Diagnostic acuuracy of cerebrospinal fluid and serum-isolated exosomes for glioblastoma: a systematic review and meta-analysis

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Abstract

Glioblastoma (GBM) is the most malignant glioma cancer with a high morbidity and mortality worldwide. Unfortunately, a routine method is not available for screening or preoperative early detection of GBM. However, early detection in a none-invasive or minimally invasive method could be beneficial and increase the survival rate. In this systematic review and meta-analysis, we aimed to examine the diagnostic accuracy of exosomal RNAs that were extracted from patients' CSF or serum for GBM diagnosis. We searched Web of Science, Scopus, PubMed (including Medline), Embase and ProQuest (as databases for grey literature) up to December 2019; we also performed backward and forward reference checking of included and relevant studies. Finally, included studies were assessed with QUADAS-2 checklist and their data extracted. We carried out a meta-analysis of included study, regarding to the diagnostic meta-analysis guidelines for obtaining pooled accuracy estimates. In addition, sensitivity analysis and meta-regression were also conducted. We retrived 1730 records from databases, nine of them included in systematic review and qualitative synthesis. Six studies were considered to statistical analysis and performed diagnostic meta-analysis. Our results suggested that the pooled sensitivity and specificity of exosomal biomarkers for GBM were 0.76 and 0.80, respectively. In addition, the pooled positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR) were 3.7, 0.30 and 12, respectively. The overall area under the curve (AUC) of exosomal biomarkers for GBM diagnosis was found to be 0.85. According to our results, the value of 0.85 for AUC, suggesting that exosomal biomarkers might serve as a high potential and non-invasive diagnostic tool for GBM.

1. Introduction

Glioblastoma or glioblastoma multiforme (GBM) is grade IV and the most malignant type of gliomas [1, 2]. For GBM cure, traditional treatment is tumor resection followed by radiotherapy and chemotherapy that is limited in effectiveness due to high rates of relapse, overall resistance to therapy, and serious neurotoxicity and neurological side effects [3, 4]. Due to lacking early detection and personalized treatment of GBM, the prognosis is often poor [5].

Detection and biopsy of tumors, especially in brain cancer, is high invasive, expensive and time-consuming. For this reason, developing of non-invasive, affordable and efficient methods for detection and grade prediction matters a great deal. In this regard, identification of biomarkers of brain cancer in the patient's body fluids, especially CSF and serum, including DNAs, miRNAs, mRNAs, lncRNAs, and proteins could be much helpful for diagnosis of GBM and other neurological cancers and diseases [5].

In the past several decades, many studies aimed to introduce new, accurate, and specific biomarkers for different types of cancers [6-8]. One of the new type of biomarkers that introduced recently, is the biomarkers that occure inside the exosomes. Exosomes are a type of extracellular nanovesicles with 30-150 nm in diameters [9]. Regarding the origin, exosomes are distinct from other types of extracellular vesicles including microvesicles (with heterogeneous size from 50 to 1000 nm in diameter) and apoptotic bodies [10, 11]. However, microvesicles are crucial in cellular communication and has been used as a

biomarker source similar to exosomes, but they are not popular as exosomes [12]. However, there is a problem in terminology of exosomes and microvesicles; sometimes, they are mistakenly used interchangeably [13].

Exosomes are the carreres of their origin biomarkers that most of the time are indistinguishable in the corresponding biological fluid as cell-free or naked biomarkers [14]. As a result of the latter property of exosomes, they can provide various sensitive and specific diagnostic biomarkers for different pathophysiological conditions [11]. At the beginning, Valadi et al. for the first time stated that exosomes, in addition to proteins, possess different types of RNAs [15]. Today it is well known that exosomes transport diverse molecular constituents (such as lipids, proteins, and nucleic acids) of their source cells and tissues [10]. Next, over important finding about exosomes biological nature and their contents, scientists made such an idea that exosomal contents as a tool for discriminating cancerous cells from healthy cells may simplify cancer diagnosis through a minimal invasive approach [16]. Interestingly, furthure studies demonstrated that the amount of exosomes in patients with cancer is higher than healthy controls [17]. Exosomal biomarkers compared to other biomarkers enable similar or higher specificity and sensitivity due to their higher stableness compared to unvesicled or cell-free biomarkers. New technical improvements in exosome isolation facilitate exosome study, and it could make exosomal diagnostics as a new method for diagnosis of diseases, especially cancers [16, 18].

2. Methods

We designated a protocol following PRISMA guideline for reporting of a systematic review, and Protocols for Reviews of Diagnostic Test Accuracy [19]. Prior to publication of the study, we were submitted this systematic review on PROSPERO on 26 Jun 2019 and registered on 17/09/2019 with CRD42019132438 ID.

2.1. Search strategy designing

To retrieving all possible studies in the area of our purpose, we aimed at developing a full search strategy. We were implemented a comprehensive systematic search which were combined (using the Boolean Operator) text-words and subject headings (MeSH or equivalent) of the following electronic databases: PubMed (including Medline), Embase, Web of Science, Scopus, and ProQuest (as databases for grey literature) through December 2019. The search was performed according to the search strategies stated in the protocol Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy [20]. For the search in electronic databases, we used the all possible keywords related to "Exosomes" and "Glioblasoma" that was extracted from MeSH database and Emtree. This strategy of search allowed us to conducting of a comprehensive search by recent published protocol [21]. The databases were searched without any restriction.

The literature search was performed independently by two investigators (AT and DJ). Any possible discordance was compared to that of an additional investigator (YM). In order to retrieve any missed related articles through database searching, in addition to the cited references in the final identified

studies, a comprehensive search was carried out for the published reviews adapted to our title. Subsequently similar to systematic screening of online databases results, the references of all these articles screened manually. This step ensures that all studies relevant to assessing the diagnostic accuracy of exosomal RNAs in the diagnosis and early detection of GBM are included in our final library. Full search strategy of PubMed, Scopus, Embase and Web of Science databases was shown in Table S1 in Aditional file 1.

2.2. Data extraction and management

2.2.1. Selection of studies

Two reviewers independently (DJ and FJ) screened the titles and abstracts of all retrieved records and then evaluated to determine whether inclusion criteria were met. Inclusion assessment of full papers was conducted by one author (DJ) and checked by a second investigator (AT). In case of disagreement, a consensus was reached by discussion or referral to a third author (YM). We conducted PRISMA diagram to illustrate the study selection process [19].

2.2.2. Inclusion criteria

We included all original articles that were surveyed diagnostic value of exosomes RNAs in human GBM patients, studies that conducted on body fluid isolated exosomes and studies that were conducted on two types of extracellular vesicles (exosomes, microvesicles).

2.2.3. Exclusion criteria

We excluded duplicate citations, non-peer-reviewed, review papers and book chapters.

2.2.4. Data collection process

The following items were collected from each article by two investigators (DJ and ER): first author, publication year, country, number participants, exosomes source, isolation and purification methods, related identified biomarkers, biomarker extraction method, biomarker analyzing/profiling method, area under the curve (AUC), confidence interval 95% (CI95%), true positive (TP), true negative (TN), false positive (FP) and false negative (FN). The TP, TN, FP and FN data extracted from the 2×2 table of studies or (if this table not provided with studies) calculated using the specificity and sensitivity. The data extracted in fully pair method from individual studies. We sought further information from the study authors if necessary. Any disagreements between data collectors were resolved through either discussion or consultation with a third author (AT).

2.3. Assessment of methodological quality and risk of bias

We also assessed the methodological quality of the included studies independently by two reviewer (DJ and AT) by using the revised Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool, recommended by the Cochrane collaboration, for risk of bias and applicability concerns [22]. It assesses

the risk of bias by scoring questions in the four domains as follows: 1. Patient Selection (the method of patient selection and the patients included), 2. Index Test (the test being studied and how it was conducted and interpreted), 3. Reference Standard (the reference standard test used and how it was conducted and interpreted), and 4. Flow and Timing (the flow of patient inclusion and exclusion, testing procedure and the interval between tests). The first three domains also assess the applicability concerns considering review question. Each of domains was categorized as high, low, or unclear and disagreements were resolved by discussion with a third reviewer (RJ).

2.4. Statistical Analysis

We used the metandi and midas modules in the STATA 11.2 (Stata Corporation, College Station, TX, USA) statistical software to perform all analyses [23, 24]. TP, FP, FN and TN data were used to calculate sensitivity, specificity, positive and negative likelihood ratio and diagnostic odds ratio. Their pooled estimates and their corresponding CI 95% for exosomes were calculated by using the bivariate and hierarchical meta-analysis that included bivariate mixed-effects regression model and the hierarchical summary ROC (HSROC) modeling [25, 26]. Results were displayed graphically on forest-plots and HSROC curve. Heterogeneity between included studies was assessed using Cochran's Q test and the inconsistency index (I^2) describing the percentage of total variation across studies due to heterogeneity rather than chance [27]. A p-value ≤ 0.05 and an I^2 value ≥ 50% would indicate substantial heterogeneity. The threshold effect was checked using Spearman's rho and potential sources of heterogeneity were explored by meta-regression. Sensitivity analysis was performed by omission of outlier studies indicated by Cook's distance and standardized predicted random effects to investigate the influence of this study on the pooled estimates [24]. We assessed publication bias using Deek's funnel plot, and a p-value < 0.1 in the Deek's asymmetry test was considered to indicate publication bias [28].

3. Results

3.1. Literature search and study selection

Initially, 1730 articles (279 from PubMed, 438 from Scopus, 400 from Web of Science and 613 from Embase) were retrieved based on the search strategies from the online databases. From wich, 900 duplicate publications were identified and removed. The remaining 830 articles were screened by titles and abstracts, led to the exclusion of 802 items, including 259 reviews and 196 conference abstracts. The full texts of the 28 remaining records studied, and 19 articles were excluded according to inclusion and exclusion criteria. Finally, nine eligible studies were included in the present systematic review from 2013 to 2019 and from four countries of Australia, China, Italy and USA (Table 1). Figure 1 shows the process of literature search and study selection as a flow diagram based on the PRISMA.

Table 1 Bibiliographic information of included primary studies regarding to publication date

NO	Title	First author	Year	Country	Ref
1	miR-21 in the Extracellular Vesicles (EVs) of Cerebrospinal Fluid (CSF): A Platform for Glioblastoma Biomarker Development	Akers	2013	USA	[29]
2	A small noncoding RNA signature found in exosomes of GBM patient serum as a diagnostic tool	Manterola	2014	China	[30]
3	Exosomal levels of miRNA-21 from cerebrospinal fluids associated with poor prognosis and tumor recurrence of glioma patients	Shi	2015	China	[31]
4	A cerebrospinal fluid microRNA signature as biomarker for glioblastoma	Akers	2017	USA	[32]
5	Serum exosomal miR-301a as a potential diagnostic and prognostic biomarker for human glioma	Lan	2017	China	[33]
6	A microRNA signature from serum exosomes of patients with glioma as complementary diagnostic biomarker	Santangelo	2018	Italy	[34]
7	Deep sequencing of circulating exosomal microRNA allows non-invasive glioblastoma diagnosis	Ebrahimkhani	2018	Australia	[35]
8	Serum long noncoding RNA HOTAIR as a novel diagnostic and prognostic biomarker in glioblastoma multiforme	Tan	2018	USA	[36]
9	Serum miR-29b as a novel biomarker for glioblastoma diagnosis and prognosis	Zhong	2019	China	[37]

3.2. Quality assessment

According to the results of quality assessment using QUADAS-2 check list in Figure 2 and Table S2 (in Aditional file 1), in the section of risk of bias, the most elements lowering the quality of studies are reference standard decription as well as the index test. In this regard, some studies did not reported ratios of diagnostic value. In addition two studies reported diagnostic potential of an exosomal biomarker for GBM from other types of disease or cancers. In the patient selection, flow and timing section totall six studies were unclear or weak. This is due to undefined patients pathophysiological condition, sex, and age as well as some technical analysis and experimental efforts.

In the case of applicability, consequently as expected, the index test is still suffering from low quality in four studies due to low applicability of developed index test for diagnosis of GBM. However, the rest of the studies have developed their index test compliance with our review question and inclusion criterias.

Alltogether, we have found that only the study of Manterola et al. [30] was perfectly meet our inclusion criterias and review questions in all parts of risk of bias and applicability assessment sections. Finally, we identified six studies that filled the thresholds to be included in meta-analysis and three studies were excluded (Figure 1).

Figure 2 Quality assessment of the included studies performed using the QUADAS-2 checklist.

3.3. The characteristics of studies included in systematic review and meta-analysis

Altogether, 487 patients histopathological and MRI diagnosed with GBM cencer were investigated for biomarker development in nine included studies. The patient population is ranged from 13-107 and the control population consisted of healthy controls, non-brain tomur patients [33] and other brain tomur patients [37].

Six studies used the serum, two studies used CSF and one study used both serum and CSF as biomarker source. In addition, one study used CSF from two different anatomical loacation (cisternal and lumbral) (Table 2).

In order to analysis of exosomal biomarkers, exosomes must first be isolated and purified from the other vesicles and molecules of the serum or CSF samples. As shown in Table 2 three studies used ultracentrifugation (UC), five studies used commercial kits and one study used size exclusion chromatography for isolation and purification of exosomes. In this regard three methods of exosome isolation was used in this studies (Table 2).

For RNA extraction from isolated exosomes, entirely all of the studies used RNA extraction commercial kits (Table 2). Besides, for profiling and analyzing the expression pattern of selected RNAs or between patients and controls, q-RT-PCR and RNA sequencing methods were used. From wich, eight studies used q-RT-PCR and only one study used sequencing.

Table 2 The methods and workflow extracted data from the included studies for GBM diagnosis.

First author	Patients	HC	Exosomes Source	Exosome Isolation	Biomarker Extraction	Biomarker Analysis
Akers (2013)	13 15	14 16	CSF	UC	mirRCURY kit	qRT-PCR
Akeres (2017)	28: 10P+12HC (17M , 5F) 53.5 (29-74) 18P +20HC (15M, 23F) 58 (27-74)		CSF-Cic CSF-Lum	UC	miRCURY™	qRT-PCR
Ebrahimkhani	12: 7M, 5F (63.3 ±11.5) VC: 3M1F (33-56)	7M, 5F(56.2 ± 12.4) V: 5F, 4M (36.2 ± 10.3)	S	SEC	Exosomal RNA Purificationi Kit	Eukaryote Total RNA chip
Lan	27: (30-72Y)	43	S	ExoQuick	mirVana kit	qRT-PCR
Manterola	25: 14M, 11F(30–75) VC 50: 30M, 20F (17–79)	14M, 11F(45-78) 14M, 16F(27-70)	S	Exoquick	Trizol	qRT-PCR
Santangelo	69: 43M, 26F (61 ± 12)	13M, 17F (41 ± 12)	S	ExoQuick	Trizol	qRT-PCR
Shi	95	50	CSF and S	UC	RNeasy Kit	RT-qPCR
Tan	43	40	S	Exosome Isolation reagent	mirVana kit	qRT-PCR
Zhong	107: 56M, 51F (45 <50 and 61 >50 mean = 50.4)	80 control 40 patients with anaplastic astrocytoma	S	Exoquick	miRNeasy	qRT-PCR

M: Male, F: Female, UC: Ultracentrifuse, SEC: Size Exclusion Chromatography, S: Serum, CSF: Cerebrospinal Fluid, C: Cohort, VC: Validation Cohort

3.4 Qualitative synthesis of diagnostic value of exosomal RNAs for GBM

In Table 3, extracted data related to diagnostic value of different biomarkers including, AUC, CI95%, TP, TN, FP and FN as well as specificity, sensitivity and their cut-offs, has been shown.

Through nine studies, 24 single RNA biomarker and there miR-panels developed for diagnosis of GBM. Among the single biomarkers, miR-21 was investigated in four individual study [29, 31, 32, 34]. In addition miR-222 , miR-29b, miR-320, miR-574-3p, miR-124-3p, RNU6 and HOTAIR biomarkers were also individually evaluated for the diagnosis of GBM in different studies. According to the studies provoded, or calculated specificity and sensitivity of these single biomarkers, they shows high diagnostic potential for GBM (Table 3). From wich, miR-21 with 85 and 100, 87 and 93, 84 and 77 for sensitivity and specificity respectively in three different GBM patient populations shows a high diagnosis value for GBM. Interestingly, in the study conducted by Lan et al. [33], they assessed the expression level of this miR-301 in healthy controls, but they did not performed any diagnostic analysis. However, they only provided diagnostic values for this miR for discriminating GBM from none-brain tomur patients.

In the context of panels, a panel consist of three RNAs (RNU6, miR-320 and miR-574-3p) showed 87% and 86%, 70% and 71% for sensitivity and specificity respectively in two groups of GBM patients [30]. Another panel with nine miRs (miR-21, miR-218, miR-193b, miR-331, miR-374a, miR-548c, miR-520f, miR-27b and miR-130b) showed 80% and 67%, 95% and 28% for sensitivity and specificity respectively in cisternal and lumbar CSF derived exosomes in two populations of GBM patient compared to healthy controls [32]. Finally another miR panel with miR-21, miR-222 and miR-124-3p showed 84% and 77% for sensitivity and specificity [34]. In addition to these panels, Ebrahimkhani et al. [35] developed six miR panels that predict GBM with perfect accuracy. Their panels including panel 1 (miR-328 and miR-485), panel 2 (miR-485 and miR-340), panel 3 (miR-182, miR-328 and miR-485), panel 4 (miR-485, miR-339 and miR-340), panel 5 (miR-543, miR-328, miR-485 and miR-340) and panel 6 (miR-543, miR-182, miR-485, miR-339 and miR-340), although have perfect accuracy but they did not reported any sensitivity and specificity for these panels.

Table 3 Diagnostic value data of included primary studies in meta-analysis

Author (Year)	Biomarker	AUC	95% CI	Tp	Fp	Fn	Tn	SEN	SPE	PLR	NLR	DOR	Accuracy	Cut-off
Akers (2013)	miR-21 *(P1)*	0.91	0.797-1	11	0	2	14	0.85	1	24.64	0.18	133.4	0.93	0.25 (Copy/EV)
	miR-21 *(P2)*			13	2	2	14	0.87	0.88	6.93	0.15	45.5	0.87	
Akers (2017)	miR-21, miR-218, miR-193b, miR-331, miR-374a, miR-548c, miR-520f, miR-27b, miR-130b *(P1)*	0.75	0.53-0.97	8	4	2	8	0.8	0.67	2.4	0.3	8	0.73	0.4 (FC)
	miR-21, miR-218, miR-193b, miR-331, miR-374a, miR-548c, miR-520f, miR-27b, miR-130b *(P2)*	0.83	0.63-0.96	5	1	13	19	0.28	0.95	5.56	0.76	7.31	0.63	0.4 (FC)
Manterola (2014)	miR-574-3p	0.73	0.58–0.89	15	10	10	15	0.6	0.6	1.5	0.67	2.25	0.60	0.454 (FC)
	miR-320	0.72	0.56–0.87	16	9	9	16	0.64	0.64	1.78	0.56	3.16	0.64	0.477 (FC)
	RNU6-1 P1	0.85	0.74–0.96	18	8	7	17	0.72	0.68	2.25	0.41	5.46	0.70	0.454 (FC)
	RNU6-1 P2	0.72	0.60–0.84	33	10	17	20	0.66	0.67	1.98	0.51	3.88	0.66	0.372 (FC)
	RNU6, miR-320, miR-574-3p *(P1)*	0.92	0.84–1	22	4	3	21	0.88	0.84	5.5	0.14	38.5	0.86	0.349 (FC)
	RNU6, miR-320, miR-574-3p *(P2)*	0.77	0.65-0.90	35	9	15	21	0.7	0.7	2.33	0.43	5.44	0.70	0.347 (FC)
Santangelo (2018)	miR-21	0.84	0.75–0.93	37	7	7	23	0.84	0.77	3.6	0.21	17.37	0.81	1.78 (FC)
	miR-222	0.80	0.69–0.89	25	0	19	30	0.57	1	35.13	0.44	79.77	0.74	129.41 (FC)
	miR-124-3p	0.78	0.67–0.89	39	11	5	19	0.89	0.63	2.42	0.18	13.47	0.78	2.95 (FC)
	miR-21, miR-222, miR-124-3p	0.87	0.78–0.95	37	7	7	23	0.84	0.77	3.6	0.21	17.37	0.81	0.90 (FC)
Tan (2018)	HOTAIR	0.91	NR	37	5	6	35	0.86	0.88	6.88	0.16	43.17	0.87	10.8 (FC)
Zhong (2019)	miR-29b	0.86	NR	89	15	18	65	0.83	0.81	4.44	0.21	21.43	0.82	NR

FC: Fold Change, AUC: Area Under Curve, Se: Sensitivity, SP: Specificity, TN: True Positive, TN: True Negative, FP: False Positive, FN: False Negative, PLR: Positive Likelihood Ratio, NLR: Negative Liklihood Ratio, DOR: Diagnostics Odds ratio, NR: Not Reported

Altogether, 24 biomarkers were analyzed for their diagnostic value in the diagnosis of GBM. These 24 biomarkers consist of one long none-coding RNA (LncRNA),one small none-coding RNA (SncRNA), one protein and 22 mi-RNA.

Table 4 The exosomal biomarkers for GBM diagnosis in the included nine studies

No	Biomarker	Type	First Author
1	miR-21	micro-RNA	Akers (2013), Akers (2017), Santangelo, Shi.
2	miR-486-5p	micro-RNA	Ebrahimkhani
3	miR-182-5p	micro-RNA	
4	miR-328-3p	micro-RNA	
5	miR-339-5p	micro-RNA	
6	miR-340-5p	micro-RNA	
7	miR-543	micro-RNA	
8	miR-485-3p	micro-RNA	
9	miR-301a	micro-RNA	Lan
10	RNU6-1	Small non-coding-RNA	Manterola
11	miR-320	micro-RNA	
12	miR-574-3p	micro-RNA	
13	miR- 222	micro-RNA	Santangelo
14	miR-124-3p	micro-RNA	
15	lncRNA HOTAIR	Long non-coding-RNA	Tan
16	miR-193b	micro-RNA	Akeres
17	miR-218	micro-RNA	
18	miR-331	micro-RNA	
19	miR-374a	micro-RNA	
20	miR-27b	micro-RNA	
21	miR-130b	micro-RNA	
22	miR-520f	micro-RNA	
23	miR-548c	micro-RNA	
24	miR-29b	micro-RNA	Zhong

3.5.1 Diagnostic accuracy

Meta-analysis was carried out on the data from six studies including 592 participants (325 GBM patients and 267 healthy controls) on 16 exosomal biomarkers. We excluded three articles from meta-analysis due to unreported TP, FP, FN and TN as well as working on patient controls [31, 33, 35].

Across all six included studies, the pooled sensitivity and specificity of exosomal biomarkers for detecting the presence of GBM were 0.76 (95% confidence interval [CI] = 0.68−0.82) and 0.80 (95% CI = 0.72−0.86), respectively. A forest plot of the included studies along with sensitivities, specificities and pooled estimates is provided in Figure 3. A significant heterogeneity was observed in the pooled sensitivity (I^2= 73.44%, P< 0.001) and specificity (I^2= 59.24%, P< 0.001).

The pooled PLR, NLR, and DOR were 3.7 (95% CI: 2.7−5.2), 0.30 (95% CI: 0.23-0.41), and 12 (95% CI: 7−21), respectively (Aditional file 1, Figure S1 and Figure S2). This statistical measures showed values of I^2> 50% (P< 0.05), suggesting substantial heterogeneity except for PLR.

The hierarchical summary receiver operating characteristic (HSROC) curve for diagnostic accuracy of exosomal biomarkers is provided in Figure 4. The area under the curve (AUC), as an overall measure for test performance, was 0.85 (95% CI: 0.81-0.87), indicating that exosomal biomarkers have good diagnostic accuracy for GBM.

3.5.2 Publication bias

The potential publication bias was examined by the Deeks' funnel plot. As displayed in Figure 5, a P-value of 0.70 indicated no evidence of publication bias in the meta-analysis.

3.5.3 Heterogeneity

Due to no threshold effect existence and given the presence of between-study heterogeneity, we conducted a meta-regression to finding source of heterogeneity. The multiple meta-regression of sensitivity and specificity indicated that source and isolation method of exosomes, RNA extraction methods, sample size and quality of study acted as the potential source of heterogeneity in the pooled estimates (Aditional file 1, Figure S3). Meta-regression result is shown in Table 5.

3.5.4 Sensitivity analysis

We found two studies including Akers 2017 (p2) and Santangelo 2018 (miR-222) as outlier by Cook's distance and standardized predicted random effects (Aditional file 1, Figure S4). When these outliers were omitted, the pooled sensitivity and specificity were 0.79 (95% CI 0.73−0.84) and 0.76 (95% CI 0.69−0.81). The omission of these studies not substantially influenced pooled estimates but reduced the extent of between-studies heterogeneity in the pooled sensitivity and specificity from I^2 73.44% to 49.25% and 59.24% to 40.68%, respectively (Aditional file 1, Figure S5).

Table 5. Results of meta-regression analysis for assessment the source of diagnostic accuracy heterogeneity

Variable	Category	Number of biomarkers	Sensitivity (CI 95%)	P-value	Specificity (CI 95%)	P-value
Source of exosomes	Serum	12	0.77 (0.70 - 0.84)	0.59	0.77 (0.69 - 0.84)	0.01
	CSF	4	0.70 (0.52 - 0.88)		0.89 (0.80 - 0.99)	
Isolation method of exosomes	EQ	11	0.76 (0.68 - 0.84)	0.18	0.75 (0.68 - 0.82)	0.001
	Other	5	0.76 (0.61 - 0.90)		0.89 (0.81 - 0.97)	
RNA extraction methods	Trizol	10	0.75 (0.66 - 0.84)	0.06	0.74 (0.66 - 0.82)	0.001
	Other	6	0.77 (0.66 - 0.89)		0.87 (0.80 - 0.94)	
Sample size	>60	8	0.79 (0.71 - 0.87)	0.29	0.80 (0.71 - 0.89)	0.07
	<60	8	0.71 (0.59 - 0.82)		0.79 (0.69 - 0.89)	
Quality of study	High	8	0.73 (0.62 - 0.84)	0.02	0.74 (0.64 - 0.84)	0.001
	Low	8	0.78 (0.69 - 0.87)		0.84 (0.76 - 0.91)	

EQ: Exoquic, **CSF**: Cerebrospinal Fluid.

4. Discussion

Cancer is a result of perturbation in cell cycle checkpoints molecules that usually occurs by mutations or other genetic changes in a single cell [38]. This molecular event of the transformation will be exist in all of a tomur cells [39]. Identifying and tracking of the molecular information of cancerous transformation could help to develop new therapies and diagnostics [40].

Malignant gliomas including GBM is a high mortality brain tomur with poor prognosis. There are no preoperative routine diagnosis method for GBM. However GBM most of the times, grew locally and rarely metastasized outside of CNS and if resected in the early stages even could completely cured [41].

In the recent few decades, plasma circulating cancer proteins [42], cell-free DNAs [43] and RNAs [44] were raised as diagnostic tools and significantly increased the prediction power of various cancers. Exosomal biomarkers possess multiple advantages compared to cell-free biomarkers. Cell-free biomarkers continuously oppose to blood degrading enzymes while exosomal biomarkers are encapsulated inside the vesicles and are more stable outside of the body after isolation so that they could be reliable than cell-free biomarkers like RNAs, that are most prone to degradation [13].

Studies showed that exosomal biomarkers, specially RNAs (miRNAs, LncRNAs and mRNAs) could be used for prediction of the cancers and their stages [45]. For instance, exosomal miR-21 with high sensitivity and specificity for discriminating multiple cancers from healthy patients has been widely studied. This exosomal biomarker has different expression in many cancers [46-50].

The present study, with aim to find the diagnostic accuracy of exosomes, is the first systematic review and meta-analysis study for aluation of overall exosomes diagnostic value for GBM. The main goal of this study was to assess how could exosomes be used as a non-invasive method for diagnosis of GBM. Regarding, we conducted a comprehensive search strategy for searching the databases for all possible exosomal biomarkers for GBM. All of the included articles to our systematic review were evaluated the exosomal RNA biomarkers and we did not find any exosomal protein, DNA, lipid or carbohydrates biomarkers that met our inclusion and exclusion criterias. It is worth mentioning that we found only exosomal protein and DNA putative biomarkers for GBM diagnosis that were not suitable for this study.

We first assessed that how these nine included studies conducted the diagnostic experiment using exosomal biomarkers. According to previous studies we found that, the method of exosome extraction, RNA profiling, and analyzing could affect the results of diagnostic evaluation [51-55]. Therefore, the workflow from the extraction of exosomes to extraction and analyzing the biomarker assessed in included studies. Recently ultracentrifugation introduced as the gold standard for exosome isolation [56]. Other methods including, affinity chromatography method [57] and specially the exosome isolation kits are mostly used for exosome isolation [58]. As discussed in detail in our previous review [13], exosomes contents are heterogeneous and dependent on the methods of isolation and purification [59]. In our included studies three methods of exosome isolation (UC, Kit and SEC) and mostly UC and exoquic were used. As downstream analysis of exosomes also are determining factors in the results of diagnostic

studies [54, 60]. We found that different RNA isolation kits, as well as two main methods were used for extraction and profiling of exosomal RNAs (q-RT-PCR and Sequencing).

According to the results of extracted data and quality assessment using QUADAS-2 checklist, we excluded three study and conducted a meta-analysis with six primary research. We calculated pooled estimates of AUC, sensiticity, specificity, DOR, PLR and NLR for overall diagnostic value of 16 exosomal biomarker for GBM diagnosis.

Our results suggest that the pooled sensitivity and specificity of exosomal biomarkers for GBM are 0.76 and 0.80, respectively. In addition, the pooled PLR, NLR, and DOR were 3.7, 0.30 and 12, respectively. The 3.7 calculated PLR shows that using this index test GBM patients had nearby 3.7-fold higher chance of a positive result than healthy population. Furthermore, 0.30 NLR, suggests that from the negative outcome of the index test results, 30 % could be GBM positive. Similarly, DOR is an index that correlates with the diagnostic performance of our index test. The higher DOR the better diagnostic performance [61]. In addition, AUC is the most important index to show the overall diagnostic power of an index test. The AUC value higher than 0.75 is considered as acceptable diagnostic performance [62]. According to our results, the value of DOR and AUC were 12 and 0.85, respectively, suggesting that exosomes might serve as a high potential diagnostic tool for GBM.

Notwithstanding, a significant heterogeneity was observed in the pooled sensitivity, specificity, DOR and NLR. In the case of publication bias, a P-value of 0.70 showed no evidence for publication bias in the meta-analysis.

Alltogether, as expected, the multivariate meta-regression of sensitivity and specificity indicated that source and isolation method of exosomes, RNA extraction methods, sample size and quality of study acted as the potential source of heterogeneity in the pooled estimates. We omitted two studies as outlier and performed a sensitivity analysis. The result of sensitivity analysis showed that, the pooled sensitivity and specificity are 0.79 and 0.76.

Regarding that exosomal diagnosis is a new emerging field in biomedical sciences, little research has conducted on every single cancer, as well as insufficient studies could find on a particular exosomal biomarker. For this reason, systematic review and meta-analyses studies conducted in this field will be associated with some inherent limitations. However, this yung field, need the secondary studies to lead and clarify the future primary studies.

The aim and the results of the present systematic review and meta-analysis and a recent meta analysis, for assessment of diagnostic value of exosomes for lung cancer are much consistent. Similar to us, they also aimed for evaluating the averall diagnostic value of exosomal biomarkers. Their results showed that the pooled sensitivity, specificity, PLR, NLR, DOR and AUC were 0.82, 0.84, 5.27,, 0.21, 25.14, and 0.90, respectively [63]. In addition, a systematic review and meta analysis of exosomal miR-21 for overall cancer detection showed that the sensitivity and specificity of pooled studies were 75% and 85%, and AUC was 0.93.

Finally, despite our complete protocols of systematic review and meta-analysis according to the latest guidelines for diagnostic studies, our study faced to some ineludable challenges and limitations. Firstly and the most important limitation of our study is heterogeneity in exosomal biomarkers. Generally, we claimed that we estimated the pooled diagnostic accuracy for 16 different biomarkers and we did not estimated that for a single particular exosomal biomarker. The latter limitation, ass discussed, is related to small number of studies on the each biomarkers. Further studies on each of these biomarker could lead a meta-analysis on single biomarker. Secondly, the number of included studies and participants were small. More studies could further validate the diagnostic performance of exosomes for GBM. Thirdly, most of the included studies were conducted in china and USA. Fourthly, the high heterogeneity should be taken into consideration. In this regard, we carried out a meta-regression, and a sensitivity analyses to detect the potential heterogeneity sources. After removing the outlier studies, sensitivity analyses were performed. Considering that the diagnostic accuracy did not changed significantly, the heterogeneity was lowered. The meta-regression analyses found some study characteristics including exosome isolation method, exosome source and RNA profiling and sample size as the source of heterogeneity.

Ultimately, despite described limitation, our study strongly suggest that exosomes have high prediction power for GBM and our results and few conducted meta-analysis show that exosomal biomarkers are a new emerging tool for cancer diagnosis that will attract much attention in the near future.

5. Conclusions

In this study, we screened the 1730 primary research article in the exosome and GBM field and finally nine primary studies remained for further analysis. We found 24 exosomal biomarker for GBM diagnosis. Six studies consit of 16 biomarker considered for meta-analysis. Our results suggest that the pooled sensitivity and specificity of 16 exosomal biomarkers for GBM are 0.76 and 0.80, respectively. In addition, the pooled PLR, NLR, and DOR were 3.7, 0.30 and 12, respectively.

Declarations

Acknowledgments

Ethics approval and consent to participate

Not applicable.

Consent for Publication

Not applicable.

Availability of Data and Material

Input data for the analyses are available from the corresponding author on request.

Competing Interests

The authors declare that they have no competing interests.

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Contributions

Conception and design of the study: Davod Jafari, **Acquisition of data**: Davod Jafari, Amir Tiyuri, Elmnaz Rezaei, **Analysis and/or interpretation:** Amir Tiyuri, Davod Jafari, **Drafting the manuscript:** Davod Jafari, Amir, Tiyuri, Rasool Jafari, Farzaneh Jokar Shoorijeh, Yousef Moradi, **Revising the manuscript critically for important intellectual content:** Davod Jafari, Amir, Tiyuri, Rasool Jafari, Mahmood barati.

References

1. Wen PY, Kesari S: **Malignant Gliomas in Adults**. *New England Journal of Medicine* 2008, **359**(5):492-507.
2. Krex D, Klink B, Hartmann C, von Deimling A, Pietsch T, Simon M, Sabel M, Steinbach JP, Heese O, Reifenberger G *et al*: **Long-term survival with glioblastoma multiforme**. *Brain : a journal of neurology* 2007, **130**(Pt 10):2596-2606.
3. Kim S-S, Harford JB, Pirollo KF, Chang EH: **Effective treatment of glioblastoma requires crossing the blood–brain barrier and targeting tumors including cancer stem cells: the promise of nanomedicine**. *Biochemical and biophysical research communications* 2015, **468**(3):485-489.
4. Huang SX, Shao K, Kuang YY, Liu Y, Li JF, An S, Guo YB, Ma HJ, He X, Jiang C: **Tumor targeting and microenvironment-responsive nanoparticles for gene delivery**. *Biomaterials* 2013, **34**(21):5294-5302.
5. Emery JD, Shaw K, Williams B, Mazza D, Fallon-Ferguson J, Varlow M, Trevena LJ: **The role of primary care in early detection and follow-up of cancer**. *Nature Reviews Clinical Oncology* 2014, **11**(1):38-48.
6. Nass D, Rosenwald S, Meiri E, Gilad S, Tabibian-Keissar H, Schlosberg A, Kuker H, Sion-Vardy N, Tobar A, Kharenko O: **MiR-92b and miR-9/9* are specifically expressed in brain primary tumors and can be used to differentiate primary from metastatic brain tumors**. *Brain pathology* 2009, **19**(3):375-383.
7. Schwartz SA, Weil RJ, Thompson RC, Shyr Y, Moore JH, Toms SA, Johnson MD, Caprioli RM: **Proteomic-based prognosis of brain tumor patients using direct-tissue matrix-assisted laser desorption ionization mass spectrometry**. *Cancer Research* 2005, **65**(17):7674-7681.

8. Khalil AA: **Biomarker discovery: a proteomic approach for brain cancer profiling**. *Cancer Sci* 2007, **98**(2):201-213.

9. Kim JH, Kim E, Lee MY: **Exosomes as diagnostic biomarkers in cancer**. *Molecular & Cellular Toxicology* 2018, **14**(2):113-122.

10. Street JM, Birkhoff W, Menzies RI, Webb DJ, Bailey MA, Dear JW: **Exosomal transmission of functional aquaporin 2 in kidney cortical collecting duct cells**. *Journal of Physiology-London* 2011, **589**(24):6119-6127.

11. Gyorgy B, Szabo TG, Pasztoi M, Pal Z, Misjak P, Aradi B, Laszlo V, Pallinger E, Pap E, Kittel A *et al*: **Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles**. *Cell Mol Life Sci* 2011, **68**(16):2667-2688.

12. Skog J, Wurdinger T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, Curry WT, Carter BS, Krichevsky AM, Breakefield XO: **Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers**. *Nature Cell Biology* 2008, **10**(12):1470-U1209.

13. Jafari D, Malih S, Eslami SS, Jafari R, Darzi L, Tarighi P, Samadikuchaksaraei A: **The relationship between molecular content of mesenchymal stem cells derived exosomes and their potentials: opening the way for exosomes based therapeutics**. *Biochimie* 2019, **165**:76-89.

14. Anastasiadou E, Slack FJ: **Malicious exosomes**. *Science* 2014, **346**(6216):1459-1460.

15. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO: **Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells**. *Nature Cell Biology* 2007, **9**(6):654-U672.

16. Del Boccio P, Raimondo F, Pieragostino D, Morosi L, Cozzi G, Sacchetta P, Magni F, Pitto M, Urbani A: **A hyphenated microLC-Q-TOF-MS platform for exosomal lipidomics investigations: Application to RCC urinary exosomes**. *Electrophoresis* 2012, **33**(4):689-696.

17. Whiteside TL: **Tumor-Derived Exosomes and Their Role in Cancer Progression**. *Advances in Clinical Chemistry* 2016, **74**:103-141.

18. Thuy MN, Kam JK, Lee GC, Tao PL, Ling DQ, Cheng M, Goh SK, Papachristos AJ, Shukla L, Wall K-L: **A novel literature-based approach to identify genetic and molecular predictors of survival in glioblastoma multiforme: Analysis of 14,678 patients using systematic review and meta-analytical tools**. *Journal of Clinical Neuroscience* 2015, **22**(5):785-799.

19. McInnes MDF, Moher D, Thombs BD, McGrath TA, Bossuyt PM, Grp P-D: **Preferred Reporting Items for a Systematic Review and Meta-analysis of Diagnostic Test Accuracy Studies The PRISMA-DTA Statement**. *Jama-Journal of the American Medical Association* 2018, **319**(4):388-396.

20. Macaskill P, Gatsonis C, Deeks J, Harbord R, Takwoingi Y: **Cochrane handbook for systematic reviews of diagnostic test accuracy**. *Version 09 0 London: The Cochrane Collaboration* 2010.

21. Gheytanchi E, Madjd Z, Janani L, Rasti A, Ghods R, Atyabi F, Asadi-Lari MH, Babashah S: **Exosomal microRNAs as potential circulating biomarkers in gastrointestinal tract cancers: a systematic review protocol**. *Systematic Reviews* 2017, **6**(1):228.

22. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, Leeflang MM, Sterne JA, Bossuyt PM: **QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies**. *Ann Intern Med* 2011, **155**(8):529-536.

23. Harbord RM, Deeks JJ, Egger M, Whiting P, Sterne JA: **A unification of models for meta-analysis of diagnostic accuracy studies**. *Biostatistics (Oxford, England)* 2007, **8**(2):239-251.

24. Dwamena BA: **midas: A program for Meta-analytical Integration of Diagnostic Accuracy Studies in Stata.** *Division of Nuclear Medicine, Department of Radiology, University of Michigan Medical School, Ann Arbor, Michigan* 2007.

25. Reitsma JB, Glas AS, Rutjes AW, Scholten RJ, Bossuyt PM, Zwinderman AH: **Bivariate analysis of sensitivity and specificity produces informative summary measures in diagnostic reviews**. *Journal of clinical epidemiology* 2005, **58**(10):982-990.

26. Rutter CM, Gatsonis CA: **A hierarchical regression approach to meta-analysis of diagnostic test accuracy evaluations**. *Statistics in medicine* 2001, **20**(19):2865-2884.

27. Higgins JP, Thompson SG, Deeks JJ, Altman DG: **Measuring inconsistency in meta-analyses**. *BMJ (Clinical research ed)* 2003, **327**(7414):557-560.

28. Deeks JJ, Macaskill P, Irwig L: **The performance of tests of publication bias and other sample size effects in systematic reviews of diagnostic test accuracy was assessed**. *Journal of clinical epidemiology* 2005, **58**(9):882-893.

29. Akers JC, Ramakrishnan V, Kim R, Skog J, Nakano I, Pingle S, Kalinina J, Hua W, Kesari S, Mao Y: **MiR-21 in the extracellular vesicles (EVs) of cerebrospinal fluid (CSF): a platform for glioblastoma biomarker development**. *PLoS One* 2013, **8**(10):e78115.

30. Manterola L, Guruceaga E, Perez-Larraya JG, Gonzalez-Huarriz M, Jauregui P, Tejada S, Diez-Valle R, Segura V, Sampron N, Barrena C *et al*: **A small noncoding RNA signature found in exosomes of GBM patient serum as a diagnostic tool**. *Neuro-Oncology* 2014, **16**(4):520-527.

31. Shi R, Wang PY, Li XY, Chen JX, Li Y, Zhang XZ, Zhang CG, Jiang T, Li WB, Ding W *et al*: **Exosomal levels of miRNA-21 from cerebrospinal fluids associated with poor prognosis and tumor recurrence of glioma patients**. *Oncotarget* 2015, **6**(29):26971-26981.

32. Akers JC, Hua W, Li HY, Ramakrishnan V, Yang ZX, Quan K, Zhu W, Li J, Figueroa J, Hirshman BR *et al*: **A cerebrospinal fluid microRNA signature as biomarker for glioblastoma**. *Oncotarget* 2017, **8**(40):68769-68779.

33. Lan FM, Qing Q, Pan Q, Hu M, Yu HM, Yue X: **Serum exosomal miR-301a as a potential diagnostic and prognostic biomarker for human glioma**. *Cell Oncol* 2018, **41**(1):25-33.

34. Santangelo A, Imbruce P, Gardenghi B, Belli L, Agushi R, Tamanini A, Munari S, Bossi AM, Scambi I, Benati D *et al*: **A microRNA signature from serum exosomes of patients with glioma as complementary diagnostic biomarker**. *J Neuro-Oncol* 2018, **136**(1):51-62.

35. Ebrahimkhani S, Vafaee F, Hallal S, Wei H, Lee MYT, Young PE, Satgunaseelan L, Beadnall H, Barnett MH, Shivalingam B *et al*: **Deep sequencing of circulating exosomal microRNA allows non-invasive glioblastoma diagnosis**. *Npj Precis Oncol* 2018, **2**(1):28.

36. Tan SK, Pastori C, Penas C, Komotar RJ, Ivan ME, Wahlestedt C, Ayad NG: **Serum long noncoding RNA HOTAIR as a novel diagnostic and prognostic biomarker in glioblastoma multiforme**. *Mol Cancer* 2018, **17**(1):74.

37. Zhong F, Huang T, Leng J: **Serum miR-29b as a novel biomarker for glioblastoma diagnosis and prognosis**. *Int J Clin Exp Pathol* 2019, **12**(11):4106-4112.

38. Bertram JS: **The molecular biology of cancer**. *Molecular Aspects of Medicine* 2000, **21**(6):167-223.

39. Hanash SM, Baik CS, Kallioniemi O: **Emerging molecular biomarkers—blood-based strategies to detect and monitor cancer**. *Nature Reviews Clinical Oncology* 2011, **8**(3):142-150.

40. Ransohoff DF: **Developing molecular biomarkers for cancer**. *Science* 2003, **299**(5613):1679-1680.

41. Bucy PC, Oberhill HR, Siqueira EB, Zimmerman HM, Jelsma RK: **Cerebral Glioblastomas Can Be Cured**. *Neurosurgery* 1985, **16**(5):714-717.

42. Enroth S, Berggrund M, Lycke M, Lundberg M, Assarsson E, Olovsson M, Stålberg K, Sundfeldt K, Gyllensten U: **A two-step strategy for identification of plasma protein biomarkers for endometrial and ovarian cancer**. *Clin Proteomics* 2018, **15**:38-38.

43. Bronkhorst AJ, Ungerer V, Holdenrieder S: **The emerging role of cell-free DNA as a molecular marker for cancer management**. *Biomol Detect Quantif* 2019, **17**:100087-100087.

44. Zaporozhchenko IA, Ponomaryova AA, Rykova EY, Laktionov PP: **The potential of circulating cell-free RNA as a cancer biomarker: challenges and opportunities**. *Expert Rev Mol Diagn* 2018, **18**(2):133-145.

45. Huang T, Deng C-X: **Current Progresses of Exosomes as Cancer Diagnostic and Prognostic Biomarkers**. *Int J Biol Sci* 2019, **15**(1):1-11.

46. Toiyama Y, Takahashi M, Hur K, Nagasaka T, Tanaka K, Inoue Y, Kusunoki M, Boland CR, Goel A: **Serum miR-21 as a Diagnostic and Prognostic Biomarker in Colorectal Cancer**. *Jnci-Journal of the National Cancer Institute* 2013, **105**(12):849-859.

47. Yaman Agaoglu F, Kovancilar M, Dizdar Y, Darendeliler E, Holdenrieder S, Dalay N, Gezer U: **Investigation of miR-21, miR-141, and miR-221 in blood circulation of patients with prostate cancer**. *Tumor Biology* 2011, **32**(3):583-588.

48. Liu XG, Zhu WY, Huang YY, Ma LN, Zhou SQ, Wang YK, Zeng F, Zhou JH, Zhang YK: **High expression of serum miR-21 and tumor miR-200c associated with poor prognosis in patients with lung cancer**. *Medical Oncology* 2012, **29**(2):618-626.

49. Gao JJ, Zhang QY, Xu JJ, Guo LJ, Li XF: **Clinical significance of serum miR-21 in breast cancer compared with CA153 and CEA**. *Chinese Journal of Cancer Research* 2013, **25**(6):743-748.

50. Wang Y, Gao XJ, Wei F, Zhang XW, Yu JP, Zhao H, Sun Q, Yan F, Yan CH, Li H *et al*: **Diagnostic and prognostic value of circulating miR-21 for cancer: A systematic review and meta-analysis**. *Gene* 2014, **533**(1):389-397.

51. Sukreet S, Silva BVRE, Adamec J, Cui J, Zempleni J: **Sonication and Short-term Incubation Alter the Content of Bovine Milk Exosome Cargos and Exosome Bioavailability (OR26-08-19)**. *Current*

Developments in Nutrition 2019, **3**(Supplement_1).

52. Kanchanapally R, Deshmukh SK, Chavva SR, Tyagi N, Srivastava SK, Patel GK, Singh AP, Singh S: **Drug-loaded exosomal preparations from different cell types exhibit distinctive loading capability, yield, and antitumor efficacies: a comparative analysis**. *International journal of nanomedicine* 2019, **14**:531-541.

53. Tang YT, Huang YY, Zheng L, Qin SH, Xu XP, An TX, Xu Y, Wu YS, Hu XM, Ping BH *et al*: **Comparison of isolation methods of exosomes and exosomal RNA from cell culture medium and serum**. *International Journal of Molecular Medicine* 2017, **40**(3):834-844.

54. Van Deun J, Mestdagh P, Sormunen R, Cocquyt V, Vermaelen K, Vandesompele J, Bracke M, De Wever O, Hendrix A: **The impact of disparate isolation methods for extracellular vesicles on downstream RNA profiling**. *Journal of Extracellular Vesicles* 2014, **3**(1):24858.

55. Taylor DD, Shah S: **Methods of isolating extracellular vesicles impact down-stream analyses of their cargoes**. *Methods* 2015, **87**:3-10.

56. Zarovni N, Corrado A, Guazzi P, Zocco D, Lari E, Radano G, Muhhina J, Fondelli C, Gavrilova J, Chiesi AJM: **Integrated isolation and quantitative analysis of exosome shuttled proteins and nucleic acids using immunocapture approaches**. 2015, **87**:46-58.

57. Stranska R, Gysbrechts L, Wouters J, Vermeersch P, Bloch K, Dierickx D, Andrei G, Snoeck RJJotm: **Comparison of membrane affinity-based method with size-exclusion chromatography for isolation of exosome-like vesicles from human plasma**. 2018, **16**(1):1.

58. Rekker K, Saare M, Roost AM, Kubo A-L, Zarovni N, Chiesi A, Salumets A, Peters MJCb: **Comparison of serum exosome isolation methods for microRNA profiling**. 2014, **47**(1-2):135-138.

59. Tauro BJ, Greening DW, Mathias RA, Ji H, Mathivanan S, Scott AM, Simpson RJ: **Comparison of ultracentrifugation, density gradient separation, and immunoaffinity capture methods for isolating human colon cancer cell line LIM1863-derived exosomes**. *Methods* 2012, **56**(2):293-304.

60. Eldh M, Lotvall J, Malmhall C, Ekstrom K: **Importance of RNA isolation methods for analysis of exosomal RNA: Evaluation of different methods**. *Molecular Immunology* 2012, **50**(4):278-286.

61. Glas AS, Lijmer JG, Prins MH, Bonsel GJ, Bossuyt PMM: **The diagnostic odds ratio: a single indicator of test performance**. *Journal of clinical epidemiology* 2003, **56**(11):1129-1135.

62. Jones CM, Athanasiou T: **Summary Receiver Operating Characteristic Curve Analysis Techniques in the Evaluation of Diagnostic Tests**. *The Annals of Thoracic Surgery* 2005, **79**(1):16-20.

63. Song Z, Wang S, Liu Y: **The diagnostic accuracy of liquid exosomes for lung cancer detection: a meta-analysis**. *Onco Targets Ther* 2018, **12**:181-192.
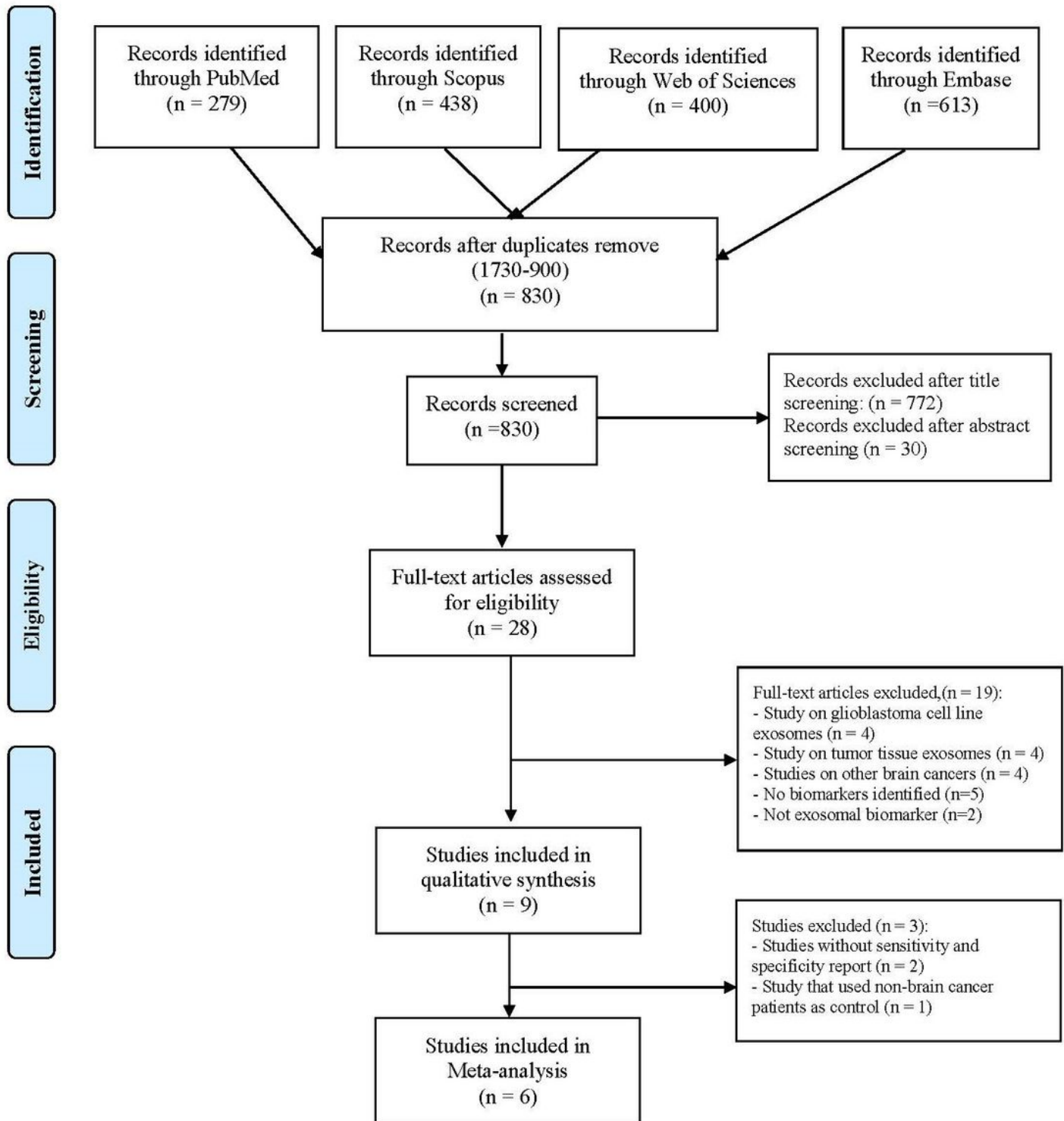
Figures

Figure 1

The literature search and study selection process for systematic review according to PRISMA
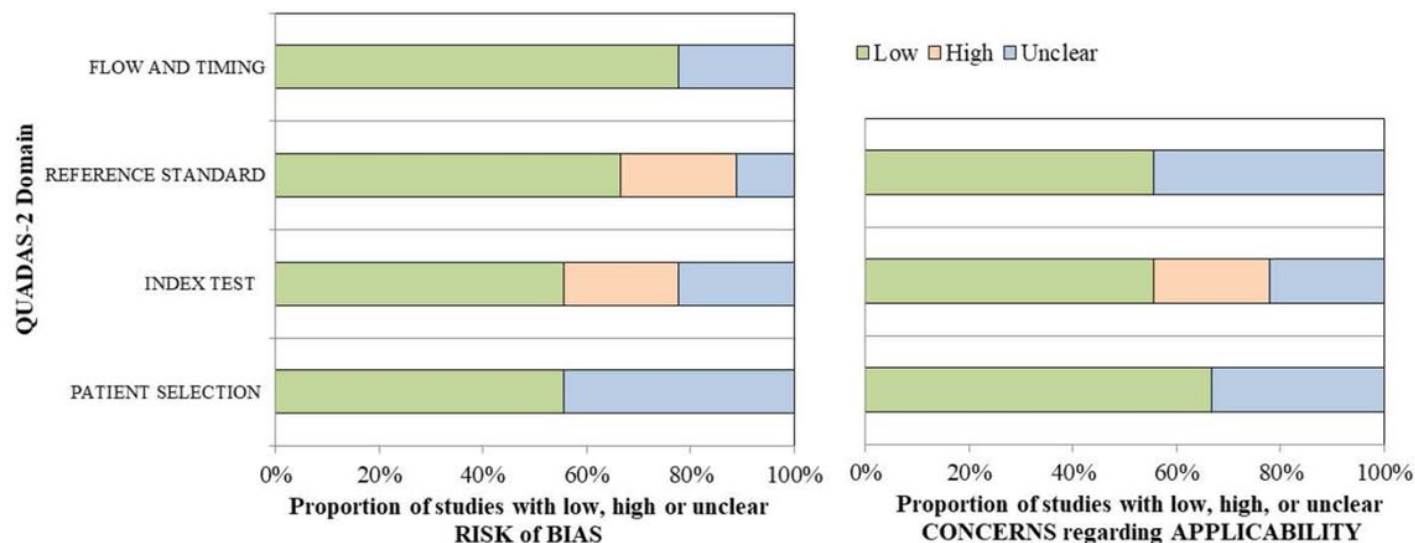
Figure 2

Quality assessment of the included studies performed using the QUADAS-2 checklist.
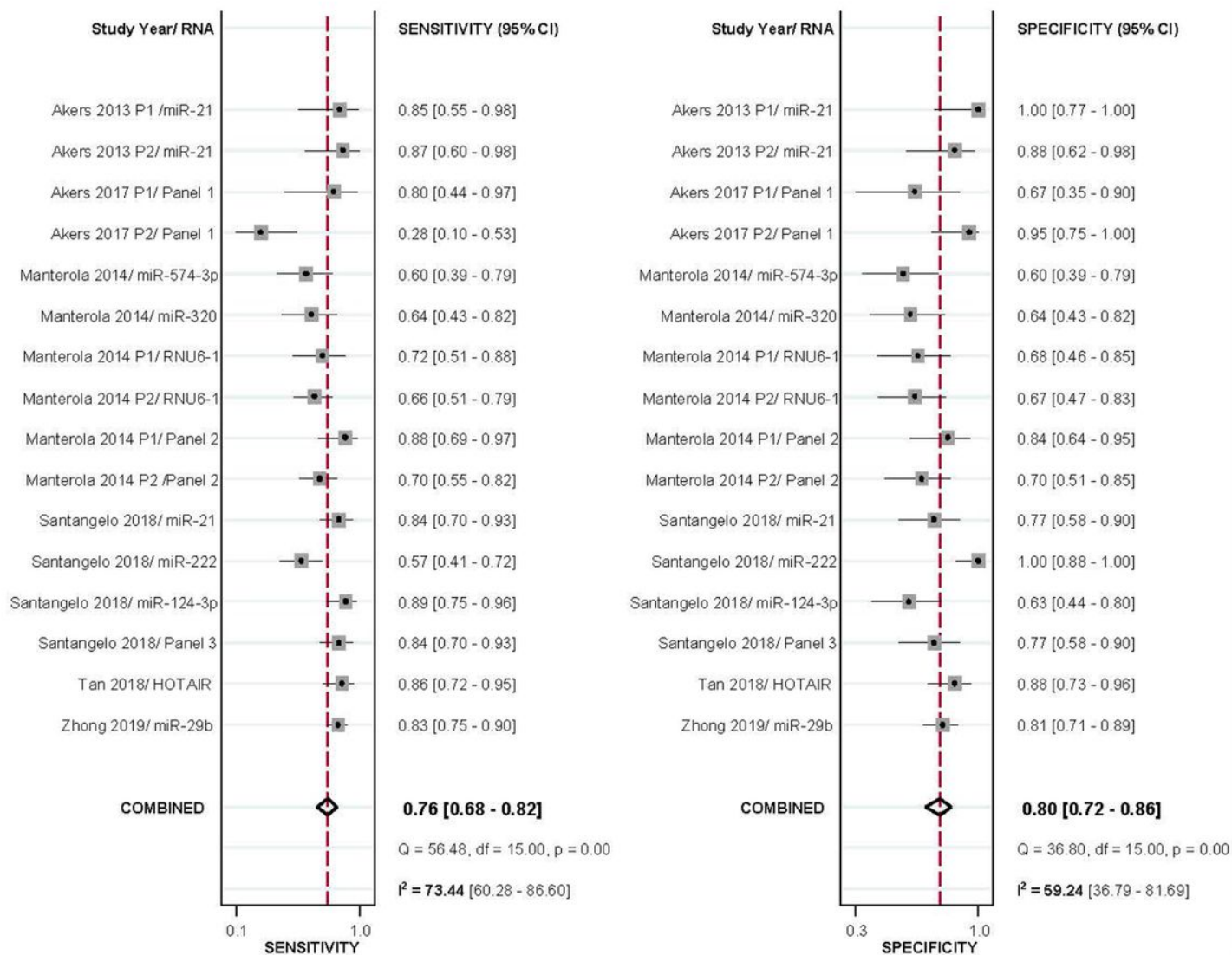
Figure 3

. Coupled forest plots show pooled estimates of sensitivity and specificity of exosomal biomarkers for glioblastoma diagnosis with corresponding heterogeneity statistics. CI: confidence interval, I2: inconsistency index.
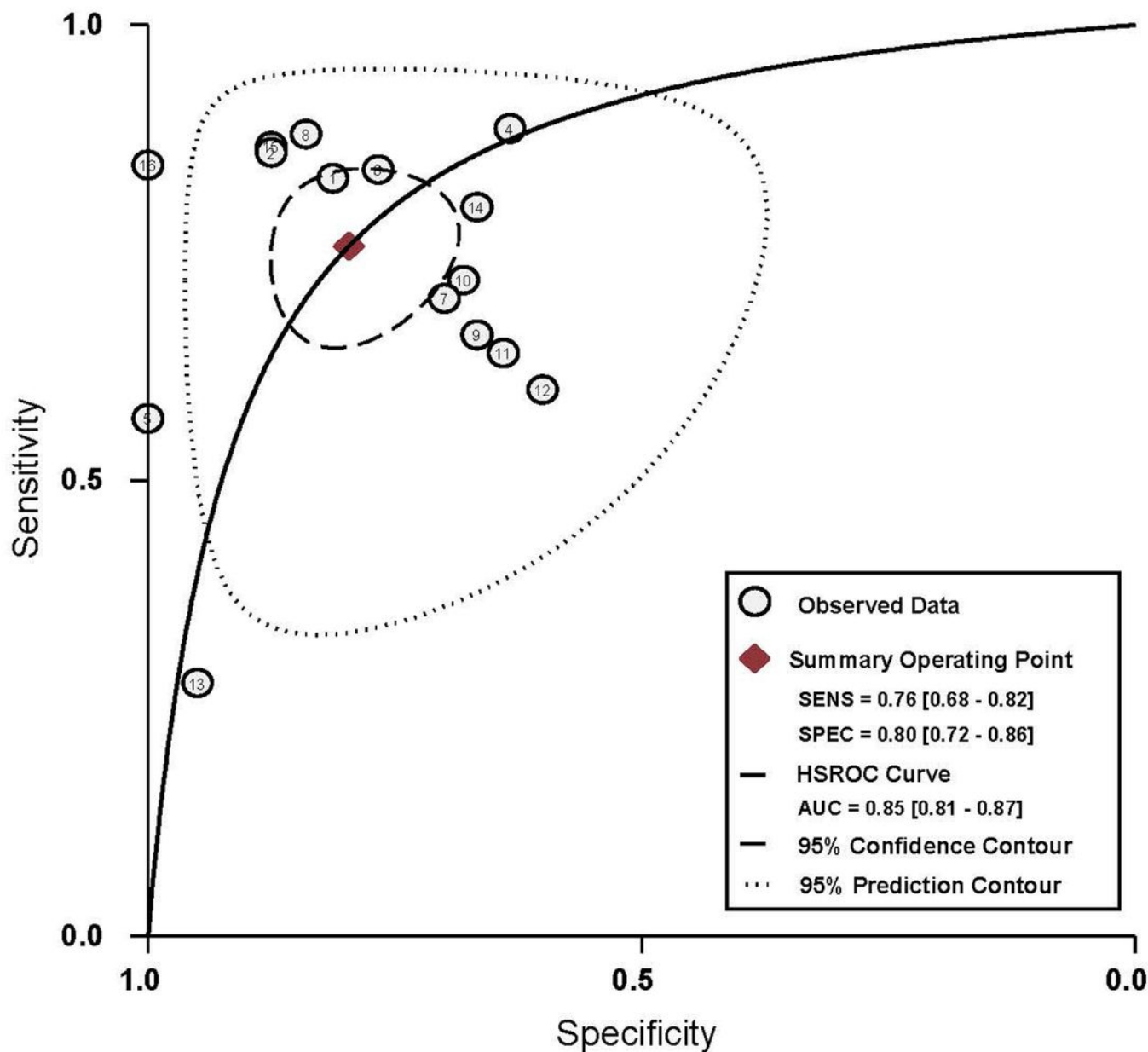

Figure 4

. Hierarchical summary receiver operating characteristic (HSROC) curve for the exosomal biomarkers performance in diagnosis of glioblastoma with confidence and prediction regions around summary operating point
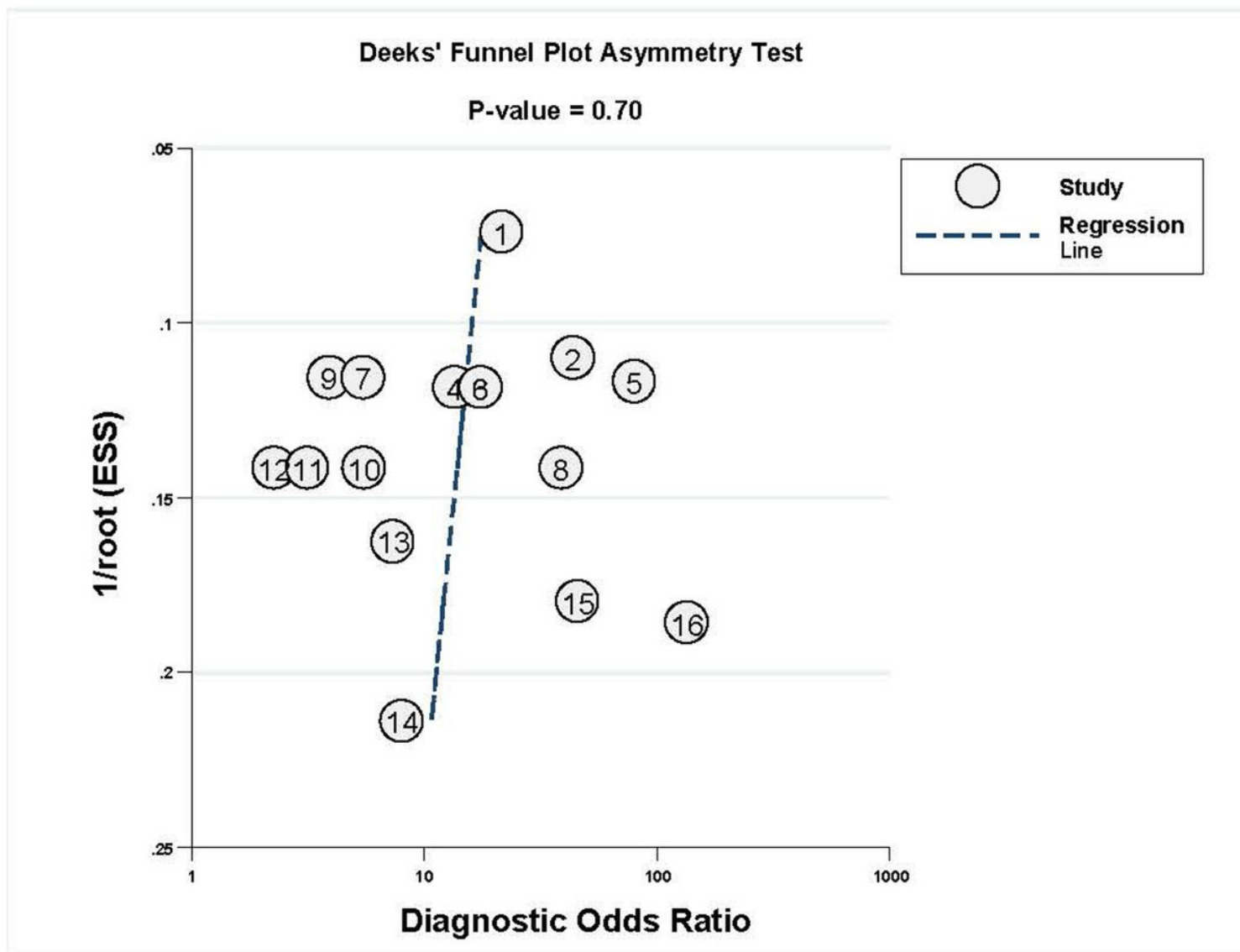
Figure 5

Deek's funnel plot and asymmetry test for evaluating potential publication bias. ESS: the effective sample size.

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