Stem-loop RT-qPCR-based Plasma MicroRNA Profiling as a Potential Diagnostic Method for Cancer: A Meta-analysis

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Abstract

Dysregulated microRNAs in plasma have been associated with cancer and the most broadly applied method for miRNA expression analysis is stem-loop quantitative reverse transcription PCR (RT-qPCR). However, the diagnostic role of stem-loop RT-qPCR-based plasma microRNA profiling remains uncertain in cancer. This meta-analysis aimed to assess the effectiveness of this method in diagnosing cancer. A comprehensive search was conducted through the PubMed, EMBASE and ScienceDirect databases to collect the relevant studies. Each eligible study was assessed for bias risk using QUADAS-2. By estimation of the pooled sensitivity, specificity, diagnostic odds ratios (DOR) and area under the receiver operating characteristic curve (AUC), the diagnostic accuracy of stem-loop-based plasma miRNA was measured in cancer. All measurements were estimated using R statistical software version 4.1.3.

127 studies from 41 articles, comprising of 10,218 cancer patients and 8,990 controls, were included in the diagnostic analysis data. The overall sensitivity, specificity and DOR were 0.77, 0.79 and 11.44 respectively. The stem-loop RT-qPCR-based plasma miRNA profiling yielded very good accuracy with 0.81 AUC; especially, the plasma miRNA panel showed excellent accuracy (AUC = 0.91) for distinguishing cancer patients from healthy individuals. Substantial heterogeneity and publication bias were observed in this study. Using stem-loop RT-qPCR, plasma miRNA profiling, especially miRNA panels, serves as a potential method for diagnosing cancer.

Keywords: Plasma microRNAs, diagnostic accuracy, stem-loop RT-qPCR, cancer detection, meta-analysis.

Introduction

Cancer has been the most dangerous disease in the world for several decades, accounting for approximately one in every six fatalities in 2020[56]. Detecting cancer early is important for improving survival rates[20]. Although pathology and imaging examination-based cancer detection could provide essential information for prognosis and treatment[52], invasive procedures, late-stage diagnosis, or radiation-related aspects limit their application[43]. Hence, efforts should be made to develop novel, sensitive and minimally invasive approaches for early cancer detection.

MicroRNAs (miRNA) are short single-stranded, non-coding RNA molecules that play a significant role in regulating cell cell differentiation, proliferation and death[19]. About 50–60% of mRNAs are reported to be controlled by miRNA expression[16] and its dysregulation has been related to the development and progression of several human cancer types[58]. Tumor-derived miRNAs have also been found in plasma and changes in the plasma miRNA profile are linked to enhanced and altered expression in cancer cells[41,69,74], indicating the significance of miRNAs as potential minimally invasive biomarkers in diagnosing early cancer.

To date, quantitative reverse transcription polymerase chain reaction (RT-qPCR) is a gold standard for detecting circulating miRNA with sensitivity, specificity and robustness[8,9], allowing detection in low amounts of miRNAs that exist in plasma. Among qPCR-based methods, stem-loop RT-qPCR is the most extensively applied for mature miRNA expression analysis because of its ability to distinguish mature miRNA from pri-miRNA and pre-miRNA as well as to differentiate closely related mature miRNAs with differences as small as one nucleotide[62] while detecting a broad dynamic range of miRNA expression. Therefore, increasing research and medical diagnoses have used the stem-loop RT-qPCR method for plasma miRNA analysis in diagnosing cancer.

Although promising results were shown in previous studies, the diagnostic performance of stem-loop RT-qPCR-based plasma miRNA profiling remains uncertain. The use of plasma as a specimen type creates the risk of affecting miRNA profiling due to components contained in plasma[27,42]. In addition, there are inconsistencies and discrepancies across studies on a certain cancer type and different cancer types as well. For instance, plasma miR-21 levels identify NSCLC patients from non-cancer individuals with high specificity and sensitivity[71]. Others found no correlation between expression level of miR-21 and clinicopathologic features of patients with NSCLC[46,63].

Furthermore, there is a substantial difference in the diagnostic accuracy of miR-21 for colorectal cancer[51],

NSCLC[46], hepatocellular carcinoma[5] and lung cancer[29]. Moreover, these studies have focused on a small number of pre-selected miRNAs with sample size limitations. The diagnostic ability of plasma miRNAs in cancer, therefore, needs to be assessed beyond the limitations of individual studies. In the present study, we validate the overall diagnostic performance of stem-loop RT-qPCR-based plasma miRNA profiling based on published case-control studies for cancer.

Material and Methods

Search strategy: Based on the preferred reporting items for a systematic review and meta-analysis of diagnostic test accuracy studies (PRISMA-DTA) guidelines[39], we carried out this study. The two investigators independently searched the PubMed, EMBASE and ScienceDirect databases to retrieve studies that used stem-loop RT-qPCR to profile plasma miRNA in cancer. The last search was on September 17, 2022, using the terms ("miRNA" OR "microRNA" OR "hsa-miR") AND ("diagnosis" OR "diagnostic" OR "test" OR "assay") and ("cancer" OR "neoplasm" OR "carcinoma" OR "malignance" OR "tumor") and "plasma." Further optimization of terms across different databases was used, with no restriction on publication status.

Inclusive and exclusive criteria: The inclusive criteria for studies included in the meta-analysis were set as follows: (i) miRNAs were investigated in any type of cancer; (ii) applying the stem-loop RT-qPCR method for measuring; (iii) expression levels of miRNAs were quantified in plasma for diagnostic accuracy analysis; (iv) all cancer patients were examined pathologically; (v) controls were cancer-free before; (vi) sensitivity, specificity and the total number of cancer patients and controls were clear to evaluate for diagnostic accuracy. Studies were removed if they exhibited the following exclusive criteria: non-English studies, duplicated publications, other types of studies such as reviews, meta-analysis studies and case reports or letters; studies on animals; studies with insufficient data.

Data extraction and quality assessment: We extracted the following information from each included study: (i) first author name, publication year and country of origin; (ii) sample size (cases and controls); (iii) miRNA profile; (iv) measurement method used in stem-loop RT-qPCR; (v) reference control; (vi) type of cancer and (vii) true positive (TP), false positive (FP), false negative (FN) and true negative (TN). Each eligible study was examined for risk of bias using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) framework[65] and the results were analyzed by Review Manager Software version 5.4.1. Four domains were evaluated by answering 14 questions about patient selection, index test, reference standard and flow and timing.

The risk of bias was then rated as "low," "high," or "unclear" when questions were answered as "yes," "no," or "unclear" respectively. Two reviewers independently performed the data extraction and quality assessment of the included studies and disagreements were resolved through consensus.

Statistical analysis: We used measurements including the pooled SENS and SPEC, positive and negative likelihood ratios (PLR and NLR), diagnostic odds ratio (DOR), summary receiver operating characteristic (SROC) and area under the curve (AUC), to evaluate the diagnostic accuracy of stem-loop RT-qPCR-base plasma miRNA for cancer. The heterogeneity across studies was tested using the Cochran-Q test and I^2 statistics. When the p-value for the Q test was less than 0.10 or the I^2 value was greater than 50%, heterogeneity was assumed to exist[28] and a random-effects model was applied for analysis; otherwise, the fixed-effects model was employed.

To investigate possible causes that lead to significant heterogeneity, we evaluated the threshold effect and subgroup analysis. The threshold effect was tested based on Spearman's correlation coefficient between sensitivity and specificity[45], while the possible differences in the diagnostic measurements were detected based on cancer type, miRNA profiles, ethnicity, the measurement method used in the stem-loop RT-qPCR and normalizer type. The potential publication bias was evaluated using Egger's regression test and a funnel plot based on the trim-and-fill method. Finally, the stability of the result was assessed using a sensitivity analysis by sequential elimination of individual studies. All statistical analyses in this meta-analysis were implemented using R version 4.1.3 and the meta, mada and metafor R packages.

Results

Study identification: The process of comprehensive literature identification is illustrated in fig. 1. A total of 11,315 articles were retrieved from PubMed, EMBASE and ScienceDirect databases and then 3,620 duplicate records were automatically eliminated after importing all the articles into EndNote X9. Additionally, the manual checking procedure excluded 326 duplicates. Another 63 articles were eliminated because they were written in a language other than English. Then, the remaining articles were screened by title and abstract; we excluded 3,570 articles, of which 1,397 were reports, reviews, meta-analysis studies and letters and 2,173 were about non-connection to miRNAs or other diseases or based on animal models.

After reviewing 3,736 full-text articles, 3,695 were further excluded because they did not use stem-loop RT-qPCR for miRNA profiling, were performed on samples other than plasma, or had insufficient data. Finally, 41 articles[1-4,13-15,21-24,26,29,30,32,34,36-38,44,46-51,54,55,57,60,61,63,64,66,68,70-73,75] were enrolled in the meta-analysis.

General characteristics of eligible studies: The characteristics of 41 eligible articles are presented in table 1. All the articles included were published between 2005 and 2020. These articles comprised of 127 studies that used

stem-loop RT-qPCR for relatively quantifying miRNA expression in plasma, with 10,218 cancer patients and 8,990 healthy individuals (Supplementary table 1) stratified by Caucasians (69 studies) and Asians (58 studies). While 106 studies applied single-miRNA assays for cancer detection, only 21 studies analyzed the diagnostic potential of panel-miRNA assays in cancer. Of 127 studies, the Taqman miRNA assay was applied for miRNA profiling in 99 studies; 25 studies were about the SYBR-green miRNA assay and three others were not measured by the method available. Their sources included breast cancer (BC) (5 studies), hepatocellular carcinoma (HCC) (24 studies), Non-Small Cell Lung Cancer (NSCLC) (13 studies), lung cancer (LC) (38 studies), colorectal cancer (CRC) (9 studies), gastric cancer (GC) (14 studies), Malignant Pleural Mesothelioma (MPM) (1 study), osteosarcomas (OS) (1 study), head and neck squamous cell carcinoma (HNSCC) (1 study), pancreatic cancer (PC) (4 studies), nasopharyngeal carcinoma (NPC) (2 study), esophageal squamous cell carcinoma (ESCC) (3 studies), extramedullary myeloma (EMM) (1 study), cervical cancer (CC) (1 study), multiple myeloma (MM) (1 study), chronic myeloid leukemia (CML) (2 study), oral cancer (OC) (3 study), prostate cancer (PCa) (2 study) and medullary thyroid carcinoma (MTC) (2 study).

Table 1
General information about the eligible studies for meta-analysis

First author, year [Ref]	Country	miRNA profile	Case/ control	Measurement method	Reference controls	Cancer type
Amr K S, 2019[1]	Egypt	miR-155, miR-10b	30/30	Taqman	RUN6B	BC
Meihong Lu, 2017[36]	China	miR-127-3p	102/90	SYBR green	U6	BC
Zeng RC, 2013 [70]	China	miRNA-30a, miR-122	100/64	SYBR green	miR-16	BC
Zanxi Fang, 2015 [15]	China	miR-24, miR-320, miR-423-5p	111/43	SYBR green	Cel-miR-39	CRC
Leping Li, 2015 [30]	China	miR-29b	200/400	Taqman	U6	CRC
Xu L, 2014 [66]	China	miR-375	88/40	TaqMan	U6	CRC
Xing-xiang Pu, 2010 [50]	China	miR-221	103/37	SYBR green	NR	CRC
A. A. Sazanov, 2016 [51]	Russia	miR-21	31/34	SYBR green	U6	CRC
Sun Y, 2016 [55]	USA	miR-96	187/47	NR	Cel-miR-39	CRC
Simonas J, 2015 [24]	Lithuanin	miR-148a-3p, miR-375, miR-223-3p	38/39	Taqman	miR-16-5p	GC
Zhu C, 2014 [75]	China	miR-16, miR-25, miR-92a, miR-451, miR-486-5p	88/142	TaqMan	Cel-miR-39	GC
Jong-Lyul Park, 2015 [48]	Korea	miR-27a	20/20	TaqMan	U6	GC
Pegah Parvaee, 2019 [49]	Iran	miR-107, miR-194, miR-210	50/50	SYBR green	U47	GC
Paola Mozzoni, 2013 [46]	Italy	miR-21, miR-486	54/46	TaqMan	miR-16	NSCLC
Wei J, 2011 [63]	China	miR-21	63/30	SYBR green	miR-16	NSCLC
Wanshuai Li, 2015 [32]	China	miR-486, miR-150	11/11	Taqman	Cel-miR-39	NSCLC
Zhang H, 2016 [71]	China	miR-145, miR-20a, miR-21, miR-223	129/83	TaqMan	miR-16	NSCLC
Wang X, 2016 [61]	China	miR-486, miR-210	59/59	TaqMan	miR-16	NSCLC
Zheng D, 2011 [73]	USA	3 miRNAs[b]	74/68	SYBR green	NR	LC
Hua Fang, 2019 [14]	China	miR-505-5p, miR-382-3p	108/50	Taqman	Cel-miR-39	LC
Qixin Leng, 2017 [29]	USA	miR-422a, miR-326, miR-324-3p, miR-103a-3p, miR-30a-5p, miR-1285, miR-1254, miR-574-5p, miR-146b-5p, miR-27a-3p, miR-27b-3p, miR-222-3p, miR-106a-3p, miR-92a-3p, miR-29c, miR-24a-3p, miR-486-5p, miR-425-5p, miR-221-3p, miR-301a-3p, miR-148a, miR-148b, miR-193a-3p, miR-21, miR-19b-3p, miR-210-3p, miR-145, miR-126-3p, miR-223-3p, miR-205-5p	92/88	Taqman	U6	LC
Amr K S, 2017 [2]	Egypt	miR-122, miR-224	40/20	Taqman	RUN6B	HCC
Dipu Bharali, 2018 [51]	India	miR-21	50/50	Taqman	U6	HCC
Wang S, 2020 [60]	China	miRNA-96, miRNA-21, miRNA-122	100/50	TaqMan	U6	HCC
Wen Y, 2015 [64]	China	miR-20a-5p, miR-25-3p, miR-30a-5p, miR-92a-3p, miR-132-3p,	67/82	TaqMan	Cel-miR-39	HCC

		miR-185-5p, miR-320a, miR-324-3p				
Niloofar Moradi, 2019 [44]	Iran	miR-214, miR-6510, miR-5193, miR-34a, miR-214, miR-5193, miR-34a	23/25	SYBR green	NR	HCC
Ya-Ching Lu, 2014 [38]	Taiwan	miR-196a, miR-196b	90/53	TaqMan	NR	OC
Ivan D. Osipov, 2016 [47]	Russia	miR-141, miR-205	48/47	TaqMan	miR-16, miR-101	PCa
Noushin Shabani, 2019 [54]	Iran	miR-144, miR-34a	50/50	TaqMan	U47	MTC
Feng Lian, 2015 [34]	China	miR-195–5p, miR-199a-3p, miR-320a, miR-374a-5p	90/90	Taqman	Cel-miR-39	OS
Kirschner, 2012 [26]	Australia	miR-625-3p	15/14	Taqman	miR-16	MPM
Yoshizawa S, 2012 [68]	Japan	miR-92a	62/113	Taqman	miR-638	ESCC
Fu-Cheng He, 2015 [21]	China	miR-20a, let-7a	70/40	SYBR green	NR	ESCC
Yongying Bai, 2016 [3]	China	miR-19a	89/125	SYBR green	Cel-miR-39	ESCC
Lenka Besse , 2015 [4]	Czech Republic	miR-130a	35/30	Taqman	miR-19b	EMM
Shengye Du, 2020 [13]	China	miR-29a, miR-25, miR-486-5p	140/140	Taqman	U6, miR-16, miR-25	CC
Cheng M Hsu, 2012 [22]	Taiwan	miR-21	50/36	Taqman	Cel-miR-39, cel-miR-54	HNSCC
Hussein, 2017 [23]	Egypt	miR-642b-3p, miR-885-5p, miR-22-3p	35/15	Taqman	miR-3196	PC
Tavano F, 2018 [57]	Italy	miR-1290	167/267	TaqMan	NR	PC
Zhang J, 2018 [72]	China	miR-451a, let-7b-5p	58/20	NR	Cel-miR-39	CML
Tianzhu Lu, 2020 [37]	China	miR-BART7-3p	483/243	Taqman	Cel-miR-39	NPC

NR not reported; *BC* breast cancer; *HCC* hepatocellular carcinoma; *NSCLC* Non-Small Cell Lung Cancer; *LC* lung cancer; *CRC* colorectal cancer; *GC* gastric cancer; *MPM* Malignant Pleural Mesothelioma; *OS* osteosarcomas; *HNSCC* head and neck Squamous cell carcinoma; *PC* pancreatic cancer; *NPC* nasopharyngeal carcinoma; *ESCC* esophageal squamous cell carcinoma; *EMM* extra medullary myeloma; *CC* cervical cancer; *MM* multiple myeloma; *CML* chronic myeloid leukemia; *OC* oral cancer; *PCa* prostate cancer; *MTC* medullary thyroid carcinoma.

Supplementary Table 1
The data for the diagnostic meta-analysis

First author, year	Cancer type	miRNA profile	Case/ control	TP	FN	FP	TN
Amr K S, 2019	BC	miR-155	30/30	30	0	3	27
		miR-10b	30/30	29	1	4	26
Meihong Lu, 2017	BC	miR-127-3p	102/90	80	22	19	71
Zeng RC, 2013	BC	miRNA-30a	100/64	74	26	22	42
		miR-122	100/64	36	14	14	36
Zanxi Fang, 2015	CRC	miR-24	111/43	87	24	7	36
		miR-320	111/43	103	8	12	31
		miR-423-5p	111/43	102	9	13	30
		miR-24, miR-320, miR-423-5p	111/43	103	8	13	30
Leping Li, 2015	CRC	miR-29b	200/400	123	77	110	290
Xu L, 2014	CRC	miR-375	88/40	68	20	14	26
Xing-xiang Pu, 2010	CRC	miR-221	103/37	89	14	22	15
A. A. Sazanov, 2016	CRC	miR-21	31/34	20	11	5	29
Sun Y, 2016	CRC	miR-96	187/47	122	65	13	34
Simonas J, 2015	GC	miR-148a-3p	38/39	22	16	26	13
		miR-375	38/39	18	20	24	15
		miR-223-3p	38/39	29	9	16	23
		miR-148a-3p, miR-375	38/39	25	13	13	26
Zhu C, 2014	GC	miR-16	88/142	66	22	11	131
		miR-25	88/142	55	33	4	138
		miR-92a	88/142	74	14	33	109
		miR-451	88/142	71	17	21	121
		miR-486-5p	88/142	62	26	11	131
		miR-16, miR-25, miR-92a, miR-451, miR-486	88/142	74	14	13	129

Jong-Lyul Park, 2015	GC	miR-27a	20/20	15	5	9	11
Pegah Parvaee, 2019	GC	miR-107	50/50	47	3	11	39
		miR-194	50/50	44	6	12	38
		miR-210	50/50	50	0	14	36
Paola Mozzoni, 2013	NSCLC	miR-21	54/46	27	27	4	42
		miR-486	54/46	38	16	5	41
Wei J, 2011	NSCLC	miR-21	63/30	48	15	9	21
Wanshuai Li, 2015	NSCLC	miR-486	11/11	10	1	2	9
		miR-150	11/11	9	2	2	9
Zhang H, 2016	NSCLC	miR-145	129/83	104	25	9	74
		miR-20a	129/83	103	26	10	73
		miR-21	129/83	100	29	12	71
		miR-223	129/83	90	39	13	70
		miR-145, miR-20a, miR-21 and miR-223	129/83	106	23	8	75
Wang X, 2016	NSCLC	miR-486	59/59	49	10	13	46
		miR-210	59/59	44	15	15	44
		miR-486, miR-210	59/59	49	10	13	46
Zheng D, 2011	LC	miR-155, miR-197, miR-182	74/68	60	14	9	59
Hua Fang, 2019	LC	miR-505-5p	108/50	90	18	3	47
		miR-382-3p	108/50	88	20	14	36
		miR-505-5p, miR-382-3p	108/50	93	15	2	48
Qixin Leng, 2017	LC	miR-422a	92/88	48	44	30	58
		miR-326	92/88	56	36	33	55
		miR-324-3p	92/88	58	34	29	59
		miR-103a-3p	92/88	54	38	35	53
		miR30a-5p	92/88	53	39	35	53
		miR-1285	92/88	61	31	29	59
		miR-1254	92/88	66	26	26	62
		miR-574-5p	92/88	47	45	41	47
		miR-146b-5p	92/88	54	38	35	53
		miR-27a-3p	92/88	53	39	23	65
		miR-27b-3p	92/88	47	45	44	44
		miR-222-3p	92/88	51	41	38	50
		miR-106a-3p	92/88	55	37	29	59
		miR-92a-3p	92/88	66	26	12	76
		miR-29c	92/88	60	32	35	53
		miR-24a-3p	92/88	55	37	35	53
		miR-486-5p	92/88	65	27	15	73
		miR-425-5p	92/88	55	37	26	62
		miR-221-3p	92/88	53	39	42	46
		miR-301a-3p	92/88	56	36	33	55
		miR-148a	92/88	54	38	29	59
		miR-148b	92/88	50	42	33	55
		miR-193a-3p	92/88	63	29	29	59
		miR-21	92/88	54	38	32	56
		miR-19b-3p	92/88	63	29	32	56
		miR-210-3p	92/88	61	31	32	56
		miR-145	92/88	66	26	23	65
		miR-126-3p	92/88	66	26	23	65
		miR-223-3p	92/88	50	42	38	50
		miR-205-5p	92/88	66	26	25	63
		miR-126, miR-145, miR-210, miR-205-5p	92/88	84	8	3	85
		miR-21, miR-210, miR-486-5p	92/88	69	23	13	75
		miR-126, miR-145, miR-210, miR-205-5p	34/30	31	3	1	29
		miR-21, miR-210, miR-486-5p	34/30	26	8	5	25

Amr K S, 2017	HCC	miR-122	40/20	35	5	1	19
		miR-224	40/20	37	3	2	18
Dipu Bharali, 2018	HCC	miR-21	50/50	37	13	12	38
Wang S, 2020	HCC	miR-96, miR-21, miR-122	100/50	96	4	1	49
Wen Y, 2015	HCC	miR-20a-5p	67/82	58	9	35	47
		miR-25-3p	67/82	37	30	17	65
		miR-30a-5p	67/82	43	24	26	56
		miR-92a-3p	67/82	51	16	26	56
		miR-132-3p	67/82	61	6	52	30
		miR-185-5p	67/82	61	6	50	32
		miR-320a	67/82	26	41	10	72
		miR-324-3p	67/82	50	17	41	41
		miR-20a-5p, miR-25-3p, miR-30a-5p, miR-92a-3p, miR- 132-3p, miR-185-5p, miR-320a, miR-324-3p	67/82	58	9	29	53
		miR-20a-5p, miR-320a, miR-324-3p, miR-375	20/40	13	7	9	31
		miR-20a-5p, miR-320a, miR-324-3p, miR-375	50/37	28	22	6	31
Niloofar Moradi, 2019	HCC	miR-214	23/25	17	6	6	19
		miR-6510	23/25	17	6	2	23
		miR-5193	23/25	22	1	0	25
		miR-34a	23/25	21	2	10	15
		miR-214	23/25	20	3	14	11
		miR-6510	23/25	19	4	15	10
		miR-5193	23/25	18	5	4	21
		miR-34a	23/25	9	14	3	22
Ya-Ching Lu, 2014	OC	miR-196a	90/53	60	30	2	51
		miR-196b	90/53	88	2	10	43
		miR-196a, miR-196b	90/53	79	11	4	49
Ivan D. Osipov, 2016	PCa	miR-141	48/47	27	21	0	47
		miR-205	48/47	32	16	11	36
Noushin Shabani, 2019	MTC	miR-144	50/50	30	20	10	40
		miR-34a	50/50	24	26	10	40
Feng Lian, 2015	OS	miR-195–5p, miR-199a-3p, miR-320a, miR-374a-5p	90/90	82	8	5	85
Kirschner, 2012	MPM	miR-625-3p	15/14	11	4	3	11
Yoshizawa S, 2012	MM	miR-92a	62/113	57	5	1	112
Fu-Cheng He, 2015	ESCC	miR-20a	70/40	45	25	10	30
		let-7a	70/40	52	18	6	34
Yongying Bai, 2016	ESCC	miR-19a	89/125	59	30	42	83
Lenka Besse , 2015	EMM	miR-130a	35/30	27	8	3	27
Shengye Du, 2020	CC	miR-29a, miR-25, miR-486-5p	140/140	122	18	15	125
Cheng M Hsu, 2012	HNSCC	miR-21	50/36	42	8	17	19
Hussein, 2017	PC	miR-642b-3p	35/15	35	0	0	15
		miR-885-5p	35/15	35	0	0	15
		miR-22-3p	35/15	34	1	0	15
Tavano F, 2018	PC	miR-1290	167/267	94	73	28	239
Zhang J, 2018	CML	miR-451a	58/20	38	20	7	13
		let-7b-5p	58/20	41	17	4	16
Tianzhu Lu, 2020	NPC	miR-BART7-3p	483/243	464	19	8	235
		miR-BART13-3p	483/243	473	10	8	235

TP: True positive; FN: false-negative; FP: false-positive; TN: true-negative; NR: not reported;
BC: breast cancer; HCC: hepatocellular carcinoma; NSCLC: Non-Small Cell Lung Cancer; LC: lung cancer; CRC: colorectal cancer; GC: gastric cancer; MPM: Malignant Pleural Mesothelioma; OS: osteosarcomas; HNSCC: head and neck Squamous cell carcinoma; PC: pancreatic cancer; NPC: nasopharyngeal carcinoma; ESCC: esophageal squamous cell carcinoma; EMM: extramedullary myeloma; CC: cervical cancer; MM: multiple myeloma; CML: chronic myeloid leukemia; OC: oral cancer; PCa: prostate cancer; MTC: medullary thyroid carcinoma

Supplementary Table 2
Results of sensitivity analyses

Study eliminated	Sensitivity		Specificity		DOR	
	Overall [95% CI]	Heterogeneity (I^2)	Overall [95% CI]	Heterogeneity (I^2)	Overall [95% CI]	Heterogeneity (I^2)
None	0.77 [0.74; 0.80]	85.1%	0.79 [0.76; 0.82]	85.1%	11.44 [8.78; 14.92]	89.4%
A. A. Sazanov, 2016 [14]	0.77 [0.74; 0.80]	85.1%	0.79 [0.76; 0.82]	85.1%	11.51 [8.81; 15.03]	89.5%
Amr K S, 2019 [59]	0.77 [0.74; 0.79]	85.0%	0.79 [0.75; 0.81]	85.2%	11.10 [8.53; 14.44]	72.1%
Cheng M Hsu, 2012 [30]	0.77 [0.74; 0.80]	85.2%	0.79 [0.76; 0.82]	85.1%	11.57 [8.86; 15.11]	89.5%
Dipu Bharali, 2018 [14]	0.77 [0.74; 0.80]	85.3%	0.79 [0.75; 0.82]	85.4%	11.60 [8.86; 15.19]	89.5%
Feng Lian, 2015 [46]	0.77 [0.74; 0.80]	84.9%	0.79 [0.76; 0.82]	84.9%	11.21 [8.62; 14.58]	89.2%
Fu-Cheng He, 2015 [42]	0.77 [0.74; 0.80]	85.3%	0.79 [0.75; 0.81]	84.9%	11.55 [8.82; 15.12]	89.5%
Hua Fang, 2019 [44]	0.77 [0.74; 0.80]	85.5%	0.78 [0.75; 0.81]	85.3%	11.11 [8.51; 14.51]	89.4%
Ivan D. Osipov, 2016 [27]	0.77 [0.74; 0.80]	85.4%	0.78 [0.75; 0.81]	85.5%	11.42 [8.74; 14.92]	89.5%
Jong-Lyul Park, 2015 [26]	0.77 [0.74; 0.80]	85.4%	0.79 [0.76; 0.82]	85.1%	11.59 [8.88; 15.13]	89.5%
AmrKS, 2017 [39]	0.77 [0.74; 0.80]	85.4%	0.78 [0.75; 0.81]	85.4%	11.14 [8.55; 14.52]	89.4%
Lenka Besse , 2015 [31]	0.77 [0.74; 0.80]	85.3%	0.79 [0.75; 0.81]	85.3%	11.41 [8.74; 14.90]	89.4%
Leping Li, 2015 [34]	0.77 [0.74; 0.80]	84.9%	0.79 [0.76; 0.82]	85.4%	11.60 [8.88; 15.15]	89.4%
Meihong Lu, 2017 [32]	0.77 [0.74; 0.80]	85.0%	0.79 [0.76; 0.82]	85.3%	11.48 [8.79; 15.00]	89.4%
Kirschner, 2012 [47]	0.77 [0.74; 0.80]	85.1%	0.79 [0.76; 0.82]	85.4%	11.51 [8.81; 15.03]	89.5%
Hussein, 2017 [55]	0.76 [0.73; 0.79]	85.2%	0.78 [0.75; 0.81]	85.4%	10.99 [8.46; 14.28]	89.5%
Niloofar Moradi, 2019 [51]	0.77 [0.74; 0.80]	85.5%	0.79 [0.76; 0.82]	85.6%	11.52 [8.74; 15.19]	89.9%
Noushin Shabani, 2019 [48]	0.77 [0.74; 0.80]	85.1%	0.79 [0.76; 0.82]	85.3%	11.64 [8.89; 15.24]	89.5%
Paola Mozzoni, 2013 [12]	0.77 [0.74; 0.80]	85.0%	0.78 [0.75; 0.81]	85.1%	11.46 [8.75; 15.00]	89.5%
Pegah Parvaee, 2019 [50]	0.76 [0.73; 0.79]	85.0%	0.79 [0.76; 0.82]	85.4%	11.12 [8.52; 14.53]	89.4%
Qixin Leng, 2017 [16]	0.81 [0.78; 0.84]	84.7%	0.82 [0.78; 0.85]	85.6%	17.32 [12.80; 23.45]	86.5%
Shengye Du, 2020 [25]	0.77 [0.74; 0.80]	84.8%	0.79 [0.75; 0.81]	85.9%	11.32 [8.68; 14.77]	89.2%
Simonas J, 2015 [24]	0.77 [0.74; 0.80]	85.2%	0.79 [0.76; 0.82]	84.8%	12.21 [9.38; 15.90]	89.2%
Sun Y, 2016 [40]	0.77 [0.74; 0.80]	85.0%	0.79 [0.76; 0.82]	85.2%	11.58 [8.86; 15.12]	89.5%
Tavano F, 2018 [38]	0.77 [0.74; 0.80]	84.8%	0.79 [0.75; 0.81]	84.5%	11.51 [8.81; 15.04]	89.4%
Tianzhu Lu, 2020 [28]	0.76 [0.73; 0.79]	80.9%	0.78 [0.75; 0.81]	83.5%	10.35 [8.13; 13.18]	86.8%
Wang S, 2020 [41]	0.77 [0.74; 0.79]	84.7%	0.78 [0.75; 0.81]	85.1%	11.13 [8.58; 14.44]	89.3%
Wang X, 2016 [57]	0.77 [0.74; 0.80]	85.1%	0.79 [0.76; 0.82]	85.4%	11.47 [8.74; 15.06]	89.6%
Wanshuai Li, 2015 [49]	0.77 [0.74; 0.80]	85.1%	0.79 [0.76; 0.82]	85.3%	11.38 [8.71; 14.87]	89.5%
Wei J, 2011 [13]	0.77 [0.74; 0.80]	85.1%	0.79 [0.76; 0.82]	85.2%	11.54 [8.83; 15.08]	89.5%
Wen Y, 2015 [56]	0.77 [0.74; 0.80]	84.8%	0.80 [0.77; 0.83]	84.0%	12.46 [9.32; 16.67]	90.2%
Xing-xiang Pu, 2010 [29]	0.77 [0.74; 0.80]	84.9%	0.79 [0.76; 0.82]	85.0%	11.59 [8.88; 15.14]	89.5%
Xu L, 2014 [43]	0.77 [0.74; 0.80]	85.0%	0.79 [0.76; 0.82]	85.2%	11.56 [8.85; 15.10]	89.5%
Ya-Ching Lu, 2014 [33]	0.77 [0.74; 0.79]	84.8%	0.78 [0.75; 0.81]	85.1%	10.91 [8.38; 14.21]	89.2%
Yongying Bai, 2016 [53]	0.77 [0.74; 0.80]	85.5%	0.79 [0.76; 0.82]	85.2%	11.61 [8.89; 15.16]	89.4%
Yoshizawa S, 2012 [36]	0.77 [0.74; 0.80]	84.9%	0.78 [0.75; 0.81]	85.0%	11.12 [8.57; 14.42]	89.3%
Zanxi Fang, 2015 [52]	0.76 [0.73; 0.79]	84.2%	0.79 [0.76; 0.82]	85.5%	11.18 [8.52; 14.68]	89.4%
Zeng RC, 2013 [37]	0.77 [0.74; 0.80]	85.2%	0.79 [0.76; 0.82]	85.3%	11.64 [8.89; 15.25]	89.5%
Zhang H, 2016 [11]	0.77 [0.74; 0.80]	85.1%	0.78 [0.75; 0.81]	84.8%	11.13 [8.46; 14.65]	89.3%
Zhang J, 2018 [35]	0.77 [0.74; 0.80]	85.2%	0.79 [0.76; 0.82]	85.3%	11.63 [8.89; 15.23]	89.5%
Zheng D, 2011 [54]	0.77 [0.74; 0.80]	85.0%	0.79 [0.76; 0.82]	85.1%	11.41 [8.74; 14.89]	89.4%
Zhu C, 2014 [45]	0.77 [0.74; 0.80]	85.3%	0.78 [0.75; 0.81]	83.6%	10.88 [8.27; 14.31]	89.0%

Methodological quality of the eligible studies: A summary of the quality of the included studies is described in fig. 2. Among these studies, only one study[64] depicted high quality, representing low bias risk and low concern for patient applicability. Studies were assessed as having a high risk of bias and high applicability concerns in the index test domain because there was knowledge of cancer by reference test and the cut-off values were determined based on the ROC curve instead of predefined. Regarding flow and timing, there was one study[13] with a high risk because of the confirmation of the diagnosis by different reference standards and eight studies with unclear risks due to inappropriate intervals between the index test and reference test. In the two remaining domains, unclear risks rarely occurred, primarily in consecutive samples and blind tests.

Diagnostic accuracy of stem-loop RT-qPCR-based plasma miRNAs in cancer: A number of 10,218 cases and 8,990 controls were provided to estimate the pooled sensitivity, specificity, PLR, NLR, DOR and AUC of plasma miRNAs in cancer detection. Due to substantial heterogeneity in sensitivity, specificity and DOR reports (sensitivity: I^2 = 85.1%, p-value < 0.001; specificity: I^2 = 85.1%, p-value < 0.001; DOR: I^2 = 89.4%, p-value < 0.001) (Supplementary Table 2), a random-effects model was applied for these analyses. The overall sensitivity and specificity were 0.77 (95% CI, 0.74–0.80) and 0.79 (95% CI, 0.76–0.82), respectively (Table 2).

The estimated DOR of 11.44 (95% CI, 8.78–14.92) and AUC of 0.81 (95% CI, 0.78–0.83) (Table 2), along with the SROC curve (Fig. 3), suggested a qualified performance of plasma miRNAs in discriminating patients with cancer from controls with moderate accuracy. The PLR and NLR were assumed to be 3.06 (95% CI, 2.74–3.31) and 0.34 (95% CI, 0.31–0.38) respectively (Table 2). These findings indicated that stem-loop RT-qPCR-based plasma miRNA profiling had moderate accuracy in diagnosing cancer.

Threshold effect and subgroup analyses: Due to substantial heterogeneity in the meta-analysis, we investigated possible sources from the threshold and the non-threshold effects. To verify whether the diagnostic threshold could be a source of heterogeneity, we determined the correlation coefficient (r) of sensitivity and specificity using Spearman's test[45]. A threshold effect with an r-value ≥ 0.6 is indicated as significant[12]. As a result, no diagnostic threshold-derived heterogeneity was observed in the meta-analysis (r-value = 0.366).

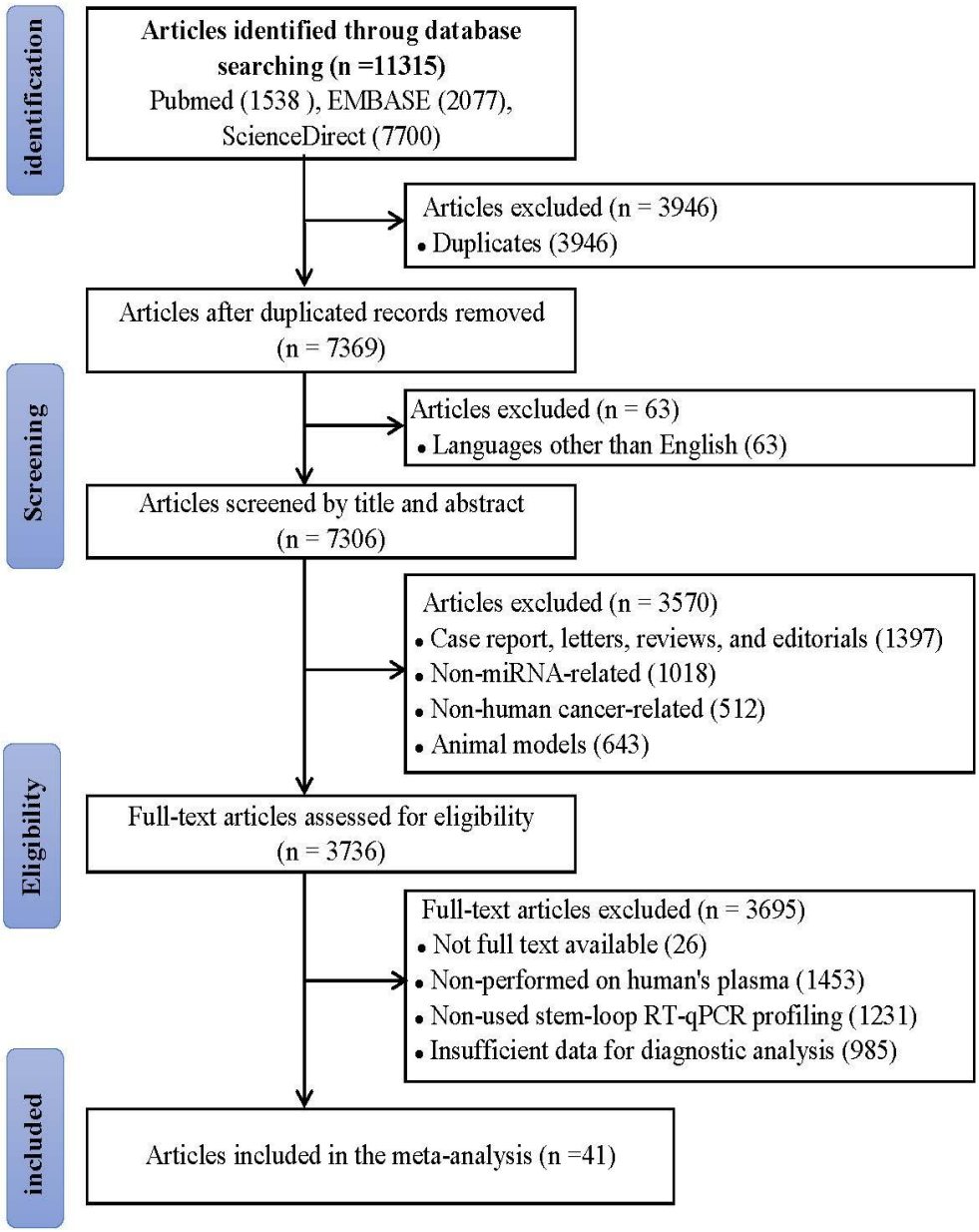


Fig. 1: Flowchart of study identification for the meta-analysis

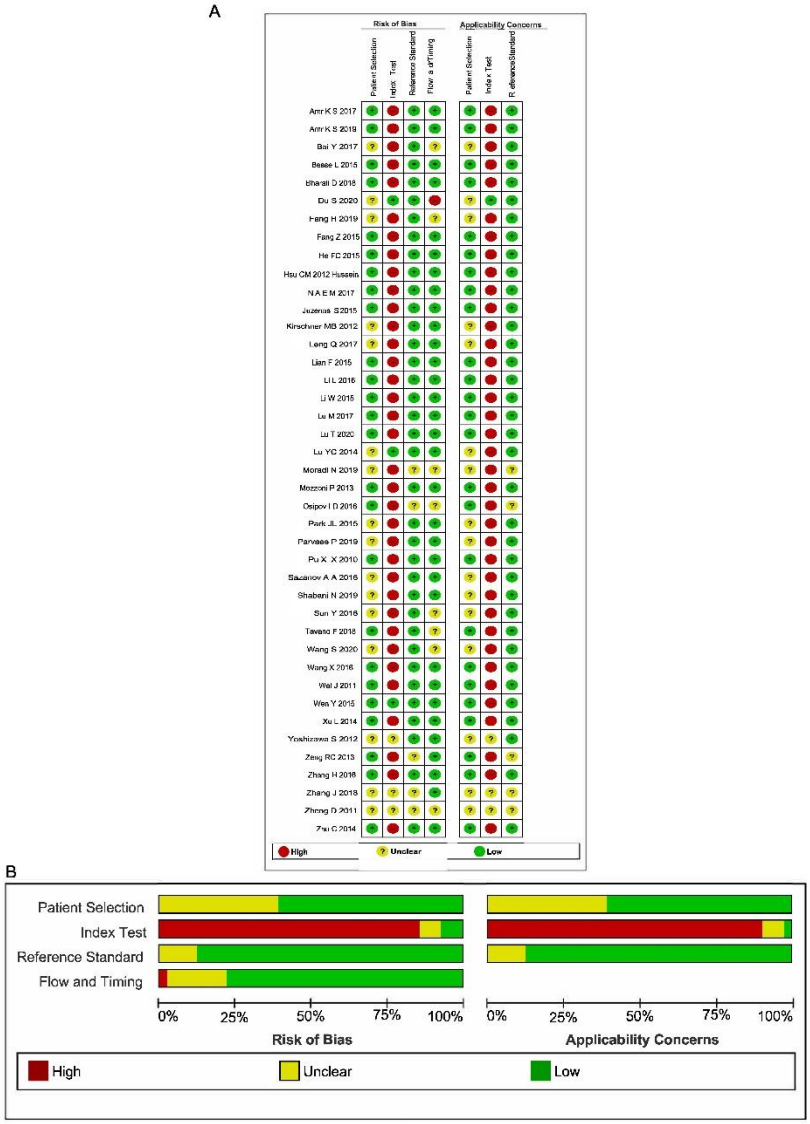


Fig. 2: The result of quality assessment for the included studies

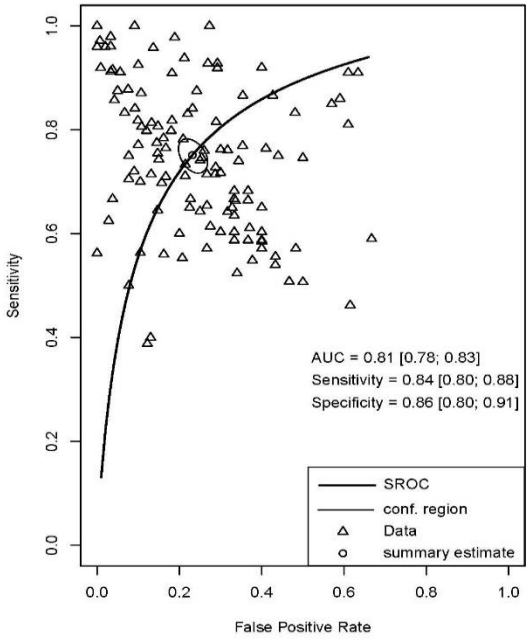


Fig. 3: The SROC curve for stem-loop RT-qPCR-based plasma miRNA profiling in cancer detection.

Table 2
The results of diagnostic measurements for plasma miRNAs profiling in cancer detection.

	Analysis	No of studies	Sensitivity [95%CI]	Specificity [95%CI]	PLR [95%CI]	NLR [95%CI]	DOR [95%CI]	AUC [95%CI]	Regression
	Overall	127	0.77 [0.74; 0.80]	0.79 [0.76; 0.82]	3.06 [2.74; 3.41]	0.34 [0.31; 0.38]	11.44 [8.78; 14.92]	0.81 [0.78; 0.83]	
	miRNA type								
	Single-miRNA	106	0.75 [0.72; 0.79]	0.77 [0.73; 0.80]	2.75 [2.46; 3.07]	0.38 [0.34; 0.42]	9.35 [7.07; 12.36]	0.79 [0.76; 0.82]	0.891
	Multiple-miRNA	21	0.84 [0.80; 0.88]	0.86 [0.80; 0.91]	5.58 [3.87; 8.04]	0.20 [0.16; 0.26]	30.04 [17.02; 53.02]	0.91 [0.89; 0.94]	0.187
	Ethnicity								
	Asian	58	0.81 [0.78; 0.84]	0.82 [0.77; 0.86]	4.09 [3.41; 4.90]	0.25 [0.21; 0.30]	18.47 [12.84; 26.57]	0.87 [0.85; 0.89]	0.079
	Caucasian	69	0.73 [0.69; 0.77]	0.75 [0.71; 0.79]	2.38 [2.10; 2.70]	0.46 [0.41; 0.51]	7.25 [5.20; 10.09]	0.76 [0.73; 0.79]	0.008
	Measurement method								
	Taqman	99	0.76 [0.72; 0.79]	0.80 [0.76; 0.83]	3.13 [2.76; 3.57]	0.35 [0.31; 0.40]	11.54 [8.33; 16.00]	0.80 [0.77; 0.83]	0.004
	SYBR-green	25	0.82 [0.77; 0.87]	0.75 [0.69; 0.80]	2.83 [2.36; 3.40]	0.29 [0.23; 0.36]	12.30 [8.51; 17.78]	0.86 [0.83; 0.89]	0.079
	Normalizer type								
	Endogenous normalizer	77	0.74 [0.70; 0.77]	0.77 [0.73; 0.80]	2.65 [2.34; 3.01]	0.40 [0.36; 0.45]	8.66 [6.21; 12.06]	0.78 [0.74; 0.81]	0.038
	Exogenous normalizer	34	0.80 [0.72; 0.87]	0.81 [0.74; 0.86]	3.97 [3.12; 5.04]	0.25 [0.19; 0.31]	17.91 [10.82; 29.66]	0.86 [0.83; 0.89]	0.357
	Cancer type								
	BC	5	0.87 [0.68; 0.96]	0.78 [0.69; 0.85]	3.51 [2.28; 5.42]	0.29 [0.18; 0.45]	21.60 [4.89; 95.33]	0.88 [0.81; 0.96]	0.090
	CRC	9	0.82 [0.72; 0.88]	0.71 [0.64; 0.78]	2.62 [2.06; 3.33]	0.27 [0.18; 0.39]	10.81 [5.98; 19.53]	0.84 [0.79; 0.90]	0.889
	GC	14	0.79 [0.70; 0.86]	0.78 [0.66; 0.86]	3.48 [2.21; 5.47]	0.34 [0.24; 0.47]	12.89 [5.37; 30.97]	0.89 [0.86; 0.93]	0.510
	HCC	24	0.79 [0.72; 0.85]	0.75 [0.66; 0.83]	2.33 [1.96; 2.76]	0.34 [0.27; 0.42]	8.86 [6.02; 13.04]	0.82 [0.78; 0.86]	0.364
	NSCLC	13	0.77 [0.72; 0.81]	0.84 [0.81; 0.88]	4.64 [3.76; 5.74]	0.29 [0.23; 0.35]	18.24 [13.28; 25.04]	0.88 [0.86; 0.90]	0.137
	LC	38	0.67 [0.63; 0.71]	0.71 [0.66; 0.76]	2.10 [1.825; 2.42]	0.49 [0.42; 0.56]	5.01 [3.41; 7.35]	0.72 [0.68; 0.76]	0.976
	PC	4	0.98 [0.62; 1.00]	0.98 [0.60; 1.00]	10.60 [3.54; 31.78]	0.06 [0.01; 0.49]	367.89 [12.68; 1067.06]	0.94 [0.36; 1.00]	0.002
	ESCC	3	0.68 [0.62; 0.74]	0.74 [0.63; 0.83]	2.66 [1.64; 4.30]	0.43 [0.32; 0.58]	6.46 [2.87; 14.51]	0.78 [0.70; 0.90]	0.022
	Others	17	0.83 [0.74; 0.90]	0.71 [0.64; 0.78]	6.69 [4.08; 10.97]	0.20 [0.13; 0.31]	43.45 [16.71; 112.94]	0.89 [0.84; 0.94]	0.349

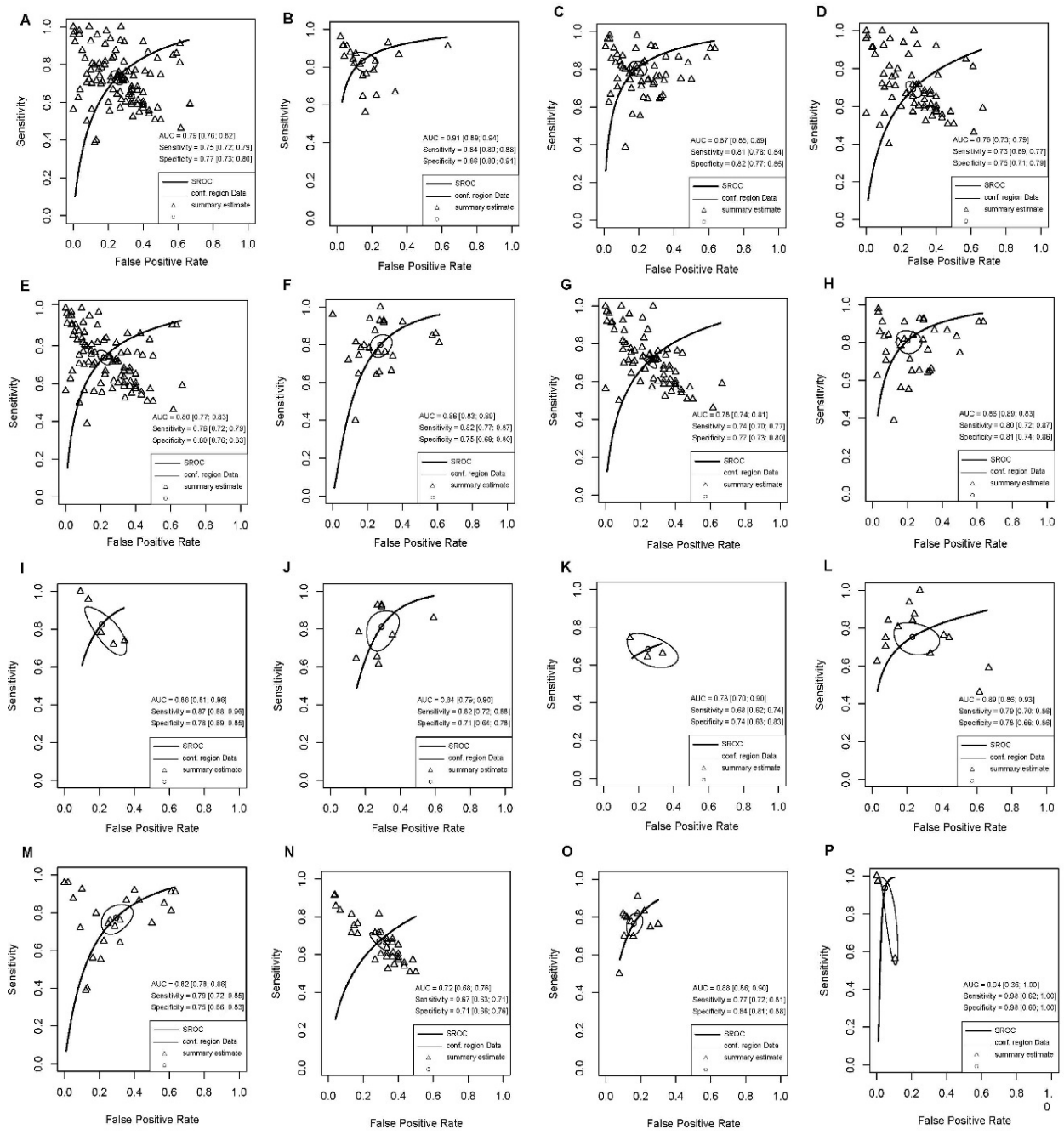
AUC area under the curve; *CI* confidence interval; *DOR* diagnostic odds ratio; *miRNA* microRNA; *NLR* negative likelihood ratio; *PLR* positive likelihood ratio; *BC* breast cancer; *HCC* hepatocellular carcinoma; *NSCLC* Non-Small Cell Lung Cancer; *LC* lung cancer; *CRC* colorectal cancer; *GC* gastric cancer; *PC* pancreatic cancer; *NPC* nasopharyngeal carcinoma; *ESCC* esophageal squamous cell carcinoma.

To address the heterogeneity in the non-threshold effect, we performed the subgroup analysis and meta-regression with the following factors: miRNA type, ethnicity, measurement method, normalizer type and cancer type (Table 2, Supplementary fig. 1). As a meta-regression result, we identified covariates including the Caucasian population, Taqman measurement, endogenous normalizer, ESCC and PC, as possible causes of heterogeneity. The miRNA type-based analysis suggested that multiple-miRNA assays significantly increased diagnostic ability compared to single-miRNA assays, with higher sensitivity (0.84 vs. 0.75), higher specificity (0.86 vs. 0.77), a higher DOR (30.04 vs. 9.35) and a higher AUC (0.91 vs. 0.79) (Table 2).

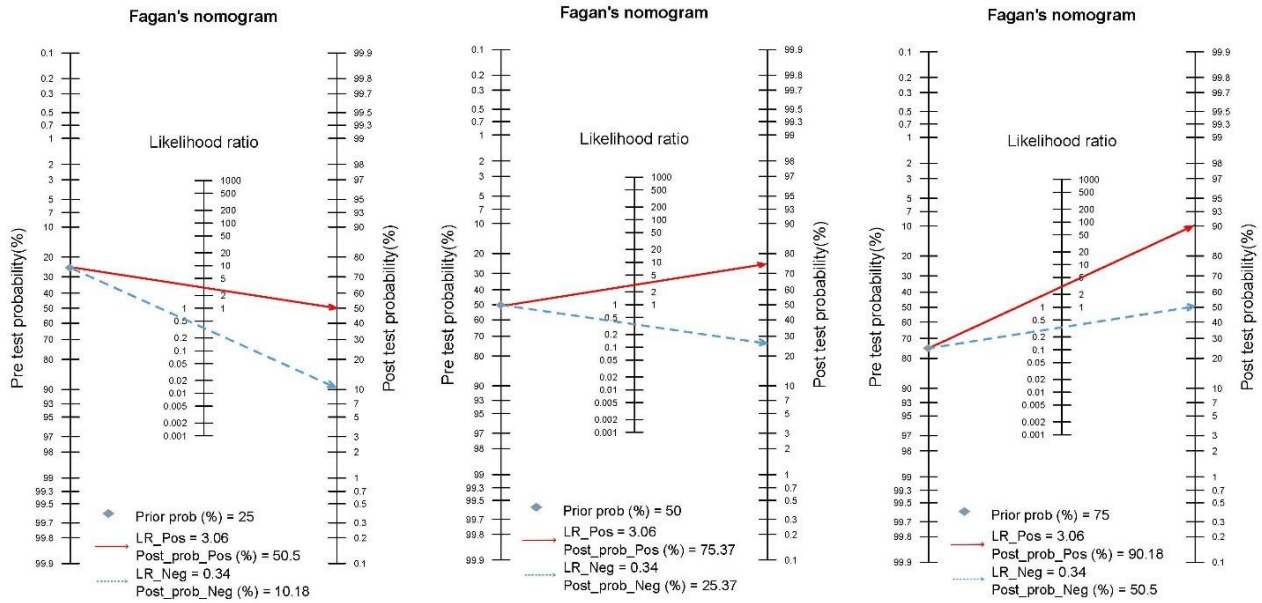
Stratified analysis by ethnicity showed that using plasma miRNAs as cancer biomarkers in Asians was better than those in Caucasians, with a sensitivity of 0.81 vs. 0.73, a specificity of 0.82 vs. 0.75, a DOR of 18.47 vs. 7.25 and an

AUC of 0.87 vs. 0.76 (Table 2). Considering stratification by groups that used endogenous and exogenous normalizers, there was a disparity in the performance of plasma miRNA among the two groups with the exogenous-based group having a higher sensitivity, DOR and AUC. The sensitivity, specificity, DOR and AUC in stem-loop RT-qPCR-based plasma miRNA profiling with exogenous normalizers were 0.80, 0.81, 17.91 and 0.86 respectively, while these values for the group with an endogenous normalizer were 0.74, 0.77, 8.66 and 0.78, respectively (Table 2).

To further investigate the impact of the measurement method used in stem-loop RT-qPCR on the diagnostic accuracy of plasma miRNAs, stratified analysis by Taqman and SYBR-green was conducted. The SYBR-green miRNA assay had slightly higher diagnostic accuracy than the Taqman miRNA assay (Table 2). Lastly, we estimated the discriminative performance of plasma miRNAs in BC, CRC, GC, ESCC, HCC, NSCLC, LC, PC and other cancer types. The subgroup results indicated that the impact of plasma miRNAs varied significantly on diagnostic performance in different types of cancer (Table 2).



Supplementary Figure 1: SROC curves of plasma-miRNA assay in cancer detection. (A) single-miRNA assay. (B) panel-miRNA assay. (C) Asians. (D) Caucasians. (E) Taqman. (F) SYBR-green. (G) endogenous control. (H) exogenous control. (I) BC detection. (J) CRC detection. (K) ESCC detection. (L) GC detection. (M) HCC detection. (N) LC detection. (O) NSCLC detection. (P) PC detection



Supplementary Figure 2: Posttest probabition of stem-loop RT-qPCR-based plasma miRNA profiling for diagnosing cancer

Plasma miRNA assay had the highest discriminative power in PC detection (sensitivity = 0.98, specificity = 0.98, AUC = 0.94) followed by very good diagnostic ability in GC, NSCLC, CRC and HCC with AUC > 0.80 and a good performance in ESCC diagnosis (AUC = 0.78). Plasma miRNAs had the lowest accuracy in discriminating LC patients from healthy persons (sensitivity = 0.67, specificity = 0.71, AUC = 0.72) (Table 2).

Identification of publication bias: We used a trim-and-fill funnel plot and the Egger test for publication bias assessment of the included studies. As a result, a p-value of 0.048 from Egger's regression test revealed possible publication bias across the studies included in the meta-analysis. Besides, the funnel plot's shape was asymmetric and some missing studies were observed in the area of high statistical significance (Fig. 4). These findings suggested that asymmetry was caused by factors other than reporting bias.

Sensitivity analysis: We performed a sensitivity analysis to explore whether our results are sensitive to eliminate each included study. As a result, we found that the lowest sensitivity (0.76, 95% CI: 0.73-0.79) and specificity (0.78, 95% CI: 0.75-0.81) were observed when eliminating study[23,37], while the highest sensitivity and specificity were assumed to be 0.81 (95% CI: 0.78-0.84), 0.82 (95% CI: 0.78-0.85) for excluding study[29] (Supplementary table 2). These results were similar to the overall results (sensitivity = 0.77, 95% CI: 0.74-0.80; specificity = 0.79, 95% CI: 0.76-0.82), suggesting robustness in our conclusions.

Discussion

Incidence and mortality rates from cancer are increasing rapidly around the world. Early cancer screening and diagnosis are critical in improving the survival rates of patients with cancer. Several current studies have shown that the expression of circulating miRNAs alters at the primary, progression and metastasis stages of tumors[7,10,23], implying their potential as cancer biomarkers. In 2008, tumor-derived miRNAs were first identified in prostate cancer plasma, providing the first evidence of plasma miRNAs' potential in cancer diagnosis[41]. Since then, growing number of plasma miRNAs have been recognized as markers for various cancer types.

Besides, RT-qPCR is a preferred approach for identifying circulating miRNA profiles as cancer biomarkers and is used for validating miRNA profiling results from other platforms[25]. Currently, the stem-loop-based RT-qPCR assay is the most popular for clinical miRNA evaluation[25,40]. With the integration of plasma miRNA and stem-loop RT-qPCR profiling as a diagnostic method, we performed a meta-analysis to validate the detection ability of this method for cancer.

The diagnostic significance of plasma miRNA has been documented in many meta-analyzed studies for various cancer types, including breast cancer[53], colorectal cancer[6], pancreatic cancer[33] and osteosarcoma[17]. Our study further extends the possible application of stem-loop RT-qPCR-based plasma miRNA profiling in diagnosing cancer.

Using stem-loop RT-qPCR profiling, plasma miRNA had a very good discriminative performance for cancer with an overall AUC of 0.81 (overall sensitivity = 0.77, overall specificity = 0.79). In addition, the overall DOR was 11.44 with a 95% CI of 8.78–14.92, indicating that the chance of individuals having cancer with a miRNA-test-positive result was 11 times higher than those having negative results. LR is widely used as a diagnostic criterion for determining or ruling out disease[11].

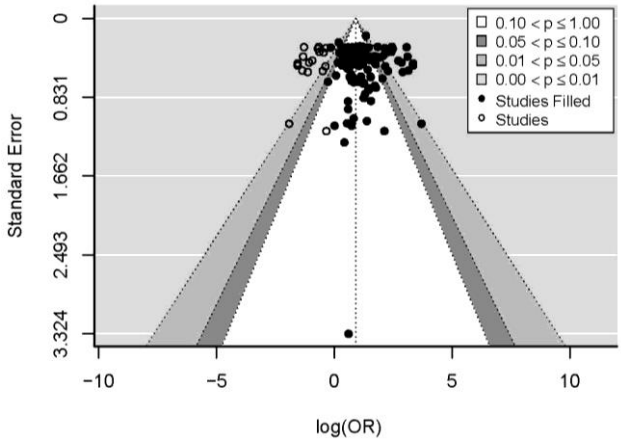


Fig. 4: The trim-and-fill funnel plot asymmetry test for publication bias for stem-loop RT-qPCR-based plasma miRNA profiling

The estimated PLR and NLR of 3.06 and 0.34 respectively revealed a moderate predictor index of stem-loop RT-qPCR-based plasma miRNA in cancer detection. When a pretest probability of 25% was used, the posttest probabilities of positive and negative stem-loop RT-qPCR-based plasma miRNA profiling were 50.5% and 10.18% respectively. These posttest probabilities were 75.37% and 25.37% respectively, when set at 50% of the pretest probability and achieved 90.18% and 50.5% respectively for a pretest probability of 75% (Supplementary fig. 2). Cancer could be confirmed through dysregulation of plasma miRNA based on the stem-loop RT-qPCR test.

However, excluding the diagnosis was insufficient information for a negative examination when the cancer prevalence was 50% or higher. These findings indicated that stem-loop RT-qPCR improved diagnostic effectiveness while having a lesser influence on clinical practice. However, cancer cannot be excluded in high-risk patients with a negative result from stem-loop RT-qPCR-based plasma miRNA profiling and needs further confirmation by other examinations. The application of stem-loop RT-qPCR-based plasma miRNA profiling was influenced by the miRNA type. Although identifying a single miRNA as a cancer biomarker is straightforward and is much more uncomplicated than setting up a panel of multiple miRNAs. The diagnostic performance of a single-miRNA assay is relatively poor.

The limitations of single-miRNA biomarkers are that cancer formation can be viewed as a complicated multistep process related to epigenetic and genetic alterations, while targeting by single-miRNA might not cover completely. The multiple miRNAs, however, generate a stable and reliable network diagnostic structure via various cancer-developed pathways[18,31,67]. Our results showed that the multiple-miRNA assay surpassed the single-miRNA assay in diagnosing cancer. The multiple-miRNA assay had 0.84 of sensitivity, 0.86 of specificity, 30.04 of DOR and 0.91 of AUC whereas these values for the single-miRNA assay were 0.75, 0.77, 9.35 and 0.79 respectively. This finding highlights the benefit of using panels of multiple miRNAs for clinical application in cancer detection.

Besides, ethnicity and normalization strategies affect the diagnostic accuracy of stem-loop RT-qPCR-based plasma miRNA profiling. Our results found that the Asian-based miRNA assay is remarkably better than the Caucasian-based one at diagnosing cancer. Likewise, an exogenous normalizer is more suitable than an endogenous normalizer for quantifying plasma miRNA expression using stem-loop RT-qPCR. With a sensitivity of 0.80, a specificity of 0.81 and an AUC of 0.86, plasma miRNA expression profiling preferred a quality control method based on an exogenous reference control. Further studies are needed to clarify the optimal normalization strategy for stem-loop RT-qPCR-based plasma miRNA profiling for cancer.

In addition, previous evidence has reported that the diagnostic potential of miRNAs was identified as a variation between different types of malignancies[35]. Likewise, our findings revealed the dependence of their performance on the type of cancer. Among the eight cancer types we investigated, the plasma miRNA assay had the highest discriminative power in PC detection (sensitivity = 0.98, specificity = 0.98, AUC = 0.94). In contrast, the lowest accuracy was for distinguishing LC patients from controls (sensitivity = 0.67, specificity = 0.71, AUC = 0.72). This difference might be addressed by the different complicated roles of miRNA in various cancers. A particular miRNA can be oncomiR in one type of tumor and suppressor in another[59]. The expression patterns have changed across cancer types, contributing to the dependence between miRNA diagnostic significance and type of cancer[35].

However, there are some limitations in this study. First, there is significant heterogeneity among the included studies, which may be caused by the differences in ethnicities of the included participants, measurement methods used in stem-loop RT-qPCR and cancer types across studies. Second, one study[29] enrolled a large number of patients, which may affect the overall result. Third, most of the studies showed a high

risk of bias due to knowledge of the reference test's result for interpreting the result of the index test and the optimal threshold for estimating sensitivity and specificity. Lastly, publication bias existed in this meta-analysis. Factors other than reporting bias, including substantive heterogeneity or chance, are possible sources of funnel plot asymmetry in our meta-analysis. Nevertheless, our results are robust in the diagnostic accuracy of plasma miRNA for cancer using stem-loop RT-qPCR for profiling.

Conclusion

Based on stem-loop RT-qPCR profiling, miRNAs in plasma, especially miRNA panels, had very good diagnostic performance and could serve as a potential method for minimally invasive cancer detection and screening. A cautionary interpretation should be performed for high-risk patients with a negative result from stem-loop RT-qPCR-based plasma miRNA profiling. Further trials nested in population cohorts are needed to assess miRNA in pre-diagnostic plasma samples to verify their diagnostic feasibility in cancer.

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