Evaluation of lateral flow immunochromatographic assay for diagnostic accuracy of cryptococcosis

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Research

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Abstract

Background: *Cryptococcus* is a conditional pathogenic fungus causing cryptococcosis, which is one of the most serious fungal diseases faced by humans. Lateral flow immunochromatographic assay (LFA) is successfully applied to the rapid detection of cryptococcal antigens.

Methods: Studies were retrieved systematically from the Embase, PubMed, Web of Science, and Cochrane Library before July 2019. The quality of the studies was assessed by Review Manager 5.0 based on the Quality Assessment of Diagnostic Accuracy Study guidelines. The extracted data from the included studies were analyzed by Meta-DiSc 1.4. Stata 12.0 software was used to detect the publication bias.

Results: A total of 15 articles with 31 fourfold tables were adopted by inclusion and exclusion criteria. The merged sensitivity and specificity in serum were 0.98 and 0.98, respectively, and those in the cerebrospinal fluid were 0.99 and 0.99, respectively.

Conclusions: Compared to the urine and other samples, LFA in serum and cerebrospinal fluid is favorable evidence for the diagnosis of cryptococcosis with high specificity and sensitivity.

Background

Cryptococcosis is caused by *Cryptococcus*, an opportunistic pathogen. *C. gattii* and *C. neoformans* are responsible for almost all cryptococcal infections in humans. People with low immunity have a high probability of being infected with *Cryptococcus*; for example, HIV patients and patients with long-term use of glucocorticoids, immunosuppressants, broad-spectrum antibiotics, and anti-tumor drugs^[1–2]. All organs of humans can be infected with *Cryptococcus*. Without complement and anti-cryptococcus growth factors in cerebrospinal fluid (CSF), cryptococcal meningitis (CM) is the main clinical manifestation of the cryptococcal infection in the central nervous system^[3]. Approximately, one million individuals die of cryptococcosis each year worldwide^[4]. Even in areas where antiretroviral therapy is available, the fatality rate of CM is high, and only about 50% of the patients survive within 6 months^[3].

Thus, cryptococcosis has become a serious global public health problem. However, the cryptococcal infection is short of specificity with diverse clinical manifestations. Cryptococcosis is frequently misdiagnosed at the early stage^[5–7]. Lateral flow immunochromatographic assay (LFA) is a rapid diagnostic method for the quantitative or qualitative detection of analytes in complex mixtures providing results within 5–30 min^[8].

The diagnosis of cryptococcosis relies on the histopathological examination, pathogenic smear, and culture of pus puncture specimens in the lesion. These methods have the advantage of high specificity, but the sensitivity is low. Moreover, these tests are time-consuming and require auxiliary equipment^[9]. LFA can detect samples without special auxiliary equipment, which can also be used for the determination of single samples and preserve the results of the test. As a rapid diagnostic method, LFA has high specificity and sensitivity under the premise of rapid reaction. The USA Food and Drug Administration has approved LFA as a semi-quantitative tool for the rapid detection of cryptococcal capsular polysaccharide antigen in the serum or CSF^[10]. Thus, this diagnostic technology has been adopted by most countries for the detection of cryptococcosis. The application of LFA for the rapid diagnosis of cryptococcosis dramatically shortens the time of detection. Furthermore, it provides a basis for the rapid faster diagnosis of cryptococcal disease and guidelines for future treatment. Herein, we collected relevant articles for the meta-analysis to assess LFA for the diagnostic accuracy of cryptococcosis.

Methods And Materials

Search strategy and source

Four investigators systematically searched all the articles about the cryptococcus and LFA before July 2019 in the Embase, PubMed, Web of Science, and Cochrane Library databases. We used the keywords “cryptococcus, torula, filobasidiella” and

"lateral flow immunochromatographic assay, LFA, colloidal gold immunochromatography: for advanced search. Geographical restrictions were not applied in these articles.

Study selection and screening criteria

Two investigators systematically screened all of the articles by pre-established screening criteria. The inclusion criteria were as follows: (1) Studies published in English. (2) The purpose of the study was related to LFA and cryptococcosis. (3) Studies limited to original research. (4) Studies related to diagnostics. (5) Data can be extracted to construct fourfold tables. The exclusion criteria were as follows: (1) Duplicate studies, abstracts, conference abstracts, case reports, reviews, editorials. (2) Studies without a reference standard or a detailed number of samples. (3) Samples not from humans. (4) LFA as the reference standard.

Data extraction

In the process of carefully reading the included articles, the investigators simultaneously extracted related data from the studies, including the name of the first author, year of article, study design, geographical distribution of strains, patient population, reference standard, sample type, true positive (TP), false positive (FP), true negative (TN), and false-negative (FN). The process of extracting data is carried out independently by the investigators, and finally, the synthesis results were compared.

Quality assessment standard

We used the Quality Assessment of Diagnostic Accuracy Study (QUADAS-2) guidelines^[11] to assess the quality of included studies. Then, we analyzed the risk of bias and applicability concerns by Review Manager 5.0, including patient selection, reference standard, index test, flow, and timing. If the assessment results conflicted, the investigators reviewed the original studies, and a third investigator would intervene to achieve consensus.

Statistical analysis

We analyzed the extracted data, such as specificity, sensitivity, negative likelihood ratio (NLR), positive likelihood ratio (PLR), and diagnostic odds ratio (DOR), from the included studies using meta-DiSc 1.4 software. Also, we analyzed the summary receiver operating characteristic (SROC) curve and calculated the area under the curve (AUC). According to the sample types, these studies were analyzed by different methods. Due to the lack of adequate data on urine and other samples in the included articles, these samples were analyzed by Review Manager 5.0 software for sensitivity and specificity. Finally, publication bias was evaluated by Stata12.0 software.

Results

Search results

A total of 167 publications were retrieved, which decreased to 82 after excluding the duplicates. Also, 18 studies were excluded after screening the abstracts. After full-text review, we included 15 qualified articles^[9-10,12-24].

Characteristics of eligible studies

Fifteen studies were published between 2011 and 2019. 13/15 articles reported data from serum samples, seven collected CSF samples, two contained urine samples, and one contained the samples of fingerprick capillary blood and whole venous

blood. A total of 9312 samples were included in the meta-analysis, with an average of 620 (range 59–3447) samples. Table 1 summarizes the characteristics of these studies.

Quality assessment

We assessed the quality of 15 articles using Review Manager 5.3. (Fig. 1)

Data analysis

We classified the studies into different categories due to the different sample types.

For serum specimens, the merged sensitivity and specificity values were 0.98 (95% CI: 0.96–0.99) and 0.98 (95% CI: 0.97–0.98), respectively. The average PLR of LFA in the serum was 45.05 (95% CI: 26.22–77.40) and the NLR was 0.04 (95% CI: 0.02–0.10). The merged DOR was 1574.65 (95% CI: 730.16–3395.87) and AUC was 0.9766. The results are shown in Figs. 2, 4A.

For CSF specimens, the merged sensitivity and specificity values were 0.99 (95% CI: 0.98–0.99) and 0.99 (95% CI: 0.99 to 0.99), respectively. The average PLR of LFA in CSF was 93.89 (95% CI: 53.54–164.64) and the NLR was 0.03 (95% CI: 0.01–0.07). The merged DOR was 3864.72 (95% CI: 1308.89–11411.28) and AUC was 0.9983. The results are shown in Figs. 3, 4B.

For other samples, the results of sensitivity and specificity are shown in Figure 5.

Publication bias

In this meta-analysis, the data of serum and CSF samples were tested by Stata 12.0 for publication bias. Deek's funnel plot asymmetry test was used to assess the potential published bias in the included studies. The results of serum and CSF samples indicated that there was no obvious publication bias (Fig. 6).

Discussion

Cryptococcosis is a disease with a high mortality rate. Therefore, a rapid diagnosis of cryptococcal infection is necessary for patients presenting appropriate clinical symptoms. A comprehensive search with stringent screening criteria retrieved 15 articles eligible for inclusion in the study. These 15 articles encompassed 3901 serum samples, 4403 CSF samples, 1125 urine samples, 1163 venous whole blood samples, and 1163 fingerprick capillary blood samples.

The results in meta-analysis showed that the combined sensitivity of LFA in serum and CSF was 0.98 (0.96–0.99) and 0.99 (0.98–0.99); specificity was 0.98 (0.97–0.98) and 0.99 (0.99–0.99); DOR was 1574.65 (730.16–3395.87) and 2509.29 (184.18–34187.48); SROC AUC was 0.9962 and 0.9983, respectively.

Among these indexes, the PLR of the serum and CSF was >10, while the NLR was <0.1. The SROC AUC of the serum and CSF was close to 1. The SROC curve was close to the upper left corner, which indicated that the area under the curve was large. Both the AUCs were >0.9, indicating that LFA had a relatively high overall diagnostic accuracy for serum and CSF. The DOR of serum and CSF was significant, indicating that the correct diagnosis is far larger than the wrong diagnosis. In conclusion, LFA has a high degree of accuracy in the diagnosis of serum and CSF.

The current analysis of these articles revealed several factors that can explain the observed heterogeneity: the differences in the reference methods in the studies; the same reference standard was not used in the study for identification; the interpretation of the results in LFA and reference methods may cause the artificial error.

Nevertheless, the current study has some limitations. Firstly, we collected all the relevant articles; however, it was difficult to ensure that no publication was missing. Secondly, we only included the articles published in the English language, which may contribute to bias. Thirdly, our study only included the articles from inception to August 2019. The difference in the reference standard might also lead to the heterogeneity of the included studies. Finally, meta-analyses of LFA for the diagnosis of cryptococcosis, only until 2015, were included. Thus, we could comprehensively analyze the accuracy of the LFA diagnosis of the cryptococcal infection.

Conclusion

In summary, our meta-analysis indicated that LFA tested in serum and CSF has high diagnostic accuracy in the diagnosis of cryptococcal infection for high-risk patients, such as HIV-infected patients. LFA performed in urine, or other samples could be a screening tool for the early diagnosis of cryptococcal infection; however, additional studies are required for the substantiation of these results.

Declarations

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Authors' contributions

Xu-Guang Guo conceived and designed the experiments. Li-Min Xie, Geng-ling Lin, Hao-Neng Dong and Ying-Xia Liao analyzed the data and made the tables. Li-Min Xie, Ye-Ling Liu and Jian-Feng Qin contributed to the production of figures by the analysis tools. All authors participated in the writing, reading, and revising of the manuscript and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Abbreviations

AUC: calculated the area under the curve; CSF: cerebrospinal fluid; CM: cryptococcal meningitis; DOR: diagnostic odds ratio; FP: false positive; FN: false negative; HIV: human immunodeficiency virus; LFA: Lateral flow immunochromatographic assay; NLR: Negative likelihood ratio; PLR: Positive likelihood ratio; QUADAS: Quality Assessment of Diagnostic Accuracy Study; SROC: Summary receiver operating characteristic; TP: true positive; TN: true negative;

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Table

Table1. Characteristics of the included studies (n = 15).

Reference no.	First author	Year	Geographical distribution of strains	Study design	Patient population	Sample type(s)	Sample size	Reference standard
1	Lindsley	2011	Thailand	prospective	HIV	Serum Urine	538	EIA
2	Binnicker	2012	USA	prospective&retrospective	SC	Serum	634	LA
3	McMullan	2012	Australia	retrospective	SC	Serum	106	Comprehensive reference ³
4	Escandón	2013	Colombia	retrospective	HIV	Serum	421	LA
5	Hansen	2013	USA	prospective	SC	Serum CSF	1000	EIA
6	Rugemalila	2013	Tanzania	prospective	SC	Serum	319	LA
7	Boulware	2014	Uganda& South Africa	prospective&retrospective	HIV SM	CSF	666	Culture
8	Lourens	2014	South Africa	prospective	HIV SM	CSF	465	Culture/LA
9	Rivet-Dañon	2015	France	prospective&retrospective	IFI ¹ SC HIV	Serum CSF	292	LA
10	Suwantarat	2015	America	retrospective&prospective	SC	Serum CSF	1047	EIA/Enhanced reference ⁴
11	Jitmuang	2016	America	retrospective	HIV-N	Serum CSF	59	LA
12	Cáceres	2017	Colombia	retrospective	CIB ²	Serum CSF	83	LA
13	Frola	2017	Argentina	prospective	HIV	Serum	123	Comprehensive reference ⁵
14	Temfack	2018	Cameroon	prospective	HIV	Serum	186	EIA
15	Drain	2019	South Africa	prospective	HIV	VWB FCB Urine	3447	EIA/Combined reference ⁶

HIV, human immunodeficiency virus; SC, suspected cryptococcosis; SM, suspected meningitis; HIV-N,HIV-negative; CSF,cerebrospinal fluid; VWB,venous whole blood; FCB,fingerprick capillary blood; LA, latex agglutination method; EIA, enzyme-linked immunoassay; 1:patients proven or probable invasive fungal infection other than cryptococcosis; 2:patients with or without diagnosis of cryptococcosis were randomly selected from a collection of biological samples stored in the CIB's biobank; 3:Cryptococcosis was proven if the organism was detected by one or more of culture, histopathology or molecular tests; 4:An enhanced reference method includes data from histopathology, cytopathology, fungal culture, and patient clinical history in addition to EIA results; 5:Pathogen identification of isolates from positive blood cultures was performed using standard microbiology methods (morphological and biochemical tests); 6:A combined reference standard for either a positive CrAg EIA or latex agglutination test.

Figures

	<u>Risk of Bias</u>				<u>Applicability Concerns</u>		
	Patient Selection	Index Test	Reference Standard	Flow and Timing	Patient Selection	Index Test	Reference Standard
Binnicker 2012	?	?	+	+	+	+	+
Boulware 2014	+	+	+	+	+	+	+
Cáceres 2017	-	+	+	+	+	+	+
Drain 2019	?	+	+	-	+	+	+
Escandón 2013	+	-	+	+	+	-	+
Frola 2017	+	+	-	+	+	+	+
Hansen 2013	+	+	+	+	+	+	+
Jitmuang 2016	+	?	+	+	+	?	+
Lindsley 2011	?	-	+	?	?	-	+
Lourens 2014	+	+	+	+	+	+	+
McMullan 2012	+	+	+	-	+	+	+
Rivet-Dañon 2015	-	?	?	+	-	+	+
Rugemalila 2013	+	+	+	+	+	+	+
Suwantarat 2015	+	?	+	-	+	?	+
Temfack 2018	+	?	?	+	+	?	+

High
Unclear
Low

Figure 1

Quality evaluation of the included studies.

Figure 2

Figure 2. Forest plots of (A) sensitivity, (B) specificity, (C) positive LR, (D) negative LR, (E) diagnostic OR of LFA for the diagnosis of cryptococcosis in serum sample.

Figure 3

Figure 3. Forest plots of (A) sensitivity, (B) specificity, (C) positive LR, (D) negative LR, (E) diagnostic OR of LFA for the diagnosis of cryptococcosis in CSF sample.

Figure 4

Forest plots of SROC curve of the sample in (A) serum sample and (B) CSF sample.

Urine

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Drain 2019	7	116	7	1021	0.50 [0.23, 0.77]	0.90 [0.88, 0.91]		
Drian* 2019	9	114	14	1014	0.39 [0.20, 0.61]	0.90 [0.88, 0.92]		
Lindsley 2011	52	0	0	22	1.00 [0.93, 1.00]	1.00 [0.85, 1.00]		

Venous whole blood

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Drain 2019	6	22	8	1127	0.43 [0.18, 0.71]	0.98 [0.97, 0.99]		
Drian* 2019	9	19	14	1121	0.39 [0.20, 0.61]	0.98 [0.97, 0.99]		

Fingerprick capillary blood

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Drain 2019	5	38	9	1111	0.36 [0.13, 0.65]	0.97 [0.95, 0.98]		
Drian* 2019	6	37	17	1103	0.26 [0.10, 0.48]	0.97 [0.96, 0.98]		

Figure 5

Forest plots of the sensitivity and specificity of LFA for the diagnosis of cryptococcal infection in urine and blood.

Figure 6

Deeks'funnel plot asymmetry test to assess publication bias in estimates of diagnostic odds ratio for LFA detection of cryptococcal infections.

Supplementary Files

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- [Flowdiagramofstudyidentificationandinclusion.docx](#)