Diagnostic value of circulating tumor DNA in molecular characterization of glioma
A meta-analysis

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Abstract
Introduction: Circulating tumor DNA (ctDNA) has provided a minimally invasive approach for the detection of genetic mutations in glioma. However, the diagnostic value of ctDNA in glioma remains unclear. This meta-analysis was designed to investigate the diagnostic value of ctDNA, compared with the current “criterion standard” tumor tissues.

Materials and methods: The included studies were collected by searching PubMed, Web of Science, Cochrane Library, and Embase databases. All statistical analyses were performed using the STATA12.0 and Meta-DiSc1.4 software.

Result: A total of 11 studies comprising 522 glioma patients met our inclusion criteria. The pooled sensitivity and specificity were 0.69 (95% confidence interval [Cl] 0.66–0.73) and 0.98 (95% Cl 0.96–0.99), respectively. The pooled diagnostic odds ratio was 23.27 (95% Cl 13.69–39.53) and the area under the curve of the summary receiver operating characteristics curve was 0.90 (95% Cl 0.89–0.92).

Conclusions: ctDNA analysis is an effective method to detect the genetic mutation status in glioma patients with high specificity and relatively moderate sensitivity. The application of high-throughput technologies, the detection of patients with high-grade glioma, and sampling from cerebrospinal fluid could have higher diagnostic accuracy. The improvement of detection methods and more large-sample case-control studies are required in the future.

Abbreviations: AUC = area under the curve, BBB = blood-brain barrier, ctDNA = circulating cell-free DNA, CI = confidence interval, CNS = central nervous system, CSF = cerebrospinal fluid, ctDNA = circulating tumor DNA, DOR = diagnostic odds ratio, dPCR = digital PCR, FN = false negative, FP = false positive, GBM = glioblastoma, LOH = loss of heterozygosity, MeSH = medical subject heading terms, MSP = methylation-specific PCR, NGS = next-generation sequencing, QUADAS 2 = quality assessment of diagnostic accuracy studies 2, ROC = receiver-operating characteristics, SNP = single-nucleotide polymorphism, SROC = summary receiver-operating characteristics, tDNA = tumor-derived DNA, TN = true negative, TP = true positive, WHO = world health organization.

Keywords: circulating tumor DNA, diagnostic value, glioma, meta-analysis

1. Introduction
Gliomas are the most common primary tumors of the central nervous system. The overall annual average incidence rate for glioma is 6.57 per 100,000 people in the United States.[1] In the past decade, remarkable achievements have been made in the molecular characterization of glioma. These molecular characteristics can be used to predict the prognosis, guide individualized treatment, and define glioma classification.[2] In the revised 2016 World Health Organization (WHO) classification of tumors of the central nervous system,[3] the molecular genetics of gliomas are incorporated with classic histological features to define disease categorization, underscoring the importance of identifying genetic and epigenetic changes in gliomas.

Circulating cell-free DNA (ctDNA) is a type of degraded DNA fragment that freely circulates in the bloodstream. ctDNA derived from tumors, which is also known as circulating tumor DNA (ctDNA), contains different fragments of tumor genes. These fragments reflect specific genetic alterations of cancer, including tumor-specific methylation alterations, single-nucleotide polymorphism (SNP), and tumor-specific loss of heterozygosity. For a wide variety of tumors including glioma, ctDNA not only provides the same genetic information as a tissue biopsy but also characterizes the genetic profile without the influence of tumor heterogeneity.[4] In addition, given its minimally invasiveness nature, ctDNA can be drawn at any time during the course of therapy, allowing for the dynamic monitoring of molecular changes, which will greatly promote personalized cancer therapy.
Through polymerase chain reaction (PCR)-based technology or sequencing analyses, this naturally occurring biological material represents a new tool for the detection and surveillance of major cancers.\(^5\)

cfDNA is a biomarker in a wide range of cancers, including glioma. Numerous studies focused on the concordance rates in genetic and epigenetic changes between cfDNA and tissues. However, variable results were reported. This meta-analysis was a comprehensive evaluation of the diagnostic accuracy of genetic mutations status in cfDNA compared with glioma tumor tissues that seeks to clarify the precise value of cfDNA in diagnosis.

2. Materials and methods

2.1. Literature research strategy

Ethics approval was not applicable for this meta-analysis. This report was prepared according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses). The following medical subject heading terms (MeSH) were used for searching: “cell-free DNA” or “circulating DNA” or “plasma DNA” or “serum DNA” or “cerebrospinal fluid DNA” or “cfDNA” or “ctDNA” and “glioma” or “glioblastoma”. These terms were used to perform a systematic literature search of PubMed, Web of Science, Cochrane Library, and Embase databases. There was no limit on the start date for published articles, and the search end date was in December 2019. Article language was limited to English. The eligibility of the potentially relevant studies was assessed by screening the titles and abstracts, and then the full texts were reviewed to determine whether the study met the inclusion criteria. Two researchers (YK and XHL) independently assessed the eligibility of the potential relevant studies, and disagreements were solved by DZK. In addition, the references of included studies and relevant reviews were also assessed to retrieve more eligible studies that were potentially missed in the initial search.

2.2. Inclusion criteria

Eligible studies were selected according to the following criteria: all glioma patients involved should be diagnosed histopathologically; all evaluation indicators were derived from cfDNA or cfDNA in plasma, serum and cerebrospinal fluid (CSF); the techniques and target gene were clearly stated in articles; the target gene was verified by detection of tumor tissues; sufficient data to construct a diagnostic 2 × 2 table, including true positive (TP), false positive (FP), false negative (FN), and true negative (TN).

2.3. Exclusion criteria

The exclusion criteria were as follows: experiments based on cell lines or animal models; studies were not written in English; duplicate publications; DNA was extracted from extracellular vesicles and circulating tumor cells; reviews, letters, technical reports, case reports, or comments.

2.4. Quality assessment

The methodological quality of the articles that met our inclusion criteria was assessed using the revised Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS 2) guidelines.\(^6\) QUADAS 2 is a tool used for the quality assessment of diagnostic accuracy studies, and comprises 4 key domains: patient selection, index test, reference standard, and flow and timing. With signaling questions, risk of bias and concerns regarding applicability were judged as “yes” (low risk/ high concern), “unclear” (unclear risk/ unclear concern), and “no” (high risk/ low concern).

2.5. Data extraction

The data extracted from the articles included the lead author’s name, research type, region, publication year, methodological quality score, country, essential characteristics of the participants, methods for detection, sample source, target mutation, TP, FP, TN, and FN.

2.6. Statistical analysis

All statistical analyses were performed using the STATA software (version 12.0, StataCorp., College Station, TX) and Meta-DiSc (version 1.4) software. Molecular pathology detected in tumor tissues was treated as the “criterion standard.” TP, FP, FN, and TN were used to calculate the pooled sensitivity, specificity, pooled diagnostic odds ratio (DOR), and corresponding 95% confidence intervals (95% CI). DOR is a measure that combined sensitivity and specificity and is calculated as: (TP/FP)/(FN/TN). Summary receiver-operating characteristics (SROC) were generated, and the area under the curve (AUC) was calculated. The Spearman correlation between the logit of sensitivity and logit of 1-specificity was calculated to determine the effect of threshold, and a P < .05 indicated a significant threshold effect. The heterogeneity caused by the nonthreshold effect was measured by the inconsistency index (I²), and P value < .05 and I² value ≥ 50% indicated significant heterogeneity.\(^7\) In the presence of significant heterogeneity, meta-regression was used to detect the source. Subgroup analyses were performed for assay method, alteration type, and sample source. All eligible studies were assessed for publication bias by Deek funnel plot with a P value < .05 showing statistical significance.\(^8\)

3. Results

3.1. Characteristics of the studies

Our search retrieved 845 records from database and 120 records from other sources, 115 of them are duplicates. After reviewing the title and abstracts, 823 records were excluded. By reviewing full-text articles, we further excluded 16 records, and 11 eligible studies\(^9\)–\(^19\) remained in the meta-analysis (Fig. 1). Only 1 study was performed in the United States. Six studies were performed in Europe, and the remaining 4 studies were all performed in Asia. The details and main characteristics of included studies are summarized in Table 1. A manual search of reference lists of eligible studies and related reviews did not identify more relevant articles. A total of 522 patients with glioma were included in the analysis. Methodological quality of eligible studies was assessed by QUADAS-2. As shown in Figure 2, the methodological quality of the eligible studies was not significantly high. The overall quality of included studies was moderate.

3.2. Diagnostic accuracy

Given the lack of a well-accepted cfDNA gene target in glioma, 11 studies including different gene targets were pooled for the meta-
analysis of diagnostic accuracy. The random-effect model was applied to determine the sensitivity and specificity of this meta-analysis. The pooled specificity was 0.98 (95% confidence interval [CI] 0.96–0.99) and the pooled sensitivity was 0.69 (95% CI 0.66–0.73) for the detection of all mutations (Fig. 3). DOR (Fig. 3) and AUC (Fig. 4) were 23.27 (95% CI 13.69–39.53) and 0.90 (95% CI 0.89–0.92), respectively, indicating that ctDNA had relatively high diagnostic accuracy.

| Author          | Year | Country | Number | Female | Assay method   | Target gene     | Alteration type | Sample source | WHO grade | Tumor type |
|-----------------|------|---------|--------|--------|----------------|-----------------|-----------------|-------------|-----------|------------|
| Kyle D.W        | 2006 | USA     | 10     | 30.00% | MSP            | p16, MGMT, p73  | Methylation     | Plasma       | II, III, IV| GBM, AA, AOA, OG |
| Toshikiko W     | 2009 | Japan   | 40     | 32.50% | MSP            | p16, MGMT       | Methylation     | Serum        | II, III, IV| AS, AA, GBM, AOA, AOG, OA, OG |
| Iris L          | 2010 | Israel  | 70     | 42.86% | LOH-PCR, MSP   | 10q, MGMT       | LOH, methylation| Serum        | III, IV    | AS, OG, GBM |
| Bo-Lin L        | 2010 | China   | 66     | 39.33% | MeDIP-qPCR     | MGMT, p16nk4a, TIMP3, THBS1 | Methylation     | CSF, serum   | II, III | AA, AOA, AO, GBM |
| Carmen B        | 2011 | Spain   | 37     | 48.57% | MSP            | MGMT            | Methylation     | Serum        | IV         | GBM        |
| Blandine B      | 2012 | France  | 39     | 44.55% | dPCR IDH1 R132H | SNP             | SNP             | Plasma       | NA        | NA         |
| Aleksandra M.C  | 2013 | Poland  | 9      | 48.57% | MSP            | MGMT, RASSF1A, p14ARF | Methylation     | Serum        | III, IV    | AA, GBM, AS, GS |
| Valentina F     | 2014 | Italy   | 48     | 34.48% | MSP            | MGMT            | Methylation     | Plasma       | II, III, IV| AS, OA, OG, AA, AG, AOA, GBM |
| Zheng W         | 2015 | China   | 89     | 40.45% | MSP            | MGMT, IDH1 R132H, R132S, IDH2 R172W, R172K, TP53, ATRX, TERTp | Methylation     | CSF, serum   | NA        | NA        |
| Francisco M.R   | 2018 | Spain   | 17     | 47.06% | dPCR           | SNP             | SNP             | CSF          | II, III, IV| GBM, GS, AA, AS, OG, GBM |
| Taneg A.J       | 2018 | Germany | 50     | 36.84% | NGS            | TERTp           | SNP             | CSF          | IV         | GBM        |

AA = anaplastic astrocytoma, AOA = anaplastic oligoastrocytoma, AOA = anaplastic oligoastrocytoma, AS = astrocytoma, CSF = cerebrospinal fluid, dPCR = digital PCR, GBM = glioblastoma, GS = gliosarcoma, LOH = loss of heterozygosity, MeDIP = methylated DNA immunoprecipitation, MSP = methylation-specific PCR, NA = not available, NGS = next-generation sequencing, OA = oligoastrocytoma, OG = oligodendroglioma, SNP = single-nucleotide polymorphism.
3.3. Heterogeneity and meta-regression analysis

Threshold effect is a major source of between study heterogeneity. Visual assessment of receiver-operating characteristics (ROC) curves reveals a nonshoulder-sleeve-shaped image, indicating nonsignificant effects (Fig. 5). The Spearman correlation coefficient was 0.154, and the P value was .426 (> .05), indicating that the threshold effect was not significant. As shown in the forest plots of sensitivity, significant heterogeneity was detected ($I^2 = 73.1\%$, $P = 0.000$). Meta-regression was performed to detect the source of heterogeneity, and country, assay method, target gene, alteration type, and sample source were analyzed to determine the effect of each on data accuracy. The result showed that assay method ($P < .001$) and sample source ($P < .001$) contributed to heterogeneity (Fig. 6). Significant heterogeneity was not detected in specificity ($I^2 = 0.0\%$, $P = .700$) and DOR ($I^2 = 0.0\%$, $P = .859$).

3.4. Stratified analysis

To investigate the effect of potential confounding factors, we conducted stratified analysis according to assay method (methylation-specific PCR [MSP] versus digital PCR [dPCR]), alteration type (SNP vs methylation), WHO grade (high grade [I-II] vs low grade [III-IV]) and sample source (serum vs plasma vs CSF). As shown in Table 2, dPCR (AUC 0.90, 95% CI 0.87–0.92) had higher diagnostic accuracy than MSP. The diagnostic accuracy of the SNP group (AUC 0.95, 95% CI 0.92–0.99) was higher than the methylation group. The diagnostic accuracy of ctDNA extracted from CSF (AUC 0.94, 95% CI 0.92–0.96) was higher than that extracted from plasma or serum. High-grade group (AUC 0.92, 95% CI, 0.89–0.93) had a higher diagnostic accuracy than the low-grade group.

3.5. Publication bias

Deek funnel plot was performed to detect potential publication bias and no significant publication bias was detected ($P = .05$) (Fig. 7).

4. Discussion

Although patients with glioma would benefit from early diagnosis, few biomarkers have satisfactory performance for clinical application. Since the advantages of genetic information in glioma diagnosis have been proven,[20] ctDNA has received increasing focus due to its minimally invasive approach for genetic mutation detection. A number of studies have investigated the diagnostic accuracy values of ctDNA in glioma, but a wide range of results have been reported. This study aims to summarize the results of individual studies investigating the value of ctDNA for glioma detection and to evaluate the overall diagnostic performance of ctDNA as a potential biomarker.
In the present study, we showed that ctDNA had moderate sensitivity (0.69) and a high degree of specificity (0.98). However, high heterogeneity found in sensitivity ($I^2 = 73.1\%$, $P < .05$) might affect the accuracy of results and the sample source was identified as the source of heterogeneity. ctDNA had fine diagnostic performance with a DOR of 23.27. According to the suggested guideline for interpretation of area under ROC, ctDNA had relatively high diagnostic accuracy (AUC = 0.90) for the detection of mutation alterations in glioma. These results
Table 2

Meta-analysis results.

| Sample source | Sensitivity (95% CI) | Specificity (95% CI) | P statistic (P) | DOR (95% CI) | F statistic (P) | AUC (95% CI) |
|---------------|---------------------|----------------------|----------------|---------------|----------------|--------------|
| Overall       | 0.69 (0.66–0.73)    | 73.1% (0.00)         | 0.08 (0.96–0.99)| 0.0% (1.00)   | 23.27 (13.69–39.53)| 0.0% (0.859) | 0.90 (0.89–0.92) |
| Assay method  |         |                     |                |               |                |              |
| MSP           | 0.56 (0.50–0.63)    | 24.5% (1.97)         | 0.09 (0.97–1.00)| 0.0% (0.642)  | 35.34 (15.29–81.70)| 0.0% (0.921) | 0.88 (0.86–0.92) |
| dPCR          | 0.67 (0.53–0.79)    | 3.6% (0.394)         | 1.00 (0.95–1.00)| 0.0% (1.000)  | 44.82 (11.63–172.78)| 0.0% (0.989) | 0.90 (0.87–0.92) |
| Alteration type |                  |                     |                |               |                |              |
| Methylation   | 0.70 (0.67–0.73)    | 73.3% (0.00)         | 0.09 (0.97–1.00)| 0.0% (0.967)  | 27.63 (14.16–53.90)| 0.0% (0.966) | 0.92 (0.90–0.94) |
| SNP           | 0.77 (0.68–0.85)    | 57.2% (0.030)        | 1.00 (0.96–1.00)| 0.0% (1.000)  | 59.69 (17.40–204.77)| 0.0% (0.951) | 0.95 (0.92–0.99) |
| Sample source |         |                     |                |               |                |              |
| Serum         | 0.64 (0.60–0.69)    | 75.4% (0.00)         | 0.07 (0.94–0.99)| 28.7% (156)   | 17.54 (8.39–36.25)| 4.1% (1.06)  | 0.89 (0.87–0.91) |
| Plasma        | 0.57 (0.45–0.68)    | 0.0% (0.963)         | 1.00 (0.91–1.00)| 0.0% (1.000)  | 20.71 (4.98–86.04)| 0.0% (0.983) | 0.89 (0.84–0.94) |
| CSF           | 0.79 (0.74–0.83)    | 65.8% (0.001)        | 1.00 (0.97–1.00)| 0.0% (1.000)  | 46.00 (17.49–120.96)| 0.0% (0.918) | 0.94 (0.92–0.96) |
| WHO grade     |         |                     |                |               |                |              |
| Low grade     | 0.48 (0.34–0.63)    | 27.2% (2.40)         | 1.00 (0.92–1.00)| 0.0% (1.000)  | 18.74 (4.49–78.21)| 0.0% (0.902) | 0.88 (0.82–0.94) |
| High grade    | 0.74 (0.71–0.78)    | 72.8% (0.00)         | 0.09 (0.96–1.00)| 0.0% (0.999)  | 30.12 (14.35–63.21)| 0.0% (0.948) | 0.92 (0.89–0.93) |

AUC = area under curve, CSF = cerebrospinal fluid, DOR = diagnostic odds ratio, dPCR = digital PCR, MSP = methylation-specific PCR, SNP = single-nucleotide polymorphism.

Figure 7. Deek funnel plot for the evaluation of potential publication bias in the value of ctDNA in glioma patients (P = .05).

revealed that ctDNAs are suitable as diagnostic biomarkers of glioma.

cDNA-related studies in glioma have suggested the potential application of ctDNA in the molecular pathological diagnosis of glioma, the evaluation of therapeutic efficacy, and monitoring of recurrence. In this study, we found that ctDNA assays have a high level of specificity, which supports their clinical use in glioma. However, given the relatively moderate sensitivity of ctDNA detection, ctDNA was only used as an auxiliary tool for molecular pathological diagnosis of glioma and cannot replace histopathologic analysis entirely. The improvement of the diagnostic accuracy of ctDNA analysis in glioma is the first prerequisite to expand its clinical application.

The main factor that influences the diagnostic accuracy of ctDNA in glioma is the presence of the blood-brain barrier (BBB); thus, only a limited number of ctDNAs could be isolated from peripheral blood. The sensitivity of traditional approaches to DNA assessment is insufficient for detection of somatic mutations in ctDNA from glioma patients. A study found that ctDNA from serum was detectable in >75% of patients with advanced pancreatic, ovarian, colorectal, bladder, gastroesophageal, breast, melanoma, hepatocellular, and head and neck cancers, but in <50% of primary brain tumors. New techniques have expanded the ability to accurately identify and quantify rare mutant molecules. Higher levels of analytical sensitivity and specificity of dPCR than traditional PCR methods have been demonstrated. Enabling high-throughput, targeted amplification of the mutant gene of interest on the background of abundant wild-type alleles and reaching limits of detection <0.1% make dPCR a promising method for ctDNA detection.

In subgroup analysis, the diagnostic performance of dPCR showed a pooled sensitivity of 0.67, specificity of 1.00, DOR of 44.82, and AUC of 0.90, which was higher than that for MSP. We also found that the diagnostic accuracy of SNP group yielded better results than methylation group. The main reason was that SNP detection was used by dPCR or next-generation sequencing (NGS) which is characterized by higher accuracy compared with traditional technologies. Although it seems that dPCR or NGS has higher accuracy to identify genetic mutation in ctDNA, its cost is the main limitation of its clinical application.

Sampling of body fluids proximal to the tumor site may yield a higher concentration of DNA of tumor origin than that found in blood, for example, urine for bladder cancers, saliva for head and neck carcinomas, or pleural effusion fluid for lung cancers. CSF is in intimate contact with central nervous system. Wang et al. studied whether the CSF was enriched for tumor-derived DNA (tDNA), and their results suggest that CSF-tDNA could be useful for the management of patients with primary tumors of the brain or spinal cord. De Mattos et al. showed that ctDNA derived from central nervous system tumors was more abundantly present in the CSF compared with plasma. Some patients with lesions adjacent to a CSF reservoir in the brain or spinal cord were much more likely to have detectable levels of CSF-tDNA. In a recent study, Miller et al. showed that the tumor-derived DNA in CSF from patients with glioma closely resembled the genomes of tumor biopsies, and was associated with disease burden and adverse outcome. Our meta-analysis results showed that the sample source was the source of heterogeneity in sensitivity, indicating that significant differences existed in ctDNA assays of CSF, plasma, and serum. In subgroup analysis, we also found that the sensitivity, specificity, and overall diagnostic performance (AUC and DOR) of CSF-tDNA were higher than blood-tDNA. This finding indicated that CSF-tDNA assays for
glioma were more encouraging than blood-based analysis. When relevant genomic alterations could not be identified through biopsy due to limited access to tumor tissues, CSF-tDNA assays for these patients may present a feasible method. Although CSF evaluation is less invasive than brain biopsy, it must be noted that lumbar puncture is still not acceptable for many patients in the clinic for the collection of CSF specimens. Blood-based ctDNA assays may be more suitable for many cases compared with CSF, especially as a monitoring marker during follow-up. Further studies should focus on exploring sensitive and precise detection methods of extremely small amounts of circulating mutant DNA sequences derived from glioma cells in blood.

In subgroup analysis, we also found that ctDNA assay in high-grade glioma had a higher diagnostic accuracy than that in low-grade group. A portion of ctDNA are derived from apoptosis or active release of the tumor cell, indicating that the patients with advanced tumors may contain more ctDNA. For this reason, ctDNA in the patients with high-grade tumor are more easily detected than that with low-grade tumor. Although some previous studies had noted that ctDNA can be identified in early tumors as well as in metastatic ones, our results proved that the application of ctDNA detection in high-grade glioma was more feasible.

Several limitations in this meta-analysis should also be highlighted. First, most included studies were small in size and the results might lead to bias. The results of subgroup analysis need to be cautiously interpreted because too few studies were included in some groups. Second, significant heterogeneity was observed in sensitivity analysis. Spearman correlation and ROC curves suggested that the heterogeneity was not caused by the threshold effect. Meta-regression was performed, and the results showed that sample source and assay method were the source of heterogeneity. Third, we did not have sufficient information for analysis of the differences in isolation methods of ctDNA, histopathologic, type and volume, which could represent the important sources of heterogeneity.

5. Conclusions

In this meta-analysis, we showed that ctDNA was an effective and promising biomarker for molecular diagnosis in glioma with high specificity and relatively moderate sensitivity. We also found that dPCR, SNP detection, high-grade glioma, and CSF-based ctDNA assays yielded better results in the subgroup analysis. The detection methods of circulating mutant DNA in glioma patients need to be improved. Due to the small sample size used in this study, further evaluation of the ctDNA with a large number of cases and control cohorts is required to confirm these findings.

Author contributions

Y.K. and D.K. designed the study; Y.K. and X.L. searched databases and collected full-text papers; X.L. extracted and analyzed data; Y.K. wrote the manuscript.

References

[1] Ostrom QT, Gittleman H, Liao P, et al. CBTRUS Statistical Report: primary brain and other central nervous system tumors diagnosed in the United States in 2010–2014. Neuro Oncol 2017;19(suppl 5):v1–v88.

[2] Nabors LB, Portnow J, Ammirati M, et al. NCCN Guidelines (R) Insights Central Nervous System Cancers, Version 1.2017 Updated Features to the NCCN Guidelines. J Natl Compr Canc Ne 2017;15:1331–45.

[3] Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol 2016;131:803–20.

[4] Diaz LA Jr, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. J Clin Oncol 2014;32:579–86.

[5] Wan JCM, Massie C, Garcia-Corbacho J, et al. Liquid biopsies come of age: towards implementation of circulating tumour DNA. Nature reviews. Cancer 2017;17:223–38.

[6] Whiting PF, Rutjes AW, Westwood ME, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med 2011;155:529–36.

[7] Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. BMJ 2003;327:557–60.

[8] 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. J Clin Epidemiol 2005;58:882–93.

[9] Weaver KD, Grossman SA, Herman JG. Methylated tumor-specific DNA as a plasma biomarker in patients with glioma. Cancer Invest 2006;24:335–40.

[10] Wakabayashi T, Natsume A, Hatano H, et al. p16 promoter methylation in the serum as a basis for the molecular diagnosis of gliomas. Neurosurgery 2009;64:455–61. discussion 461-452.

[11] Lavon I, Refael M, Zelikovich B, et al. Serum DNA can define tumor-specific genetic and epigenetic markers in gliomas of various grades. Neuro Oncol 2010;12:173–80.

[12] Liu BL, Cheng JX, Zhang W, et al. Quantitative detection of multiple gene promoter hypermethylation in tumor tissue, serum, and cerebrospinal fluid predicts prognosis of malignant gliomas. Neuro Oncol 2010;12:540–8.

[13] Bilana C, Carrato C, Ramirez JL, et al. Tumour and serum MGMT promoter methylation and protein expression in glioblastoma patients. Clin Transl Oncol 2011;13:677–85.

[14] Butscher B, Gallego Perez-Larraya J, Rossetto M, et al. Detection of IDH1 mutation in the plasma of patients with glioma. Neurology 2012;79:1693–8.

[15] Majchrzak-Celinska A, Paluszczak J, Kleszcz R, et al. Detection of MGMT, RASSF1a, p15INK4B, and p14ARF promoter methylation in circulating tumor-derived DNA of central nervous system cancer patients. J Appl Genet 2013;54:335–44.

[16] Fiano V, Trevisan M, Trevisan E, et al. MGMT promoter methylation in plasma of glioma patients receiving temozolomide. J Neurooncol 2014;117:347–57.

[17] Wang Z, Jiang W, Wang Y, et al. MGMT promoter methylation in serum and cerebrospinal fluid as a tumor-specific biomarker of glioma. Biomed Rep 2015;3:543–8.

[18] Martinez-Ricarte F, Mayor R, Martinez-Saez E, et al. Molecular diagnosis of diffuse gliomas through sequencing of cell-free circulating tumor DNA from cerebrospinal fluid. Clin Cancer Res 2018;24:2812–9.

[19] Juratli TA, Stasik S, Zolal A, et al. Detection of MGMT promoter mutation detection in cell-free tumor-derived DNA in patients with IDH wild-type glioblastoma: a pilot prospective study. Clin Cancer Res 2018;24:3282–91.

[20] Reifenberger G, Wirsching HG, Knobbe-Thomsen CR, et al. Advances in the molecular genetics of gliomas—implications for classification and therapy. Nat Rev Clin Oncol 2017;14:434–52.

[21] Salkeni MA, Zarzour A, Ansay TY, et al. Detection of EGFRvIII mutant DNA in the peripheral blood of brain tumor patients. J Neurooncology 2013;115:27–35.

[22] Pentsova EI, Shah RH, Tang J, et al. Evaluating cancer of the central nervous system through next-generation sequencing of cerebrospinal fluid. J Clin Oncol 2016;34:2404–15.

[23] De Mattos-Arruda L, Mayor R, Ng CK, et al. Cerebrospinal fluid-derived circulating tumour DNA better represents the genomic alterations of brain tumours than plasma. Nat Commun 2015;6:8839.

[24] Li Y, Pan W, Connolly ID, et al. Tumor DNA in cerebral spinal fluid reflects clinical course in a patient with melanoma leptomeningeal brain metastases. J Neurooncol 2016;128:93–100.

[25] Bettgeowda C, Sausen M, Leary RJ, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med 2011;3:57.

[26] Fruza T, Diquor M, Smits J, et al. Liquid biopsy: opportunities, challenges, and recent advances in detection technologies. Lab Chip 2018;18:1174–96.
[28] Millholland JM, Li S, Fernandez CA, et al. Detection of low frequency FGFR3 mutations in the urine of bladder cancer patients using next-generation deep sequencing. Res Rep Urol 2012; 4:33–40.

[29] Li Y, Zhou X, St John MA, et al. RNA profiling of cell-free saliva using microarray technology. J Dental Res 2004;83:199–203.

[30] Soh J, Toyooka S, Aoe K, et al. Usefulness of EGFR mutation screening in pleural fluid to predict the clinical outcome of gefitinib treated patients with lung cancer. Int J Cancer 2006;119:2353–8.

[31] Wang Y, Springer S, Zhang M, et al. Detection of tumor-derived DNA in cerebrospinal fluid of patients with primary tumors of the brain and spinal cord. Proc Natl Acad Sci U S A 2015;112:9704–9.

[32] Huang TY, Piunti A, Lulla RR, et al. Detection of Histone H3 mutations in cerebrospinal fluid-derived tumor DNA from children with diffuse midline glioma. Acta Neuropathol Commun 2017;5:28.

[33] Miller AM, Shah RH, Pentsova EI, et al. Tracking tumour evolution in glioma through liquid biopsies of cerebrospinal fluid. Nature 2019; 565:654–8.

[34] Cohen PA, Flowers N, Tong S, et al. Abnormal plasma DNA profiles in early ovarian cancer using a non-invasive prenatal testing platform: implications for cancer screening. BMC Med 2016;14:126.

[35] Beaver JA, Jelovac D, Balukrishna S, et al. Detection of cancer DNA in plasma of patients with early-stage breast cancer. Clin Cancer Res 2014;20:2643–50.