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Review Article

Potential diagnostic value of circulating miR-30a-3p for non-small cell lung cancers: meta-analysis from microarray datasets

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Abstract: *Background and objective:* Aberrant microRNAs (miRNAs) have been reported to play vital roles in the tumorigenesis and progression of non-small cell lung cancers (NSCLCs). Several circulating miRNAs have also been demonstrated as potential biomarkers in early diagnosis of NSCLCs. However, the clinical diagnostic value of circulating microRNA-30a-3p (miR-30a-3p) of NSCLCs has not been clarified. Thus, the current meta-analysis was carried out to assess the possibility of circulating miR-30a-3p as a biomarker with microarray datasets for early detection of NSCLCs. *Material and Methods:* The NCBI Gene Expression Omnibus (GEO) database and EBI ArrayExpress database were searched to collect NSCLC–related miRNA microarray datasets for detection of circulating miRNA levels until 30th September, 2015. Limma package and ExiMiR package in R were used to evaluate the quality control of the data output. Standardized mean difference (SMD) together with 95% confidence intervals (CIs) of miR-30a-3p level from included datasets was pooled with STATA 12.0. Heterogeneity was evaluated by Cochran's Q test and the I^2 statistic. A p value<0.005 or I^2>50% was regarded as significant heterogeneity. Additionally, sensitivity analysis was performed to estimate the stability of the pooled results. *Results:* Six miRNA datasets (GSE61741, GSE46729, GSE40738, GSE24709, GSE17681 and GSE27486) from blood samples were selected, including 250 NSCLC patients and 242 healthy controls. A marginal alteration of circulating miR-30a-3p level was observed between NSCLC cases and control groups; however, the p value did not reach the statistically significant standard (SMD = 0.169; 95% CI, -0.012 to 0.350; P = 0.067). No significant heterogeneity was generated by random-effects model (P = 0.186, I^2 = 33.4%). Furthermore, sensitivity analysis showed stable results of the current meta-analysis. Conclusions: MiR-30a-3p expression levels in whole blood and peripheral blood cells show no significant differences between NSCLC patients and healthy controls; thus it might be ineffective to detect circulating miR-30a-3p expression for an early diagnosis of lung cancer. However, larger cohorts are required to verify this finding.

Keywords: Biomarker, non-small cell lung cancer, meta-analysis, microarray datasets, miR-30a-3p

Introduction

Non-small cell lung cancer (NSCLC) is one of the most common leading causes of cancer related death worldwide [1, 2]. The determining factors of the high mortality of NSCLC include a late clinical manifestation, tumor heterogeneities from histological and molecular subtypes, and the insufficient understanding of the tumor biology [3]. The early diagnosis has been one of the key elements in the clinic management of NSCLCs, which can be realized with the discovery of sensitive and specific biomarkers [4].

MicroRNAs (miRNAs), a family of short endogenous non-coding RNAs, have been confirmed to play significant roles in cell growth, cell differentiation, and tumorigenesis and progress of many classes of cancers [5-7]. Several reviews have summarized that circulating miRNAs have been demonstrated as stable and potential biomarkers in early diagnosis of NSCLCs [8-10], for instance, miR-21, miR-25, miR-126, miR-141, miR-155, etc. However, it is still premature to apply these candidate miRNAs in clinical process due to lack of potent clinical confirmation. Thus, it is still necessary to explore new candi-

Table 1. Characteristics of hsa-miR-30a-3p expression profiling datasets included in the current meta-analysis

Series	Country	Tissue	Platform	Lung cancer types	Sample of Lung Cancer Patients	Sample of Healthy Controls	Citation (ref.)	Year
GSE61741	Germany	Peripheral blood	GPL9040	NSCLC	73	109	Keller A.	2014
GSE46729	USA	Serum	GPL8786	NSCLC	24	24	Godrey A, et al.	2014
GSE40738	USA	Whole blood	GPL16016	NSCLC	86	59	Patnaik SK, et al.	2012
GSE27486	USA	Whole blood	GPL11432	NSCLC*	22	12	Patnaik SK, et al.	2011
GSE24709	Germany	Peripheral blood	GPL9040	NSCLC	28	19	Keller A, et al.	2011
GSE17681	Germany	Peripheral blood	GPL9040	NSCLC	17	19	Keller A, et al.	2009

NSCLC: non-small cell lung carcinoma. *Only adenocarcinomas were involved.

Table 2. Forest plot of studies evaluating standard mean difference (SMD) of hsa-miR-30a-3p expression between NSCLC and control group (a fixed-effects model)

	Experimental			Control				
Study	Mean	SD	Total	Mean	SD	Total	Weight	SMD. Fixed [95% CI]
GSE61741	4.4827158	2.0731199	73	3.9908385	2.3510626	109	37.01%	0.219 [-0.078, 0.516]
GSE46729	3.9741756	0.2633923	24	3.8569575	0.2539839	24	9.95%	0.453 [-0.120, 1.026]
GSE40738	-1.057073	0.1310346	86	-1.050453	0.1238929	59	29.79%	-0.052 [-0.383, 0.280]
GSE24709	5.5639448	1.0051026	28	5.4563445	1.5529064	19	9.63%	0.086 [-0.497, 0.669]
GSE17681	1.4177732	1.9339732	17	1.5744519	1.9675208	19	7.63%	-0.080 [-0.735, 0.574]
GSE27486	-0.127729	0.1082534	22	-0.226394	0.1002277	12	5.98%	0.935 [0.195, 1.674]
Total [95% CI]			250			242	100.00%	0.169 [-0.012, 0.350]

Heterogeneity: $Chi^2 = 7.51$; df = 5 (P = 0.186); $I^2 = 33.4\%$. Test for overall effect: Z = 1.83 (P = 0.067).

date biomarkers for the precise early diagnosis for NSCLC patients.

The relationship between miR-30a-3p and NSCLC has been investigated by only one research group [11]. Cazzoli R *et al.* [11] studied the predictive power of miRNAs with 2 patient sets: 1 training set with 10 adenocarcinomas of lung, 10 granulomas of lung and 10 former smokers as healthy controls, and another validation set with 50 cases of lung adenocarcinomas, 30 cases of lung granulomas and 25 former smokers. Among the aberrant miRNAs, miR-30a-3p was found to be one of the best microRNAs by CfsSubsetEval analysis, which was discovered by Cazzoli R *et al.* [11] with microRNA Ready-to-Use PCR, Human panel I+II, V2.M and quantitative RT-PCR, respectively. MiR-30a-3p was slightly downregulated in lung granulomas, while it was moderately upregulated in lung adenocarcinomas. However, the small sample size in this individual study [11] has restricted the reliability of any conclusion that miR-30a-3p expression level in blood samples can be considered as a diagnostic marker for NSCLCs. Hence, the diagnostic effect of circulating miR-30a-3p on NSCLC remains unclear.

Several microarray datasets from blood samples are available to detect the differential levels of miRNAs between NSCLCs and non-cancer controls. The two frequently used databases are the National Center of Biotechnology Information (NCBI) Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) and the European Bioinformatics Institute (EBI) Array Express (http://www.ebi.ac.uk/arrayexpress/). Thus, the current meta-analysis was carried out to assess the possibility of circulating miR-30a-3p as a biomarker with microarray datasets for early detection of NSCLCs.

Materials and methods

Data acquisition

Two databases of GEO and Array Express were searched for NSCLC-related miRNA microarray datasets from blood samples until 30th September 2015. The following keywords were applied in the study screening of the meta-analysis: lung OR bronchi OR bronchioles OR pulmonary OR alveoli OR respiratory, cancer OR carcinoma OR tumor OR neoplas* OR malignan* OR adenocarcima, ser* OR plasma OR blood OR circulating, microRNA OR miRNA OR non-coding RNA.

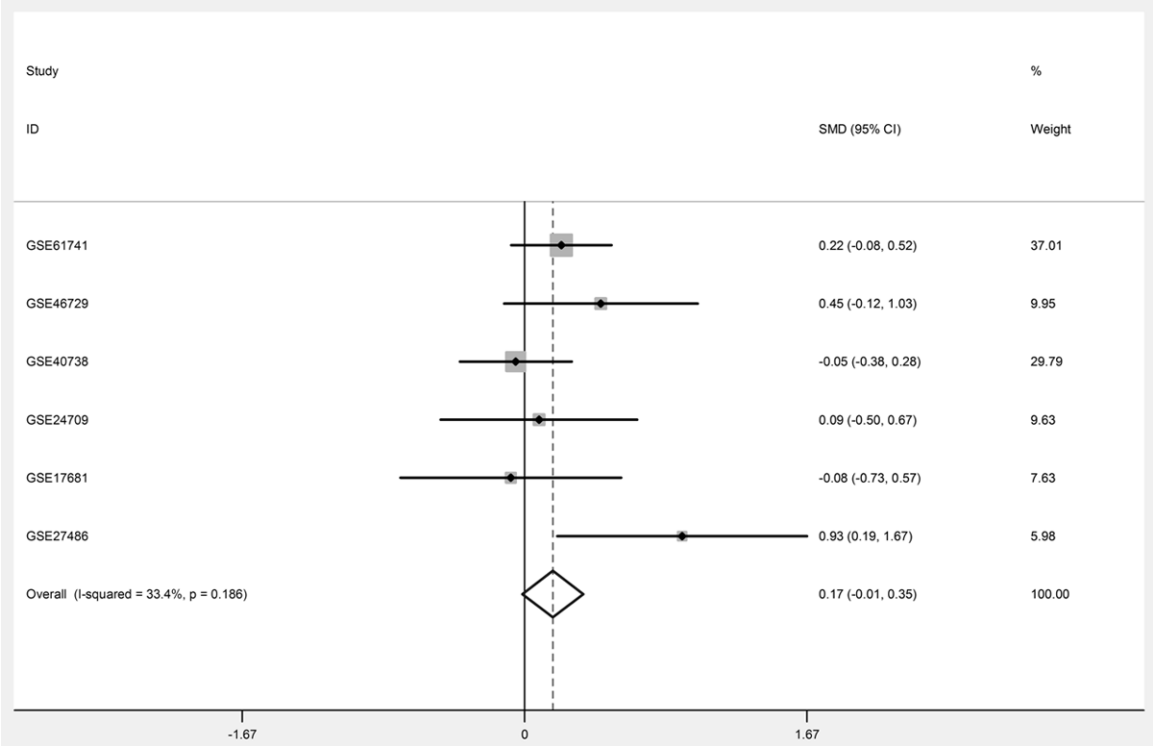

Figure 1. Forest plot of meta-analysis of the diagnostic value of miR-30a-3p expression for patients with NSCLCs. Random effects model was applied when combining standardized mean difference (SMD). The combined SMD displayed in this figure suggested that high level of circulating miR-30a-3p might contain a certain potential value to diagnose NSCLC, however, the *p* value did not reach the statistically significant level (P = 0.067).

Inclusion criteria

Eligible datasets were included if they met the criteria as follows: (i) both NSCLC patients and non-cancerous healthy controls were included in each dataset, and each group contained at least 3 samples; (ii) the original expression profiling data of miRNAs from both of the case and control groups were available or could be calculated; (iii) the subjects involved in the current meta-analysis were only humans.

Quality control and data extraction

Two investigators (Xing-gu Lin and Gang Chen) extracted the data independently from all qualified datasets according to the aforementioned requirements. Disagreements were resolved via cautious deliberation with the third and fourth authors (Rong-quan He and Li-hua Yang). Quality control was conducted with Limma package and ExiMiR package in R, including background correction and normalization processing [12, 13]. Expression values of miR-30a-3p and sample size were extracted in both case and control groups. If multiple probes were mapped to a single miRNA, average value of these levels was regarded as the expression value of a certain miRNA. Furthermore, means and standard deviations (SD) of these values were calculated.

Statistical analysis

The meta package in R was employed to perform the current meta-analysis [12, 14]. Continuous outcomes were presented as standard mean difference (SMD) with 95% confidence interval (CI), and effect sizes were pooled with random- or fixed-effects model according to different conditions. Heterogeneity across studies was assessed with the chi-square test of Q and the I^2 statistic [12, 15, 16]. A p value<0.05 or I^2>50% was considered as heterogeneous, if so, the random-effects model (DerSimonian-Laird method) would be selected to calculate the pooled SMD. Or else, the fixed-effects model (Mantel-Haenszel method) was preferred for the pooling process [17].

To further elucidate whether the pooled result was achieved due to one large study or a single

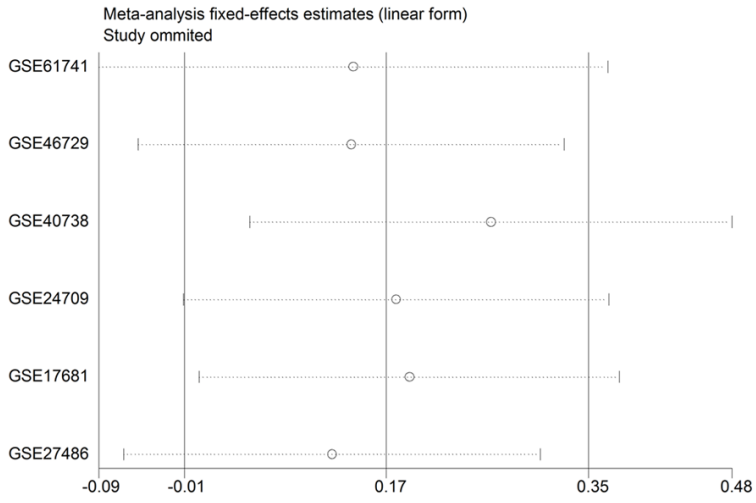

Figure 2. Sensitivity analysis of the value of miR-30a-3p on the early diagnosis of NSCLC. No studies had the most negative or positive influence on the summary SMD.

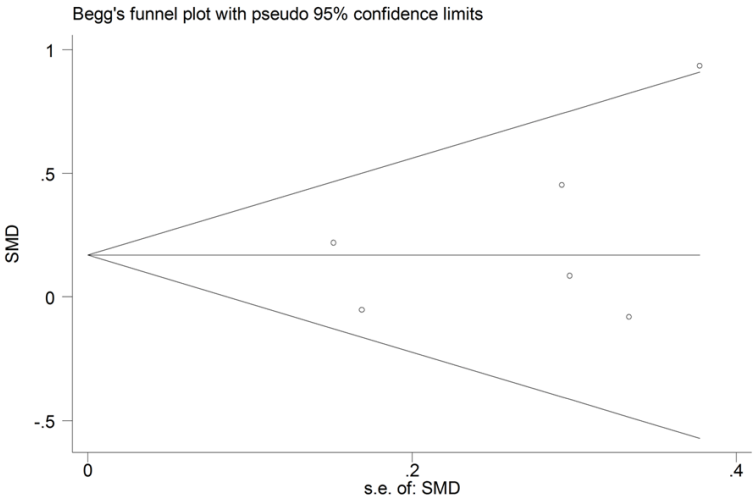

Figure 3. Begg's funnel plot for the assessment of potential publication bias. No potential publication bias was found.

study with an extremely divergent result, sensitivity analysis was applied to omit one study at a time. In addition, the potential publication bias was assessed with Begg's and Egger's tests. When P<0.05, the results would be regarded as publication bias.

Results

Features of the included datasets

The features of the enrolled datasets were shown in **Table 1**. In total, 6 eligible datasets, including GSE61741 (Germany), GSE46729 (USA), GSE40738 (USA), GSE27486 (USA), GSE24709 (Germany), and GSE17681 (Germany), were involved in the meta-analysis. The data for GSE61741 (Germany), GSE24709 (Germany), and GSE17681 (Germany) were all derived from peripheral blood cells, and the data for GSE46729 (USA) was from serum, while the data for GSE40738 (USA) and GSE27486 (USA) were from whole blood. Altogether, 250 NSCLC patients and 242 healthy controls were enrolled in these 6 datasets.

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The means and SD of both experimental and control groups were calculated for all 6 included microarray datasets (**Table 2**). The SMD ranged from -0.080 to 0.935 (**Table 2**). According to the heterogeneity test, no significant heterogeneity was found among these individual datasets (P = 0.186, I^2 = 33.4%, **Table 2**; **Figure 1**). Thus, the fixed-effects model was selected to calculate the pooled SMD and 95% CI.

Though no remarkable change was observed between NSCLC and non-cancerous control group (SMD = 0.169; 95% CI, -0.012 to 0.350; P = 0.067), the *p* value was close to 0.05. The results suggested that high circulating miR-30a-3p expression might have potential to distinguish NSCLC patients from non-cancer controls. However, this hypothesis needs to be verified with larger cohorts.

Sensitivity analysis and publication bias assessment

According to sensitivity analysis, the results indicated that no studies had the most negative or positive influence on the summary SMD

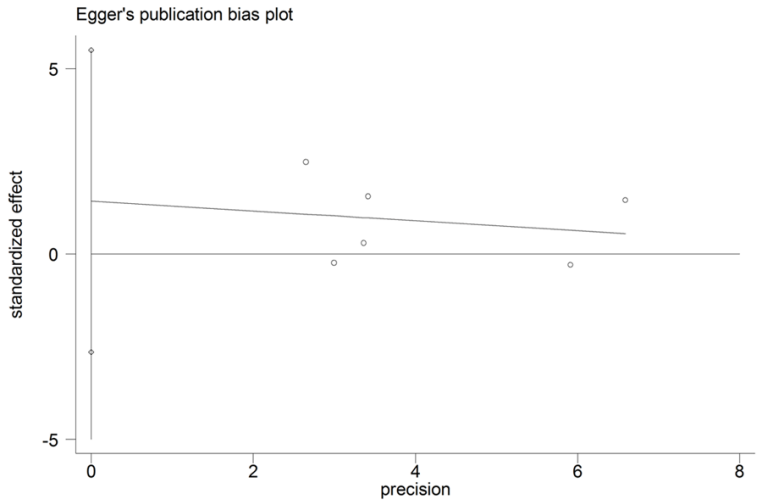

Figure 4. Egger's funnel plot for the assessment of potential publication bias. No potential publication bias was observed.

(**Figure 2**). The results of the meta-analysis were stable. Thus, no study omission was performed for the sensitivity analysis. Similarly, the Begg's funnel plot showed symmetry distribution and no obvious publication bias was found in this meta-analysis (**Figure 3**, P = 0.707). Additionally, Egger's test revealed no potential publication bias (**Figure 4**, P = 0.388).

Discussion

Lung cancer, including NSCLC and SCLC, is a risk factor in cancer death and late diagnosis is one of the most essential causes for the high mortality rate [1, 2, 18]. Circulating miRNAs represent stable and reproducible markers for various solid tumors, including NSCLCs [19-21]. Hence, circulating miRNAs in serum, plasma or whole peripheral blood have been hypothesized as non-invasive diagnostic markers for NSCLC [19, 22, 23]. However, no extremely effective miRNA has been discovered as biomarker in clinical settings for the early diagnosis of NSCLC [8, 19, 22].

In addition to some miRNA candidates, the clinical significance of miR-30a-3p in NSCLCs was first investigated by the group of Cazzoli R *et al.* [11]. In a training set with 30 frozen plasma samples, including 10 NSCLCs (adenocarcinomas), 20 non-cancerous controls (10 granulomas and 10 healthy smokers), Cazzoli R *et al.* [11] performed microRNA Ready-to-Use PCR and found that miR-30a-3p was slightly downregulated in lung granulomas and on the contrary, miR-30a-3p was upregulated moderately in lung adenocarcinomas. Furthermore, consistent expression pattern of miR-30a-3p was confirmed in another validation set with another 50 NSCLCs and 55 non-cancerous controls with quantitative RT-PCR. Student t-test comparing the difference of miR-30a-3p between controls and NSCLCs showed the significance of 8 miRNAs in the exploitation of the diagnostic test and miR-30a-3p was one of the candidates. However, this has been the only study by far to explore the diagnostic value of circulating miR-30a-3p for NSCLCs. Due to the small number of patients involved, the validity of the conclusion was questioned. Therefore, the current meta-analysis was carried out to explore the clinical significance of circulating miR-30a-3p for the early detection of NSCLCs.

In the present meta-analysis, a total of 6miRNA expression profiling datasets including 250 NSCLC patients and 242 healthy controls were enrolled. The pooled SMD revealed that miR-30a-3p expression in patients' whole blood, peripheral blood cells and serum showed no significant difference between NSCLC patients and healthy controls. However, the pooled SMD was 0.169 and *p* value was a borderline as 0.067, which suggested a potential trend that high level of circulating miR-30a-3p occurred more likely in NSCLCs, as compared to non-cancer people. This showed the concordant direction with the report of Cazzoli R *et al.* [11]. Of course, the diagnostic significance of circulating miR-30a-3p level needs further verification.

To the best of our knowledge, this is the first meta-analysis so far to assess the suitability of miR-30a-3p as a blood-based biomarker for early revealing of NSCLCs based on the microarray datasets. Two meta-analyses were conducted to summarize the known different microRNA expression profiles between lung cancer tissues and non-tumor lung by vote-counting strategy with miRNA microarray assays [24, 25]. Both of these 2 meta-analyses

demonstrated that miR-30a-5p, but not miR-30a-3p, was among the most frequent lowly expressed miRNAs in NSCLC tissues rather than in non-cancerous lung tissues. However, the miR-30a-3p level in NSCLC tissues and its clinical significance remain yet uncertain. Either, the correlation between miR-30a-3p level in tissues and in body fluid has not been clarified. Further experiments are required to figure out the clinical role of miR-30a-3p in NSCLC tissue samples.

Nevertheless, several studies have been performed to investigate the characteristic and molecular mechanism of miR-30a-3p in different malignancies. Most of the studies revealed a lower expression of miR-30a-3p in the tumor tissues and regarded miR-30a-3p as a suppressor miRNA. For example, in breast cancer tissues, downregulation of miR-30a-3p was identified [26, 27] and miR-30a-3p was also downregulated in tumors from breast cancer patients with early recurrence [26]. In clear cell renal cell carcinoma tissues, miR-30a-3p downregulation promoted increased expression of HIF2α [28]. The expression of miR-30a-3p was also significantly downregulated in tumors in HCC patients compared to adjacent normal tissues. Downregulation of miR-30a-3p was associated with notably higher occurrence of portal vein tumor thrombus in HCC. Furthermore, miR-30a-3p overexpression *in vitro* showed a suppressive effect on cell proliferation, an induced effect on apoptosis and an increased effect on arrest of cells in the S phase in HCC, via targeting vimentin, MMP3 and E-cadherin in HCC [29]. Among different types of ovarian carcinomas, miR-30a-3p expression was found to be the lowest in mucinous carcinoma, but highest in clear cell carcinoma. Also, expression level of miR-30a-3p was up-regulated in well-differentiated tumor as compared with poorly differentiated ones of ovarian carcinomas [30]. Additionally, the expression of miR-30a-3pwas decreased in bladder cancer, colorectal cancer, as well as esophageal squamous cell carcinoma tissues [31-33]. Being inconsistent with aforementioned studies, upregulation of miR-30a-3p was reported in squamous cell carcinoma of the tongue [34]. Thus, miR-30a-3p may act as a tumor-specific miRNA, playing various roles in different cancers.

In addition to the level of miR-30a-3p in tumor tissues, another study demonstrated that circulating level of miR-30a-3p in plasma significantly decreased after hysterectomy of endometrioid endometrial carcinoma [35]. Due to the non-invasiveness and stability, detection of circulating miRNAs gains the potential value in clinical application for tumor early diagnosis. Further research is needed to study the clinical role of circulating miR-30a-3p level in different classes of malignancies.

Several limitations should be noted in the current meta-analysis. Firstly, the small sample size limited the robustness of the meta-analysis. Only 6 datasets were included. The power of the funnel plots in estimating publication bias might be misguided owing to the limited number of qualified studies for meta-analysis. Further studies involving large sample size should be designed to confirm miR-30a-3p expression levels in NSCLCs. Secondly, because the miR-30a-3p expression data were only extracted from microarray assays without extra confirmation by more accurate methods (for instance, real time RT-qPCR), the level of circulating miR-30a-3p needs to be further validated. Thirdly, additional source of a relevant bias might include the regions involved in the meta-analysis, since only data sets from USA and Germany were included. Therefore, the current result has to be interpreted cautiously.

In conclusion, even though high level of circulating miR-30a-3p expression showed a potential relationship with the risk of NSCLC, the evidence of significant difference of circulating miR-30a-3p between NSCLC patients and healthy controls is insufficient. Further studies of high quality in the future are desired to evaluate the predictive power of circulating miR-30a-3p in larger cohorts of samples with validated detection methods.

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Disclosure of conflict of interest

None.

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