Online Appendix B3 BTS Guideline for Pleural Disease

Section B Investigation of the undiagnosed pleural effusion

Question B3 Evidence Review and Protocol

B3 What is the optimal volume and container for a pleural aspiration sample when diagnosing unilateral pleural effusion in adults?

Contents

Question Evidence Review

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Background

Increasing the volume of pleural fluid may aid in the cytological diagnosis of malignancy and understanding the optimal volume and container may allow optimisation of clinical pathways for the diagnosis of pleural malignancy and infection.

Outcomes

Diagnostic accuracy of optimal volume and optimal container type for pleural aspiration samples

Evidence Review

The literature search identified eight relevant studies, but no studies directly answered the question. Two studies directly investigated if different volumes from the same patient had an impact on diagnostic yield[1,2] and one prospective comparative study of relevance looked into the optimal container for pleural aspiration samples.[3] A further three studies retrospectively examined the effect of different volumes on diagnostic yield[4-6] and two case series investigated the effects of using different sample containers[7,8]

Optimal volume

Abouzgheib et al performed a prospective study on 44 patients undergoing diagnostic thoracentesis for possible MPE, comparing the diagnostic yield of the first 50ml sent for analysis to a subsequent 'large-volume' sent (890 ± 375 ml, mean ± SD). Results showed 100% concordance in diagnosing malignancy between the 50 ml and large-volume aspirate (23/44 cases in both).[1]

Swiderek et al prospectively examined 121 pleural samples from 102 patients with suspected or known malignancy. Fluid was collected in three aliquots, 10 ml, 60 ml and ≥150 ml (Table B3a) and demonstrated little increased diagnostic yield above 60 ml.[2]

Other studies have retrospectively examined the effect of different volumes on diagnostic yield. Sallach examined 374 pleural samples in 282 patients who underwent diagnostic thoracentesis. Samples were classified into broad quartiles based on fluid volumes (6.2 ± 3.0 ml, 36.0 ± 16.8 ml, 397.1 ± 189.1 ml, 1129.7 ± 355.9 ml, mean ± SD), but there was no statistical difference between the diagnostic sensitivities of each group.[4]

Thomas et al examined 2155 pleural samples from 2155 patients. Narrow volume thresholds of 5 ml were used between 0-100 ml. The majority of initial sample (71%) had a volume of less than 30 ml and the study determined that a plateau in proportionate yield of positive malignancy was reached with a minimum of 25 ml pleural fluid with less than 25 ml appearing to double false negative rates compared to greater than 25 ml (from 48% to 22% respectively in 81 patients who had a pleural biopsy). Thresholds of >50 ml were reported as not improving sensitivity.[5]

Finally, Rooper et al performed a retrospective review of 2540 pleural fluid specimens from 1943 patients and data were divided into 9 group depending on volume (<5 ml, 5-10 ml, 10-25 ml, 25-50 ml, 50-75 ml, 75-300 ml, 300-600 ml, 600-900 ml and >900 ml). The study reported that there was no minimal volume below which malignant cell could not be found. Diagnostic yield gradually increased from 10.1% (<5 ml sent) to 23.3% (50-75 ml sent) (p <0.001 compared to <5 ml), but then dropped to 20.3% in the 75-300 ml group. Further between-group statistical comparisons were not reported and the overall sensitivity for detecting malignancy was 20.1%.[6]

Table B3a: Comparison of processing technique and sample volume from Swiderek et al for diagnosing unilateral pleural effusion in adults[2]

Process	Samples (n)	Volume (ml)	Sensitivity (%)	Specificity (%)
Direct smear/cytospin	107	10	48.7	96.6
Direct smear/cytospin	107	60	62.8	89.7
Direct smear/cytospin	107	≥150	69.2	86.2
*Combined	71	10	75.9	94.1
*Combined	71	60	79.6	97.7
*Combined	71	≥150	87.0	94.0

Pairwise comparison *p* values

	Direct smear/cytospin			*Combined		
Volume (ml)	**10**	**60**	**≥150**	**10**	**60**	**≥150**
10	-	0.0058	<0.0001	-	NS	0.0099
60	-	-	NS	-	-	NS

* Combined – Direct smear/cytospin and cell block formation combined

NS – not significant

Optimal container

In the Menzies et al study, patients suspected of having pleural infection had pleural fluid samples aliquoted into a sterile container for standard Gram stain and culture. If pleural fluid volume allowed, 2, 5 and 10 ml pleural fluid samples were also aliquoted into BACTEC PLUS blood culture bottles (aerobic and anaerobic). The addition of blood culture bottles increased diagnostic yield from 37.7% to 58.5% (Table B3b).[3]

Table B3b: Frequency of bacteria isolation in standard sterile containers and BACTEC PLUS blood culture bottles

Container	Frequency of bacteria isolation
Standard sterile container	20/53 (37.7%)
BACTEC PLUS blood culture bottle	31/53 (58.5%)

Some bacterial isolates (e.g. *Streptococcus pneumoniae* and anginosus group) were potentially more likely to be identified by standard sterile container sample. In the patients who had all three volumes of pleural fluid inoculated into blood culture bottles there was no evidence of increasing yield with larger volumes.[3]

Two additional large case series were identified, but neither directly compared yield with BACTEC bottles against sterile white top/plain container. The first examined the microbiological features of patients with pleural effusions, where approximately half were suspected of having infection. Comparing blood culture bottles against an anaerobic transport vial and separate sterile tube reserved for mycobacterial culture, a higher frequency of microbiologic positivity was found in patients who had pleural fluid inoculated into blood culture bottles at the bedside. Only 36/237 were classed as having non-tuberculous empyema, with 25/36 having a positive microbiological diagnosis. In 9/25, growth was detected exclusively in the blood culture bottle sample.[7]

A further case series looked solely at tuberculous infection. The prevalence of tuberculosis (TB) was very high in this cohort (63/155) and non-tuberculous pleural infection was not described as a final diagnosis in any patients. Bedside inoculation was direct into BACTEC bottles (12B – 1 ml and 13A – 5 ml), alongside a plain, sterile container and a sterile heparinised container, which were subsequently inoculated into BACTEC 12A

and 12B media in the laboratory. 41/63 patients had samples in all three containers and the total number of positive cultures per container type are summarised in Table B3c.[8]

Table B3c: Comparison of laboratory inoculation recovery rates for differing pleural fluid sample containers

Container	Positive cultures	Identified by single container type alone
BACTEC bottle	17/41 patients	3/41 patients
Sterile container	10/41 patients	1/41 patients
Sterile heparinised container	16/41 patients	2/41 patients

Evidence statements

The evidence does not support an optimal pleural fluid volume of for initial cytological diagnosis but suggests that increasing pleural fluid volume above 50ml provides no diagnostic benefit (**Ungraded**)

The evidence supports the use of aerobic blood culture bottles, anaerobic blood culture bottles and plain ("white top") containers when investigating suspected pleural infection (**Ungraded**)

Recommendations

- 25-50 ml of pleural fluid should be submitted for cytological analysis in patients with suspected MPE (**Strong** – by consensus)
- Pleural fluid should be sent in both plain and blood culture bottle tubes in patients with suspected pleural infection (**Strong** – by consensus)

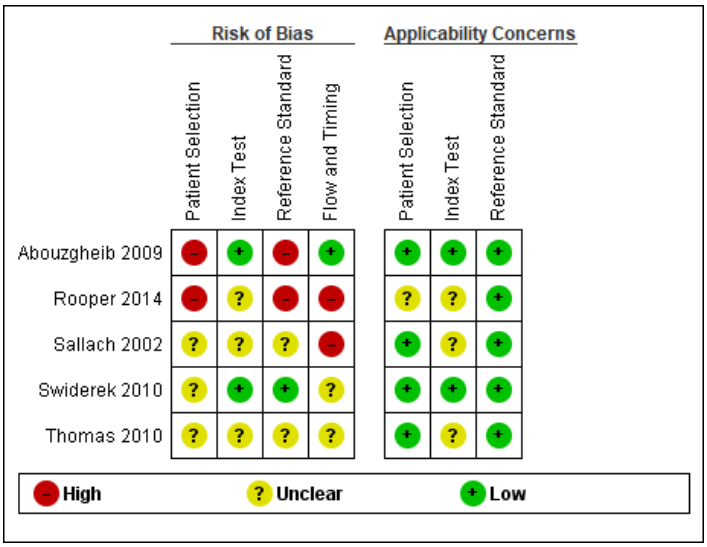
Good Practice Points

- ✓ At least 25 ml, and where possible 50 ml, of pleural fluid should be sent for initial cytological examination.
- ✓ If volumes of ≥ 25ml cannot be achieved, smaller volumes should be sent, but clinicians should be aware of the reduced sensitivity.
- ✓ If small volume aspirate (<25 ml) has been non-diagnostic, a larger volume should be sent, if achievable, except when there is high suspicion of a tumour type associated with low pleural fluid cytology sensitivity (especially mesothelioma)
- ✓ Pleural fluid samples should be processed by direct smear and cell block preparation
- ✓ In patients with an undiagnosed pleural effusion where pleural infection is possible and volume of fluid sample available allows, microbiological samples should be sent in both white top containers and volumes of 5-10 ml inoculated into (aerobic and anaerobic) blood culture bottles
- ✓ In cases where volume available does not allow 5-10 ml inoculation, volumes of 2-5 ml should be prioritised to blood culture bottles rather than a plain, sterile container

Research Recommendation

- Further research is needed into the optimal volume required to establish "actionable cytology" based on currently available molecular profiling, in particular in relation to tumour types associated with higher sensitivity of pleural fluid cytology

Risk of bias summary



References

1. Abouzgheib W, Bartter T, Dagher H, Pratter M, Klump W. A prospective study of the volume of pleural fluid required for accurate diagnosis of malignant pleural effusion. *Chest.* 2009;135(4):999-1001.
2. Swiderek J, Morcos S, Donthireddy V, et al. Prospective study to determine the volume of pleural fluid required to diagnose malignancy. *Chest.* 2010;137(1):68-73.
3. Menzies SM, Rahman NM, Wrightson JM, et al. Blood culture bottle culture of pleural fluid in pleural infection. *Thorax.* 2011;66(8):658-662.
4. Sallach SM, Sallach JA, Vasquez E, Schultz L, Kvale P. Volume of pleural fluid required for diagnosis of pleural malignancy. *Chest.* 2002;122(6):1913-1917.
5. Thomas SC, Davidson LR, McKean ME. An investigation of adequate volume for the diagnosis of malignancy in pleural fluids. *Cytopathology.* 2011;22(3):179-183.
6. Rooper LM, Ali SZ, Olson MT. A minimum fluid volume of 75 mL is needed to ensure adequacy in a pleural effusion: a retrospective analysis of 2540 cases. *Cancer Cytopathology.* 2014;122(9):657-665.
7. Ferrer A, Osset J, Alegre J, et al. Prospective clinical and microbiological study of pleural effusions. *European Journal of Clinical Microbiology & Infectious Diseases.* 1999;18(4):237-241.
8. Cheng AF, Tai VH, Li MS, et al. Improved recovery of Mycobacterium tuberculosis from pleural aspirates: bedside inoculation, heparinized containers and liquid culture media. *Scand J Infect Dis.* 1999;31(5):485-487.

Question Protocol

Field	Content
Review Question	What is the optimal volume and container for a pleural aspiration sample when diagnosing unilateral pleural effusion in adults?
Type of review question	Diagnostic accuracy
Objective of the review	To assess pleural fluid volume for the diagnosis of malignant effusion, and the use of plane and culture bottles for the diagnosis of infectious effusion
Eligibility criteria – population / disease / condition / issue / domain	Adults with unilateral pleural effusion 18+.
Eligibility criteria – index test(s)	Pleural aspiration sample in a Bactec container Pleural aspiration sample in a plain tube Pleural aspiration sample in a Biochem tube
Eligibility criteria – gold standard	Clinico-pathology
Outcomes and prioritisation	Diagnostic accuracy
Eligibility criteria – study design	RCTs Prospective comparative studies Case series of >100 patients
Other inclusion /exclusion criteria	Non-English language excluded unless full English translation Conference abstracts, Cochrane reviews, systematic reviews, reviews Cochrane reviews and systematic reviews can be referenced in the text, but **DO NOT** use in a meta-analysis
Proposed sensitivity / subgroup analysis, or meta-regression	Volume <10 ml Volume 10-20 ml Volume >20 ml
Selection process – duplicate screening / selection / analysis	Agreement should be reached between Guideline members who are working on the question. If no agreement can be reached, a decision should be made by the Guideline co-chairs. If there is still no decision, the matter

	should be brought to the Guideline group and a decision will be made by consensus
Data management (software)	RevMan5 — Meta-analysis data input. Evidence review/considered judgement. Storing Guideline text, tables, figures, etc. MetaDTA — Data meta-analyses Gradepro — Quality of evidence assessment / Recommendations
Information sources – databases and dates	MEDLINE, Embase, PubMED, Central Register of Controlled Trials and Cochrane Database of Systematic Reviews 1966 - present
Methods for assessing bias at outcome / study level	RevMan5 diagnostic accuracy full review template (based on QUADAS2) (follow instructions in '*BTS Guideline Process Handbook - Diagnostic Accuracy*')
Methods for quantitative analysis – combining studies and exploring (in)consistency	If 3 or more relevant studies: RevMan5 for forest plots, summary ROC plot MetaDTA to combine studies (pooled specificity, sensitivity, likelihood ratios, diagnostic odds ratio and confidence intervals) and calculate RevMan parameters for summary ROC plot (follow instructions in '*BTS Guideline Process Handbook - Diagnostic Accuracy*')
Meta-bias assessment – publication bias, selective reporting bias	GRADEpro — Diagnostic accuracy quality of evidence assessment for each index test (follow instructions in '*BTS Guideline Process Handbook - Diagnostic Accuracy*')
Rationale / context – what is known	A minimum fluid volume increases the diagnostic yield of malignant effusion. Use of Bactec blood culture bottles increases diagnostic yield in pleural infection for pleural fluid