The Diagnostic Accuracy of Direct Agglutination Test for Serodiagnosis of Human Visceral Leishmaniasis: A Systematic Review with Meta-analysis

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Abstract

Background: agglutination test (DAT) as simple, accurate and non-expensive tool that has been used widely for serodiagnosis of visceral leishmaniasis (VL) during the last three decades. We conducted a systematic review and meta-analysis to evaluate the diagnostic accuracy of DAT for serodiagnosis of human VL.

Methods: Electronic databases, including MEDLINE (via PubMed), SCOPUS, Web of Science, SID and Mag Iran (two Persian scientific search engines) were searched from December 2004 to April 2019.

The study quality was evaluated using the QUADAS checklist. We determined the sensitivities and specificities across studies, calculated positive and negative likelihood ratios (LR+ and LR-), and constructed summary receiver operating characteristic(ROC) curves parameters.

Results: Of the 2928 records identified in the mentioned electronic databases and through articles' reference lists, 25 articles met inclusion criteria and enrolled into the systematic review and among them 22 records were qualified for meta-analysis. The pooled sensitivity and specificity of DAT was 96% [(95% CI, 93–98])and 95% [(95 % CI, 88–98]), respectively. The likelihood ratio of a positive test (LR+) was found to be 19.8 [CI95%, 7.6–51.8] and the likelihood ratio of a negative test (LR−) was found to be 0.04 [CI95%, 0.02–0.08]. The combined estimate of the diagnostic odds ratio for DAT was high [454)136-1561])].We found that the summary receiver operating characteristic curve (SROC) is positioned near the upper left corner of the curve and the area under curve (AUC) was 0.98 (95% CI, 0.97 to 0.99).

Conclusion: Based on our analysis, we find DAT can be considered as valuable tool for the serodiagnosis and seroprevalence of human VL with high sensitivity and specificityrates. As DAT is simple, accurate ,non-invasive and efficient serological test, it can be used for serodiagnosis of human VL particularly in endemic areas of the disease.

Background

Visceral leishmaniasis (VL) is one of the most important neglected tropical diseases that is caused by *Leishmania donovani* and *L. infantum/ chagasi* in both humans and canines. (1-4). The morbititidy and mortality of VL are estimated 200,000 to 400,000 new cases and approximately 20,000 to 40,000 deaths occur annually(4). More than 90% of all VL cases occur in 6 countries including India, Bangladesh, Brazil, Ethiopia, Sudan, and South Sudan (5).

Since fatality rate of VL is high and it can be reached to 100% when it was not treat accurately, a diagnostic test with both highly validity and reproducibility rates is required(1,6). The gold standard for diagnosing VL is mainly parasitological examinations including the demonstration of parasites by microscopic examination of splenic or bone-marrow aspiration (1,4, 6,7).

Sample preparation for parasitological examinations of VL is highly invasive. In spite of high specificity of parasitological examinations, the sensitivity of these methods is depended on the type of kind and prepared samples (7). Moreover, the accuracy and precision of parasitological examinations also assiciates on the experience of laboratory microscopist. Molecular methods including Polymerase chain reaction (PCR)-based methods have been developed and assessed for human and canine VL using various target genes and different clinical specimens (8). The pooled sensitivity and specificity of PCR for detection of *L.infantum/ chagasi* infections in humans on peripheral blood samples was reported 93.1% and 95.6%, respectively(7,8). The most limitation of PCR assays is the lack of standardization due to the high number of different administrative protocols(7). In addition, the specificity of the molecular methods was significantly lower in various studies because in some studies parasitological methods are not able to identify true-positive patients (7). Moreover, these methods need to sophisticated and expensive equipments and materials and also the performance of molecular methods particularly in remote areas is very difficult.

Several serological tests are used for sero diagnosis of VL, including the indirect fluorescent antibody test (IFAT), the enzyme linked immunosorbent assay (ELISA), the Latex agglutination test (LAT), immunoblotting, the direct agglutination test (DAT) and the rK39 rapid diagnostic test. Among which, the direct agglutination test (DAT) and rK39 are simple and don't need to sophisticated materials and time consuming thus,they are usually used in the field studies as well as in laboratories.

One of the major drawbacks of serological tests is they cannot be able to detect relapses among VL cases because anti-*Leishmania* antibodies remain a long time after clinical cure (9, 10) and many people are living in endemic areas have anti-*Leishmania* antibody titers due to high exposure to asymptomatic infections individuals(11). In the previous meta-analysis of the diagnostic performance of the DAT for VL, the pooled sensitivities and specificitie rates were calculated 94.8% and 86%, respectively(12). This study aimed to update the diagnostic accuracy of the DAT for human VL diagnosis.

Methods

Study design

This study has systematically searched all the studies related to diagnostic performance of DAT from December 2004 to April 2019.

Selection was made independently by two reviewers (SSH and AB) and discrepancies were solved by consensus after discussion.

This systematic review with meta-analysis was conducted as per PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) guidelines (13).

Search strategy

Electronic databases, including MEDLINE (via PubMed), SCOPUS, Web of Science and two Persian scientific search engines "Scientific Information Database" (www.sid.ir) and "Mag Iran" (www.magiran.com) were searched systematically with various combinations of the following scientific keywords: "Visceral leishmaniasis", "*Leishmania infantum*", "DAT", "Direct Agglutination Test", "Parasitology", "Microscopy", "Specificity" and "Sensitivity" using "OR" and/or "AND". The reference lists of selected articles were also screened manually for possibly relevant articles. Abstracts of articles which published in congresses were not explored.

Case definition

Patients in VL-endemic countries with pathogonomonic signs and symtomes such as fever >2 weeks, hepatosplenomegaly, progress weakness, anaemia or pancytopenia , and lymphadenopathy with confirmation of following testsparticularly in VL endemic areas were considered as VL cases.

1-Parasitological diagnosis

a. Microscopic examination of bone marrow aspirate, lymph node aspirate, and/or spleen aspirate using direct semi-quantified microscopic examination of Giemsa-stained smears for detection of *leishmania* amastigotes.
b. Culture of bone marrow aspirate, lymph node aspirate, and/or spleen aspirate aspirate in Novy–MacNeal–Nicolle (NNN) media. Demonstration of at least one amastigote upon microscopic examination of tissue smears or one promastigote in culture is sufficient for diagnosis.

2- Serological tests

DAT test was done twice on each sample in standardized conditions, and each test was read by two readers who were blind to other test results.

Study selection and data extraction

Titles and abstracts of all articles were screened by one reviewer, and eligibility of the screened articles was assessed by two independent investigators using the following criteria.

Inclusion criteria were: a) have a full description of accuracy of DAT as a diagnostic test for the detection of VL in patients. b) Articles were published from December 2004 to April 2019. c) include both DAT test and Parasitological examinations (direct demonstration,culture) as the confirmatory diagnostic method for VL.

Exclusion criteria were: a) insufficient primary and/or secondary data information of (lack information about sensitivity and specificity), b) unavailable full text article, and c) written in a language other than English or Persian.

Eligibility of all explored papers was assessed by three reviewers. The discrepancies among studies were obviated by discussion and consensus. Afterward, data of interest were gathered using a pre-designed data extraction form containing all the descriptive variables and test results. The following information was extracted: authors, year and country in which the study was carried out, diagnostic methods applied, reference test used, cut off, characteristics of the participants, quality of the study and sample size.

Assessment of Study Quality

We assessed the quality of studies using the Quality Assessment of Studies of Diagnostic Accuracy Approach-QUADAS (14).

Data Synthesis and Statistical Analysis

All meta-analysis methods were performed using STATA (Release 12. statistical software. College Station, Texas: STATA Corp LP). Sensitivity and specificity were calculated when available data were presented. To calculate sensitivity and specificity values for the tests, we cross-tabulated each result against the reference standard. Whenever possible, we extracted raw data from primary studies to fill in the four cell values of a diagnostic 2×2 table: true positives, false positives, true negatives, and false negatives.

In many articles the numbers of true positive, false negative, true negative, and false positive observations were available. If not, we derived the numbers from the marginal totals and the reported sensitivity and specificity.

Meta-analyses were accomplished by using random-effects inverse-variance weights. Results of the meta-analysis were illustrated by a forest plot diagram, which demonstrated the estimated prevalence and their relevant 95% confidence interval (CI).

The Cochran's Q and I^2 statistics were used for assessment of the between-study inconsistency and heterogeneity, respectively. The I^2 values of 25%, 50%, and 75% were representatives of low, moderate and high heterogeneity, respectively.

Publication bias was evaluated through Egger's test(15).

Results

Of the 2928 records identified in the mentioned electronic databases and through articles' reference lists (Fig1), 25 articles met inclusion criteria and enrolled into the systematic review and among them 22 records were qualified for meta-analysis. The characteristics of the included studies are summarized in Table 1.

Table 1. The characteristics of the included studies

Author	Country	Year	Sample size	Diagnostic method	Cut off	DAT+ Parasitology +	DAT+ Parasitology -	DAT- Parasitology +	DAT- Parasitology -	QUADAS score
Bimal (21)	India	2005	799	Bone marrow and spleen aspiration	≥1:800	99	0	9	691	12
Silva(17)	Brazil	2005	16	Bone marrow aspiration	≥1:800	16	0	0	0	11
Sundar(25)	India	2006	329	Spleen aspiration	≥1:3200	227	3	5	94	13
Sundar(26)	India	2006	508	Spleen aspiration	≥1:1600	144	54	6	304	13
Sundar(26)	India	2006	508	Spleen aspiration	≥1:1600	146	45	4	313	13
Kumar(22)	India	2006	548	Bone marrow and spleen aspiration	≥1:800	63	4	17	464	10
El Mutasim(34)	Sudan	2006	302	Lymph nodes, bone marrow or spleen aspiration	≥1:3200	92	16	7	187	13
Gavgani(29)	Iran	2007	12	Bone marrow aspiration	≥1:3200	12	0	0	0	9
Mansour(35)	Sudan	2007	174	Lymph node aspirate	≥1:3200	24	1	1	148	11
Taran-Angel(39)	Venezuela	2007	54	Bone marrow or spleen aspiration	>1:1:600	26	0	0	28	10
Taran(31)	Iran	2007	15	Bone marrow aspiration	≥1:3200	12	3	0	0	12
Sinha(24)	India	2008	18	Spleen or bone marrow aspiration	≥1:800	6	2	0	10	11
Sinha(24)	India	2008	20	Spleen or bone marrow aspiration	≥1:800	10	0	0	10	11
Kilic(38)	Turkey	2008	59	Bone marrow aspiration - culture	≥1:1600	24	4	0	31	12
Mandal(23)	India	2008	57	Spleen and bone marrow aspiration	≥1:800	16	1	0	40	10
Ghatei(30)	Iran	2009	90	Bone marrow or spleen aspiration	≥1:3200	27	0	2	61	12
Hailu(19)	Ethiopia	2009	137	Spleen or lymph node aspiration - cultures	≥1:1600	41	20	43	33	10
Mansour(36)	Sudan	2009	322	Lymph node aspirate	≥1:3200	114	17	11	180	11
Ter Horst(20)	Ethiopia	2009	699	Spleen aspiration	≥1:3200	144	338	9	208	10
Gani(28)	Iraq	2010	57	Bone marrow aspiration	≥1:800	10	0	47	0	11
Topno(27)	India	2010	355	Bone marrow or spleen aspiration	≥1:800	5	34	0	316	12
Canavate(18)	Ethiopia	2011	102	Spleen aspiration - culture	≥1:3200	32	1	3	66	12
Abass(33)	Sudan	2011	183	Lymph node aspiration	≥1:3200	100	6	0	77	11
El. Moamly(32)	Saudi Arabia	2012	33	Bone marrow aspiration - culture	≥1:1600	9	1	1	22	12
El. Moamly(32)	Saudi Arabia	2012	65	Bone marrow aspiration - culture	≥1:1600	24	2	1	38	12
Machado(16)	Brazil	2012	356	Bone marrow smear	≥1:3200	192	41	21	102	8

Junior(15)	Brazil	2015	7	Bone marrow aspiration	≥1:3200	3	4	0	0	10
Osman(37)	Sudan	2016	135	Lymph node aspirate	≥1:3200	92	0	1	42	11
Osman(37)	Sudan	2016	141	Lymph node aspirate	≥1:3200	95	0	4	42	11

In overall, studies had a wide geographical distribution and were carried out in different countries including Brazil (16-18), Ethiopia (19-21), India (22-28), Iraq (29), Iran (30-32), Saudi Arabia (33), Sudan (34-38), Turkey (39) and Venezuela (40).

The reference method for diagnosis of VL in all studies was a positive result on microscopic examination and/or culture of lymph node, bone marrow, or spleen aspirate. Diagnosis was confirmed by detection of parasites in culture (19, 20, 33, 39).

Some studies performed DAT using both freeze-dried (FD) and aqueous (AQ) antigen (27, 33, 38). No significant difference in the sensitivity of the DAT (FD and AQ) was found. In one study (38), relatively higher sensitivity (99%) was recorded for the LQ-DAT than for the FD-DAT (96%) that might be due to the use of the endemic autochthonous *Leishmania donovani* isolate as the antigen.

Cut off dilution for a positive test on DAT reported to vary from 1:800 to 1:3200 (Table 2).

Table 2. Diagnostic accuracy of DAT for diagnosis of VL based on different cut off values

Parameter	Cut off			
	All	1:800	1:1600	1:3200
Sensitivity	96 [93-98]	98 [67-100]	97 [87-99]	95 [93-97]
Specificity	95 [88-98]	98 [91-100]	88 [79-94]	93 [70-99]
Positive Liklihood Ratio	19.8 [7.6-51.8]	53.5 [10.1- 282.1]	8.2 [4.3- 15.8]	14.7 [2.7-79.8]
Negative Liklihood Ratio	0.04 [0.02-0.08]	0.02 [0- 0.47]	0.03 [0.01- 0.17]	0.05 [0.03-0.08]
Diagnostic Odds Ratio	454 [136- 1516]	3271 [116-92224]	267 [26-2712]	303 [41-2226]

Figures 1 shows the results of individual and combined sensitivity and specificity estimates of the DAT test for diagnosis of VL.

The pooled sensitivity of the 25 studies evaluating the DAT was 96% [CI95% 93–98] and the pooled specificity was 95% [CI95% 88–98] (Figure 2). The likelihood ratio of a positive test (LR+) was found to be 19.8 [CI95% 7.6–51.8] and the likelihood ratio of a negative test (LR−) was found to be 0.04 [CI95% −0.02–0.08]. Among studies which considered DAT cut off value of 1:800, the pooled sensitivity and specificity was 98% [CI95% 67–100] and 98% [CI95% 91-100], respectively. With considering this cut off value, the LR+ was found to be 53.5 [CI95% 10.1-282.1] that was more higher than LR+ obtained from analysis of studies with cut off value of 1:1600 and 1:3200. Although the pooled sensitivity of DAT with cut off value of 1:1600 and 1:3200 was remained stable??% (CI95% 95-97%), the pooled specificity was declined to 88% [CI95%79-94] with cut off value of 1:1600 and 93% [CI95% 70-99] with cut off value of 1:3200, respectively.

The combined estimate of the diagnostic odds ratio for DAT was high (454 [136-1561]).

We found that the summary receiver operating characteristic curve (SROC) is positioned near the upper left corner of the curve and the area under curve (AUC) was 0.98 (95 % CI, 0.97 to 0.99) (Figure 3).

The Deeks' funnel plots for publication bias [16] also showed no asymmetry (Figure 4). The evaluation of publication bias showed no potential for publication bias (p=0.65).

Discussion

Until the 1990, VL diagnosis was needed to parasitological confirmation including microscopy or culture of the spleen,bone-marrow, lymph nodes and sometimes peripheral blood specemens. The invasiveness and sometimes fatal complications particularly associated with splenic aspiration caused to develop of non-invasive serological tests such as DAT (41,42).

Although the IFAT and ELISA are two important serological methods for diagnosis of human VL but they require specific materials and equipments (1,43).

Other rapid diagnostic tests or immunochromatograhic diagnostic methods such as recombinant *Leishmania donovani* complex antigens (rK39, rK26, rKe16, have some limitations including low specificity in sub-clinical and asymptomatic forms of *L.donovani* infections particularly among Sudanese population(22,42,44).

Among the available serological tests for the diagnosis of VL, DAT is simple, highly specific and sensitive, reliable and cost-effective test can be used in field as well as laboratory studies(41,45-49).

According to our knowledge, this is the second systematic review and meta-analysis about diagnostic accuracy of DAT for diagnosis of VL. Chappuis *et al.* included 30 relevant studies that evaluated DAT from January 1986 to December 2004 (12) and reported the pooled sensitivities and specificities of 94.8% and 86%, respectively. In the present study, 25 eligible studies from April 2004 to December 2019 were evaluated DAT for the diagnosis VL in immunocompetent patients by the systematic review. Our meta-analysis showed DAT is still as a validated serodiagnostic test with high pooled sensitivity and specificity of 96% [CI95%,93–98] and 95% [CI95%,88–98], respectively while 1:1600 or 1:3200 were considered as a cut-off titer.Highier pooled sensitivity 98% (95% CI, 67–100) and specificity 98% (95% CI, 91-100) were found at 1:800 cut-off titer.

Specific *Leishmania* antibodies at a titer of 1:800 showed visceral leishmanial infection(43) whereas *Leishmania* antibodies at a titer of 1:3200 with pathogonomonic clinical signs such as hepatosplenomegaly, dromedary fever, anemia and progress weakness reflected active visceral leishmaniasis (45-48).

DAT can be performed on serum, plasma or even urine samples (41,50-53) with high diagnostic accuracy.

According to the results of meta-analysis that compared DAT with rK39 strip test, almost 1% more sensitivity and 2% more specificity than rK39 strip test was found.

Although we did not enough information about human immunodeficiency viruses status of the cases, it has been reported that DAT has acceptable sensitivity in the diagnosis of VL in HIV-positive patients (16,21,55,56).

Early diagnosis and prompt treatment are essential role for reducing mortality rate of VL (1). As the clinical manifestations of VL are not completely pathognomonic and have low specificity, confirmatory tests are required to recognize VL patients which need to treat. These tests not only must be sensitive, but they also need to be specific because the current anti-*Leishmania* drugs preccriped to treat VL are highly toxic (1,7).

The most important limitation for the evaluation of serological tests is the absence of an appropriate gold standard test. Confirmation of VL depends on the finding of Leishman bodies of *Leishmania* sp. in samples prepared from bone marrow or spleen, lymph nodes and liver. This procedure is highly invasive, and thus it can be done only in suspected cases of VL. Moreover,the sensitivity rate of this method is varied (1,7,51).

In some endemic areas for VL, DAT is prepared and used routinely for the diagnosis and sero-epidemiological studies of VL because it is simple and highly sensitive (41,43).

The performance of DAT is neither *Leishmania* species-specific nor region dependent (51).

Major shortcomings of DAT are long incubation time for reading the results, batch to batch variability of the antigen and cross-reactivity with *Trypanosoma cruzi* infection(41,45,51). DAT titer decline over time falling below the cut off (1:800) at the ninth month of cure but still remains positive for relatively long time (up to 5 years in more than 50% of VL cases) after the cure. Thus, the test cannot be used for follow up of treatment or for the diagnosis of disease relapse (54). To overcome the problem of long incubation time, a fast agglutination screening test (FAST) has been introdust, which uses only one serum dilution and require only three hours of incubation (57). The validity of FAST for the detection of *L. infantum* infection in the field was compared with the conventional DAT in Iran and the results showed a sensitivity of 95.4% and specificity of 88.5% for fast DAT in comparison with conventional DAT (57).

Conclusion

Based on our analysis, DAT can be used as the best choice for early detection of acute and ,chronic clinical forms of VL as well as asymptomatic form of the infections in immunocompenent individuals. Further research on DAT antigen standardization in the laboratories located in endemic areas of VL is recommended.

Abbreviations

DAT: direct agglutination test

VL: visceral leishmaniasis

FAST: fast agglutination screening test

PCR: Polymerase chain reaction

IFAT: indirect fluorescent antibody test

ELISA: enzyme linked immunosorbent assay

FD: freeze-dried

AQ: aqueous antigen

SROC: summary receiver operating characteristic curve

AUC: area under curve

Declarations

Authors' contributions

MM: involved in designing,interpretations and writing of the manuscript. HK, SM and GH: participated in interpretation of the DAT results. SS,BA and AB:involved in gathering and grouping the articles regarding DAT for visceral leishmaniasis and drafted the manuscript. SM.involved in data analysis and writing of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data obtained

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

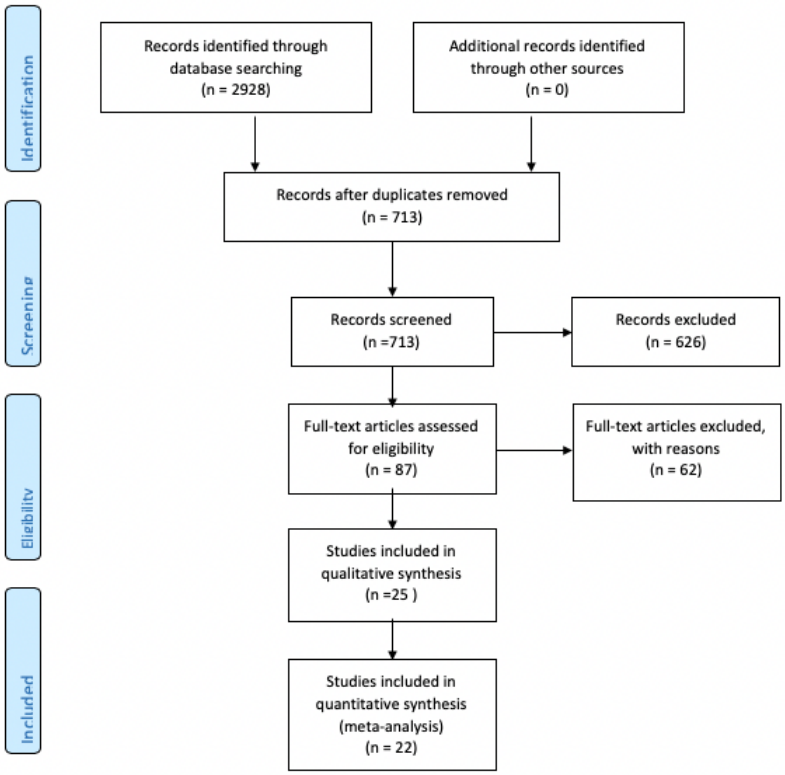

Figure 1

Flowchart of study selection

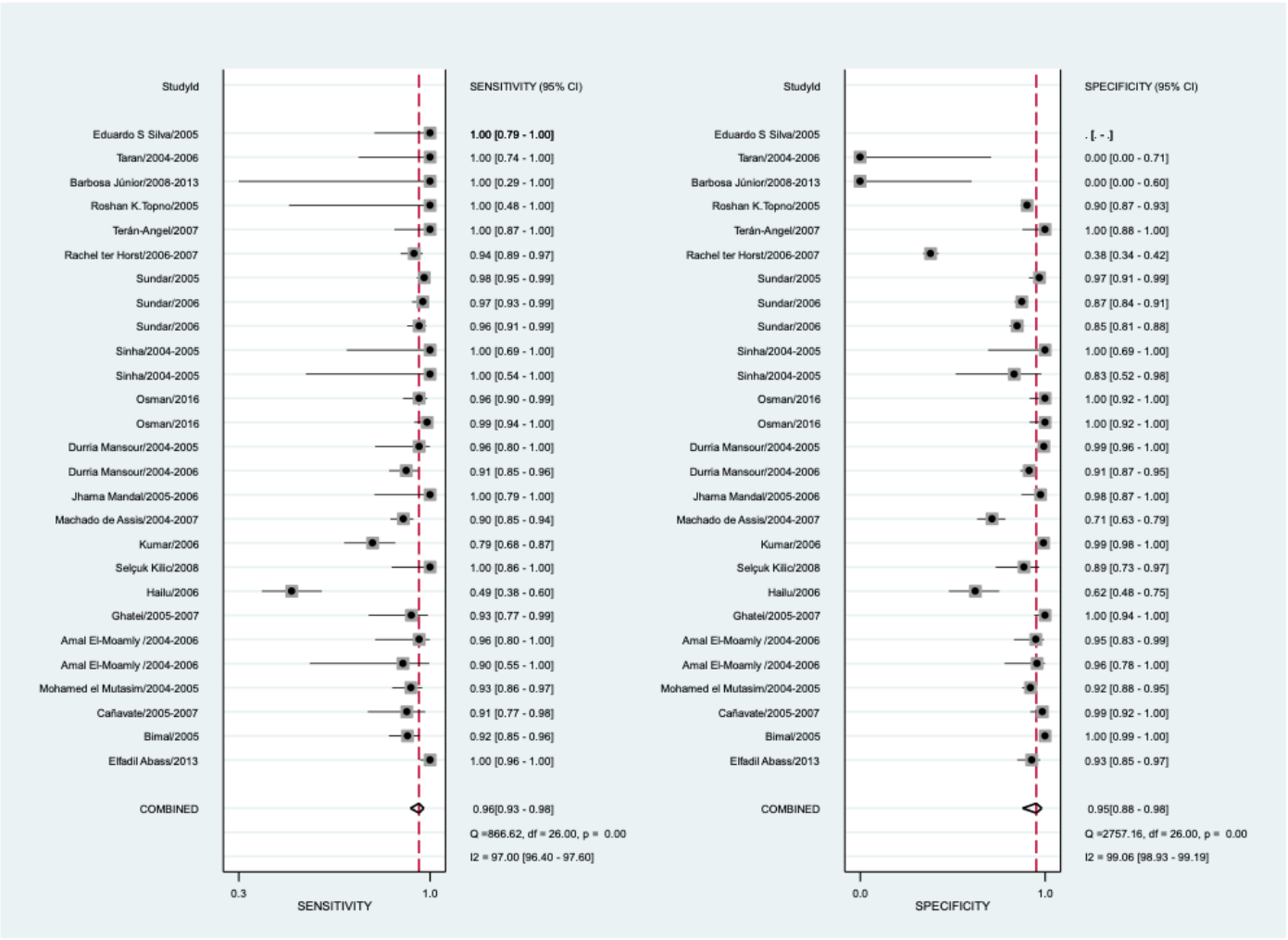

Figure 2

Forest plot showing the sensitivity and specificity of DAT in the diagnosis of VL

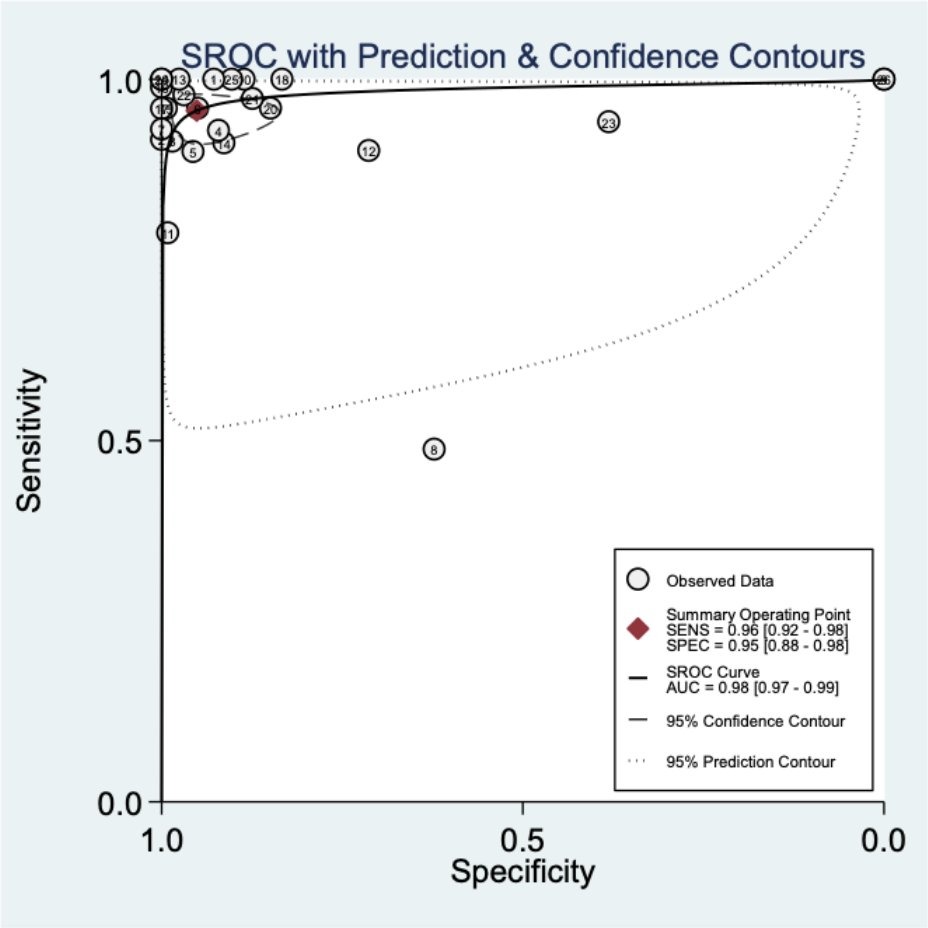

Figure 3

Summary receiver operating characteristic (SROC) curve for assessment of the diagnostic accuracy of DAT for the diagnosis of VL

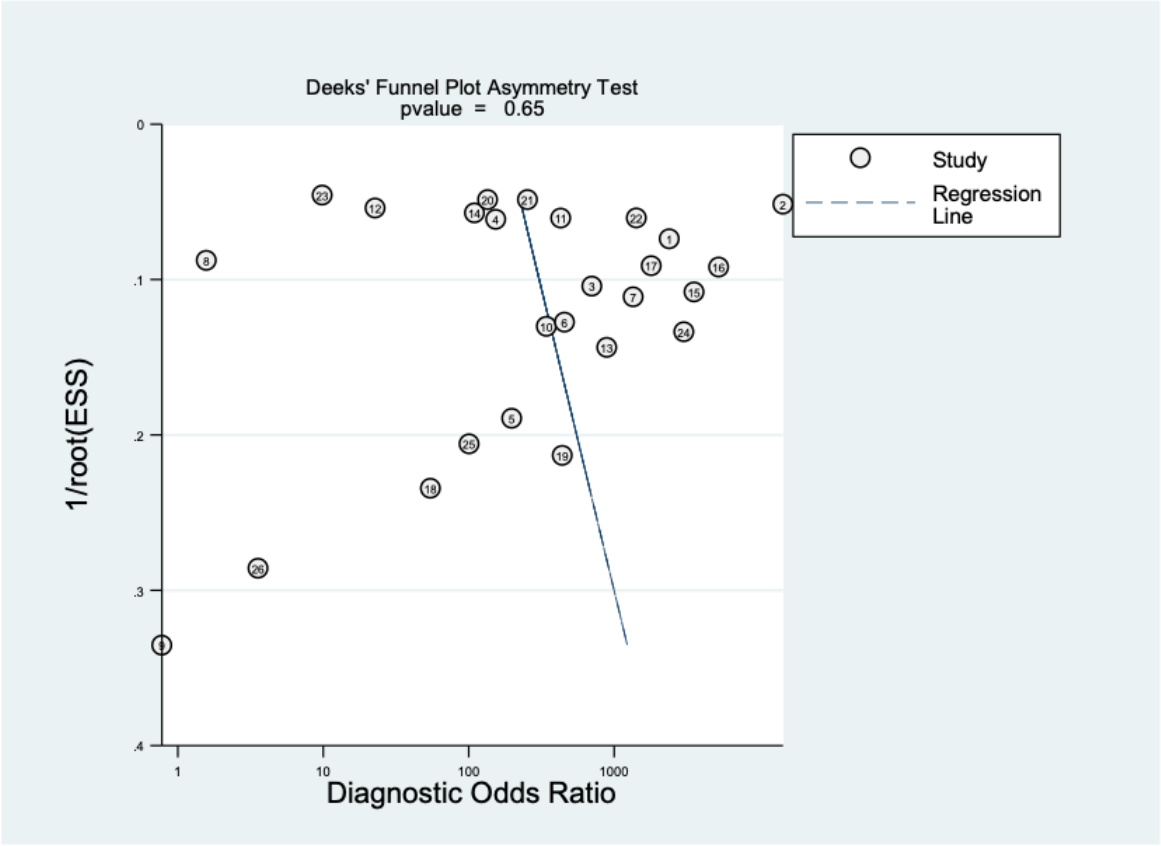

Figure 4

Deeks' funnel plots for publication bias