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Blood-based microRNAs as biomarkers for the diagnosis of colorectal cancer: a systematic review and meta-analysis

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Background: Colorectal cancer (CRC) is common and associated with significant mortality. Current screening methods for CRC lack patient compliance. microRNAs (miRNAs), identified in body fluids, are negative regulators of gene expression and are dysregulated in many cancers, including CRC. This paper summarises studies identifying blood-based miRNAs dysregulated in CRC compared with healthy controls in an attempt to evaluate their use as a screening tool for the diagnosis of CRC.

Methods: A search of electronic databases (PubMed and EMBASE) and grey literature was performed between January 2002 and April 2016. Studies reporting plasma or serum miRNAs in the diagnosis of CRC compared with healthy controls were selected. Patient demographics, type of patient sample (serum or plasma), method of miRNA detection, type of normalisation, and the number of significantly dysregulated miRNAs identified were recorded. Statistical evaluation of dysregulated miRNAs using sensitivity, specificity, and area under the curve (AUC) was performed.

Results: Thirty-four studies investigating plasma or serum miRNAs in the diagnosis of CRC were included. A total of 31 miRNAs were found to be either upregulated ($n = 17$) or downregulated ($n = 14$) in CRC cases as compared with controls. Fourteen studies identified panels of $\geqslant 2$ dysregulated miRNAs. The highest AUC, 0.943, was identified using a panel of 4 miRNAs with 83.3% sensitivity and 93.1% specificity. Meta-analysis of studies identifying a single dysregulated miRNA in CRC cases compared with controls was performed. Overall sensitivity and specificity of 28 individual miRNAs in the diagnosis of CRC were 76% (95% CI 72%–80%) and 76% (95% CI 72%–80%), respectively, indicating good discriminative ability of miRNAs as biomarkers for CRC. These data did not change with sensitivity analyses.

Conclusions: Blood-based miRNAs distinguish patients with CRC from healthy controls with high sensitivity and specificity comparable to other common and invasive currently used screening methods for CRC. In future, miRNAs may be used as a relatively non-invasive blood-based marker for detection of CRC.

Colorectal cancer (CRC) is a common cancer with substantial mortality. A significant proportion of CRC arises from preexisting colorectal adenomas (CAA; polyps) through the adenoma–carcinoma sequence. In this sequence, a stepwise pattern of genetic changes drive normal colonic epithelium to invasive cancer. Many deaths could be avoided if precancerous adenomas or early-stage cancers could be identified and treated prior to the development of more advanced malignancy (Muller and Sonnenberg, 1995; Zauber *et al*, 2012). Current screening tools for detection of CRC include colonoscopy, faecal-based tests (occult blood testing and DNA-based stool tests), and plasma-based assays. As these methods each have disadvantages, including morbidity (Lohsiriwat, 2010),

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low sensitivity and specificity (Hundt *et al*, 2009), expense (Calderwood and Jacobson, 2013), and poor patient compliance (Logan *et al*, 2012), there is need for a more accurate, less invasive screening tool to permit early detection and intervention.

Biological markers or biomarkers are defined as 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention' (Naylor, 2003). Biomarkers, such as blood, urine, and cerebrospinal fluid, can be used for screening, diagnosis, and predicting prognosis in human diseases (Mayeux, 2004). Plasma and serum are components of blood and are commonly used in diagnostic assays. Serum is of similar composition to plasma except for lacking clotting factors. Both plasma and serum contain hormones, glucose, electrolytes, antibodies, antigens, and nutrients making them ideal media in diagnostic testing (Maton *et al*, 1993). The most commonly used blood-based CRC biomarker, carcinoembryonic antigen, which is used for postoperative surveillance and for monitoring response to therapy, lacks sensitivity and specificity for screening or for the detection of recurrent CRC (Fakih and Padmanabhan, 2006).

microRNAs (miRNAs) are small, non-protein-coding RNA molecules that regulate gene expression by complementary binding to the 3′ untranslated region of target mRNA. They cause target degradation, translational repression, or gene silencing and thus affect subsequent protein expression. miRNAs exhibit important regulatory functions related to cell differentiation, development, and growth (Meltzer, 2005; Croce, 2008). They have also been shown to be dysregulated in a number of cancers, including CRC, by influencing oncogenes and tumour-suppressor genes (Zhang *et al*, 2007; Chiang *et al*, 2012). miRNAs have been identified in body fluids, such as plasma, saliva, urine, and faeces (Chevillet *et al*, 2014). They have been shown to be actively released from cells in microvesicles, exosomes, or bound to proteins. miRNAs are inherently stable accounting for the emerging use as potential biomarkers for human disease and as targets for disease intervention (Kanaan *et al*, 2012; Mishra, 2014).

The purpose of this systematic review and meta-analysis is to assess published studies describing the use of plasma- or serum-based miRNAs as biomarkers for the diagnosis of CRC, to assess the accuracy of these miRNAs as biomarkers, and to identify those miRNAs for which there is the most evidence of potential use as a specific and sensitive blood-based assay for the diagnosis of CRC.

MATERIALS AND METHODS

This study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analysis guidelines (Mahid *et al*, 2006; Moher *et al*, 2009). Two reviewers (JC, NG) were independently involved with study selection, data extraction, and quality assessment.

Study selection. An electronic search of PubMed (NCBI, Bethesda, MD, USA) and EMBASE (Elsevier, Amsterdam, The Netherlands) databases was performed for relevant articles published between January 2002 and April 2016 using the following medical subject heading terms: ('plasma' OR 'serum') AND ('microRNA' OR 'miRNAs' OR 'miR*') AND ('colorectal cancer' OR 'colorectal carcinoma' OR 'colon cancer' OR 'colorectal adenoma'). This time period was based on a preliminary search that revealed no serum or plasma miRNA studies in patients with CRC undertaken prior to 2002. A grey literature search of meeting proceedings and abstracts was performed to detect any relevant unpublished work (Mahid *et al*, 2006). Meeting proceedings from the following organisations were searched for abstracts relevant to plasma miRNA and CRC for the period 2010 and 2016: American Society for Clinical Oncology, the American Society of Colon and Rectal Surgeons, the American Association for Cancer Research, and the Society for Surgical Oncology.

No language restrictions were imposed. Duplicates were removed. Based on the title and abstract, manuscripts of interest were obtained for full-text review. Only full-text manuscripts were included. Additional articles were identified by manually searching the references of original and review articles.

Inclusion and exclusion criteria. Inclusion and exclusion criteria were developed by the investigative team. Human studies investigating plasma- or serum-based miRNAs as a method for diagnosis of CRC using patients with CRC as compared with healthy controls were included. Studies evaluating treatment response were excluded, as were studies evaluating tissue miRNA or miRNA in other body fluids. The primary outcome measure was determination of significantly dysregulated serum or plasma miRNA in patients with CRC as compared with healthy controls.

Data extraction, meta-analysis, and quality assessment. Each manuscript was assessed independently by two investigators (JC and NG). Disagreements among reviewers were resolved by consensus. Data extracted included the following: authors, publication year, country, type of blood-based fluid (serum or plasma), characteristics of the study population (both case and control), study design (qRT-PCR detection method), whether miRNA screening was performed, number of miRNAs assessed, listing of the specific dysregulated miRNAs in patients with CRC as compared with controls, and outcome of statistical analyses including details of miRNA analysis, such as type of reference miRNA utilised. Studies reporting on single miRNA were included in the meta-analysis and were evaluated according to the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) checklist (Whiting *et al*, 2011). QUADAS-2 is designed to assess the risk of bias and applicability of studies of diagnostic accuracy. It consists of four domains: patient selection, index test, reference standard, and flow and timing. Each is assessed with respect to the risk of bias and the first three domains assessed with respect to applicability.

Statistical analysis. Analysis was based on the accuracy of the identified miRNAs to diagnose the presence of CRC as determined using Receiver Operator Characteristic (ROC) curves as Area Under the Curve (AUC) value and sensitivity and specificity, where available (Carter *et al*, 2016a). Studies that identified the sensitivity and specificity of individual miRNA in diagnosing CRC were included in the meta-analysis. Owing to the presumed heterogeneity of studies, a random-effects model (DerSimonian–Laird method) was used (Mahid *et al*, 2006). Forest plots were constructed using STATA version 11 (StataCorp LP, College, Station, TX, USA). The heterogeneity of included studies was assessed using I^2 and χ^2 statistics and was judged to be significant if I^2 was $>50\%$ or $P<0.05$. Sensitivity analyses were performed for (1) studies judged to have low risk and low concern following QUADAS-2 assessment (see above), (2) based upon the type of sample used (i.e., plasma or serum), (3) the method of qRT-PCR detection (TaqMan or SYBR Green), and (4) the type of reference control used for normalisation (internal or external).

RESULTS

Three hundred and ninety-nine records from the database search and 3 from the grey literature were initially identified (Figure 1); 282 records remained after duplicates were removed and a further 190 were considered not relevant to the review topic. Ninety-two records were screened, and after abstract review, 33 were subsequently excluded owing to a lack of reporting on miRNA, results using cancers other than CRC, non-human subjects, and/or

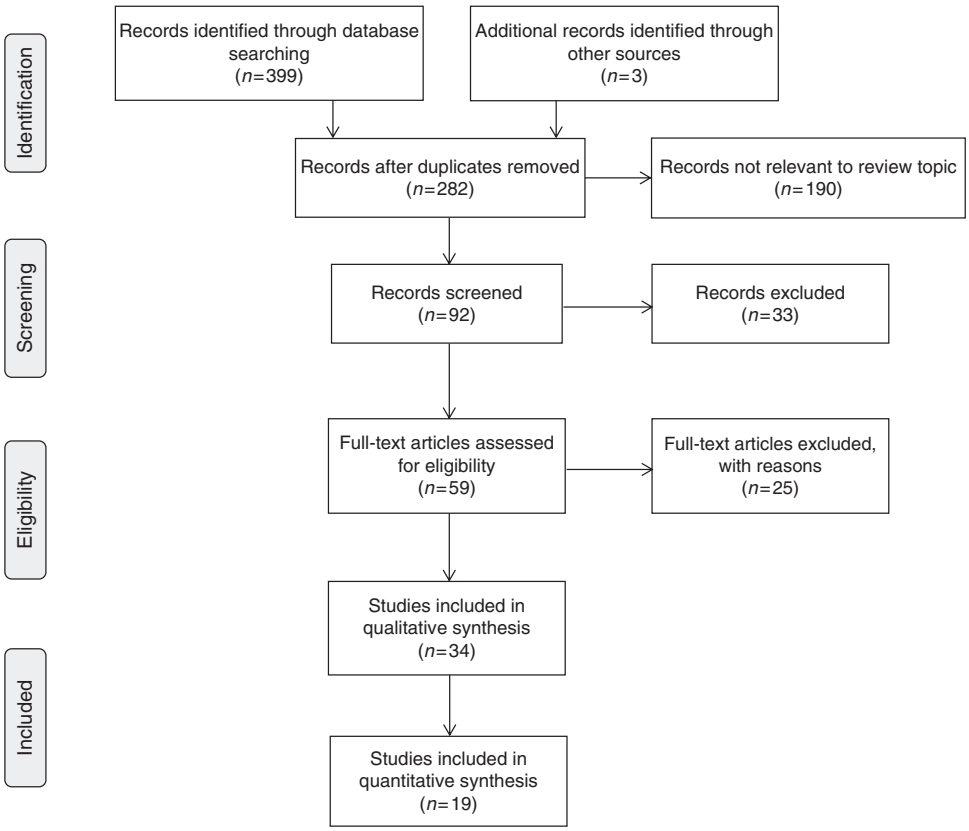

Figure 1. Flow diagram of the literature search process and study inclusion.

inability to obtain full abstracts or articles. Full-text articles were obtained for the remaining 59 articles that were deemed relevant. A further 25 articles were excluded for the following reasons: review article ($n=14$), abstract only ($n=2$), not plasma or serum ($n=1$), not diagnostic ($n=5$), and not CRC ($n=3$). Thirty-four studies investigating plasma or serum miRNAs in the diagnosis of CRC were included for the final systematic review (Ng *et al*, 2009; Huang *et al*, 2010; Pu *et al*, 2010; Ahmed *et al*, 2012; Kanaan *et al*, 2012; Wang and Zhang, 2012; Wang *et al*, 2012b; Brunet Vega *et al*, 2013; Giraldez *et al*, 2013; Hofsli *et al*, 2013; Kanaan *et al*, 2013; Liu *et al*, 2013; Luo *et al*, 2013; Toiyama *et al*, 2013; Yu *et al*, 2013; Zhang *et al*, 2013; Basati *et al*, 2014; Du *et al*, 2014; Perilli *et al*, 2014; Xu *et al*, 2014; Zanutto *et al*, 2014; Zheng *et al*, 2014; Basati *et al*, 2015; Chen *et al*, 2015; Fang *et al*, 2015; Ghanbari *et al*, 2015; Ho *et al*, 2015; Ramzy *et al*, 2015; Wang *et al*, 2015; Li *et al*, 2015; Li *et al*, 2016; Sun *et al*, 2016; Wang *et al*, 2016; Yu *et al*, 2016) and of these 19 were included in the meta-analysis (Ng *et al*, 2009; Huang *et al*, 2010; Pu *et al*, 2010; Kanaan *et al*, 2012; Wang and Zhang, 2012; Wang *et al*, 2012b; Liu *et al*, 2013; Toiyama *et al*, 2013; Zhang *et al*, 2013; Basati *et al*, 2014; Du *et al*, 2014; Xu *et al*, 2014; Basati *et al*, 2015; Chen *et al*, 2015; Fang *et al*, 2015; Li *et al*, 2016; Sun *et al*, 2016; Wang *et al*, 2016; Yu *et al*, 2016). Of these 34 studies, 20 (59%) used plasma (Ng *et al*, 2009; Huang *et al*, 2010; Pu *et al*, 2010; Ahmed *et al*, 2012; Kanaan *et al*, 2012; Wang *et al*, 2012b; Giraldez *et al*, 2013; Kanaan *et al*, 2013; Luo *et al*, 2013; Zhang *et al*, 2013; Du *et al*, 2014; Perilli *et al*, 2014; Xu *et al*, 2014; Zanutto *et al*, 2014; Chen *et al*, 2015; Fang *et al*, 2015; Ghanbari *et al*, 2015; Wang *et al*, 2015; Li *et al*, 2016; Sun *et al*, 2016) and 14 (41%) used serum samples (Wang and Zhang, 2012; Brunet Vega *et al*, 2013; Hofsli *et al*, 2013; Liu *et al*, 2013; Toiyama *et al*, 2013; Yu *et al*, 2013; Basati *et al*, 2014; Zheng *et al*, 2014; Basati *et al*, 2015; Ho *et al*, 2015; Ramzy *et al*, 2015; Li *et al*, 2015; Wang *et al*, 2016; Yu *et al*, 2016). Studies were conducted in North America, South East Asia, or Europe. Study characteristics are shown in Table 1.

A total of 6010 patients were included; 3454 CRC patients, and 2556 healthy controls. Seven studies also included evaluation of 420 patients with CAA, the precursor lesion to CRC. From the studies that reported patient demographics, 1792 men and 1410 women were included among CRC cases and 1198 men and 942 women were included among healthy controls (Table 1).

All patients included in this review had the diagnosis of CRC confirmed by histopathology. Patients were excluded from individual studies if they had a diagnosis of familial adenomatous polyposis, hereditary non-polyposis CRC or other inflammatory condition or if they received preoperative radiotherapy or chemotherapy. Of the 19 studies included in meta-analysis, 18 studies staged CRC according to either the American Joint Committee on Cancer or the Union for International Cancer Control Tumor-Node-Metastasis staging system. The remaining study staged CRC cases according to the Dukes' classification system.

Identification of dysregulated miRNAs in the plasma or serum. Sixteen of the 34 studies performed miRNA screening studies (Ng *et al*, 2009; Ahmed *et al*, 2012; Kanaan *et al*, 2012; Wang *et al*, 2012b; Brunet Vega *et al*, 2013; Giraldez *et al*, 2013; Hofsli *et al*, 2013; Kanaan *et al*, 2013; Luo *et al*, 2013; Zheng *et al*, 2014; Xu *et al*, 2014; Ghanbari *et al*, 2015; Ho *et al*, 2015; Wang *et al*, 2015; Li *et al*, 2015; Sun *et al*, 2016) in order to identify dysregulated miRNAs in the plasma or serum of CRC cases compared with healthy controls, whereas the remaining 18 studies identified miRNAs for assessment based on the literature or based on their own prior studies (Huang *et al*, 2010; Pu *et al*, 2010; Wang and Zhang, 2012; Liu *et al*, 2013; Yu *et al*, 2013; Toiyama *et al*, 2013; Zhang *et al*, 2013; Basati *et al*, 2014; Du *et al*, 2014; Perilli

Table 1. Characteristics of studies included in systematic review

			Study population					
First author	Year	Country	CRC (no. of patients)	Control (no. of patients)	Age (mean ± s.d./(range)), years		Gender male: female	Specimen
Ng	2009	China	130	75	CRC Control	71 (42–91) 69 (45–85)	68 : 62 39 : 36	Plasma
Huang	2010	China	100	59	CRC Control	61 ± 11 58 ± 12	51 : 49 31 : 28	Plasma
Pu	2010	China	103	37	CRC Control	58 (39–84) 32 (17–77)	66 : 37 19 : 18	Plasma
Kanaan	2012	USA	50	50	CRC Control	60 ± 11 61 ± 9	21 : 29 21 : 29	Plasma
Wang Q	2012	China	100	68	CRC Control	61 (21–84) 57 (36–85)	50 : 50 35 : 33	Plasma
Wang B	2012	China	32	39	CRC Control	63 (45–80) 46	17 : 15 30 : 9	Serum
Ahmed	2012	USA	10	5	CRC Control	— —	— —	Plasma
Kanaan	2013	USA	45	26	CRC Control	64 60 ± 11	28 : 17 11 : 15	Plasma
Zhang G	2012	China	78	86	CRC Control	61.4 ± 13.6 60.3 ± 11.8	43 : 35 53 : 33	Plasma
Luo	2013	Germany	130	244	CRC Control	67.55 61.93	70 : 60 105 : 139	Plasma
Giraldez	2013	Spain	63	73	CRC Control	— 61.35	— 37 : 36	Plasma
Brunet Vega	2013	Spain	30	26	CRC Control	68.1 ± 11 64.1 ± 7.4	18 : 12 13 : 13	Serum
Liu	2013	China	200	80	CRC Control	57.09 (20–89) 57.71 (28–89)	126 : : 74 42 : 38	Serum
Yu H	2013	China	30	30	CRC Control	— —	— —	Serum
Toiyama	2013	USA	186	53	CRC Control	67.5 ± 7.5 64 ± 12.9	106 : 80 27 : 26	Serum
Hofsli	2013	Norway	70	20	CRC Control	70.5 ⩾50	32 : 38 10 : 10	Serum
Perilli	2014	Italy	20	10	CRC Control	— —	— —	Plasma
Basati	2014	Iran	40	40	CRC Control	55.35 ± 10.13 55.00 ± 10.35	21 : 19 22 : 18	Serum
Zheng	2014	China	307	226	CRC Control	57.68 53.15	180 : 127 128 : 98	Serum
Du	2014	China	49	49	CRC Control	— —	— —	Plasma
Xu	2014	China	94	46	CRC Control	64.9 65.8	54 : 40 22 : 24	Plasma
Zanutto	2014	Italy	65	70	CRC Control	— —	— —	Plasma
Chen	2015	China	100	79	CRC Control	58.91 60.20	60 : 40 44 : 35	Plasma
Ramzy	2015	Egypt	25	10	CRC Control	— —	— —	Serum
Ho	2015	USA	11	10	CRC Control	68.0 54.5	8 : 3 4 : 6	Serum
Li J	2015	China	175	130	CRC Control	58.7 54.1	113 : 62 70 : 50	Serum
Li L	2015	China	200	400	CRC Control	66.3 ± 11.8 65.5 ± 10.8	135 : 65 283 : : 117	Plasma
Fang	2015	China	111	130	CRC Control	60 —	59 : 52 —	Plasma
Basati	2015	Iran	55	55	CRC Control	58.52 ± 10 57.87 ± 10.15	30 : 25 31 : 24	Serum

Table 1. (Continued)

			Study population					
First author	Year	Country	CRC (no. of patients)	Control (no. of patients)	Age (mean ± s.d./(range)), years		Gender male: female	Specimen
Ghanbari	2015	Iran	61	24	CRC Control	64.13 ± 8.67 61.96 ± 8.67	34 : 27 14 : 10	Plasma
Wang S	2015	China	124	117	CRC Control	64 43	54 : 70 65 : 52	Plasma
Wang W	2016	China	268	102	CRC Control	58 (49–66) 56 (48–66)	151 : 117 —	Serum
Sun	2016	USA	227	57	CRC Control	55 ± 7.8 54 ± 6.3	119 : 108 27 : 30	Plasma
Yu J	2016	China	165	30	CRC Control	60.7 ± 11.6 59.4 ± 14.5	78 : 87 15 : 15	Serum

Abbreviation: CRC, colorectal cancer.

et al, 2014; Zanutto *et al*, 2014; Basati *et al*, 2015; Chen *et al*, 2015; Fang *et al*, 2015; Ramzy *et al*, 2015; Li *et al*, 2016; Wang *et al*, 2016; Yu *et al*, 2016). A total of 617 miRNAs were differentially expressed between CRC and healthy controls in these screening studies, and of these, 69 miRNAs were found to be significantly dysregulated in validation or subsequent studies. A further 36 miRNAs were shown to have either increased or decreased expression in CRC, but no statistical analyses were performed (Ahmed *et al*, 2012; Hofsli *et al*, 2013) (Supplementary Table S1).

Significantly dysregulated miRNAs in the plasma or serum of CRC cases. Thirty-one miRNAs were found to be either upregulated ($n = 17$, miR-17-3p, miR-18a, miR-19a-3p, miR-20a, miR-21, miR-29a, miR-92, miR-96, miR-106a, miR-182, miR-200c, miR-206, miR-210, miR-221, miR-223-3p, miR-372, miR-378) (Ng *et al*, 2009; Huang *et al*, 2010; Pu *et al*, 2010; Kanaan *et al*, 2012; Wang and Zhang, 2012; Brunet Vega *et al*, 2013; Liu *et al*, 2013; Toiyama *et al*, 2013; Zhang *et al*, 2013; Basati *et al*, 2014; Du *et al*, 2014; Perilli *et al*, 2014; Xu *et al*, 2014; Zanutto *et al*, 2014; Zheng *et al*, 2014; Chen *et al*, 2015; Yamada *et al*, 2015; Li *et al*, 2015; Sun *et al*, 2016; Wang *et al*, 2016; Yu *et al*, 2016) or downregulated ($n = 14$, miR-24, miR-26a-5p, miR-29b, miR-30b, miR-142-3p, miR-145, miR-194, miR-218, miR-320a, miR-375, miR-422a, miR-423-5p, miR-601, miR-760) (Wang *et al*, 2012b; Yu *et al*, 2013; Xu *et al*, 2014; Zheng *et al*, 2014; Basati *et al*, 2015; Fang *et al*, 2015; Ghanbari *et al*, 2015; Ho *et al*, 2015; Ramzy *et al*, 2015; Li *et al*, 2015; Li *et al*, 2016) in CRC cases as compared with controls (Table 2). Six upregulated miRNAs (miR-17-3p, miR-18a, miR-21, miR-29a, miR-92, miR-106a) and two downregulated miRNAs (miR-29b, miR-145) were identified by more than one study (Ng *et al*, 2009; Huang *et al*, 2010; Kanaan *et al*, 2012; Wang and Zhang, 2012; Brunet Vega *et al*, 2013; Liu *et al*, 2013; Toiyama *et al*, 2013; Zhang *et al*, 2013; Basati *et al*, 2014; Du *et al*, 2014; Zanutto *et al*, 2014; Zheng *et al*, 2014; Basati *et al*, 2015; Chen *et al*, 2015; Ramzy *et al*, 2015; Yamada *et al*, 2015; Li *et al*, 2015; Li *et al*, 2016). miR-21 was the most frequently identified dysregulated miRNA (Kanaan *et al*, 2012; Wang and Zhang, 2012; Liu *et al*, 2013; Toiyama *et al*, 2013; Basati *et al*, 2014; Du *et al*, 2014; Zanutto *et al*, 2014; Yamada *et al*, 2015).

Seven studies identified a two miRNA panel (Huang *et al*, 2010; Wang *et al*, 2012b; Giraldez *et al*, 2013; Kanaan *et al*, 2013; Liu *et al*, 2013; Zhang *et al*, 2013; Xu *et al*, 2014), five studies identified a three miRNA panel (Giraldez *et al*, 2013; Fang *et al*, 2015; Ho *et al*, 2015; Wang *et al*, 2015; Li *et al*, 2015), and five studies identified a panel consisting of four or more miRNAs (Ahmed *et al*, 2012; Wang *et al*, 2012b; Giraldez *et al*, 2013; Hofsli *et al*, 2013; Luo *et al*, 2013) that was utilised in the diagnosis of CRC (Table 2).

Area under the curve, sensitivity, and specificity. The most common method of reporting diagnostic accuracy of dysregulated miRNAs was using AUC and sensitivity and specificity as determined from ROC curves. Of the identified miRNAs, AUC values ranged from 0.590 to 0.943, sensitivity ranged from 46% to 92.79% and specificity from 41% to 93.2%. The highest AUC, sensitivity, and specificity combination was reported for a panel of four miRNAs, miR-29a, miR-92a, miR-601, and miR-760 (AUC 0.943, sensitivity 83.3%, specificity 93.1%) (Wang *et al*, 2012b) (Table 2).

Quality assessment. Quality assessment results of the studies reporting upon single miRNA included in meta-analysis are shown in Figures 2A and B using the QUADAS-2 evaluation tool.

Meta-analysis. Studies that identified a single dysregulated miRNA in CRC cases compared with controls, along with the corresponding AUC and sensitivity and specificity for the identified miRNA, were included in the meta-analysis. Using these criteria, 28 individual miRNAs were identified in 19 studies (Supplementary Table S2). The sensitivity (±95% confidence intervals (CIs)) and specificity (±95% CIs) for each miRNA are shown in the corresponding forest plots (Figures 3A and B). Overall sensitivity and specificity of the 28 individual miRNAs in the diagnosis of CRC were 76% (95% CI 72–80%) and 76% (95% CI 72–80%), respectively. These results indicate good discriminative ability of the use of miRNAs as biomarkers for the detection of CRC. Sensitivity analyses were performed for studies of good quality (Figures 4A and B), type of patient sample used (plasma *vs* serum, Supplementary Figures S1a and b and S2a and b), type of miRNA detection method (SYBR green *vs* TaqMan, Supplementary Figures S3a and b and S4a and b), and type of miRNA reference used for normalisation (internal *vs* external, Supplementary Figures S5a and b and S6a and b). Compared with the sensitivity and specificity obtained when including all studies, sensitivity analysis using only high-quality studies did not significantly alter sensitivity and specificity (77% *vs* 76% and 77% *vs* 76%, respectively). This was also true for the remaining sensitivity analyses, where there was no improvement in sensitivity or specificity.

DISCUSSION

This systematic review of 34 manuscripts that utilise plasma- or serum-based miRNAs in the diagnosis of CRC has identified 617 miRNAs reported to be dysregulated in 3454 cases of CRC as compared with 2556 healthy controls. A total of 31 single miRNAs

Table 2. Significantly dysregulated miRNAs in the plasma and serum of patients with CRC as compared with controls

miRNA	Expression	Area under the curve (AUC)	Sensitivity	Specificity	Study
Single miRNA					
miR-17-3p	Upregulated	0.717 0.781	64% —[a]	70% —	Ng *et al*, 2009 Li *et al*, 2015
miR-18a	Upregulated	0.804 —	73.1% —	79.1% —	Zhang *et al*, 2013 Brunet Vega *et al*, 2013
miR-19a-3p	Upregulated	0.849	—	—	Zheng *et al*, 2014
miR-20a	Upregulated	0.590	46%	73.42%	Chen *et al*, 2015
miR-21	Upregulated	0.910 0.850 0.802 0.927 0.870 0.877 0.647	90% 87.5% 65% 82.8% 77% 76.2% —	90% 74.4% 85% 90.6% 78% 93.2% —	Kanaan *et al*, 2012 Wang and Zhang, 2012 Liu *et al*, 2013 Toiyama *et al*, 2013 Basati *et al*, 2014 Du *et al*, 2014 Zanutto *et al*, 2014
miR-29a	Upregulated	0.844 —	69% —	89.1% —	Huang *et al*, 2010 Brunet Vega *et al*, 2013
miR-92 miR-92a miR-92a-3p	Upregulated	0.885 0.838 0.786 0.890	89% 84% 65.5% —	70% 71.2% 82.5% —	Ng *et al*, 2009 Huang *et al*, 2010 Liu *et al*, 2013 Zheng *et al*, 2014
miR-96	Upregulated	0.740	65.4%	73.3%	Sun *et al*, 2016
miR-106a	Upregulated	0.605 0.808	74% —	44.4% —	Chen *et al*, 2015 Li *et al*, 2015
miR-182	Upregulated	—	—	—	Perilli *et al*, 2014
miR-200c	Upregulated	0.749	64.1%	73.3%	Zhang *et al*, 2013
miR-206	Upregulated	0.705	—	—	Xu *et al*, 2014
miR-210	Upregulated	0.821	74.6%	73.5%	Wang *et al*, 2016
miR-221	Upregulated	0.606	86%	41%	Pu *et al*, 2010
miR-223-3p	Upregulated	0.871	—	—	Zheng *et al*, 2014
miR-372	Upregulated	0.854	81.9%	73.3%	Yu *et al*, 2016
miR-378	Upregulated	0.796	—	—	Zanutto *et al*, 2014
miR-24	Downregulated	0.839	78.38%	83.85%	Fang *et al*, 2015
miR-26a-5p	Downregulated	0.670	—	—	Ghanbari *et al*, 2015
miR-29b	Downregulated	0.743 0.870	61.4% 77%	72.5% 75%	Li *et al*, 2015 Basati *et al*, 2015
miR-30b	Downregulated	—	—	—	Ho *et al*, 2015
miR-142-3p	Downregulated	0.710	—	—	Ghanbari *et al*, 2015
miR-145	Downregulated	— 0.874	— —	— —	Ramzy *et al*, 2015 Li *et al*, 2015
miR-194	Downregulated	0.850	72%	80%	Basati *et al*, 2015
miR-218	Downregulated	—	—	—	Yu *et al*, 2013
miR-320a	Downregulated	0.886	92.79%	73.08%	Fang *et al*, 2015
miR-375	Downregulated	0.749	76.92%	64.63%	Xu *et al*, 2014
miR-422a	Downregulated	0.843	—	—	Zheng *et al*, 2014
miR-423-5p	Downregulated	0.833	91.89%	70.77%	Fang *et al*, 2015
miR-601	Downregulated	0.747	69.2%	72.4%	Wang *et al*, 2012b
miR-760	Downregulated	0.788	80%	72.4%	Wang *et al*, 2012b
Two miRNA panels					
miR-19a, -19b	Upregulated	0.820	78.57%	77.36%	Giraldez *et al*, 2013
miR-21, -92a	Upregulated	0.847	68%	91.2%	Liu *et al*, 2013
miR-29a, -92a	Upregulated	0.883	83%	84.7%	Huang *et al*, 2010
miR-200c, -18a	Upregulated	0.839	84.6%	75.6%	Zhang *et al*, 2013
miR-431, -139-3p	Upregulated	0.829	91%	57%	Kanaan *et al*, 2013
miR-601, -760	Downregulated	0.792	83.3%	69.1%	Wang *et al*, 2012b
miR-375, -206	Upregulated and Downregulated[b]	0.846	—	—	Xu *et al*, 2014

Table 2. (Continued)

miRNA	Expression	Area under the curve (AUC)	Sensitivity	Specificity	Study
Three miRNA panels					
miR-19a, -19b, -15b	Upregulated	0.840	78.57%	79.25%	Giraldez *et al*, 2013
miR-486, -25, -1180	Upregulated	—	—	—	Ho *et al*, 2015
miR-24, -320a, -423-5p	Downregulated	0.899	92.79%	70.77%	Fang *et al*, 2015
miR-145, -106a, -17-3p	Upregulated and Downregulated	0.886	78.5%	82.8%	Li *et al*, 2015
miR-409-3p, -7, -93	Upregulated and Downregulated	0.897	82%	89%	Wang *et al*, 2015
Panels with ⩾4 miRNA					
miR-18a, -19a, -19b, -15b, 29a, -335	Upregulated	0.70–0.80	—	—	Giraldez *et al*, 2013
miR-18a, -20a, -21, -29a, -92a, -106b, -133a, -143, -145	Upregulated	0.745	—	—	Luo *et al*, 2013
miR-423-5p, -210, -720, -320a, -378, -92a, -29a, 155	Upregulated	—	—	—	Hofsli *et al*, 2013
miR-7, -17-3p, -20a, -21, -92a, -96, -183, -196a, -214	Upregulated	—	—	—	Ahmed *et al*, 2012
miR-29a, -92a, -601, -760	—	0.943	83.3%	93.1%	Wang *et al*, 2012b
miR-124, -127-5p, -138, -143, -146a, -222	Downregulated	—	—	—	Ahmed *et al*, 2012
miR-106a, -143, -103, -199-3p, -151-5p, -107, -191, -423-3p, -221, -382, -409-3p, -652, let 7d	Downregulated	—	—	—	Hofsli *et al*, 2013

Abbreviations: CRC = colorectal cancer; miRNA = microRNA.
[a]Not reported.
[b]Upregulated and downregulated—where the individual miRNAs were either upregulated or downregulated in CRC compared with healthy controls.

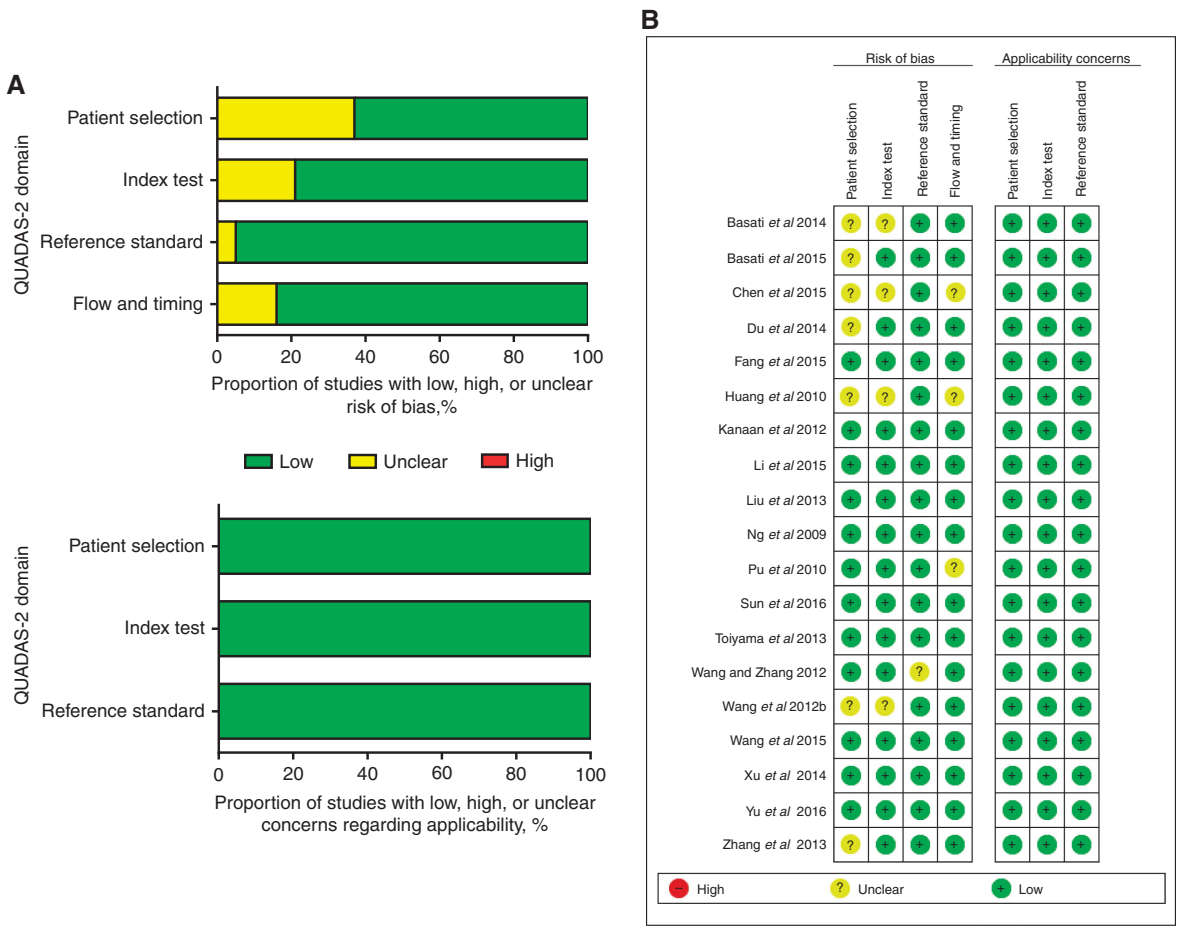

Figure 2. QUADAS-2 assessment. Investigators' assessment regarding each domain for included studies. Risk of bias and applicability concerns' (**A**) graph and (**B**) summary.

were identified as significantly dysregulated between CRC cases and controls. Eight of the dysregulated miRNAs were identified and validated by more than one study; six were upregulated: miR-17-3p, miR-18a, miR-21, miR-29a, miR-92, and miR-106a and two were downregulated: miR-29b and miR-145. A further 23 significantly dysregulated miRNAs were identified by only one

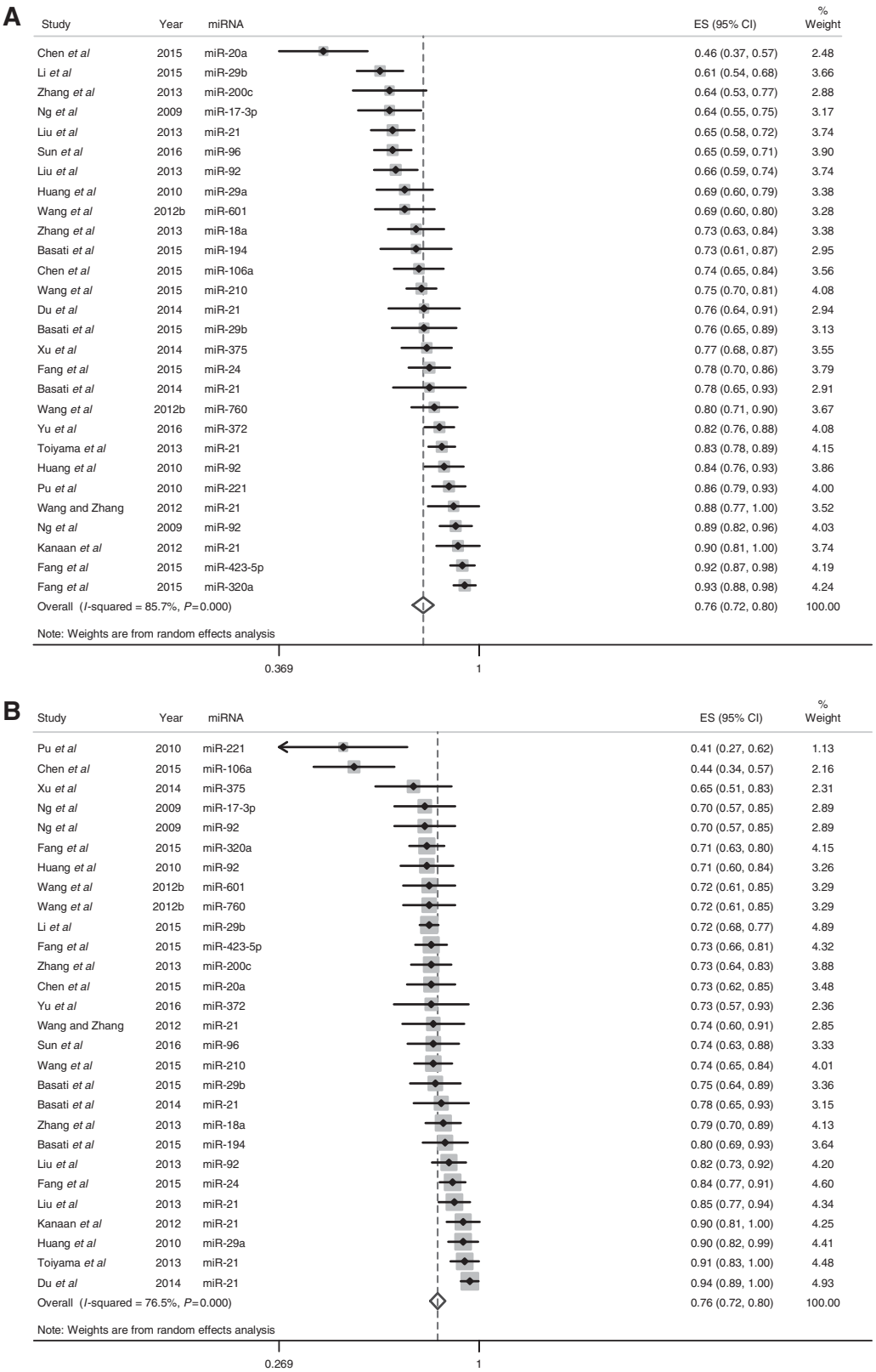

Figure 3. Forest plots for studies on individual miRNA assays used in the diagnosis of CRC among the 19 studies included in the meta-analysis. (**A**) Estimates of sensitivity. (**B**) Estimates of specificity. A full color version of this figure is available at the *British Journal of Cancer* journal online.

study. Conceptually, the increased expression of specific miRNAs in the presence of disease is accepted, assuming that the cancer is the source of the dysregulated miRNA export into the blood. However, the source of the miRNA dysregulation is not necessarily known, and miRNAs that are consistently downregulated only in the presence of disease should not necessarily be discounted as reliable biomarkers, provided there is an acceptable negative predictive value for the elevated levels of that miRNA in the absence of disease. This would, for example, be true of a miRNA whose target was a known oncogene.

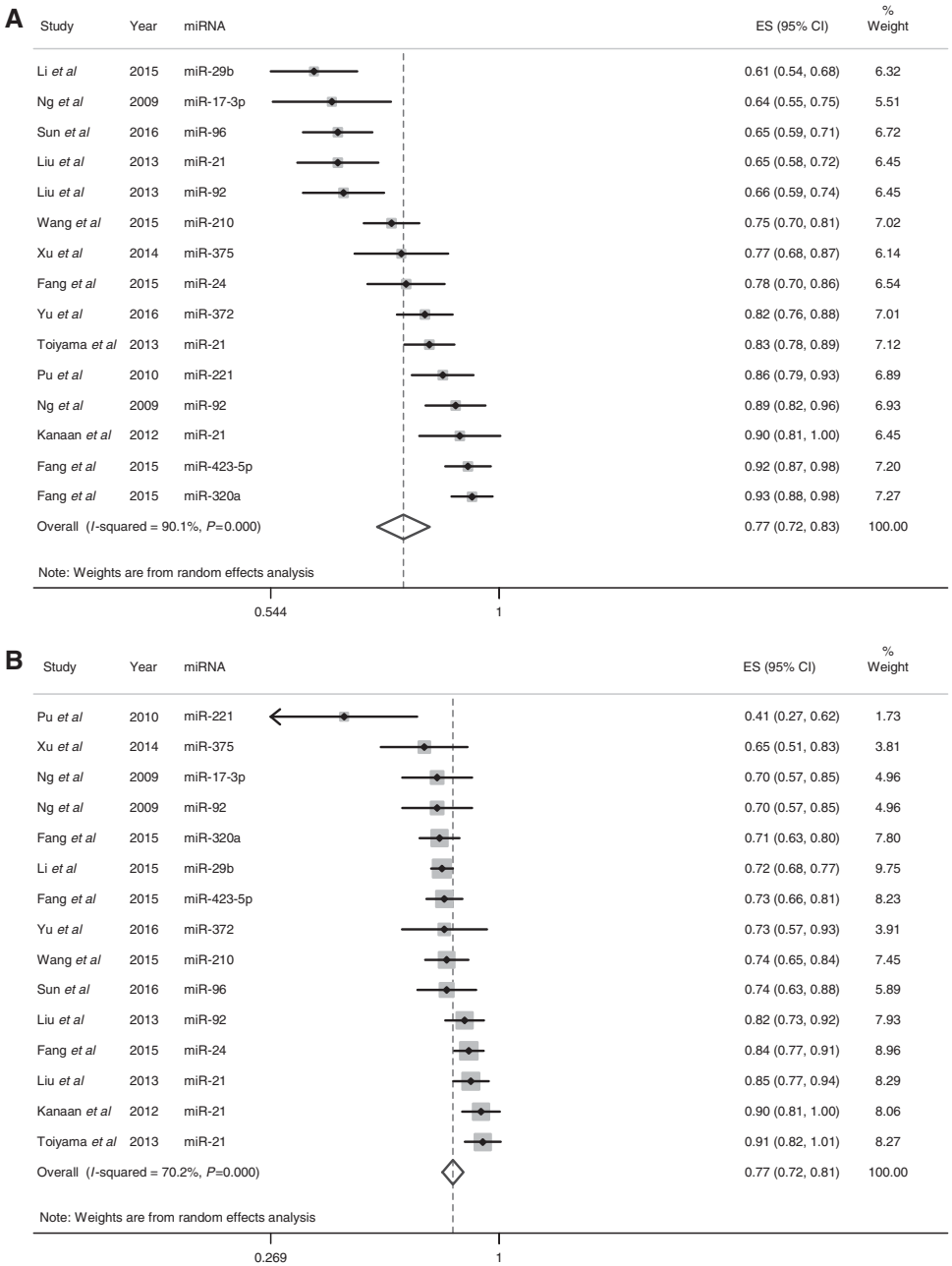

Figure 4. Analysis performed for low-risk/low-concern studies with respect to QUADAS-2 quality assessment. (**A**) Quality sensitivity analysis. Overall sensitivity was 77% (95% CI 72–83%). (**B**) Quality specificity analysis. Overall specificity was 77% (95% CI 72–81%). A full color version of this figure is available at the *British Journal of Cancer* journal online.

Significant advances have occurred in the field of tumour-associated miRNAs since their first discovery in plasma (Mitchell *et al*, 2008; Chevillet *et al*, 2014) as the number of studies investigating their expression has markedly increased in recent years. Although colonoscopy remains the gold standard screening test for the diagnosis of CRC (Muller and Sonnenberg, 1995; Citarda *et al*, 2001; Zauber *et al*, 2012; Nishihara *et al*, 2013), the evaluation of miRNA within plasma and/or serum of circulating blood has provided a new area for biomarker research.

microRNAs are small, naturally occurring, non-protein-coding RNA that are transcribed in the nucleus as large RNA precursors called primary miRNAs (pri-miRNAs) (Lee *et al*, 2004). These pri-miRNAs are enzymatically cleaved in the nucleus by the enzyme Drosha into precursor miRNA (pre-miRNA) (Han *et al*, 2004). The resulting approximately 70-nucleotide pre-miRNA are folded into stem-loop structures. These pre-miRNAs are then exported into the cytoplasm by the GTP-dependent transport protein exportin 5 (Yi *et al*, 2003). Once in the cytoplasm, they undergo additional processing by the enzyme Dicer to generate the mature double-stranded miRNA approximately 22 nucleotides in length. Dicer also initiates the formation of the RNA-induced silencing complex (RISC) (Hammond, 2005). It is in this final part of miRNA biogenesis that the leading miRNA strand is incorporated into the RISC complex, which is then guided into the complementary 3′ or 5′ untranslated region of the target mRNA. The RISC is responsible for the gene silencing observed owing to miRNA expression and RNA interference (Filipowicz *et al*, 2005).

miRNAs are stable in extracellular fluid as they are protected from RNases by virtue of being bound to argonaute proteins (Meister, 2013). Discovery of dysregulated miRNA expression has been identified in oesophageal, lung, liver, pancreatic, bladder,

ovarian, and gastric cancers (Chiang *et al*, 2012). miRNAs have been identified in numerous body fluids, such as plasma, saliva, faeces, and urine, where they are actively released from cells within microvesicles, exosomes, or bound to proteins (Kanaan *et al*, 2012; Chevillet *et al*, 2014). These factors suggest that miRNAs can be used as biomarkers for cancer and other disease states (Turchinovich *et al*, 2011).

miRNAs can function as either tumour suppressors or oncogenes. Overexpression of miRNAs in cancer may function as oncogenes and promote oncogenesis by downregulating tumour suppressors or other genes involved in cell differentiation. In contrast, underexpressed miRNAs in cancer may function as tumour suppressors by inhibiting oncogenesis via downregulation of proteins with oncogenic qualities (Zhang *et al*, 2007; Shenouda and Alahari, 2009). The miR-17-92 cluster, also known as oncomiR-1 was the first miRNA oncogene to be described (Li *et al*, 2014). Six miRNAs form this miR-17-92 cluster: miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1 and miR-92a-1, and are known to be dysregulated in many cancers (Mogilyansky and Rigoutsos, 2013). miR-17-92 has also been found to be over-expressed in both the tissue and serum of CRC patients (Dews *et al*, 2006; Ng *et al*, 2009). In our systematic review, five of the six members of this cluster, with the exception of miR-19b-1, were found to be significantly upregulated in the serum or plasma of CRC patients as compared with controls in eight studies (Ng *et al*, 2009; Huang *et al*, 2010; Brunet Vega *et al*, 2013; Liu *et al*, 2013; Zhang *et al*, 2013; Zheng *et al*, 2014; Chen *et al*, 2015; Li *et al*, 2015). The most predictive miRNA was miR-92a, with AUC values ranging from 0.786 to 0.890, from 65.5% to 89% sensitivity, and from 70% to 82.5% specificity. The remaining member of the cluster, miR-19b-1, was also significantly upregulated in CRC patients when combined as a two miRNA panel together with miR-19a, with an AUC of 0.82, 78.57% sensitivity, and 77.36% specificity (Giraldez *et al*, 2013). These data support the oncogenic role of the miR-17-92 cluster in CRC.

We identified seven different studies that demonstrated significant upregulation of miR-21 in CRC patients as compared with healthy controls. Kanaan *et al* (2012) identified miR-21 as a biomarker for CRC. Validated CRC tissue miRNAs were evaluated in a plasma test set consisting of 30 CRC patients and 30 healthy controls. The most dysregulated tissue miRNAs were then validated in different cohort of 20 CRC patients with 20 age- and race-matched subjects without CRC. In the plasma test group, miR-21 differentiated CRC patients from controls with AUC 0.910 and 90% specificity and sensitivity (Kanaan *et al*, 2012). Following this, 6 groups reproduced similar results with AUCs ranging from 0.647–0.927, 65–87.5% sensitivity, and 74.4–93.2% specificity. Although these data were promising, dysregulation of miR-21 has also been described in many other cancers (Chan *et al*, 2005; Iorio *et al*, 2005; Volinia *et al*, 2006; Iorio *et al*, 2007; Lui *et al*, 2007; Meng *et al*, 2007; Tetzlaff *et al*, 2007; Hu *et al*, 2011). There is therefore need for a plasma miRNA profile specific for CRC.

miR-29a is another miRNA that has been reported to be upregulated in CRC patients in three studies (Huang *et al*, 2010; Brunet Vega *et al*, 2013; Yamada *et al*, 2015). miR-29a is part of the miR-29 family (miR-29a, miR-29b, miR-29c). Interestingly, another member of the miR-29 family, miR-29b, was observed to be downregulated in CRC compared with control subjects in two studies (Basati *et al*, 2015; Li *et al*, 2016). Members of the miR-29 family exhibit differential regulation, implying that their functional relevance may not be identical (Kriegel *et al*, 2012). miR-29b has been shown to function as a tumour suppressor in various cancers and diseases (Yan *et al*, 2015), while miR-29a has been shown to act as a tumour suppressor in lung cancer (Fabbri *et al*, 2007) but as an oncogene in CRC, ovarian, and breast cancer (Gebeshuber *et al*, 2009; Resnick *et al*, 2009).

In order to reduce CRC-associated mortality, the ideal biomarker would be able to identify individuals with both early-stage CRC and its precursor lesion, the colorectal advanced adenoma (CAA), from healthy control subjects. CAA have previously been defined as adenomas with a villous component or with high-grade dysplasia >0.75 cm size in diameter (Carter *et al*, 2016b). In the studies included in this review, seven studies evaluated miRNAs found to be significantly dysregulated in CRC patients and in 420 patients with a diagnosis of CAA. Five of these seven studies reported miRNA panels significantly upregulated in CAA as compared with healthy controls (Huang *et al*, 2010; Kanaan *et al*, 2013; Liu *et al*, 2013; Toiyama *et al*, 2013; Zheng *et al*, 2014). One study also identified a panel of 5 miRNAs that distinguished between CRC and CAA with AUC 0.856 (Kanaan *et al*, 2013). The remaining two studies found no significant differences in miRNA profile between CAA and healthy controls (Giraldez *et al*, 2013; Luo *et al*, 2013) (Supplementary Table S3).

Commercially available non-invasive screening tests have recently been developed in an attempt to have the same detection accuracy of CRC compared with the current gold standard screening method of colonoscopy (2016; Imperiale *et al*, 2014; Marshall *et al*, 2010; Nichita *et al*, 2014). These tests, both blood based and faecal based, measure validated markers of CRC and include DNA markers and gene expression profiles. To date, none have a good ability to detect the precursor to CRC, CAA. We have developed a plasma-based miRNA screening assay comprising of combinations of 7 miRNAs, which is able to detect any neoplasia from controls with AUC 0.91, colorectal neoplasia from other cancers with AUC 0.79, and CRC from CAA with AUC 0.98. In addition, our assay is able to differentiate CRC from controls with AUC 0.98, sensitivity 90%, and specificity 100%, CAA from controls with AUC 0.823, sensitivity 65%, and specificity 95%, and CRC and CAA from controls with AUC 0.835, sensitivity 80%, and specificity 85% (Carter *et al*, 2016b). This miRNA panel, although in its infancy, provides much promise in the development of a non-invasive screening test for detection of CRC.

Inevitably, studies included in the systematic review and meta-analysis will vary with regards to clinical characteristics, study design, and type of study, and these factors contribute sources of heterogeneity. Clinical characteristics of control subjects were often poorly defined. Of the individual studies included in the meta-analysis, five defined healthy controls by colonoscopic confirmed absence of CRC, including absence of adenomas. The remaining 14 studies defined healthy controls as having the absence of malignancy or inflammatory condition or a benign physical examination without other qualification or elaboration. Similarly, 11 studies reported no significant differences in age or sex between CRC cases and healthy controls, 5 reported to age-sex matching between cases and controls, and 3 did not comment on age-sex matching status.

There was significant heterogeneity of the studies included in this review (I^2 85.7% for sensitivity, and 76.5% for specificity, $P<0.001$). Heterogeneity is expected in meta-analyses, one reason being differing sample sizes between studies (Higgins, 2008). Many of the studies included a relatively small number of participants. For the studies included in meta-analysis, the study populations ranged from 32 to 268 for CRC cases and from 30 to 400 for control cases. This may reflect the strict inclusion criteria adopted by investigators but potentially carries the risk of type I error. In future, studies with larger numbers of patients, along with different populations of patients in terms of genetics, geography and race, will increase the validity of our conclusions regarding the reliability of plasma miRNA assays. In addition, reference miRNA controls used varied between studies, with both internal and external miRNAs used for normalisation of results. This may account for the differing miRNAs identified. Similarly, miRNAs assessed were extracted from either plasma or serum samples. Some have reported differing miRNA concentrations depending on whether serum or

plasma are used as the source material (Wang *et al*, 2012a). Unlike previous studies, miRNAs measured in the serum of patient samples yielded better results than for plasma in this review. Although there is little difference in composition between plasma and serum, for the development of a blood-based biomarker, a standard blood component is preferred. A limitation to this review is the validity of the results given the significant heterogeneity of the studies. We believe it is, however, still reasonable to interpret these results as the overwhelming majority of the studies trend towards high sensitivity and specificity of miRNAs as biomarkers for the diagnosis of CRC.

Despite the large numbers of studies investigating plasma or serum miRNAs in patients with CRC or CAA, there still remains variation as to which significantly dysregulated miRNAs are identified. Some miRNAs were found to be significantly dysregulated in multiple studies, yet not all of these studies performed in miRNA screening prior to testing and validation, for example, miR-21, was significantly dysregulated in seven studies; however, only one of these performed initial miRNA screening. In addition, of the 28 miRNAs included in the meta-analysis, only 3 were reported as significantly dysregulated by more than 1 study: miR-21, miR-29b and miR-92.

The majority of studies were performed in either North America or South East Asia. It is possible that genetics, geography, and race may affect miRNA expression differently in these groups of patients, resulting in the observed differences in significantly dysregulated miRNAs. With the recent availability of blood-based screening tests for CRC, it is important to focus on quality and study reporting standards.

CONCLUSION

This systematic review identifies numerous miRNAs associated with colorectal carcinogenesis. miRNAs can distinguish patients with CRC from healthy controls with high sensitivity and specificity, however; no single stand-alone miRNA has yet been identified as an ideal biomarker for the diagnosis of CRC. In order to develop a diagnostic test for the detection of CRC, we propose that a panel of miRNAs with high sensitivity and specificity should be chosen and tested in a large population of subjects. This would include a combination of the most commonly identified miRNA, those miRNA most predictive of CRC, and the most unique miRNA to CRC, in order to develop a non-invasive test with sensitivity and specificity comparable to current screening methods for CRC. In addition, standardisation of study methodology such as the use of serum samples, TaqMan qRT-PCR detection, and an external miRNA reference control should be implemented in future studies.

With deaths from CRC among the leading cause of cancer deaths worldwide, there is an emphasis to increase screening, and therefore identify early, less invasive stage of disease, to improve survival. For this purpose, miRNAs have the potential in the future to be used as a relatively non-invasive, inexpensive, blood-based marker for detection of CRC.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

(2016) Test: About ColoVantage [Internet]. Questdiagnostics.com[Online]. Available at http://www.questdiagnostics.com/home/physicians/testing-services/by-test-name/colovantage/about (accessed 16 March 2016).

Ahmed FE, Amed NC, Vos PW, Bonnerup C, Atkins JN, Casey M, Nuovo GJ, Naziri W, Wiley JE, Allison RR (2012) Diagnostic microRNA markers to screen for sporadic human colon cancer in blood. *Cancer Genomics Proteomics* **9**: 179–192.

Basati G, Emami Razavi A, Abdi S, Mirzaei A (2014) Elevated level of microRNA-21 in the serum of patients with colorectal cancer. *Med Oncol* **31**: 205.

Basati G, Razavi AE, Pakzad I, Malayeri FA (2015) Circulating levels of the miRNAs, miR-194, and miR-29b, as clinically useful biomarkers for colorectal cancer. *Tumour Biol* **37**: 1781–1788.

Brunet Vega A, Pericay C, Moya I, Ferrer A, Dotor E, Pisa A, Casalots A, Serra-Aracil X, Oliva JC, Ruiz A, Saigi E (2013) microRNA expression profile in stage III colorectal cancer: circulating miR-18a and miR-29a as promising biomarkers. *Oncol Rep* **30**: 320–326.

Calderwood AH, Jacobson BC (2013) Colonoscopy quality: metrics and implementation. *Gastroenterol Clin North Am* **42**: 599–618.

Carter JV, Pan J, Rai SN, Galandiuk S (2016a) ROC-ing along: evaluation and interpretation of receiver operating characteristic curves. *Surgery* **159**: 1638–1645.

Carter JV, Roberts HL, Pan J, Rice JD, Burton JF, Galbraith NJ, Eichenberger MR, Jorden J, Deveaux P, Farmer R, Williford A, Kanaan Z, Rai SN, Galandiuk S (2016b) A highly predictive model for diagnosis of colorectal neoplasms using plasma microRNA: improving specificity and sensitivity. *Ann Surg* **264**: 575–584.

Chan JA, Krichevsky AM, Kosik KS (2005) MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. *Cancer Res* **65**: 6029–6033.

Chen WY, Zhao XJ, Yu ZF, Hu FL, Liu YP, Cui BB, Dong XS, Zhao YS (2015) The potential of plasma miRNAs for diagnosis and risk estimation of colorectal cancer. *Int J Clin Exp Pathol* **8**: 7092–7101.

Chevillet JR, Lee I, Briggs HA, He Y, Wang K (2014) Issues and prospects of microRNA-based biomarkers in blood and other body fluids. *Molecules* **19**: 6080–6105.

Chiang Y, Song Y, Wang Z, Liu Z, Gao P, Liang J, Zhu J, Xing C, Xu H (2012) microRNA-192, -194 and -215 are frequently downregulated in colorectal cancer. *Exp Ther Med* **3**: 560–566.

Citarda F, Tomaselli G, Capocaccia R, Barcherini S, Crespi M. Italian Multicentre Study Group (2001) Efficacy in standard clinical practice of colonoscopic polypectomy in reducing colorectal cancer incidence. *Gut* **48**: 812–815.

Croce CM (2008) Oncogenes and cancer. *N Engl J Med* **358**: 502–511.

Dews M, Homayouni A, Yu D, Murphy D, Sevignani C, Wentzel E, Furth EE, Lee WM, Enders GH, Mendell JT, Thomas-Tikhonenko A (2006) Augmentation of tumor angiogenesis by a Myc-activated microRNA cluster. *Nat Genet* **38**: 1060–1065.

Du M, Liu S, Gu D, Wang Q, Zhu L, Kang M, Shi D, Chu H, Tong N, Chen J, Adams TS, Zhang Z, Wang M (2014) Clinical potential role of circulating microRNAs in early diagnosis of colorectal cancer patients. *Carcinogenesis* **35**: 2723–2730.

Fabbri M, Garzon R, Cimmino A, Liu Z, Zanesi N, Callegari E, Liu S, Alder H, Costinean S, Fernandez-Cymering C, Volinia S, Guler G, Morrison CD, Chan KK, Marcucci G, Calin GA, Huebner K, Croce CM (2007) MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. *Proc Natl Acad Sci USA* **104**: 15805–15810.

Fakih MG, Padmanabhan A (2006) CEA monitoring in colorectal cancer. What you should know. *Oncology (Williston Park)* **20**: 579–587.

Fang Z, Tang J, Bai Y, Lin H, You H, Jin H, Lin L, You P, Li J, Dai Z, Liang X, Su Y, Hu Q, Wang F, Zhang ZY (2015) Plasma levels of microRNA-24, microRNA-320a, and microRNA-423-5p are potential biomarkers for colorectal carcinoma. *J Exp Clin Cancer Res* **34**: 86.

Filipowicz W, Jaskiewicz L, Kolb FA, Pillai RS (2005) Post-transcriptional gene silencing by siRNAs and miRNAs. *Curr Opin Struct Biol* **15**: 331–341.

Gebeshuber CA, Zatloukal K, Martinez J (2009) miR-29a suppresses tristetraprolin, which is a regulator of epithelial polarity and metastasis. *EMBO Rep* **10**: 400–405.

Ghanbari R, Mosakhani N, Asadi J, Nouraee N, Mowla SJ, Yazdani Y, Mohamadkhani A, Poustchi H, Knuutila S, Malekzadeh R (2015)

Downregulation of plasma miR-142-3p and miR-26a-5p in patients with colorectal carcinoma. *Iran J Cancer Prev* **8**: e2329.
Giraldez MD, Lozano JJ, Ramirez G, Hijona E, Bujanda L, Castells A, Gironella M (2013) Circulating microRNAs as biomarkers of colorectal cancer: results from a genome-wide profiling and validation study. *Clin Gastroenterol Hepatol* **11**: 681–8 e3.
Hammond SM (2005) Dicing and slicing: the core machinery of the RNA interference pathway. *FEBS Lett* **579**: 5822–5829.
Han J, Lee Y, Yeom KH, Kim YK, Jin H, Kim VN (2004) The Drosha-DGCR8 complex in primary microRNA processing. *Genes Dev* **18**: 3016–3027.
Higgins JP (2008) Commentary: Heterogeneity in meta-analysis should be expected and appropriately quantified. *Int J Epidemiol* **37**: 1158–1160.
Ho GY, Jung HJ, Schoen RE, Wang T, Lin J, Williams Z, Weissfeld JL, Park JY, Loudig O, Suh Y (2015) Differential expression of circulating microRNAs according to severity of colorectal neoplasia. *Transl Res* **166**: 225–232.
Hofsli E, Sjursen W, Prestvik WS, Johansen J, Rye M, Trano G, Wasmuth HH, Hatlevoll I, Thommesen L (2013) Identification of serum microRNA profiles in colon cancer. *Br J Cancer* **108**: 1712–1719.
Hu Y, Correa AM, Hoque A, Guan B, Ye F, Huang J, Swisher SG, Wu TT, Ajani JA, Xu XC (2011) Prognostic significance of differentially expressed miRNAs in esophageal cancer. *Int J Cancer* **128**: 132–143.
Huang Z, Huang D, Ni S, Peng Z, Sheng W, Du X (2010) Plasma microRNAs are promising novel biomarkers for early detection of colorectal cancer. *Int J Cancer* **127**: 118–126.
Hundt S, Haug U, Brenner H (2009) Comparative evaluation of immunochemical fecal occult blood tests for colorectal adenoma detection. *Ann Intern Med* **150**: 162–169.
Imperiale TF, Ransohoff DF, Itzkowitz SH, Levin TR, Lavin P, Lidgard GP, Ahlquist DA, Berger BM (2014) Multitarget stool DNA testing for colorectal-cancer screening. *N Engl J Med* **370**: 1287–1297.
Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S, Magri E, Pedriali M, Fabbri M, Campiglio M, Menard S, Palazzo JP, Rosenberg A, Musiani P, Volinia S, Nenci I, Calin GA, Querzoli P, Negrini M, Croce CM (2005) MicroRNA gene expression deregulation in human breast cancer. *Cancer Res* **65**: 7065–7070.
Iorio MV, Visone R, Di Leva G, Donati V, Petrocca F, Casalini P, Taccioli C, Volinia S, Liu CG, Alder H, Calin GA, Menard S, Croce CM (2007) MicroRNA signatures in human ovarian cancer. *Cancer Res* **67**: 8699–8707.
Kanaan Z, Rai SN, Eichenberger MR, Roberts H, Keskey B, Pan J, Galandiuk S (2012) Plasma miR-21: a potential diagnostic marker of colorectal cancer. *Ann Surg* **256**: 544–551.
Kanaan Z, Roberts H, Eichenberger MR, Billeter A, Ocheretner G, Pan J, Rai SN, Jorden J, Williford A, Galandiuk S (2013) A plasma microRNA panel for detection of colorectal adenomas: a step toward more precise screening for colorectal cancer. *Ann Surg* **258**: 400–408.
Kriegel AJ, Liu Y, Fang Y, Ding X, Liang M (2012) The miR-29 family: genomics, cell biology, and relevance to renal and cardiovascular injury. *Physiol Genomics* **44**: 237–244.
Lee Y, Kim M, Han J, Yeom KH, Lee S, Baek SH, Kim VN (2004) MicroRNA genes are transcribed by RNA polymerase II. *EMBO J* **23**: 4051–4060.
Li J, Liu Y, Wang C, Deng T, Liang H, Wang Y, Huang D, Fan Q, Wang X, Ning T, Liu R, Zhang CY, Zen K, Chen X, Ba Y (2015) Serum miRNA expression profile as a prognostic biomarker of stage II/III colorectal adenocarcinoma. *Sci Rep* **5**: 12921.
Li L, Guo Y, Chen Y, Wang J, Zhen L, Guo X, Liu J, Jing C (2016) The diagnostic efficacy and biological effects of microRNA-29b for colon cancer. *Technol Cancer Res Treat* **15**: 772–779.
Li M, Guan X, Sun Y, Mi J, Shu X, Liu F, Li C (2014) miR-92a family and their target genes in tumorigenesis and metastasis. *Exp Cell Res* **323**: 1–6.
Liu GH, Zhou ZG, Chen R, Wang MJ, Zhou B, Li Y, Sun XF (2013) Serum miR-21 and miR-92a as biomarkers in the diagnosis and prognosis of colorectal cancer. *Tumour Biol* **34**: 2175–2181.
Logan RF, Patnick J, Nickerson C, Coleman L, Rutter MD, Von Wagner C. English Bowel Cancer Screening Evaluation Committee (2012) Outcomes of the Bowel Cancer Screening Programme (BCSP) in England after the first 1 million tests. *Gut* **61**: 1439–1446.
Lohsiriwat V (2010) Colonoscopic perforation: incidence, risk factors, management and outcome. *World J Gastroenterol* **16**: 425–430.
Lui WO, Pourmand N, Patterson BK, FIRE A (2007) Patterns of known and novel small RNAs in human cervical cancer. *Cancer Res* **67**: 6031–6043.
Luo X, Stock C, Burwinkel B, Brenner H (2013) Identification and evaluation of plasma microRNAs for early detection of colorectal cancer. *PLoS One* **8**: e62880.
Mahid SS, Hornung CA, Minor KS, Turina M, Galandiuk S (2006) Systematic reviews and meta-analysis for the surgeon scientist. *Br J Surg* **93**: 1315–1324.
Marshall KW, Mohr S, Khettabi FE, Nossova N, Chao S, Bao W, Ma J, Li XJ, Liew CC (2010) A blood-based biomarker panel for stratifying current risk for colorectal cancer. *Int J Cancer* **126**: 1177–1186.
Maton A, Hopkins J, Mclaughlin C, Johnson S, Warner M, Lahart D, Wright J (1993) *Human Biology and Health*. Prentice Hall: Englewood Cliffs, New Jersey, USA.
Mayeux R (2004) Biomarkers: potential uses and limitations. *NeuroRx* **1**: 182–188.
Meister G (2013) Argonaute proteins: functional insights and emerging roles. *Nat Rev Genet* **14**: 447–459.
Meltzer PS (2005) Cancer genomics: small RNAs with big impacts. *Nature* **435**: 745–746.
Meng F, Henson R, Wehbe-Janek H, Ghoshal K, Jacob ST, Patel T (2007) MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. *Gastroenterology* **133**: 647–658.
Mishra PJ (2014) Non-coding RNAs as clinical biomarkers for cancer diagnosis and prognosis. *Expert Rev Mol Diagn* **14**: 917–919.
Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A, Lin DW, Urban N, Drescher CW, Knudsen BS, Stirewalt DL, Gentleman R, Vessella RL, Nelson PS, Martin DB, Tewari M (2008) Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci USA* **105**: 10513–10518.
Mogilyansky E, Rigoutsos I (2013) The miR-17/92 cluster: a comprehensive update on its genomics, genetics, functions and increasingly important and numerous roles in health and disease. *Cell Death Differ* **20**: 1603–1614.
Moher D, Liberati A, Tetzlaff J, Altman DG. Group, P. (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *J Clin Epidemiol* **62**: 1006–1012.
Muller AD, Sonnenberg A (1995) Prevention of colorectal cancer by flexible endoscopy and polypectomy. A case-control study of 32 702 veterans. *Ann Intern Med* **123**: 904–910.
Naylor S (2003) Biomarkers: current perspectives and future prospects. *Expert Rev Mol Diagn* **3**: 525–529.
Ng EK, Chong WW, Jin H, Lam EK, Shin VY, Yu J, Poon TC, Ng SS, Sung JJ (2009) Differential expression of microRNAs in plasma of patients with colorectal cancer: a potential marker for colorectal cancer screening. *Gut* **58**: 1375–1381.
Nichita C, Ciarloni L, Monnier-Benoit S, Hosseinian S, Dorta G, Ruegg C (2014) A novel gene expression signature in peripheral blood mononuclear cells for early detection of colorectal cancer. *Aliment Pharmacol Ther* **39**: 507–517.
Nishihara R, Wu K, Lochhead P, Morikawa T, Liao X, Qian ZR, Inamura K, Kim SA, Kuchiba A, Yamauchi M, Imamura Y, Willett WC, Rosner BA, Fuchs CS, Giovannucci E, Ogino S, Chan AT (2013) Long-term colorectal-cancer incidence and mortality after lower endoscopy. *N Engl J Med* **369**: 1095–1105.
Perilli L, Vicentini C, Agostini M, Pizzini S, Pizzi M, D'Angelo E, Bortoluzzi S, Mandruzzato S, Mammano E, Rugge M, Nitti D, Scarpa A, Fassan M, Zanovello P (2014) Circulating miR-182 is a biomarker of colorectal adenocarcinoma progression. *Oncotarget* **5**: 6611–6619.
Pu XX, Huang GL, Guo HQ, Guo CC, Li H, Ye S, Ling S, Jiang L, Tian Y, Lin TY (2010) Circulating miR-221 directly amplified from plasma is a potential diagnostic and prognostic marker of colorectal cancer and is correlated with p53 expression. *J Gastroenterol Hepatol* **25**: 1674–1680.
Ramzy I, Hasaballah M, Marzaban R, Shaker O, Soliman ZA (2015) Evaluation of microRNAs-29a, 92a and 145 in colorectal carcinoma as candidate diagnostic markers: an Egyptian pilot study. *Clin Res Hepatol Gastroenterol* **39**: 508–515.
Resnick KE, Alder H, Hagan JP, Richardson DL, Croce CM, Cohn DE (2009) The detection of differentially expressed microRNAs from the serum of ovarian cancer patients using a novel real-time PCR platform. *Gynecol Oncol* **112**: 55–59.
Shenouda SK, Alahari SK (2009) MicroRNA function in cancer: oncogene or a tumor suppressor? *Cancer Metastasis Rev* **28**: 369–378.

Sun Y, Liu Y, Cogdell D, Calin GA, Sun B, Kopetz S, Hamilton SR, Zhang W (2016) Examining plasma microRNA markers for colorectal cancer at different stages. *Oncotarget* **10**: 11434–11449.

Tetzlaff MT, Liu A, Xu X, Master SR, Baldwin DA, Tobias JW, Livolsi VA, Baloch ZW (2007) Differential expression of miRNAs in papillary thyroid carcinoma compared to multinodular goiter using formalin fixed paraffin embedded tissues. *Endocr Pathol* **18**: 163–173.

Toiyama Y, Takahashi M, Hur K, Nagasaka T, Tanaka K, Inoue Y, Kusunoki M, Boland CR, Goel A (2013) Serum miR-21 as a diagnostic and prognostic biomarker in colorectal cancer. *J Natl Cancer Inst* **105**: 849–859.

Turchinovich A, Weiz L, Langheinz A, Burwinkel B (2011) Characterization of extracellular circulating microRNA. *Nucleic Acids Res* **39**: 7223–7233.

Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, Prueitt RL, Yanaihara N, Lanza G, Scarpa A, Vecchione A, Negrini M, Harris CC, Croce CM (2006) A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci USA* **103**: 2257–2261.

Wang B, Zhang Q (2012) The expression and clinical significance of circulating microRNA-21 in serum of five solid tumors. *J Cancer Res Clin Oncol* **138**: 1659–1666.

Wang K, Yuan Y, Cho JH, Mcclarty S, Baxter D, Galas DJ (2012a) Comparing the microRNA spectrum between serum and plasma. *PLoS One* **7**: e41561.

Wang Q, Huang Z, Ni S, Xiao X, Xu Q, Wang L, Huang D, Tan C, Sheng W, Du X (2012b) Plasma miR-601 and miR-760 are novel biomarkers for the early detection of colorectal cancer. *PLoS One* **7**: e44398.

Wang S, Xiang J, Li Z, Lu S, Hu J, Gao X, Yu L, Wang L, Wang J, Wu Y, Chen Z, Zhu H (2015) A plasma microRNA panel for early detection of colorectal cancer. *Int J Cancer* **136**: 152–161.

Wang W, Qu A, Liu W, Liu Y, Zheng G, Du L, Zhang X, Yang Y, Wang C, Chen X (2016) Circulating miR-210 as a diagnostic and prognostic biomarker for colorectal cancer. *Eur J Cancer Care (Engl)*; e-pub ahead of print 22 February 2016; doi:10.1111/ecc.12448.

Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, Leeflang MM, Sterne JA, Bossuyt PM. QUADAS-2 Group (2011) QUADAS-2: a revised tool for the Quality Assessment of Diagnostic Accuracy Studies. *Ann Intern Med* **155**: 529–536.

Xu L, Li M, Wang M, Yan D, Feng G, An G (2014) The expression of microRNA-375 in plasma and tissue is matched in human colorectal cancer. *BMC Cancer* **14**: 714.

Yamada A, Horimatsu T, Okugawa Y, Nishida N, Honjo H, Ida H, Kou T, Kusaka T, Sasaki Y, Yagi M, Higurashi T, Yukawa N, Amanuma Y, Kikuchi O, Muto M, Ueno Y, Nakajima A, Chiba T, Boland CR, Goel A (2015) Serum miR-21, miR-29a, and miR-125b are promising biomarkers for the early detection of colorectal neoplasia. *Clin Cancer Res* **21**: 4234–4242.

Yan B, Guo Q, Fu FJ, Wang Z, Yin Z, Wei YB, Yang JR (2015) The role of miR-29b in cancer: regulation, function, and signaling. *Onco Targets Ther* **8**: 539–548.

Yi R, Qin Y, Macara IG, Cullen BR (2003) Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes Dev* **17**: 3011–3016.

Yu H, Gao G, Jiang L, Guo L, Lin M, Jiao X, Jia W, Huang J (2013) Decreased expression of miR-218 is associated with poor prognosis in patients with colorectal cancer. *Int J Clin Exp Pathol* **6**: 2904–2911.

Yu J, Jin L, Jiang L, Gao L, Zhou J, Hu Y, Li W, Zhi Q, Zhu X (2016) Serum miR-372 is a diagnostic and prognostic biomarker in patients with early colorectal cancer. *Anticancer Agents Med Chem* **16**: 424–431.

Zanutto S, Pizzamiglio S, Ghilotti M, Bertan C, Ravagnani F, Perrone F, Leo E, Pilotti S, Verderio P, Gariboldi M, Pierotti MA (2014) Circulating miR-378 in plasma: a reliable, haemolysis-independent biomarker for colorectal cancer. *Br J Cancer* **110**: 1001–1007.

Zauber AG, Winawer SJ, O'Brien MJ, Lansdorp-Vogelaar I, Van Ballegooijen M, Hankey BF, Shi W, Bond JH, Schapiro M, Panish JF, Stewart ET, Waye JD (2012) Colonoscopic polypectomy and long-term prevention of colorectal-cancer deaths. *N Engl J Med* **366**: 687–696.

Zhang B, Pan X, Cobb GP, Anderson TA (2007) microRNAs as oncogenes and tumor suppressors. *Dev Biol* **302**: 1–12.

Zhang GJ, Zhou T, Liu ZL, Tian HP, Xia SS (2013) Plasma miR-200c and miR-18a as potential biomarkers for the detection of colorectal carcinoma. *Mol Clin Oncol* **1**: 379–384.

Zheng G, Du L, Yang X, Zhang X, Wang L, Yang Y, Li J, Wang C (2014) Serum microRNA panel as biomarkers for early diagnosis of colorectal adenocarcinoma. *Br J Cancer* **111**: 1985–1992.

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