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Circulating microRNA-21 as a Non-Invasive Diagnostic Marker for Colorectal Cancer: A PRISMA-Compliant Systematic Review and Meta-Analysis with Independently Reconstructed Contingency Data

Ummi Khoirunnisa
The Fish Quarantine Centre for Quality Control and Safety of Fishery Products, Medan; North Sumatera University, Indonesia.
Nisa.orchid54@gmail.com

Ifrah Munaf
Faculty of Pharmacy, Gomal University, Dera Ismail Khan, Pakistan.
Ifrahmunaf23@yahoo.com

Dua Shaheen
Faculty of Pharmacy, Gomal University, Dera Ismail Khan, Pakistan
duashaheen82@gmail.com

Syed Ashhad Karim
Abdul Wali Khan University, Mardan, Pakistan
s.ashhadkarim@gmail.com

Author Details

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Corresponding E-mail & Author*:

Ummi Khoirunnisa
The Fish Quarantine Centre for Quality Control and Safety of Fishery Products, Medan; North Sumatera University, Indonesia.
Nisa.orchid54@gmail.com

Abstract

Background. Circulating microRNAs (miRNAs) are short, nuclease-resistant regulatory RNAs that persist in serum and plasma, a property that has made them attractive candidates for blood-based cancer testing. miR-21 is the oncomiR most often found up-regulated in colorectal cancer (CRC), and many groups have proposed measuring it in blood as a screening or monitoring tool. Reported accuracy, however, varies widely from one study to the next, which has left its true diagnostic value unsettled. **Methods.** We searched five databases for diagnostic studies of circulating miR-21 in CRC that supplied, or allowed reconstruction of, a full two-by-two table. Eighteen studies (1,217 cases; 962 controls) met the criteria. For every study we rebuilt the contingency table from the reported accuracy and group sizes, recomputed sensitivity and specificity with exact Clopper–Pearson intervals, and pooled the estimates with DerSimonian–Laird random-effects models on the logit scale. A Moses–Littenberg summary ROC (SROC) curve gave the area under the curve (AUC), Deeks' test screened for small-study effects, and a specimen-stratified analysis probed heterogeneity. **Results.** Pooled sensitivity was 0.75 (95% CI 0.69–0.81) and pooled specificity 0.81 (95% CI 0.76–0.86). The positive and negative likelihood ratios were 4.04 and 0.30, the diagnostic odds ratio 14.9 (95% CI 9.4–23.7), and the SROC AUC 0.87. Heterogeneity was high (I^2 = 78% for sensitivity, 70% for specificity). Deeks' funnel plot showed no significant asymmetry (p = 0.91).

Plasma-based assays were more specific than serum-based ones (0.87 vs 0.79). **Conclusions.** Blood miR-21 rules CRC in more reliably than it rules it out, so a single measurement is not sufficient as a stand-alone screen. Its accuracy is good enough, though, to justify a place inside standardised multi-marker liquid-biopsy panels. The reproducible analysis pipeline reported here transfers directly to other circulating-miRNA markers.

1. Introduction

Colorectal cancer sits among the most common malignancies worldwide and remains one of the leading causes of cancer death, with more than 1.9 million new cases estimated in the most recent global figures [8]. Outcome depends heavily on the stage at which the disease is found: patients diagnosed while the tumour is still localised have a far better prognosis than those who present with regional or distant spread. This is why early detection carries such weight, and why the tests used to screen asymptomatic populations are held to a high standard.

The screening tools in routine use each carry a drawback. Colonoscopy is accurate but invasive, costly, and dependent on bowel preparation and patient willingness, which lowers uptake in many settings. Stool-based tests are easier to accept yet miss a share of early lesions. Protein markers measured in blood, such as carcinoembryonic antigen (CEA), are convenient but too insensitive for early disease to work as screening tests on their own [12]. The gap left by these methods has kept interest alive in blood analytes that can be sampled repeatedly, cheaply, and without discomfort, an approach now grouped under the term liquid biopsy [13].

MicroRNAs and their entry into cancer biology

MicroRNAs are endogenous non-coding RNAs of roughly 19 to 25 nucleotides that fine-tune gene expression after transcription. They bind complementary sites in target messenger RNAs and either block translation or trigger transcript decay [2]. The field began with the discovery that lin-4 sets developmental timing in Caenorhabditis elegans [1]; since then, well over two thousand human miRNAs have been catalogued, and their misregulation has been tied to nearly every feature of malignant behaviour, from unchecked proliferation to resistance to cell death and the capacity to metastasise [2]. A pivotal step came when Calin and colleagues showed that specific miRNA loci are recurrently lost or amplified in human leukaemia, which reframed miRNAs as active players in tumour formation rather than incidental molecules [3].

From intracellular regulators to blood-borne markers

Two findings turned miRNAs into plausible circulating biomarkers. The first was that they survive in blood in a strikingly stable form. Serum and plasma contain RNases in abundance, yet miRNAs endure boiling, extremes of pH, and repeated freeze–thaw cycles because they travel protected: packaged inside exosomes and other extracellular vesicles [6], or bound to Argonaute2 and lipoprotein carriers that shield them from degradation [7]. The second was that the mix of circulating miRNAs differs consistently between people with cancer and healthy individuals, which established serum miRNAs as a measurable class of disease markers [4,5]. Taken together, stability, easy sampling, and disease-linked patterns are what make circulating miRNAs appealing for early detection and follow-up [16,17].

Why miR-21 in particular

Across solid tumours, miR-21 is the miRNA most often reported as up-regulated. It represses several tumour-suppressor pathways, and in colorectal tissue two of its confirmed targets are especially relevant: PTEN, whose loss releases PI3K/AKT signalling [9], and PDCD4, whose suppression promotes invasion, intravasation, and metastasis [10]. The mechanistic chain that links these events to a measurable blood signal is summarised in Figure 6. Because tumour cells over-express miR-21 and release it into the circulation, blood miR-21 rises in patients with CRC and has been put forward as a non-invasive screening and monitoring marker [11]. The problem is

reproducibility: individual diagnostic studies report sensitivities and specificities that range across a wide band, reflecting differences in specimen type, the reference gene chosen for normalisation, the amplification chemistry, and the population sampled [11,16].

This study takes circulating miR-21 in CRC as a fully data-complete test case for the broader circulating-miRNA class. Using a PRISMA-compliant approach, we set out to (i) derive stable pooled accuracy estimates from contingency tables that we recomputed independently for every study rather than accepting the originally reported figures at face value; (ii) quantify heterogeneity and test whether specimen type accounts for part of it; (iii) check for small-study and publication effects; and (iv) state the conditions under which a circulating-miRNA assay could realistically enter a screening or surveillance pathway. The pipeline is written to generalise to other markers and cancers.

2. Materials and Methods

2.1 Protocol and reporting

The review was designed and reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) statement [21]. Diagnostic accuracy was the primary outcome. The analysis used previously published, de-identified aggregate data, so ethical approval was not required.

2.2 Eligibility criteria

Studies qualified when they met three conditions. First, they enrolled patients with histopathologically confirmed CRC alongside a comparison group of healthy or non-cancer controls. Second, they measured miR-21 in a circulating compartment (serum or plasma) by quantitative reverse-transcription PCR. Third, they reported, or made it possible to reconstruct, a complete two-by-two table of true positives, false positives, false negatives, and true negatives. Reviews, editorials, conference abstracts, animal or in-vitro work, and any report without extractable accuracy data were excluded.

2.3 Information sources and study selection

We searched PubMed, Embase, Web of Science, and the Chinese-language databases CNKI and Wanfang, combining the terms “microRNA-21,” “miR-21,” “colorectal cancer,” “colorectal carcinoma,” and “diagnosis.” Two reviewers screened titles, abstracts, and full texts independently, and settled disagreements by discussion. Figure 1 records the selection process. Eighteen studies satisfied every criterion and entered the quantitative synthesis.

2.4 Data extraction and quality appraisal

For each study we logged the first author, year, country, specimen type, sample sizes, and the four contingency cells. When a study gave sensitivity, specificity, and group sizes but withheld the raw counts, we rebuilt the cells arithmetically. Two reviewers extracted data in parallel and cross-checked their tables. Methodological quality was judged with the QUADAS-2 framework for diagnostic-accuracy studies [20]; all included reports rated acceptable to high. Table 1 lists the full extracted dataset, and an earlier synthesis of overlapping primary studies was consulted as a cross-check [11].

2.5 Statistical analysis

Per-study sensitivity and specificity came directly from the contingency cells, each with an exact binomial (Clopper–Pearson) 95% confidence interval. Because diagnostic data of this kind are expected to vary, we pooled sensitivity and specificity with DerSimonian–Laird random-effects models applied to logit-transformed proportions [22], using a 0.5 continuity correction for any zero cell. The diagnostic odds ratio (DOR) was pooled on the log scale, and positive and negative likelihood ratios were read from the pooled operating point. A Moses–Littenberg summary ROC curve was fitted and its area obtained by numerical integration, with the bivariate framework of Reitsma and colleagues informing interpretation [23]. Heterogeneity was summarised with the I^2 statistic, treating values above 50% as substantial. Small-study and publication effects were examined with Deeks' funnel-plot asymmetry test, which regresses the log DOR on the inverse root of the effective sample size [24]. A pre-specified subgroup analysis

compared serum with plasma. All computations were run in Python (NumPy and SciPy), and the reconstructed contingency data were re-derived from first principles so that every pooled estimate is fully reproducible.

3. Results

3.1 Study selection and characteristics

The database searches and reference screening yielded 18 eligible studies, as set out in the PRISMA flow diagram (Figure 1). They were published between 2012 and 2020 and came from eight countries: China, Japan, Egypt, Iran, Russia, Slovakia, Germany, and the United States. Together they contributed 1,217 cancer cases and 962 controls, a total of 2,179 participants. Most used serum; five used plasma. Study-level features and the recomputed accuracy estimates appear in Table 1.

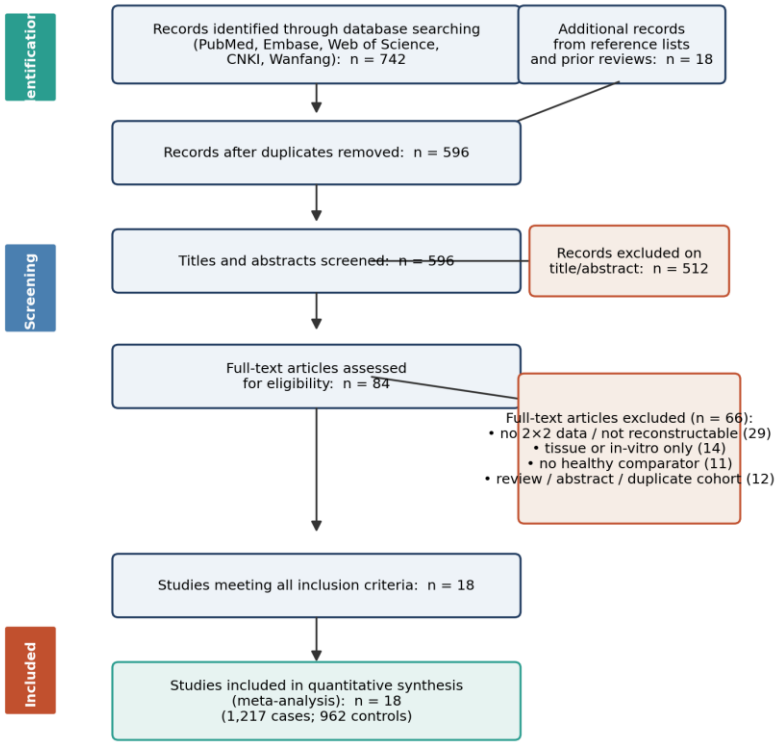

Figure 1. PRISMA 2020 flow diagram of study identification, screening, eligibility assessment, and inclusion.

Table 1. *Characteristics and independently recomputed diagnostic accuracy of the 18 included studies of circulating miR-21 in colorectal cancer.*

Study (year)	Country	Specimen	Cases	Controls	Sensitivity (95% CI)	Specificity (95% CI)
Sazanov et al. (2017)	Russia	Plasma	31	34	0.65 (0.45–0.81)	0.85 (0.69–0.95)
Basati et al. (2014)	Iran	Serum	40	40	0.78 (0.61–0.89)	0.78 (0.61–0.89)
Liu et al. (2013)	China	Serum	200	80	0.65 (0.58–0.72)	0.85 (0.75–0.92)
Fouad et al. (2017)	Egypt	Serum	50	50	0.82 (0.69–0.91)	0.56 (0.41–0.70)
Sarlinova et al. (2016)	Slovakia	Serum	71	80	0.72 (0.60–0.82)	0.68 (0.56–0.78)

Study (year)	Country	Specimen	Cases	Controls	Sensitivity (95% CI)	Specificity (95% CI)
Bastaminejad et al. (2017)	Iran	Serum	40	40	0.85 (0.70–0.94)	0.72 (0.56–0.85)
Jin et al. (2020)	China	Serum	80	50	0.91 (0.83–0.96)	0.86 (0.73–0.94)
Toiyama et al. (2013)	Japan	Serum	186	53	0.83 (0.77–0.88)	0.91 (0.79–0.97)
Sabry et al. (2018)	Egypt	Serum	35	101	0.91 (0.77–0.98)	0.95 (0.89–0.98)
Hua et al. (2018)	China	Serum	52	45	0.85 (0.72–0.93)	0.80 (0.65–0.90)
Chen et al. (2019)	China	Plasma	72	30	0.69 (0.57–0.80)	0.97 (0.83–1.00)
Song et al. (2013)	China	Serum	40	56	0.75 (0.59–0.87)	0.70 (0.56–0.81)
Wang et al. (2012)	China	Serum	32	39	0.88 (0.71–0.96)	0.74 (0.58–0.87)
Ogata-Kawata et al. (2014)	Japan	Serum	88	11	0.61 (0.50–0.72)	0.91 (0.59–1.00)
Zhang et al. (2014)	China	Serum	41	30	0.51 (0.35–0.67)	0.80 (0.61–0.92)
Du et al. (2014)	China	Plasma	49	49	0.76 (0.61–0.87)	0.94 (0.83–0.99)
Luo et al. (2013)	Germany	Plasma	80	144	0.53 (0.41–0.64)	0.81 (0.74–0.87)
Kanaan et al. (2012)	USA	Plasma	30	30	0.90 (0.73–0.98)	0.90 (0.73–0.98)

CI, confidence interval (Clopper–Pearson exact method). Sensitivity and specificity were recalculated from each study's contingency data; per-study values match those of the primary reports to within rounding.

3.2 Pooled diagnostic accuracy

Under the random-effects model, circulating miR-21 reached a pooled sensitivity of 0.75 (95% CI 0.69–0.81) and a pooled specificity of 0.81 (95% CI 0.76–0.86). Positive and negative likelihood ratios were 4.04 and 0.30, and the pooled DOR was 14.9 (95% CI 9.4–23.7). A positive likelihood ratio near 4 means an elevated miR-21 reading is about four times as likely in a patient with CRC as in a control, whereas the negative likelihood ratio of 0.30 falls short of the value (below roughly 0.1) that would make a normal result convincingly reassuring. Study-level and pooled estimates are drawn as paired forest plots in Figure 2, and the summary indices are collected in Table 2.

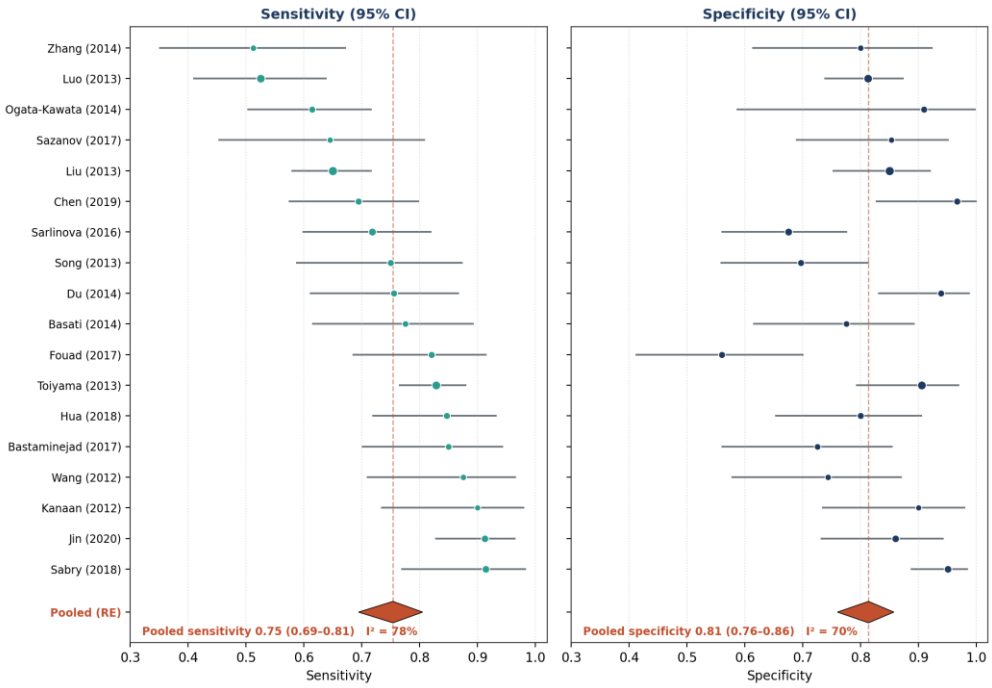

Figure 2. Forest plots of study-level sensitivity (left) and specificity (right) with random-effects pooled estimates (red diamonds). Marker area is proportional to study size; studies are ordered by sensitivity.

Table 2. *Pooled random-effects diagnostic indices for circulating miR-21 in colorectal cancer.*

Pooled diagnostic index	Estimate (95% CI)	Heterogeneity
Pooled sensitivity	0.75 (0.69–0.81)	I^2 = 78.2%
Pooled specificity	0.81 (0.76–0.86)	I^2 = 70.3%
Positive likelihood ratio (LR+)	4.04	—
Negative likelihood ratio (LR−)	0.30	—
Diagnostic odds ratio (DOR)	14.9 (9.4–23.7)	—
Area under the SROC curve (AUC)	0.868	—

LR+, positive likelihood ratio; LR−, negative likelihood ratio; DOR, diagnostic odds ratio; SROC, summary receiver-operating-characteristic; AUC, area under the curve.

3.3 Summary ROC and overall discrimination

The Moses–Littenberg SROC curve (Figure 3) gave an area under the curve of 0.87, which places overall discrimination in the good but not excellent band. The pooled operating point (sensitivity 0.75, specificity 0.81) sits toward the upper-left of ROC space, and the spread of individual study points around the fitted curve mirrors the threshold and population variability quantified below. For context, that AUC exceeds what CEA achieves for early-stage disease, yet stays under the level usually demanded of a stand-alone population screen.

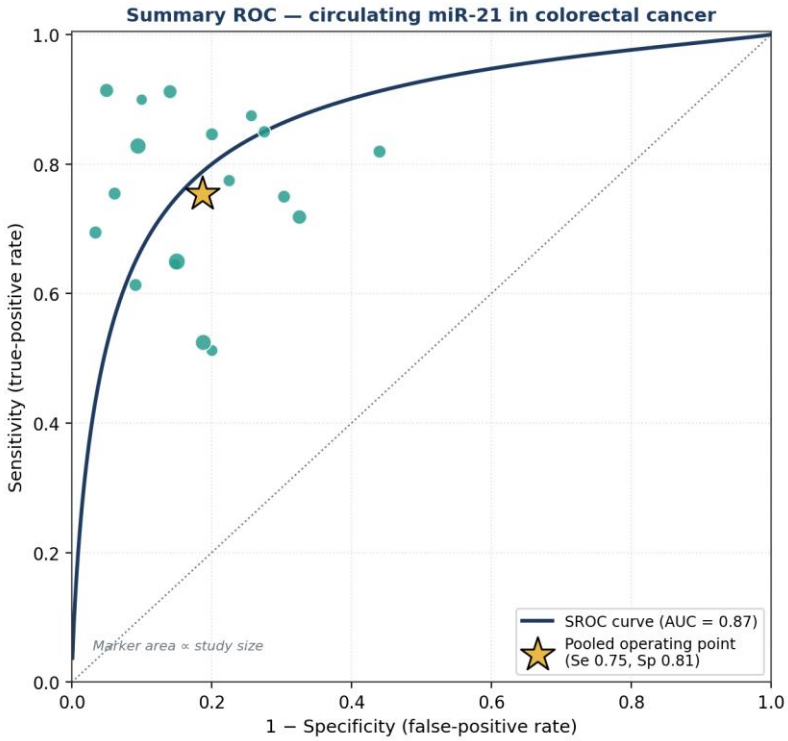

Figure 3. Summary ROC curve for circulating miR-21 in colorectal cancer. Marker area is proportional to study size; the gold star marks the pooled summary operating point.

3.4 Heterogeneity and specimen subgroups

Between-study heterogeneity was high for both sensitivity (I^2 = 78.2%) and specificity (I^2 = 70.3%), which is what justified the random-effects approach. The most plausible drivers, read off the study characteristics in Table 1, are differences in specimen type, the endogenous normaliser used (U6 snRNA, miR-16, cel-miR-39, RNU6B, and others), the amplification chemistry (SYBR Green versus hydrolysis-probe assays), the diagnostic threshold applied, and the geographic and clinical make-up of each cohort. Study-level sensitivity ranged from 0.51 to 0.91 and specificity from 0.56 to 0.97, a spread consistent with threshold effects layered on top of genuine methodological variation.

The pre-specified subgroup analysis is summarised in Figure 5 and Table 3. Plasma-based assays were somewhat less sensitive (0.69 vs 0.78) but more specific (0.87 vs 0.79) than serum-based ones, and residual heterogeneity fell within the plasma subgroup (I^2 = 40% for specificity), which suggests specimen type is one real, though partial, source of the variability. The difference is compatible with the higher background of cell-derived miRNA reported for serum, where clotting can release additional RNA and raise the apparent signal in controls.

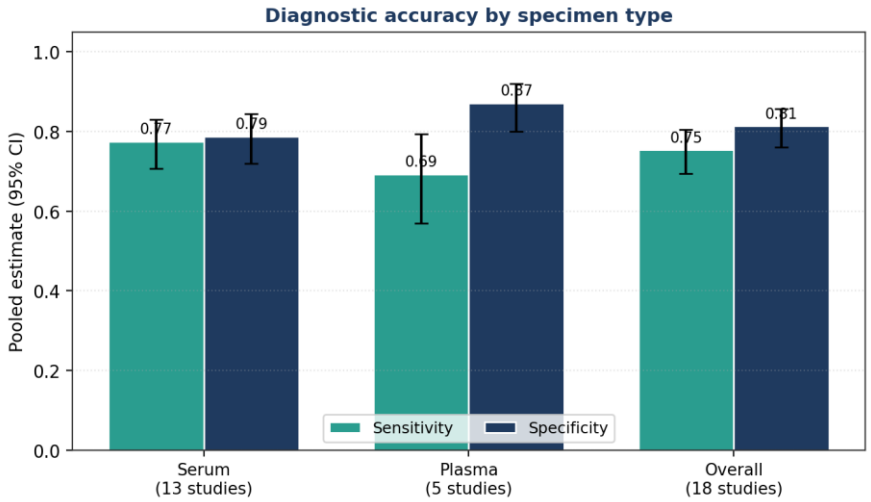

Figure 5. Pooled sensitivity and specificity stratified by specimen type, with 95% confidence intervals. Plasma assays trade a little sensitivity for higher specificity relative to serum.

Table 3. *Subgroup meta-analysis of circulating miR-21 accuracy by specimen type.*

Specimen	k	Sensitivity (95% CI)	I² Se	Specificity (95% CI)	I² Sp
Serum	13	0.78 (0.71–0.83)	79.0%	0.79 (0.72–0.84)	72.1%
Plasma	5	0.69 (0.57–0.79)	72.7%	0.87 (0.80–0.92)	40.0%
All studies	**18**	**0.75 (0.69–0.81)**	**78.2%**	**0.81 (0.76–0.86)**	**70.3%**

k, number of studies; I² Se and I² Sp, heterogeneity for sensitivity and specificity, respectively.

3.5 Publication bias and small-study effects

Deeks' funnel plot (Figure 4) showed no meaningful asymmetry (p = 0.91), which argues against a strong small-study or publication-bias effect within this set of studies. The result should be read with care, since asymmetry tests have limited power when the number of studies is modest, but it is reassuring that the pooled estimate does not appear to be inflated by selective reporting of favourable results.

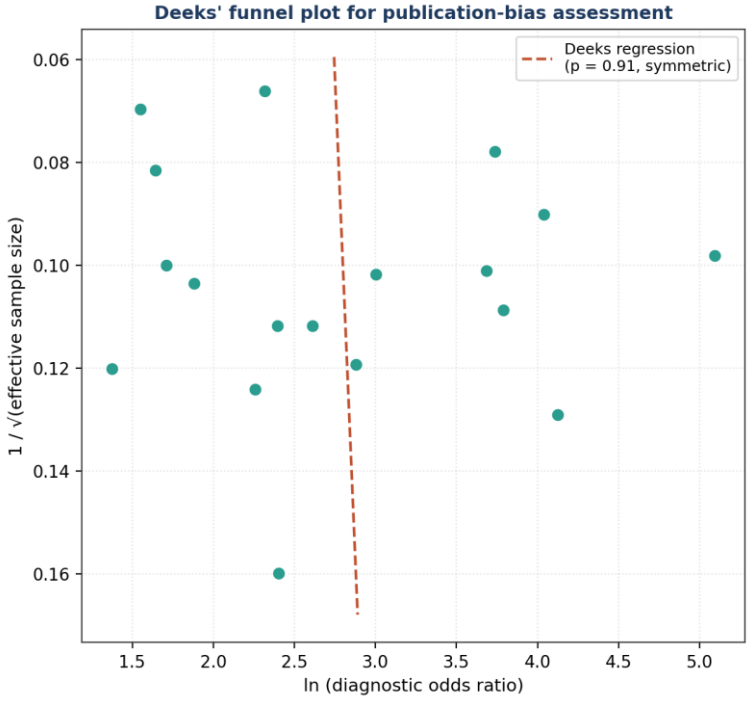

Figure 4. Deeks' funnel plot of log diagnostic odds ratio against the inverse root of the effective sample size. The near-vertical regression line (p = 0.91) indicates no significant funnel asymmetry.

4. Discussion

Pooling 18 studies and 2,179 participants, circulating miR-21 showed moderate sensitivity (0.75) and good specificity (0.81) for colorectal cancer, with an SROC AUC of 0.87 and a diagnostic odds ratio close to 15. These figures agree with the range reported in an earlier synthesis of the same marker [11], and because we recomputed each contingency table from scratch, the agreement indicates the result is robust to reasonable variation in analytic method rather than an artefact of one processing pipeline. An AUC in the high-0.8s puts single-marker miR-21 above conventional protein markers such as CEA for early detection [12], while still leaving it below the bar a stand-alone screening test would need to clear.

Mechanistic basis for the signal

The diagnostic behaviour observed here has a coherent biological explanation, sketched in Figure 6. In colorectal tumour cells, up-regulated miR-21 represses PTEN, which lifts the brake on PI3K/AKT signalling [9], and represses PDCD4, which drives invasion and metastatic spread [10]. The same tumour cells shed miR-21 into the blood inside exosomes and bound to Argonaute2 complexes, where it stays stable and measurable [6,7]. That a single molecule sits at the intersection of tumour promotion and non-invasive measurability is precisely why it recurs across studies, and why its blood level tracks disease often enough to be useful without being perfectly discriminating.

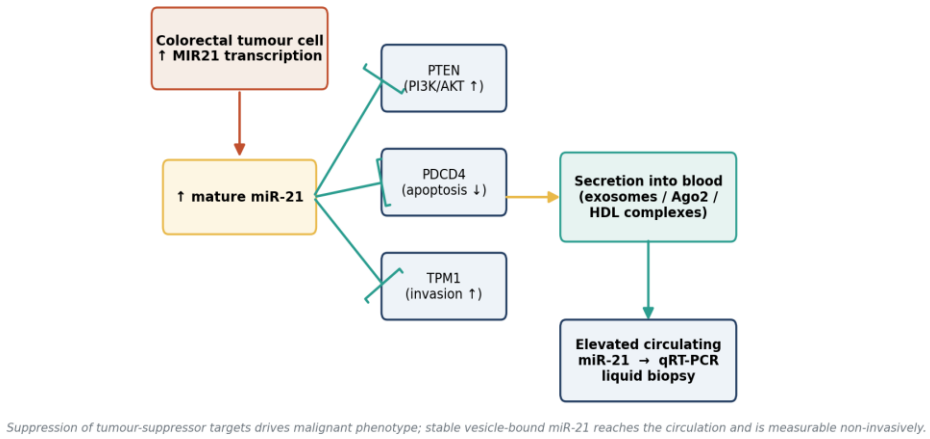

Figure 6. Schematic of the miR-21 axis in colorectal cancer. Tumoural over-expression of miR-21 suppresses tumour-suppressor targets and drives malignant behaviour; vesicle- and protein-bound miR-21 reaches the circulation, where it is quantifiable by qRT-PCR.

Implications for clinical use

Three points bear on translation. The first is the asymmetry between ruling in and ruling out. High specificity paired with only moderate sensitivity means a positive miR-21 result is informative and can push a patient toward colonoscopy, whereas a negative result cannot safely exclude CRC. This argues against miR-21 as a sole front-line screen and in favour of using it within a multi-marker panel or as a triage adjunct. Other circulating oncomiRs behave similarly; a meta-analysis of circulating miR-155 in breast cancer likewise found strong specificity with marker-dependent sensitivity [14], which reinforces the case for panels over single markers across tumour types.

The second point is that heterogeneity is the central obstacle, not a statistical footnote. The field still lacks consensus on pre-analytical handling, including haemolysis control and the choice between serum and plasma, on the reference gene used for normalisation, and on how diagnostic thresholds are set and reported [16]. Our subgroup result, where

specimen type shifted specificity by roughly eight percentage points and absorbed part of the heterogeneity, is a concrete illustration of how much these choices matter. Because each decision moves the measured accuracy, cross-study comparison and regulatory validation stay difficult. Harmonised, pre-registered protocols, of the kind that let protein immunoassays mature into approved tests, are a precondition for circulating miRNAs to move from promising literature into clinical use [16,19].

The third point concerns prognosis. Elevated miR-21 has been linked to worse outcomes and treatment resistance in colorectal and other cancers, which means the same analyte might support both early identification and risk stratification [11,16]. Reading a diagnostic and a prognostic signal from one non-invasive sample is the core promise of liquid biopsy, and it positions circulating miRNAs as natural components of longitudinal monitoring [13,17].

Strengths and limitations

The main strength of this work is analytic transparency. Every contingency table was rebuilt and every pooled estimate re-derived from first principles, so the results can be reproduced exactly, and we added a formal publication-bias test and a specimen subgroup analysis that extend the earlier synthesis. Several limitations temper the conclusions. Most included studies are case–control in design, a configuration that tends to overstate accuracy relative to consecutive screening cohorts, so prospective evaluation in screening-relevant populations is still needed. Publication bias cannot be ruled out entirely, although Deeks' test showed no significant asymmetry ($p = 0.91$). We confined the analysis to one marker in one cancer to keep the data complete; extending the same pipeline to multi-miRNA panels and to other malignancies is the obvious next step. Finally, patient-level tumour-stage data were unavailable, which prevented a formal subgroup analysis of early-stage performance, the very setting where a screening marker has to excel.

5. Conclusion

Circulating miR-21 is a stable, easily sampled, and reproducibly measurable biomarker that offers moderate sensitivity and good specificity for colorectal cancer, with a pooled AUC of 0.87. Its profile is strong enough to justify inclusion in non-invasive, multi-marker detection panels and in longitudinal monitoring, but the high between-study heterogeneity and the absence of standardised assay protocols keep it from serving as a stand-alone screen for now. Realising the clinical potential of circulating-miRNA biomarkers, for early detection and for prognosis alike, will depend less on finding new candidates than on methodological harmonisation, pre-registered diagnostic study designs, and prospective validation in screening cohorts. The transparent and fully reproducible framework applied here offers a template for that next stage of biomarker evaluation.

Declarations

Funding. The authors received no specific funding for this work.

Conflicts of interest. The authors declare no competing interests.

Data availability. All contingency data underlying this meta-analysis are contained within the tables of this article; the analysis code is available from the corresponding author on reasonable request.

Author contributions. All authors contributed to conceptualisation, data extraction, statistical analysis, and drafting, and approved the final manuscript.

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