 354–363 (2017).

21. Mowat, C. *et al.* Faecal haemoglobin and faecal calprotectin as indicators of bowel disease in patients presenting to primary care with bowel symptoms. *Gut***65**, 1463–1469 (2016).

22. Kok, L. *et al.* Diagnostic accuracy of point-of-care fecal calprotectin and immunochemical occult blood tests for diagnosis of organic bowel disease in primary care: The cost-effectiveness of a decision rule for abdominal complaints in primary care (CEDAR) study. *Clin. Chem.***58**, 989–998 (2012).

23. Alhadi, S. C. *et al.* The use of M2-pyruvate kinase as a stool biomarker for detection of colorectal cancer in tertiary teaching hospital: A comparative study. *Ann. Coloproctol.***36**, 409–414 (2021).

24. Rigi, F. *et al.* Expression of tumor pyruvate kinase M2 isoform in plasma and stool of patients with colorectal cancer or adenomatous polyps. *BMC Gastroenterol.***20**, (2020).

25. Dabbous, H. K. *et al.* Evaluation of Fecal M2PK as a Diagnostic Marker in Colorectal Cancer. *J. Gastrointest. Cancer***50**, 442–450 (2019).

26. Sithambaram, S., Hilmi, I. & Goh, K. L. The diagnostic accuracy of the M2 pyruvate kinase Quick stool test-A rapid office based assay test for the detection of colorectal cancer. *PLoS One***10**, (2015).

27. Wang, H. P., Wang, Y. Y., Pan, J., Cen, R. & Cai, Y. K. Evaluation of specific fecal protein biochips for the diagnosis of colorectal cancer. *World J. Gastroenterol.***20**, 1332–1339 (2014).

28. Wei, C. Value of combined detection of tumor type M2 pyruvate kinase, tissue polypeptide specific antigen and carcino-embryonic antigen in diagnosis of colorectal cancer. *Int J Lab Med***35**, 2775–2778 (2014).

29. Abdullah, M., Rani, A., Simadibrata, M., Fauzi, A. & Syam, A. The value of fecal tumor M2 pyruvate kinase as a diagnostic tool for colorectal cancer screening. *Acta Med Indones***44**, 94–99 (2012).

30. LI, Y. & WANG, J. Clinical significance of blood and fecal tumor M2-pyruvate kinase expression in patientswith colorectal cancer. *J South Med Univ***31**, 2087–2089 (2011).

31. Haug, U., Hundt, S. & Brenner, H. Sensitivity and specificity of faecal tumour M2 pyruvate kinase for detection of colorectal adenomas in a large screening study. *Br. J. Cancer***99**, 133–135 (2008).

32. Haug, U. *et al.* Tumour M2-PK as a stool marker for colorectal cancer: Comparative analysis in a large sample of unselected older adults vs colorectal cancer patients. *Br. J. Cancer***96**, 1329–1334 (2007).

33. Koss, K., Maxton, D. & Jankowski, J. A. Z. Faecal dimeric M2 pyruvate kinase in colorectal cancer and polyps correlates with tumour staging and surgical intervention. *Color. Dis.***10**, 244–248 (2008).

34. Zhang, Y., Gu, W., Lin, Z. & Zhiuha, R. Significance of Fecal Tumor M2 Pyruvate Kinase for Detection of Gastrointestinal Cancers. *Chin J Gastroenterol***12**, 465–468 (2007).

35. Shastri, Y. M. *et al.* Prospective multicenter evaluation of fecal tumor pyruvate kinase type M2 (M2-PK) as a screening biomarker for colorectal ecoplasia. *Int. J. Cancer***119**, 2651–2656 (2006).

36. Tonus, C., Neupert, G. & Sellinger, M. Colorectal cancer screening by non-invasive metabolic biomarker fecal tumor M2-PK. *World J. Gastroenterol.***12**, 7007–7011 (2006).

37. Naumann, M. *et al.* Faecal pyruvate Kinase type M2 valid screening parameter for colorectal cancer? Preliminary results from a multicenter comparative study. *Dtsch Med Wochenschr***129**, 1806–1807 (2004).

38. Hardt, P. D. *et al.* Faecal tumour M2 pyruvate kinase: A new, sensitive screening tool for colorectal cancer. *Br. J. Cancer***91**, 980–984 (2004).

39. Khoshbaten, M. *et al.* Diagnostic value of fecal calprotectin as a screening biomarker for gastrointestinal malignancies. *Asian Pacific J. Cancer Prev.***15**, 1667–1670 (2014).

40. Meucci, G. *et al.* Diagnostic value of faecal calprotectin in unselected outpatients referred for colonoscopy: A multicenter prospective study. *Dig. Liver Dis.***42**, 191–195 (2010).

41. Damms, A. & Bischoff, S. C. Validation and clinical significance of a new calprotectin rapid test for the diagnosis of gastrointestinal diseases. *Int. J. Colorectal Dis.***23**, 985–992 (2008).

42. Hoff, G. *et al.* Testing for faecal calprotectin (PhiCal) in the Norwegian Colorectal Cancer Prevention trial on flexible sigmoidoscopy screening: Comparison with an immunochemical test for occult blood (FlexSure OBT). *Gut***53**, 1329–1333 (2004).

43. Carroccio, A. *et al.* Diagnostic accuracy of fecal calprotectin assay in distinguishing organic causes of chronic diarrhea from irritable bowel syndrome: A prospective study in adults and children. *Clin. Chem.***49**, 861–867 (2003).

44. Costa, F. *et al.* Role of faecal calprotectin as non-invasive marker of intestinal inflammation. *Dig. Liver Dis.***35**, 642–647 (2003).

45. Summerton, C. B., Longlands, M. G., Wiener, K. & Shreeve, D. R. Faecal calprotectin: A marker of inflammation throughout the intestinal tract. *Eur. J. Gastroenterol. Hepatol.***14**, 841–845 (2002).

46. Tibble, J. A., Sigthorsson, G., Foster, R., Forgacs, I. & Bjarnason, I. Use of surrogate markers of inflammation and Rome criteria to distinguish organic from nonorganic intestinal disease. *Gastroenterology***123**, 450–460 (2002).

47. B. Johne, O. Kronborg, H. I. Tøn, J. A New Fecal Calprotectin Test for Colorectal Neoplasia: Clinical Results and Comparison with Previous Method. *Scand. J. Gastroenterol.***36**, 291–296 (2001).

48. Kristinsson, J. *et al.* Screening of first degree relatives of patients operated for colorectal cancer: Evaluation of fecal calprotectin vs. Hemoccult II. *Digestion***64**, 104–110 (2001).

49. Tibble, J. *et al.* Faecal calprotectin and faecal occult blood tests in the diagnosis of colorectal carcinoma and adenoma. *Gut***49**, 402–408 (2001).

50. Tibble, J. *et al.* A simple method for assessing intestinal inflammation in Crohn's disease. *Gut***47**, 5006–513 (2000).

51. Røseth, A. G. *et al.* Faecal calprotectin: A novel test for the diagnosis of colorectal cancer? *Scand. J. Gastroenterol.***28**, 1073–1076 (1993).

52. Turvill, J. *et al.* Faecal calprotectin in patients with suspected colorectal cancer: A diagnostic accuracy study. *Br. J. Gen. Pract.***66**, e499–e506 (2016).

53. Li, R., Liu, J., Xue, H. & Huang, G. Diagnostic value of fecal tumor M2-pyruvate kinase for CRC screening: A systematic review and meta-analysis. *Int. J. Cancer***131**, 1837–1845 (2012).

54. Tinmouth, J., Lansdorp-Vogelaar, I. & Allison, J. E. Faecal immunochemical tests versus guaiac faecal occult blood tests: What clinicians and colorectal cancer screening programme organisers need to know. *Gut***64**, 1327–1337 (2015).

55. Lee, J. K., Liles, E. G., Bent, S., Levin, T. R. & Corley, D. A. Accuracy of fecal immunochemical tests for colorectal cancer: Systematic review and meta-analysis. *Ann. Intern. Med.***160**, 171–181 (2014).

56. Hirai, H. W. *et al.* Systematic review with meta-analysis: Faecal occult blood tests show lower colorectal cancer detection rates in the proximal colon in colonoscopy-verified diagnostic studies. *Aliment. Pharmacol. Ther.***43**, 755–764 (2016).

57. Lu, M., Luo, X., Li, N., Chen, H. & Dai, M. Diagnostic accuracy of fecal occult blood tests for detecting proximal versus distal colorectal neoplasia: A systematic review and meta-analysis. *Clin. Epidemiol.***11**, 943–954 (2019).

58. Huang, J. X. *et al.* Tumor M2-pyruvate kinase in stool as a biomarker for diagnosis of colorectal cancer: A meta-analysis. *J. Cancer Res. Ther.***10**, C225–C228 (2014).

59. Uppara, M. *et al.* A systematic review and meta-analysis of the diagnostic accuracy of pyruvate kinase M2 isoenzymatic assay in diagnosing colorectal cancer. *World J. Surg. Oncol.***13**, 48 (2015).

60. Haug, U. *et al.* Tumor M2 pyruvate kinase as a stool marker for colorectal cancer: Stability at room temperature and implications for application in the screening setting. *Clin. Chem.***52**, 782–784 (2006).

61. Ye, X., Huai, J. & Ding, J. Diagnostic accuracy of fecal calprotectin for screening patients with colorectal cancer: A meta-analysis. *Turkish J. Gastroenterol.***29**, 397–405 (2018).

Tables

Table 1. Characteristics of included studies

† Colorectal Cancer

‡ Advanced Adenoma

£ Advanced Neoplasms

₸ LAIT: *Latex Agglutination Immunoturbidimetry; ELISA:* Enzyme-Linked Immunosorbent Assay

¥ C: Cohort, CC: Case-Control

£ Not Available

€ Five patients did not undergo colorectal resection. In those cases, the tumor invasion could not be determined.

 iFOB was performed by two different commercial kits.

₡ This unit was transformed to µg/g.

Table 2. Diagnostic accuracy of fecal biomarkers and their comparisons

†P-Se: Pooled-Sensitivity; **P-Sp:** Pooled-Specificity; **P-LR:** Pooled-Likelihood Ratio; **P-DOR:** Pooled-Diagnostic Odds Ratio; **AUC:** Area Under the Curve; **TE:** P-Value of threshold effect; **PB:** P-Value of Publication Bias; **CA:** Clinical Applicability.

‡iFOB: Immunochemical Fecal occult blood tests; **PK-M2:** Pyruvate kinase-M2; **FC:** Fecal Calprotectin; **A:** applicable; **NA:** not applicable; **RDOR:** relative-DOR.

Table 3. Subgroup analysis

Study ID	Language	Country	Study design[¥]	Study Population			Number of population			Cutoff Value	Method[₹]	%Distal	%Late
				Sample	%Male	Age	CRC[†]	AA[‡]	AN[£]				
Immunochemical Fecal occult blood tests (iFOBT)													
Turvill 2018 [7]	English	UK	C	515	50	69	27	_	_	7 µg/g	*LAIT*	NA[£]	NA
Högberg 2017 [8]	English	Sweden	C	373	36.4	63	8	8	16	25 µg/g	Lateral flow	NA	NA
Zaccaro 2017 [15]	English	Italy	C	127	50.3	63	_	_	11	20 [₡] µg/g	*LAIT*	NA	NA
Widlak 2017 [20]	English	UK	C	430	49	67	24	1	5	7 µg/g	*LAIT*	76	NA
Caviglia 2016 [18]	English	Italy	C	572	66.2	66	7	19	26	20 µg/g	*LAIT*	NA	NA
Cho 2016 [19]	English	Korea	C	236	67.8	47	1	3	4	7 [₡] µg/g	*LAIT*	NA	NA
Rutka 2016 [16]	English	Hungary	C	95	40	67	19	_	_	3 [₡] µg/g	Lateral flow	89	31.5
Kim 2015 [10]	English	Korea	CC	323	57	62	139	_	_	10 [₡] µg/g	Lateral flow	NA	55.4
Mowat 2016 [21]	English	UK	C	755	45.3	64	28	40	68	10 µg/g	*LAIT*	NA	NA
Kok 2012 [22]	English	Netherland	C	382	46.3	60	19	16	35	6 µg/g	Lateral flow	NA	NA
Parente 2012 [17]	English	Italy	C	280	56	67	47	85	132	20 [₡] µg/g	*LAIT*	NA	NA
Karl 2008 [9]	English	Germany	CC	551	47.2	65.6	186	_	_	12.27 µg/g	ELISA	NA	NA
Shastri 2008 [12]	English	Germany	C	640	41.4	52.6	55	21	76	2 [₡] µg/g	Lateral flow	61.8	34.5
Mulder[₪] 2007 [13]	English	Netherland	C	181	50.2	58	52	22	74	30 µg/g	Lateral flow	65	81[€]
Mulder[₪] 2007 [13]	English	Netherland	C	181	50.2	58	52	22	74	10 µg/g	Lateral flow	65	81[€]
Guan-Fu 2006 [11]	Chinese	China	CC	86	NA	NA	43	_	_	2 [₡] µg/g	Lateral flow	74.4	51.1
Vogel 2005 [14]	German	Germany	CC	138	44.2	58	22	_	_	30 µg/g	Lateral flow	NA	NA
Pyruvate kinase-M2 (PK-M2)													

Alhadi 2021 [23]	English	Malaysia	C	85	58.8	56.8	17	_	_	4 U/mL	Lateral flow	NA	NA
Rigi 2020 [24]	English	Iran	C	226	NA	NA	39	_	_	4 U/mL	ELISA	53.8	NA
Dabbous 2019 [25]	English	Egypt	CC	60	71.6	52	20	_	_	4 U/mL	ELISA	NA	NA
Zaccaro 2017 [15]	English	Italy	C	127	50.3	63	_	_	11	4 U/mL	ELISA	NA	NA
Caviglia 2016 [18]	English	Italy	C	572	66.2	66	7	19	26	4 U/mL	ELISA	NA	NA
Cho 2016 [19]	English	Korea	C	236	67.8	47	1	3	4	4 U/mL	Lateral flow	NA	NA
Rutka 2016 [16]	English	Hungary	C	95	40	67	19	20	39	4 U/mL	Lateral flow	89	31.5
Kim 2015 [10]	English	Korea	CC	323	57	62	40	_	_	4 U/mL	ELISA	NA	NA
Kim 2015 [10]	English	Korea	CC	323	57	62	139	94	233	4 U/mL	Lateral flow	NA	55.4
Sithambaram 2015 [26]	English	Malaysia	CC	300	52.7	62.5	100	_	_	4 U/mL	Lateral flow	86	NA
Wang 2014 [27]	English	China	CC	40	60	67.5	19	_	_	114 U/ml	ELISA	65	55
Wei 2014 [28]	Chinese	China	CC	134	61.2	55.2	74	_	_	166.7 μkat /L	ELISA	NA	NA
Abdullah 2012 [29]	English	Indonesia	C	328	60.1	53.4	42	_	_	4 U/mL	ELISA	NA	NA
Parente 2012 [17]	English	Italy	C	280	56	67	47	85	132	4 U/mL	ELISA	NA	NA
Li 2011 [30]	Chinese	China	CC	66	NA	NA	44	_	_	4 U/mL	ELISA	NA	NA
Haug 2008 [31]	English	Germany	C	1082	50	63	_	106	_	4 U/mL	ELISA	NA	NA
Shastri 2008 [12]	English	Germany	C	640	41.4	52.6	55	21	76	4 U/mL	ELISA	61.8	34.5
Koss 2008 [33]	English	UK	CC	55	67.2	66.3	32	5	37	4 U/mL	ELISA	NA	37.5
Haug 2007 [32]	English	Germany	CC	982	44.2	63.5	65	_	_	4 U/mL	ELISA	75.3	58.4
Zhang	Chinese	China	CC	95	73.6	48.6	31	_	_	4	ELISA	NA	54.8

2007 [34]										U/mL			
Mulder 2007 [13]	English	Netherland	C	181	50.2	58	52	22	74	4 U/mL	ELISA	65	81[€]
Guan-Fu 2006 [11]	Chinese	China	CC	86	NA	NA	43	_	_	4 U/mL	ELISA	74.4	51.1
Shastri 2006 [35]	English	Germany	C	317	47.9	56	74	10	84	4 U/mL	ELISA	NA	NA
Tonus 2006 [36]	English	Germany	CC	96	56.2	66	54	_	_	4 U/mL	ELISA	NA	NA
Vogel 2005 [14]	German	Germany	CC	138	44.2	58	22	_	_	4 U/mL	ELISA	NA	NA
Naumann 2004 [37]	German	Germany	C	232	NA	NA	27	_	_	4 U/mL	ELISA	NA	NA
Hardt 2004 [38]	English	Germany	CC	204	NA	NA	60	_	_	4 U/mL	ELISA	NA	55
Fecal Calprotectin													
Turvill 2018 [7]	English	UK	C	515	50	69	27	_	_	118 µg/g	ELISA	NA	NA
Högberg 2017 [8]	English	Sweden	C	373	36.4	63	8	8	16	50 µg/g	ELISA	NA	NA
Rutka 2016 [16]	English	Hungary	C	95	40	67	19	_	_	128.5 µg/g	ELISA	89	31.5
Turvill 2016 [52]	English	UK	C	654	44	69	39	_	_	50 µg/g	ELISA	NA	NA
Widlak 2017 [20]	English	UK	C	430	49	67	24	1	25	50 µg/g	ELISA	76	NA
Mowat 2016 [21]	English	UK	C	755	45.3	64	28	41	69	50 µg/g	ELISA	NA	NA
Khoshbaten 2014 [39]	English	Iran	CC	100	65	47	50	_	_	75.8 µg/g	ELISA	NA	NA
Wang 2014 [27]	English	China	CC	40	60	67.5	19	_	_	144 lU/ml	ELISA	65	55
Kok 2012 [22]	English	Netherland	C	382	46.3	60	19	16	35	50 µg/g	ELISA	NA	NA
Kok 2012 [22]	English	Netherland	C	382	46.3	60	19	16	35	50 µg/g	Lateral flow	NA	NA
Parente 2012 [17]	English	Italy	C	280	56	67	47	85	132	50 µg/g	ELISA	NA	NA
Meucci	English	Italy	C	870	47.5	59.1	21	_	_	50	ELISA	NA	NA

2010 [40]										µg/g			
Damms 2008 [41]	English	Germany	CC	140	44.2	58	8	_	_	50 µg/g	ELISA	NA	NA
Damms 2008 [41]	English	Germany	CC	140	44.2	58	8	_	_	50 µg/g	Lateral flow	NA	NA
Karl 2008 [9]	English	Germany	CC	551	47.2	65.6	186	_	_	50 µg/g	ELISA	NA	NA
Hoff 2004 [42]	English	Norway	C	2321	49	58	16	195	206	50 µg/g	ELISA	NA	NA
Carroccio 2003 [43]	English	Italy	C	80	43.7	62.5	3	_	_	50 µg/g	ELISA	NA	NA
Costa 2003 [44]	English	Italy	CC	239	46.4	46.3	18	8	26	50 µg/g	ELISA	NA	NA
Summerton 2002 [45]	English	UK	C	134	NA	NA	8	_	_	50 µg/g	ELISA	NA	NA
Tibble 2002 [46]	English	UK	C	346	NA	NA	7	_	_	50 µg/g[℄]	ELISA	NA	NA
John 2001 [47]	English	Norway	C	453	51	66	154	_	_	50 µg/g	ELISA	NA	NA
Kristinsson 2001 [48]	English	Norway	C	253	39.5	60	5	4	9	50 µg/g[℄]	ELISA	NA	NA
Tibble 2001 [49]	English	UK	C	295	NA	NA	66	22	88	50 µg/g[℄]	ELISA	NA	NA
Tibble 2000 [50]	English	UK	C	220	28.6	43	2	_	_	50 µg/g[℄]	ELISA	NA	NA
Røseth 1993 [51]	English	Norway	CC	206	NA	61.6	53	_	_	50 µg/g[℄]	ELISA	66	NA

Diagnostic accuracy of fecal protein biomarkers									
Test‡	P-Se (95% CI) ($\%I^2$)	P-Sp (95% CI) ($\%I^2$)	P-LR+ (95% CI)	P-LR- (95% CI)	P-DOR (95% CI)	AUC (95% CI)	TE	PB	CA†
Colorectal cancer									
iFOBT	0.83 (0.74-0.89) (87.6%)	0.86 (0.81- 0.90) (95.2%)	6.10 (4.5-8.2)	0.20 (0.13-0.31)	30 (18-49)	0.91 (0.89-0.94)	0.32	0.72	A
PK-M2	0.82 (0.76- 0.87) (82.1%)	0.73 (0.65-0.80) (95.1%)	3 (2.3-4.0)	0.24 (0.18-0.33)	12 (8-20)	0.85 (0.82-0.88)	0.51	0.73	A
FC	0.85 (0.80-0.89) (77.9%)	0.65 (0.57-0.71) (97.5%)	2.4 (2.0-2.9)	0.23 (0.18-0.30)	10 (7-15)	0.85 (0.81-0.87)	0.40	0.53	A
Advanced adenoma									
Ifob	0.53 (0.35-0.70) (82.3%)	0.81 (0.70-0.89) (97%)	2.8 (1.7-4.4)	0.58 (0.41-0.83)	5 (2-10)	0.74 (0.70-0.78)	0.66	0.05	NA
PK-M2	0.46 (0.34-0.58) (84.7%)	0.64 (0.48-0.77) (98.4)	1.3 (0.8-2.0)	0.85 (0.64-1.13)	1 (1-3)	0.54 (0.49-0.58)	0.33	0.83	NA
FC	0.45 (0.35-0.55) (84.2%)	0.56 (0.45-0.66) (98.5%)	1.0 (0.9-1.2)	0.99 (0.89-1.10)	1 (1-1)	0.50 (0.45-0.54)	0.58	0.10	NA
Advanced neoplasm									
Ifob	0.72 (0.58-0.83) (90.2%)	0.88 (0.80-0.92) (96%)	5.9 (4.1-8.4)	0.31 (0.21-0.47)	19 (12-28)	0.88 (0.85-0.91)	0.49	0.53	A
PK-M2	0.68 (0.63-0.73) (56.72%)	0.76 (0.65-0.84) (95.9%)	2.9 (2.0-4.2)	0.42 (0.36-0.48)	7 (4-11)	0.73 (0.69-0.77)	<0.01	0.22	A
FC	0.70 (0.58-0.80) (92.3%)	0.59 (0.49-0.70) (98.3%)	1.7 (1.4-2.2)	0.50 (0.36-0.70)	3 (2-6)	0.69 (0.65-0.73)	0.78	0.01	NA

Comparison of fecal biomarkers diagnostic accuracies		
Comparison	RDOR (95% CI)	P-Value
Colorectal cancer		
iFOBT versus PK-M2	2.49 (1.12-5.54)	0.02
iFOBT versus FC	2.96 (1.50-5.85)	<0.01
PK-M2 versus FC	1.07 (0.56-2.05)	0.83
Advanced neoplasm		
iFOBT versus PK-M2	2.84 (0.88-9.21)	0.07
iFOBT versus FC	3.44 (1.22-9.68)	0.02
PK-M2 versus FC	1.03 (0.35-3.04)	0.95
Advanced neoplasm		
iFOBT versus PK-M2	2.70 (1.42-5.12)	<0.01
iFOBT versus FC	6.41 (2.75-14.96)	<0.01

PK-M2 versus FC	1.70(0.75-3.83)	0.19

Test	Subgroup†		No. of study	Sensitivity (95% CI)	Specificity (95% CI)	RDOR (95% CI)	P-value
Colorectal cancer							
iFOBT	Method of measurement	LAIT	6	0.77 (0.60-0.93)	0.90 (0.86-0.95)	1.76	0.24
		LF	9	0.84 (0.75-0.94)	0.81 (0.74-0.88)	(0.65-4.80)	
	Cut off	≥ 20 μg/g	5	0.86 (0.73-0.98)	0.83 (0.74-0.92)	0.84	0.77
		< 20 μg/g	11	0.81 (0.72-0.91)	0.88 (0.83-0.93)	(0.24-2.96)	
	Study type	C	12	0.85 (0.77-0.93)	0.85 (0.80-0.91)	1.01	0.98
		CC	4	0.76 (0.59-0.92)	0.90 (0.83-0.97)	(0.28-3.65)	
	Patient selection	Low	12	0.85 (0.77-0.93)	0.85 (0.80-0.91)	0.99	0.98
		High/Unclear	4	0.76 (0.59-0.92)	0.90 (0.83-0.97)	(0.27-3.57)	
	Index test	Low	11	0.83 (0.74-0.92)	0.86 (0.81-0.92)	0.96	0.94
		High/Unclear	5	0.81 (0.67-0.95)	0.87 (0.79-0.95)	(0.29-3.15)	
	Reference standard	Low	11	0.81 (0.71-0.91)	0.89 (0.85-0.93)	1.85 (0.57-6.07)	0.28
		High/Unclear	5	0.86 (0.74-0.97)	0.79 (0.69-0.89)		
	Flow and timing	Low	10	0.83 (0.74-0.93)	0.86 (0.80-0.92)	0.95 (0.30-2.97)	0.92
		High/Unclear	6	0.82 (0.69-0.94)	0.87 (0.80-0.94)		
PK-M2	Method of measurement	ELISA	20	0.78 (0.73-0.83)	0.72 (0.63-0.81)	0.14 (0.04-0.48)	<0.01
		LF	5	0.95 (0.90-0.99)	0.76 (0.61-0.91)		
	Cut off	> 4 U/mL	2	0.70 (0.46-0.93)	0.64 (0.31-0.97)	3.71 (0.56-24.49)	0.16
		4 U/mL	23	0.83 (0.78-0.88)	0.73 (0.66-0.81)		
	Study type	C	11	0.86 (0.79-0.93)	0.70 (0.58-0.82)	0.86 (0.27-2.66)	0.77
		CC	14	0.79 (0.72-0.86)	0.75 (0.66-0.85)		
	Patient selection	Low	11	0.86 (0.79-0.93)	0.70 (0.58-0.82)	1.17 (0.38-3.64)	0.77
		High/Unclear	14	0.79 (0.72-0.86)	0.75 (0.66-0.85)		
	Index test	Low	14	0.87 (0.82-0.91)	0.71 (0.61-0.81)	1.68	0.34
		High/Unclear	11	0.75 (0.68-0.83)	0.75 (0.64-0.87)	(0.55-5.09)	
	Reference standard	Low	20	0.82 (0.76-0.88)	0.75 (0.68-0.83)	1.66	0.43
		High/Unclear	5	0.83 (0.73-0.94)	0.62 (0.42-0.81)	(0.44-6.32)	
	Flow and timing	Low	19	0.80 (0.74-0.86)	0.74 (0.66-0.83)	0.79	0.72
		High/Unclear	6	0.88 (0.81-0.96)	0.69 (0.51-0.86)	(0.21-3.07)	
FC	Method of measurement	ELISA	23	0.85 (0.81-0.89)	0.65 (0.58-0.72)	1.64	0.49
		LF	2	0.86 (0.70-1.00)	0.57 (0.31-0.83)	(0.37-7.32)	
	Cut off	> 50 μg/g	4	0.83 (0.72-0.94)	0.78 (0.65-0.92)	1.22	0.68
		50 μg/g	21	0.85 (0.81-0.90)	0.62 (0.55-0.69)	(0.44-3.37)	
	Study type	C	18	0.84 (0.78-0.90)	0.61 (0.52-0.69)	3.47	0.03
		CC	7	0.90 (0.83-0.97)	0.74 (0.63-0.85)	(1.58-7.60)	
	Patient selection	Low	18	0.84 (0.78-0.90)	0.61 (0.52 - 0.69)	0.29	0.03
		High/Unclear	7	0.90 (0.83-0.97)	0.74 (0.63 - 0.85)	(0.13-0.63)	
	Index test	Low	15	0.86 (0.80-0.91)	0.60 (0.51 - 0.69)	0.65 (0.30-1.40)	0.25
		High/Unclear	10	0.84 (0.78-0.91)	0.72 (0.62 - 0.81)		
	Reference standard	Low	24	0.85 (0.81-0.89)	0.64 (0.57 - 0.71)	0.53 (0.04-6.64)	0.60

		High/Unclear	1	0.88 (0.63-1.00)	0.72 (0.42 - 1.00)		
	Flow and timing	Low	13	0.88 (0.83-0.93)	0.64 (0.54 - 0.74)	1.48 (0.72-3.04)	0.26
		High/Unclear	12	0.83 (0.77-0.89)	0.65 (0.55 - 0.75)		
Advanced adenoma							
iFOBT	Method of measurement	LAIT	5	0.40 (0.19-0.61)	0.88 (0.82-0.95)	1.50 (0.14-16.54)	0.69
		LF	5	0.66 (0.48-0.83)	0.70 (0.56-0.84)		
	Cut off	≥ 20 μg/g	4	0.60 (0.33-0.87)	0.75 (0.58-0.92)	0.87 (0.12-6.36)	0.87
		< 20 μg/g	6	0.48 (0.23-0.72)	0.84 (0.75-0.94)		
	Index test	Low	8	0.53 (0.34-0.72)	0.83 (0.73-0.92)	2.08 (0.20-21.12)	0.47
		High/Unclear	2	0.50 (0.10-0.90)	0.73 (0.47-0.98)		
	Reference Standard	Low	7	0.47 (0.28-0.65)	0.87 (0.81-0.92)	4.58 (0.39-54.43)	0.18
		High/Unclear	3	0.71 (0.49-0.94)	0.60 (0.43-0.77)		
	Flow and Timing	Low	7	0.46 (0.24-0.68)	0.81 (0.70-0.92)	0.45 (0.08-2.63)	0.31
		High/Unclear	3	0.62 (0.35-0.89)	0.81 (0.65-0.98)		
PK-M2	Method of measurement	ELISA	7	0.39 (0.27-0.50)	0.68 (0.52-0.85)	1.72 (0.13-22.98)	0.63
		LF	3	0.64 (0.46-0.82)	0.53 (0.24-0.81)		
	Study type	C	8	0.40 (0.29-0.51)	0.38 (0.08-0.68)	2.31 (0.07-74.63)	0.58
		CC	2	0.70 (0.49-0.90)	0.70 (0.56-0.83)		
	Patient selection	Low	8	0.40 (0.29-0.51)	0.70 (0.56-0.83)	0.43 (0.01-14.02)	0.58
		High/Unclear	2	0.70 (0.49-0.90)	0.38 (0.08-0.68)		
	Index test	Low	8	0.44 (0.30-0.58)	0.67 (0.51-0.83)	0.90 (0.06-13.03)	0.92
		High/Unclear	2	0.53 (0.24-0.81)	0.51 (0.15-0.87)		
	Reference standard	Low	8	0.44 (0.30-0.58)	0.69 (0.55-0.83)	1.83 (0.13-24.82)	0.60
		High/Unclear	2	0.52 (0.27-0.78)	0.42 (0.10-0.75)		
	Flow and timing	Low	8	0.40 (0.29-0.51)	0.61 (0.44-0.78)	0.20 (0.03-1.33)	0.08
		High/Unclear	2	0.62 (0.42-0.81)	0.76 (0.50-1.00)		
FC	Method of measurement	ELISA	9	0.43 (0.33-0.53)	0.56 (0.45-0.68)	0.41 (0.10-1.58)	0.16
		LF	1	0.69 (0.36-1.00)	0.51 (0.16-0.85)		
	Study type	C	9	0.42 (0.33-0.52)	0.40 (0.08-0.72)	3.84 (0.18-81.07)	0.33
		CC	1	0.88 (0.62-1.00)	0.58 (0.47-0.68)		
	Patient selection	Low	9	0.42 (0.33-0.52)	0.58 (0.47-0.68)	0.26 (0.01-5.50)	0.33
		High/Unclear	1	0.88 (0.62-1.00)	0.40 (0.08-0.72)		
	Index test	Low	7	0.45 (0.28-0.62)	0.59 (0.47-0.71)	1.11 (0.26-4.71)	0.87
		High/Unclear	3	0.56 (0.29-0.83)	0.48 (0.29-0.68)		
	Reference standard	Low	9	0.46 (0.35-0.56)	0.54 (0.43-0.65)	0.41 (0.04-3.77)	0.37
		High/Unclear	1	0.50 (0.06-0.94)	0.71 (0.44-0.98)		
	Flow and timing	Low	5	0.39 (0.22-0.56)	0.57 (0.42-0.72)	0.62 (0.30-1.24)	0.14
		High/Unclear	5	0.48 (0.32-0.64)	0.55 (0.39-0.70)		
Advanced neoplasm							
iFOBT	Method of measurement	LAIT	6	0.61 (0.44-0.79)	0.91 (0.86-0.97)	1.07 (0.34-3.33)	0.89
		LF	5	0.82 (0.70-0.93)	0.82 (0.72-0.93)		

	Cut off	≥ 20 µg/g	5	0.77 (0.60-0.94)	0.84 (0.74-0.94)	1.65 (0.58-4.65)	0.29
		< 20 µg/g	6	0.68 (0.49-0.86)	0.90 (0.84-0.96)		
	Index test	Low	8	0.71 (0.57-0.85)	0.89 (0.83-0.95)	1.17 (0.28-4.81)	0.80
		High/Unclear	3	0.74 0.49-0.99)	0.83 (0.69-0.97)		
	Reference Standard	Low	7	0.61 (0.48-0.74)	0.92 (0.88-0.95)	1.13 (0.22-5.74)	0.86
		High/Unclear	4	0.87 (0.78-0.96)	0.76 (0.64-0.87)		
	Flow and Timing	Low	8	0.73 (0.58-0.88)	0.89 (0.83-0.95)	1.83(0.66-5.04)	0.20
		High/Unclear	3	0.71 (0.47-0.96)	0.84 (0.70-0.97)		
PK-M2	Method of measurement	ELISA	7	0.67 (0.61-0.72)	0.78 (0.68-0.89)	1.28 (0.62-2.65)	0.44
		LF	3	0.72 (0.65-0.80)	0.71 (0.51-0.90)		
	Study type	C	8	0.67 (0.61-0.72)	0.76 (0.65-0.86)	1.40 (0.60-3.28)	0.38
		CC	2	0.72 (0.64-0.81)	0.80 (0.59-1.00)		
	Patient selection	Low	8	0.67 (0.61-0.72)	0.76 (0.65-0.86)	0.72 (0.30-1.68)	0.38
		High/Unclear	2	0.72 (0.64-0.81)	0.80 (0.58-1.00)		
	Index test	Low	7	0.70 (0.66-0.74)	0.76 (0.74-0.78)	1.55 (0.82-2.92)	0.15
		High/Unclear	3	0.66 (0.58-0.73)	0.73 (0.67-0.78)		
	Reference standard	Low	7	0.68 (0.62-0.73)	0.78 (0.67-0.89)	0.98 (0.49-1.95)	0.93
		High/Unclear	3	0.70 (0.62-0.77)	0.72 (0.53-0.91)		
	Flow and timing	Low	8	0.69 (0.64-0.74)	0.72 (0.62-0.81)	0.85 (0.38-1.93)	0.66
		High/Unclear	2	0.64 (0.53-0.75)	0.88 (0.78-0.98)		
FC	Method of measurement	ELISA	9	0.70 (0.58-0.81)	0.60 (0.49-0.71)	1.14 (0.10-12.94)	0.89
		LF	1	0.75 (0.44-1.00)	0.52 (0.18-0.87)		
	Study type	C	9	0.67 (0.56-0.78)	0.61 (0.51-0.72)	3.21 (0.14-74.04)	0.40
		CC	1	0.93 (0.80-1.00)	0.43 (0.10-0.76)		
	Patient selection	Low	9	0.67 (0.56-0.78)	0.61 (0.51-0.72)	0.31 (0.01-7.20)	0.40
		High/Unclear	1	0.93 (0.80-1.00)	0.43 (0.10-0.76)		
	Index test	Low	7	0.67 (0.54-0.81)	0.63 (0.51-0.74)	1.08 (0.19-6.09)	0.91
		High/Unclear	3	0.76 (0.59-0.94)	0.52 (0.32-0.71)		
	Reference standard	Low	9	0.70 (0.59-0.82)	0.58 (0.47-0.69)	0.53 (0.04-6.74)	0.57
		High/Unclear	1	0.69 (0.31-1.00)	0.73 (0.46-0.99)		
	Flow and timing	Low	5	0.74 (0.61-0.88)	0.63 (0.49-0.77)	2.07 (0.55-7.75)	0.23
		High/Unclear	5	0.64 (0.48-0.81)	0.56 (0.41-0.71)		

†iFOB: Immunochemical Fecal occult blood tests; **PK-M2:** Pyruvate kinase-M2; **FC:** Fecal Calprotectin; **LAIT:** *Latex agglutination immunoturbidimetry;* ***LF:*** *lateral flow;* ***C:*** *cohort study design;* ***CC:*** *case-control study design.*

Table 4. Univariate meta-regression.

Test†		Covariate	No. of study	Sensitivity (95% CI)	P-value	Specificity (95% CI)	P-value
iFOBT	CRC	Age	16	0.84 (0.75-0.90)	0.89	0.86 (0.81-0.90)	0.89
		%Male	16	0.81 (0.73-0.88)	0.78	0.87 (0.83-0.91)	0.85
		%Distal	6	0.90 (0.81-0.95)	0.95	0.86 (0.76-0.92)	0.95
		%Late	6	0.86 (0.70-0.94)	0.99	0.85 (0.77-0.90)	0.95
	AA	Age	10	0.54 (0.36-0.72)	0.90	0.80 (0.70-0.88)	0.89
		%Male	10	0.52 (0.35-0.68)	0.89	0.82 (0.73-0.88)	0.87
	AN	Age	11	0.73 (0.59-0.84)	0.90	0.87 (0.80-0.92)	0.88
		%Male	11	0.72 (0.61-0.81)	0.90	0.88 (0.82-0.92)	0.92
PK-M2	CRC	Age	22	0.83 (0.77-0.87)	0.93	0.74 (0.65-0.82)	0.83
		%Male	22	0.82 (0.76-0.87)	0.98	0.73 (0.63-0.81)	1.00
		%Distal	8	0.87 (0.75-0.94)	0.99	0.80 (0.68-0.89)	0.92
		%Late	10	0.81 (0.74-0.87)	1.00	0.71 (0.64-0.78)	0.99
	AA	Age	10	0.45 (0.34-0.58)	0.91	0.64 (0.48-0.78)	0.99
		%Male	10	0.47 (0.35-0.60)	0.97	0.65 (0.49-0.78)	0.95
	AN	Age	10	0.68 (0.64-0.73)	0.90	0.77 (0.67-0.85)	0.89
		%Male	10	0.68 (0.64-0.73)	0.97	0.77 (0.66-0.84)	0.97
FC	CRC	Age	22	0.85 (0.79-0.89)	0.92	0.65 (0.57-0.73)	0.97
		%Male	21	0.83 (0.77-0.87)	0.96	0.65 (0.57-0.73)	0.93
		%Distal	4	0.88 (0.71-0.95)	0.91	0.80 (0.59-0.92)	0.97
	AN	Age	9	0.69 (0.55-0.80)	0.89	0.59 (0.47-0.70)	0.93
		%Male	9	0.69 (0.56-0.80)	0.93	0.57 (0.46-0.68)	0.91

†iFOB: Immunochemical Fecal occult blood tests; **PK-M2:** Pyruvate kinase-M2; **FC:** Fecal Calprotectin; **CRC:** Colorectal Cancer; **AA:** Advanced Adenoma; **AN:** Advanced Neoplasms.

Figures

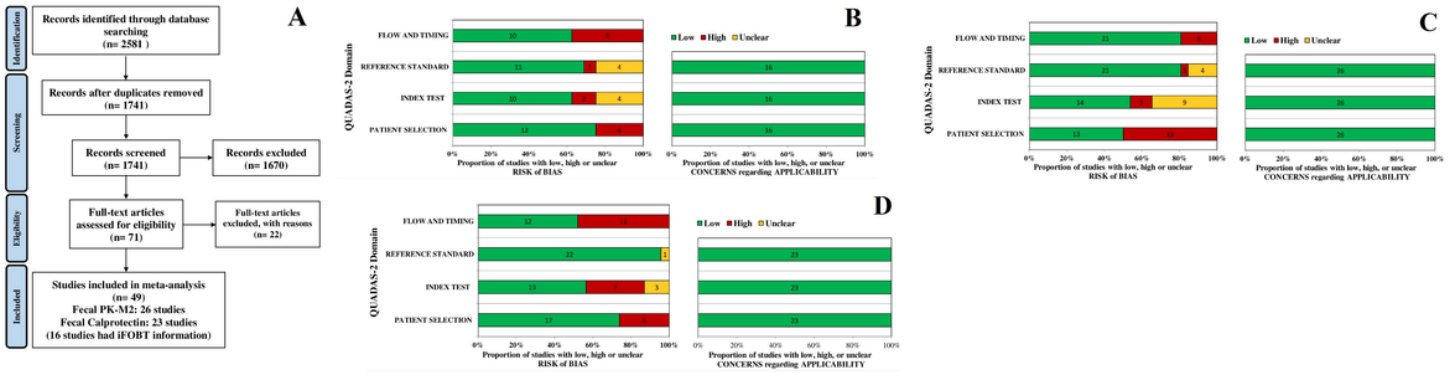

Figure 1

Flowchart diagram of study selection and quality assessment of included studies utilizing the QUADAS-2. A) Flowchart diagram of study selection based on the inclusion and exclusion criteria; B) QUADAS-2 diagram for iFOBT; C) QUADAS-2 diagram for PK-M2; D) QUADAS-2 diagram for FC. iFOB: Immunochemical Fecal occult blood tests; PK-M2: Pyruvate kinase-M2; FC: Fecal Calprotectin; QUADAS-2: Quality Assessment of Diagnostic AccuracyStudies-2.

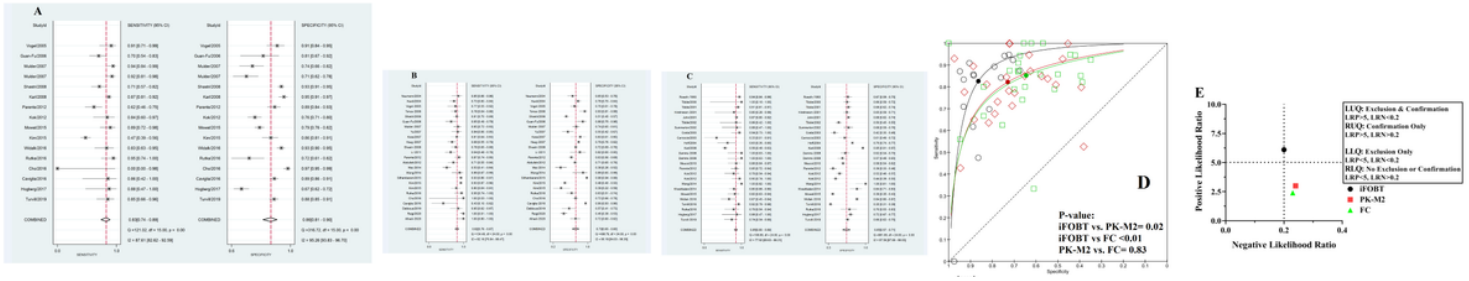

Figure 2

Forest plot, HSROC and LR scattergram of clinically applicable fecal protein biomarkers for CRC diagnosis. A: forest plot of iFOBT; B: forest plot PK-M2; C: forest plot of FC; D: comparison HSROC of clinically applicable fecal protein biomarkers; E: LR scattergram of clinically applicable fecal protein biomarkers. CRC: colorectal cancer; iFOBT: immunochemical Fecal occult blood tests; PK-M2: pyruvate kinase-M2; FC: fecal calprotectin; LUQ: left upper quadrant; RUQ: right upper quadrant; LLQ: left lower quadrant; RLQ: right lower quadrant.

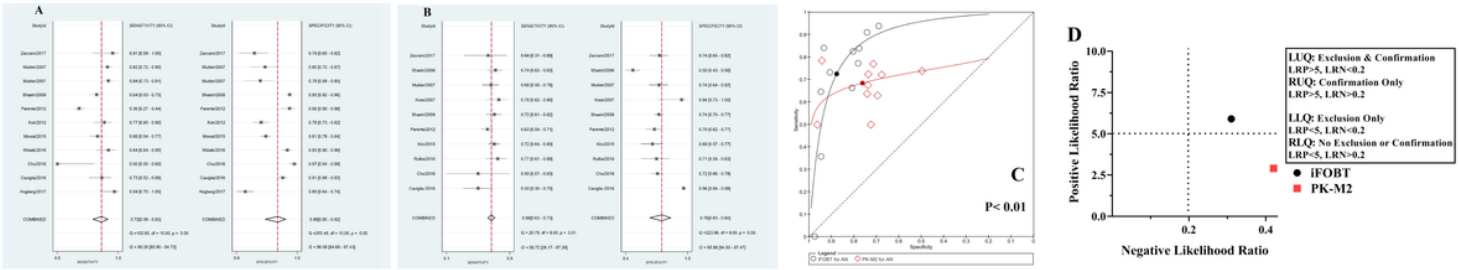

Figure 3

Forest plot, HSROC and LR scattergram of clinically applicable fecal protein biomarkers for AN diagnosis. A: forest plot of iFOBT; B: forest plot PK-M2; C: comparison HSROC of clinically applicable fecal protein biomarkers; C: LR scattergram of clinically applicable fecal protein biomarkers. AN: advanced neoplasms; iFOBT: immunochemical fecal occult blood tests; PK-M2: pyruvate kinase-M2; LUQ: left upper quadrant; RUQ: right upper quadrant; LLQ: left lower quadrant; RLQ: right lower quadrant.

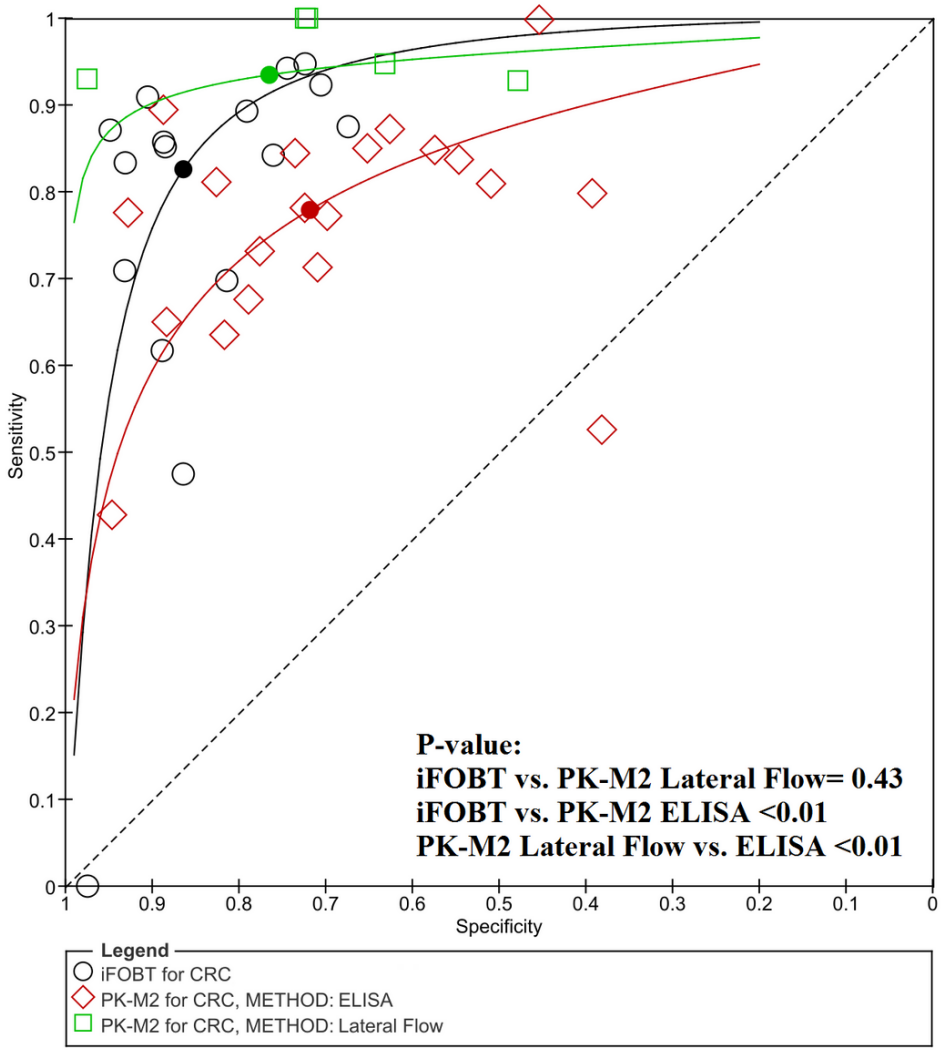

Figure 4

comparison HSROC of PK-M2 different methods of measurement. iFOBT: immunochemical fecal occult blood tests; PK-M2: pyruvate kinase-M2; ELISA: enzyme-linked immunosorbent assay.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryinformation.pdf