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REVIEW

Endobronchial ultrasound-guided transbronchial needle aspiration for diagnosis of sarcoidosis in clinically unselected study populations

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ABSTRACT

Literature suggests that ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) has excellent performance characteristics for diagnosis of sarcoidosis. However, many authors challenge the external validity of EBUS-TBNA results, as most studies were performed in referral centres by highly experienced investigators, and included populations with very high sarcoidosis prevalence.

We performed a systematic review and meta-analysis to estimate the role of EBUS-TBNA for diagnosis of sarcoidosis in studies enrolling consecutive patients with lymphadenopathy detected at imaging studies, regardless of the suspected underlying clinical aetiology. The Pubmed, Embase, Cinahl, Web of Science and Cochrane Library databases were screened to identify the pertinent literature. Quality of eligible studies was assessed by Quality Assessment, Data Abstraction and Synthesis-2 criteria. Pooled diagnostic yield, sensitivity and specificity were calculated, and a summary receiver operating characteristic curve was constructed. Subgroup analysis was planned to identify possible sources of study heterogeneity. Fourteen studies, collectively involving 2097 patients, fulfilled eligibility criteria. The median prevalence of sarcoidosis was 15%. EBUS-TBNA had a pooled diagnostic yield of 0.79 (standard deviation, 0.24), a pooled sensitivity of 0.84 (95% confidence interval (CI), 0.79–0.88) and a pooled specificity of 1.00 (95% CI, 0.99–1.00). Only subgroup analysis exploring the influence of study design seemed to influence the observed inter-study heterogeneity for sensitivity, retrospective studies showing worst sensitivity than prospective ones. The results of EBUS-TBNA for diagnosis of sarcoidosis in clinically unselected populations are excellent and compare favourably with published results from studies conducted in selected populations. High-quality trials would be needed to evaluate factors possibly explaining the observed heterogeneity in sensitivity.

Key words: bronchoscopy, endobronchial ultrasound-guided transbronchial needle aspiration, interventional technique, sarcoidosis.

Abbreviations: CT, computed tomography; EBUS-TBNA, endobronchial ultrasound-guided transbronchial needle aspiration; IQR, interquartile range; PET, positron emission tomography; QUADAS-2, Quality Assessment, Data Abstraction and Synthesis-2; ROSE, rapid on-site cytology evaluation.

INTRODUCTION

Convex-probe endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is increasingly used for pathologic confirmation of the clinical suspect of sarcoidosis owing to its excellent success rate. Most individual studies[1–8] and the only meta-analysis[9] of EBUS-TBNA in sarcoidosis published up to now, in fact, have reported sensitivity values higher than 80%.

However, several authors have cast doubt on the external validity of these results mainly on the basis of two different concerns.[10–15] First, studies on the role of EBUS-TBNA for diagnosis of sarcoidosis were derived from study populations with high pre-test probability, as suggested by their very high prevalence of the disease (>90%), and might not be reproducible in groups of patients with more heterogeneous causes underlying the lymphadenopathy.[10–14] Second, most such studies were carried out in tertiary medical centres by bronchoscopists and pathologists with large experience on execution of EBUS-TBNA and sample interpretation, respectively.[10–15]

Ideally, the usefulness of EBUS-TBNA should be assessed in populations including patients for whom sarcoidosis is only one of the possible diagnostic alternatives, after clinical and radiological inclusion criteria have been prospectively defined.[14] As no such study exists in the literature, the only possible

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alternative to analysing the results of EBUS-TBNA for diagnosis of sarcoidosis in selected populations consists in extrapolating its performance characteristics from studies where all patients undergoing EBUS-TBNA for the diagnosis of lymphadenopathy were analysed in a given time frame.

We performed a systematic review and meta-analysis to estimate the test performance of EBUS-TBNA for diagnosis of sarcoidosis in studies that enrolled consecutive patients with intrathoracic lymphadenopathy detected at imaging studies (computed tomography (CT) and/or positron emission tomography (PET)), whatever the clinical entity suspected of causing them.

METHODS

Literature search

A systematic search of the medical literature was performed in the first week of May 2013, and updated in the first week of November 2013, to identify all studies that evaluated the diagnostic role of EBUS-TBNA in consecutive patients undergoing the procedure for diagnosis of intrathoracic lymphadenopathy identified by CT and/or PET.

The search was constructed and performed by a professional medical librarian (V.S.) from January 2003 (the year of first publication regarding convex-probe EBUS-TBNA in mediastinal lymphadenopathies)[16] through October 2013. The following databases were screened: Pubmed, Embase, Cinahl, Web of Science and Cochrane Library (the full search strategy is listed in the Supplementary Table S1). There were no language restrictions.

Selection of studies

All references identified by our search strategy were independently assessed by two authors (R.T. and L.L.A.), first by title and abstract, then by review of the complete paper, as indicated. Additional articles were sought through review of reference lists.

Studies were eligible for inclusion if they fulfilled the following criteria: (i) convex-probe EBUS-TBNA was used in consecutive patients with lymph nodes having short axis > 1 cm at CT and/or PET; (ii) Histopathology analysis and/or clinical-radiological follow-up for at least 6 months was used as reference standard. (iii) Sarcoidosis patients were present in the study population, regardless of their absolute number. (iv) For per-patient statistics, sufficient data were presented to calculate at least the diagnostic yield of EBUS-TBNA in patients with sarcoidosis.

We excluded papers that were not about convex-probe EBUS-TBNA for diagnosis of lymphadenopathy; review articles, case reports, letters and editorials; and studies that included clinically selected study populations (i.e. suspected lung cancer staging/restaging, suspected sarcoidosis, suspected tuberculosis).

Discrepancies were resolved by consensus. If agreement could not be reached, a third reviewer (M.P.) was consulted, and the majority opinion was used for analysis.

Data extraction

Two Authors (R.T. and L.A.) extracted the following key data on a standard data extraction form: (i) publication details; (ii) patient enrollment (prospective or retrospective); (iii) imaging method used to identify the lymphadenopathy; (iv) type of sedation used (moderate or deep), (v) size of EBUS-TBNA needle (21-gauge or 22-gauge), (vi) availability of rapid on-site cytology evaluation (ROSE); (vii) processing method for EBUS-TBNA cytologic samples; (viii) prevalence of sarcoidosis; (ix) diagnostic yield, true positives, true negatives, false positives, false negatives; (x) reference standard; (xi) complications.

The authors of studies not reporting sufficient data were contacted to request for additional information.

Quality assessment

The quality of the eligible studies was evaluated through the Quality Assessment, Data Abstraction and Synthesis-2 (QUADAS-2) tool, which comprises four domains: patient selection, index test, reference standard and flow and timing. Each domain is assessed in terms of risk of bias, and the first three domains are also assessed in terms of concerns regarding applicability. Signalling questions, which must be tailored to adequately cover any issue of the review, are included to help judging the risk of bias. For this specific review, tailoring of some signalling question was performed in the 'index test' and in the 'reference standard' domains, while the 'patient selection' and 'flow and timing' domains were retained in their entirety (the complete QUADAS-2 checklist used in the present review is outlined in Supplementary Table S2).

Data synthesis and statistical analysis

We assessed the possibility of publication bias by examining asymmetry of funnel plot of estimates of diagnostic *d* against corresponding precision (its variance).[17] As the informal examination for asymmetry of funnel plot is subjective, so that different observers may interpret the same graph differently, we also formally evaluated asymmetry of funnel plots by using Begg's test, which calculates the Spearman's adjusted rank correlation *rho* to assess the association between test accuracy estimates and their variances. The deviation of Spearman's *rho* values from zero provides an estimate of funnel plot asymmetry. Positive values indicate a trend towards higher levels of test accuracy in studies with smaller sample sizes.

Diagnostic yield, sensitivity and specificity were pooled with weighted averages applied, in which the weight of each study was its sample size. As no diagnostic threshold exists for histological diagnoses, symmetrical summary receiver operating characteristic curve, as described by Moses *et al.*,[18] was constructed to summarize the results regarding sensitivity quantitatively.

Study heterogeneity was assessed by the I^2 index, which describes the percentage of total variation across studies that is due to heterogeneity rather than chance. A value of > 50% may be considered indicative

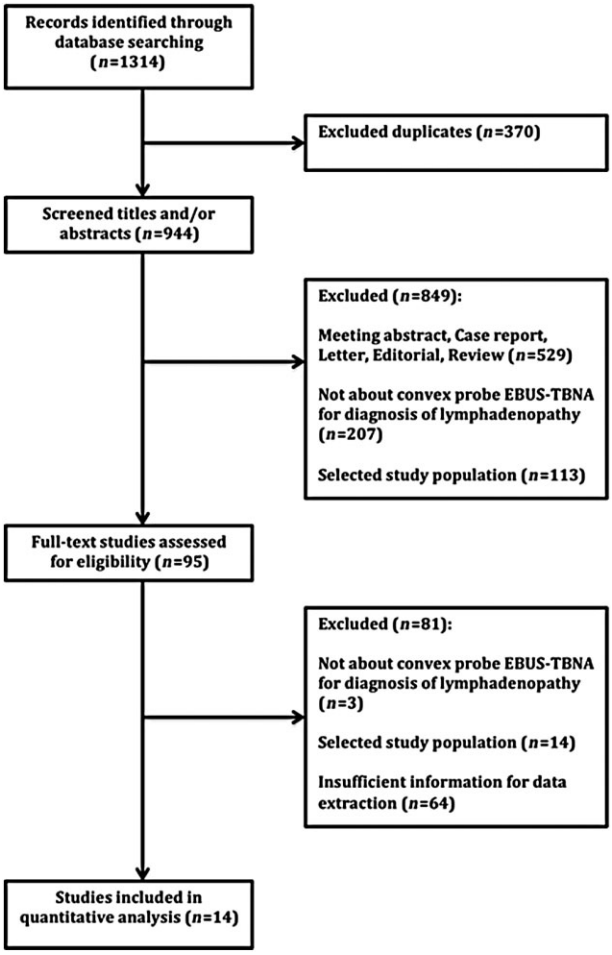

Figure 1 PRISMA flow diagram of the literature search.

of significant heterogeneity. If heterogeneity was demonstrated, subgroup analysis was performed according to common methodological and clinical features of the studies to identify possible sources of heterogeneity.

All tests were two sided, and a P-value < 0.05 was considered to be statistically significant. Data analysis was performed with STATA statistical package (release 13.1, 2013, Stata Corporation, College Station, Texas, USA). Meta-analysis was performed using Meta-DiSc (Version 1.4).[19]

RESULTS

Study selection

Our search strategy yielded 1314 papers for consideration (Fig. 1). Following elimination of the 370 duplicates, 944 titles and/or abstract were reviewed. Figure 1 shows the flow of study selection. A total of 14 studies were finally deemed eligible for inclusion and were submitted to quantitative analysis.[20–33]

Study description, publication bias and quality assessment

Study characteristics are summarized in Table 1. Studies were conducted in 11 different countries and collectively enrolled 2097 patients (median (interquartile range (IQR)) number of participants per study: 114 (56–191)). The overall number of patients diagnosed with sarcoidosis was 269, with a median (IQR) number of sarcoidosis patients per study of 9.5[6–21] and a median prevalence of sarcoidosis of 15% (0.4–20.0).

Low inter-study variation of the EBUS method was noted (Table 2). All studies used a convex-probe ultrasound bronchoscope produced by Olympus Ltd, and most of them (13/14, 92.8%) used 22-gauge needles. ROSE was available for all enrolled patients in a minority of studies (5/14, 35.7%). EBUS specimens were processed as smears in 10 studies, and as smears and cell-block in three studies, whereas the processing method was not described in one study.

The funnel plot was not asymmetric, as demonstrated by both visual inspection and by formal Begg's test (rho = 0.33, P = 0.26), indicating the absence of an important publication bias (Fig. 2). Assessment of study quality by applying QUADAS-2 criteria raised some potential methodological limitations (Table 3). For instance, no study specified who interpreted the results of the index test (EBUS-TBNA), which are *per se* not specific to sarcoidosis. All of the studies were therefore deemed to carry an unclear risk of bias in the 'index test domain'. Furthermore, patients with EBUS-TBNA findings suggesting a diagnosis 'reactive lymphadenopathy' did not receive the same reference standard in most studies. In only four studies (28.6%), in fact, the investigators confirmed with a surgical procedure, on a regular basis, the diagnosis of reactive lymphadenopathy suggested by the EBUS findings. In the remaining 10 studies (71.4%), the diagnosis of reactive lymphadenopathy suggested by EBUS was often confirmed or refuted based on clinical and radiological follow-up. The latter 10 studies were deemed to carry a high risk of bias in the 'flow and timing domain'.

Diagnostic performance, heterogeneity and subgroup analysis

EBUS-TBNA had a pooled mean (standard deviation (SD)) diagnostic yield of 0.79 (0.24), a pooled sensitivity of 0.84 (95% CI, 0.79–0.88; Fig. 3) and a pooled specificity of 1.00 (95% CI 0.99–1.00). The area under the summary receiver operating characteristic curve was 0.998 (standard error 0.001).

On I^2 statistics, no heterogeneity in specificity was found (I^2=0; P = 1.000), whereas significant heterogeneity between sensitivity of individual studies was demonstrated (I^2 = 80.4%; P = 0.0001). To explore heterogeneity in sensitivity, we performed several subgroup analyses even though statistical tests between subgroups were not performed, as they would likely yield unreliable results owing to significant heterogeneity within studies of each subgroup (Table 4). First, we evaluated the influence of the prevalence of sarcoidosis by comparing the sensitivity of EBUS-TBNA in studies with prevalence values below or above the median (15%). Sensitivity was lower in studies with lower prevalence (0.69; 95% CI 0.58–0.79) than in studies with higher prevalence (0.89; 95% CI

Table 1 Study characteristics

Author	Year	Country	Study design	Imaging test used for patients' enrollment	Eligible patients (No.)	Mean age (years)	Sex (Male %)	No. of sarcoidosis patients/prevalence (%)
Yasufuku[20]	2004	Japan	Prospective	CT	70	64.3	74%	3/4.3%
Herth[21]	2006	Germany, Denmark	Prospective	CT	502	58.9	63%	6/1.2%
Szlubowski[22]	2008	Poland	Retrospective	CT	149	56.7	73%	21/14.1%
Garcia-Olive[23]	2009	Spain	Prospective	CT	128	62	81%	5/3.9%
Bizekis[24]	2010	USA	Retrospective	CT and/or PET	51	62	67%	8/15.7%
Tian[25]	2010	China	Retrospective	CT	52	52.3	63%	16/31%
Cetinkaya[26]	2011	Turkey	Prospective	CT	287	50.2	56%	105/36.6%
Jernlas[27]	2011	Sweden	Retrospective	CT	243	63	58%	28/11.5%
Mohan[28]	2011	India	Retrospective	CT	191	65	54%	5/2.6%
Saji[29]	2011	Japan	Prospective	CT and/or PET	56	65	66%	11/19.6%
Gurioli[30]	2012	Italy	Prospective	CT	94	62	67%	18/19.1%
Lange[31]	2012	Germany	Retrospective	CT	100	58	71%	7/7.0%
Yang[32]	2012	China	Retrospective	PET/CT	45	55	73%	7/15.6%
Gindesgaard[33]	2013	Denmark	Retrospective	CT	129	61.1	50%	29/22%

Table 2 Procedural details and yield of ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) for diagnosis of sarcoidosis

Author	EBUS scope	Needle size	Sedation	Availability of ROSE	Processing method for cytologic material	Complication rate	Diagnostic yield for sarcoidosis
Yasufuku[25]	Olympus	22-gauge	Moderate	Yes	Smears	0%	100%
Herth[20]	Olympus	22-gauge	Moderate or Deep	No	Smears	0%	33%
Szlubowski[28]	Olympus	22-gauge	Moderate	No	Smears	0%	85.7%
Garcia-Olive[21]	Olympus	22-gauge	Deep	Yes	Smears	0%	100%
Bizekis[22]	Olympus	22-gauge	Deep	Some cases	Smears and Cell Block	0%	50%
Tian[30]	Olympus	22-gauge	Moderate	Not reported	Smears	0%	56%
Cetinkaya[24]	Olympus	22-gauge	Moderate	No	Smears and Cell Block	0.34%	96%
Jernlas[26]	Olympus	22-gauge	Moderate	Some cases	Smears	0.8%	53.4%
Mohan[29]	Olympus	22-gauge	Moderate or Deep	No	Smears and Cell Block	9.3%	100%
Saji[31]	Olympus	21- or 22-gauge	Moderate	Yes	Smears	0%	91%
Gurioli[23]	Olympus	22-gauge	Deep	Yes	Smears	0%	100%
Lange[27]	Olympus	22-gauge	Deep	No	Smears	0%	57%
Yang[32]	Olympus	22-gauge	Moderate	Not reported	Smears	Not reported	100%
Gindesgaard[33]	Olympus	Not reported	Deep	Yes	Not reported	0%	82.7%

EBUS, endobronchial ultrasound; ROSE, rapid on-site cytology evaluation.

0.84–0.93). However, further analysis using robust linear regression (Fig. 4) failed to confirm the relationship between prevalence of sarcoidosis and EBUS-TBNA sensitivity (rho = 0.10; $P = 0.724$). Second, we assessed the influence of the study design (prospective versus retrospective enrollment). Interestingly, the diagnostic sensitivity of EBUS-TBNA for sarcoidosis was much higher in prospective than in retrospective studies (94% vs 71%, respectively). Third, we explored the possible influence of the size of the study population on sensitivity. By using the cut-off value of 100 patients, very close to the median (IQR) number of patients per study among eligible studies (114 (56–191)), we found a marginal advantage in terms of sensitivity in larger (0.85; 95% CI 0.80–0.90) than in smaller studies (0.79; 95% CI 0.67–0.87). Fourth, no differences were found between studies that carried out a systematic surgical confirmation of a diagnosis of reactive lymphadenopathy made at EBUS (0.77; 95% CI 0.60–0.90) versus studies that did not (0.85; 95% CI 0.79–

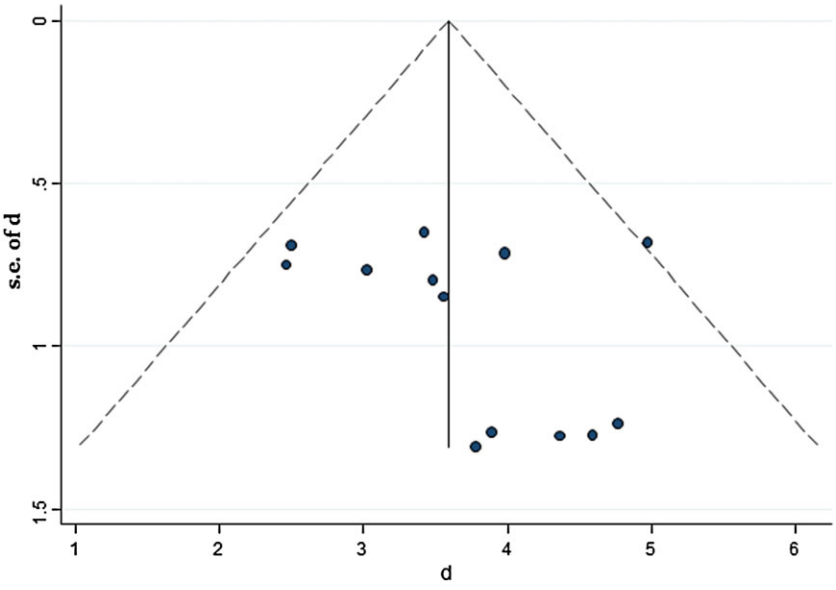

Figure 2 The funnel plot of included studies was not asymmetric both by visual inspection and by formal evaluation through Begg's test (rho = 0.33, $P = 0.26$), a result suggesting the lack of significant publication bias (d: measure of the discriminative ability; SE: standard error).

Table 3 Study quality assessment by Quality Assessment, Data Abstraction and Synthesis-2 (QUADAS-2) criteria

	Risk of BIAS				Applicability CONCERNS		
Study	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Yasufuku[20]	L	U	L	L	L	L	L
Herth[21]	L	U	L	L	L	L	L
Szlubowski[22]	L	U	L	H	L	L	L
Garcia-Olive[23]	L	U	L	H	L	L	L
Bizekis[24]	L	U	L	L	L	L	L
Tian[25]	U	U	L	H	L	L	L
Cetinkaya[26]	L	U	L	H	L	H	L
Jernlas[27]	L	U	L	H	L	L	L
Mohan[28]	L	U	L	H	L	L	L
Saji[29]	L	U	L	H	L	L	L
Gurioli[30]	H	U	L	L	L	L	L
Lange[31]	L	U	L	H	L	L	L
Yang[32]	U	U	L	H	L	L	L
Gindesgaard[33]	L	U	L	H	L	L	L

H, high risk; L, low risk; U, unclear risk.

0.89). Finally, no difference was seen between studies that employed moderate versus deep sedation, as well as between studies that employed ROSE versus those that did not (Table 4). We had also planned a subgroup analysis by sarcoidosis stage, as literature suggests that characteristics of lymph nodes and sensitivity of EBUS-TBNA are actually different in stage I as compared to stage II, but we could not complete this analysis due to inconsistent reporting of data regarding sarcoidosis stage across studies.

DISCUSSION

The results of our study indicate that EBUS-TBNA has very good test performance for diagnosis of sarcoidosis even in clinically unselected cohorts of patients with intrathoracic lymphadenopathy. In spite of the low overall median prevalence of sarcoidosis (15%) in the 14 studies included in the present analysis, the pooled diagnostic yield (79%) and sensitivity (84%) were excellent and compared favourably with those reported in the only available meta-analysis as well as in individual studies performed in selected populations characterized by a prevalence of the disease uniformly higher than 90%.[1–9]

It is important that these results be interpreted keeping into account the strengths and limitations of the present study. The decision to skip studies enrolling selected populations is certainly important, as they may overestimate the success rate of EBUS-TBNA for diagnosis of sarcoidosis through several

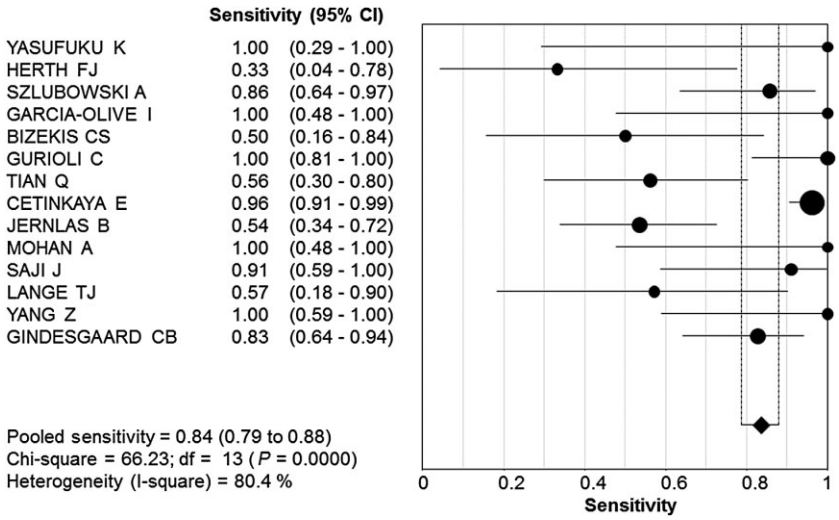

Figure 3 Forest plot of sensitivity.

Table 4 Results of pooled analysis and heterogeneity

	Studies (No.)	Patients (No.)	Pooled sensitivity (95% CI)	Pooled specificity (95% CI)	AUC (SE)	Likelihood ratio (I^2)	Chi square test (*P*-value)
Sarcoidosis prevalence							
>15%	7	194	0.89 (0.84–0.93)	1.00 (0.99–1.00)	0.999 (0.001)	81.6	32.65 (<0.001)
<15%	7	75	0.69 (0.58–0.79)	1.00 (0.99–1.00)	0.998 (0.003)	69.0	19.36 (0.004)
Study population size							
≤100	7	70	0.79 (0.67–0.87)	1.00 (0.99–1.00)	0.999 (0.002)	74.4	23.46 (0.001)
>100	7	199	0.85 (0.80–0.90)	1.00 (0.99–1.00)	0.999 (0.001)	85.4	41.08 (<0.001)
Study design							
Prospective	6	148	0.94 (0.89–0.97)	1.00 (0.99–1.00)	0.998 (0.002)	74.4	19.51 (0.002)
Retrospective	8	121	0.71 (0.62–0.79)	1.00 (0.99–1.00)	0.997 (0.006)	65.7	20.42 (0.005)
ROSE							
Available	5	66	0.90 (0.81–0.96)	1.00 (0.99–1.00)	0.999 (0.003)	41.6	6.85 (0.144)
Unavailable	5	144	0.90 (0.84–0.94)	1.00 (0.99–1.00)	0.999 (0.001)	82.9	23.44 (<0.001)
Outliers removed†	3	59					
Sedation							
Deep	5	67	0.82 (0.70–0.90)	1.00 (0.99–1.00)	0.999 (0.001)	74.5	15.67 (0.003)
Moderate	6	184	0.84 (0.78–0.89)	1.00 (0.99–1.00)	0.998 (0.003)	87.0	38.42 (<0.001)
Outliers removed‡	2	18					
Reactive lymph nodes							
Surgical confirmation	4	35	0.77 (0.60–0.90)	1.00 (0.99–1.00)	0.997 (0.005)	84.1	18.90 (<0.001)
Clinical follow-up	10	234	0.85 (0.79–0.89)	1.00 (0.99–1.00)	0.999 (0.002)	80.5	46.18 (<0.001)

† Outliers: studies where the use of ROSE was not described, and studies where ROSE was unavailable for all patients.
‡ Outliers: studies where the type of sedation was not described.
AUC, area under the curve; ROSE, rapid on-site cytology evaluation; SE, standard error.

mechanisms. Studies conducted in patients selected based on a strong clinical–radiological suspect of sarcoidosis, for instance, may overestimate the test performance of EBUS-TBNA as they usually include more than 90% of sarcoidosis patients in their population, leading to an unrealistically high pre-test probability of the disease.[1–8] Likewise, studies conducted in patients selected based on the presence of isolated hilar/mediastinal lymphadenopathy may overestimate the success rate of EBUS-TBNA, as they inevitably include only patients with stage I sarcoidosis.[34–36] Literature, in fact, suggests that the success rate of both conventional and EBUS-TBNA is higher in stage I than in stage II sarcoidosis,[1–8,37–40] probably due to a higher density of granulomas in lymph nodes of stage I patients.[41] Conversely, the idea to focus our review on studies enrolling consecutive patients being tested for lymphadenopathy detected at imaging tests, regardless of the suspected underlying clinical aetiology, has probably some advantages. Patients enrolled in this fashion account for the average cohort of patient submitted to EBUS-TBNA

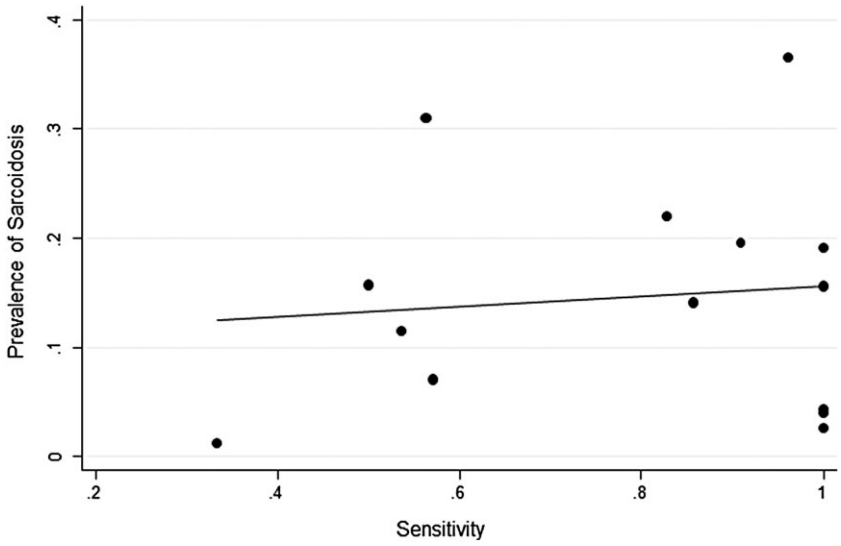

Figure 4 Linear regression analysis showing the absence of correlation between prevalence of sarcoidosis and ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) sensitivity (rho = 0.10; $P = 0.724$).

for diagnostic purposes in everyday clinical practice. This is demonstrated by the fact that the prevalence of malignancy in the studies included in the analysis was much higher than that of both sarcoidosis and infectious causes, just like it happens in real life. Second, these studies are significantly more likely to include also 'unusual' cases of sarcoidosis, which might not be enrolled in clinically selected study populations owing to clinical and/or radiological findings not suggesting sarcoidosis as a likely diagnostic option at the time of trial enrollment.

Another important strength of the present meta-analysis is that included studies took place in 11 countries from five different continents, and were carried out by authors/centres with different expertise and annual EBUS-TBNA volumes, as suggested by the wide variation in the size of study populations. This should have allowed us to assess and combine the results of EBUS-TBNA from both low-volume and high-volume centres. This is particularly important as the results of the American College of Chest Physicians AQuIRE Bronchoscopy Registry demonstrate that the diagnostic yield is associated with annual TBNA volume, and that the excellent results obtained in high-volume centres may not be generalizable.[42]

Some potentially important limitations of our review also deserve mention. In the first place, patients with EBUS-TBNA results suggesting a diagnosis of 'reactive/benign' lymphadenopathy did not receive the same reference standard in all studies. While in a minority of cases, the status of the lymphadenopathy was further evaluated through a surgical procedure, in most of them the reactive/benign nature of the lymphadenopathy was confirmed as such if the radiological follow-up showed regression or stability of the lymphadenopathy at 6 months. This conduct may have led to overestimation of EBUS-TBNA performance, as lymphadenopathy in the setting of sarcoidosis may remain stable or even regress spontaneously.

Second, as none of the studies included in the present meta-analysis were specifically designed to evaluate the role of EBUS for suspected sarcoidosis, only three of them provided minimal diagnostic criteria,[26,29,31] and none of them stated who adjudicated the diagnosis of sarcoidosis. Even though a single reference standard for diagnosis of sarcoidosis does not exist, it is common opinion that the diagnosis is more reliable if it is established in the setting of multidisciplinary discussion/meeting, or at least by an expert adjudicator.[13,14] For studies regarding index tests whose results require some degree of subjective interpretation, such as EBUS-TBNA for diagnosis of sarcoidosis, it is particularly important that the operator performing the procedure or the study personnel not assign the final diagnosis, as they might be more prone to interpret the EBUS-TBNA results towards a diagnosis sarcoidosis, leading to overestimation of test performance.

Lastly, we found a significant inter-study heterogeneity in sensitivity, and we could not apply statistical tests to the subgroup analysis due to significant heterogeneity within each subgroup. With this limitation in mind, we found that only subgroup analysis by study design seemed to influence the diagnostic sensitivity, prospective studies showing a higher sensitivity than retrospective ones. Interestingly, Agarwal *et al*. observed the same finding, even though most studies included in their meta-analysis on EBUS-TBNA in sarcoidosis were performed in clinically selected study populations.[9] This feature is not unexpected, as retrospective studies are more likely to carry substantial bias. Rigorous prospective trials would be needed to assess a number of possible factors possibly explaining the observed heterogeneity in sensitivity that we could not assess due to inconsistent or missing reporting in the studies included in the meta-analysis. For instance, the pathologist's experiences in interpreting the EBUS-TBNA samples, as well as the processing method of EBUS-TBNA specimens, are factors of particular importance that we could not assess. In a randomized trial aimed at comparing the success rates of EBUS-TBNA versus conventional TBNA in sarcoidosis,[15] Tremblay *et al*.

demonstrated that the yield for the identification of granulomas was fairly different when two pathologists with different experience in lung cytology interpreted the same slides from the same patients.[15] In particular, when a research pathologist reviewed the samples first seen by the assigned cytopathologist, the yield rose from 53.8% to 73.1% in the conventional TBNA group and from 83.3% to 95.8% in the EBUS-TBNA group.[15] The processing method for specimens retrieved with EBUS-TBNA can also be important. Schwartz *et al*. did recently review the cytological material retrieved with EBUS-TBNA from the mediastinum of 25 sarcoidosis patients.[43] Interestingly, in no case were granulomas seen on the material directly smeared onto slides, on cytospins, and on ThinPrep® (Cytyc Corporation, Marlborough, MA, USA) preparations, whereas the yield of the cell-block preparations was extremely high (24 of 25 cases, 96%). However, until a study is specifically designed to evaluate the impact of various preparation methods in this setting, the extent to which any observed differences are true rather than related to local preference and experience with a given method is difficult to establish.

In conclusion, the present review provides preliminary evidence that EBUS-TBNA can be a valuable option for diagnosis of sarcoidosis even in clinically unselected study populations. There is urgent need for studies that evaluate the usefulness of EBUS-TBNA in patient cohorts' for whom sarcoidosis is only one of the possible diagnostic alternatives after clinical and radiological inclusion criteria have been prospectively defined. These studies should also carefully examine the role of factors possibly influencing the diagnostic sensitivity.

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Supplementary Information

Additional Supplementary Information can be accessed via the *html* version of this article at the publisher's web-site.

Supplementary Table S1 Bibliographic search strategy.

Supplementary Table S2 Quality Assessment of Diagnostic Accuracy Studies, version 2 (QUADAS-2) checklist used in the present systematic review and meta-analysis.

