Investigating Unilateral Pleural Effusions: The role of cytology.

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Take home message: Largest prospective study investigating unilateral pleural effusions; the value of cytology depends on the primary.

Abstract

The vast majority of undiagnosed unilateral pleural effusions have fluid sent for cytological analysis. Despite widespread use, there is uncertainty about its sensitivity to diagnose malignant pleural effusions (MPEs). Our aim was to ascertain the utility of cytology using a large prospective cohort.

Consecutive patients presenting with an undiagnosed unilateral pleural effusion were recruited to this UK-based study. All had pleural fluid sent for cytological analysis. Cytological sensitivity was based on the final diagnosis at 12 months, confirmed by two consultants.

Over 8-years, 921 patients were recruited, of which 515 had a MPE. Overall sensitivity of fluid cytology to diagnose malignancy was 46% (95%CI 42-58). There was variation in sensitivity depending on cancer primary, with mesothelioma (6%) and haematological malignancies (40%), being significantly lower than adenocarcinomas (79%). MPEs secondary to ovarian cancer had high pick-up rates (95%). In asbestos-exposed males with exudative effusions, the risk of MPE was 60%, but cytological sensitivity was 11%.

This is the largest prospective study of pleural fluid cytology and informs discussions with patients about the likely requirement for investigations following thoracentesis. In patients presenting with a clinical suspicion of mesothelioma, cytological sensitivity is low, so more definitive investigations could be performed sooner.

Introduction

Pleural fluid analysis with cytological assessment is a fundamental part of the investigation of unilateral pleural effusions. In Europe and North America, one of the commonest causes is primary or secondary pleural malignancy[1]. Identifying malignancy from pleural fluid cytology alone can spare patients from more invasive investigations, reduces healthcare costs, is important for staging, and allows earlier progression to treatment. However, it has several drawbacks including an uncertain sensitivity, and extending the time (routinely between 5 to 7 days) before further investigations are organised [2].

The estimates of sensitivity for detecting malignancy from pleural fluid cytology vary greatly within guidelines, ranging from 40-87%[1, 3]. The reason for this variation is due to retrospective study designs[4-7], selective study inclusion criteria[8, 9] and a variation in cytopathological methods. Additionally, most studies of cytological yield cited in guidelines are over 20 years old. There has been a significant advance in immunohistochemical methods since then.

Better knowledge of the discriminative ability of pleural fluid cytology would allow, not only more informed consultations with patients, but better planning of further investigations. This study uses a large prospective cohort of patients with undiagnosed unilateral pleural effusions to assess cytological sensitivity depending on cancer type and patient factors. It aims to inform practice for respiratory physicians when diagnosing malignant pleural effusions.

Methods

Patients

Consecutive patients referred to a single centre pleural service with an undiagnosed unilateral pleural effusion were recruited to this prospective observational study. All patients had a diagnostic thoracentesis as part of normal clinical care and consented to having their demographic data, blood and pleural fluid results stored. The study received ethical approval from the South West regional ethics committee (REC number 08/H0102/11). All patients were followed up to 12 months or death (whichever occurred first) and were assigned a final diagnosis as to the pathology or pathologies most likely to be the cause of their effusion. The final diagnosis was agreed by two independent consultant respiratory physicians based on all the available clinical, histological and radiological information. Any areas of contention were re-examined till consensus was reached.

Serum and pleural fluid analysis

All patients had routine pleural fluid analysis at baseline, including protein, glucose, LDH, pH, microbiology culture, and cytology. Light's criteria were used to distinguish exudative from transudative effusions[10]. Predominant pleural fluid cell types were defined based on British Thoracic Society guidelines[1]. A lymphocyte or neutrophil predominant effusion was defined as the presence of over 50% of that cell type in the absence of ≥10% eosinophils, in which case the effusion was deemed eosinophilic. Any effusion not meeting any of the above criteria was classed 'non-specific', i.e. both lymphocytes and neutrophils<50%, eosinophils <10%, with another cell type predominating (e.g. mesothelial, blood or atypical cells). Routine baseline blood tests were also performed. The serum neutrophil/lymphocyte ratio (a widely used indicator of poor prognosis for malignancy[11]) was calculated by dividing the serum neutrophils (10^9/L) by serum lymphocytes.

Pleural fluid cytology and immunohistochemistry

As per guidelines, 40ml of pleural fluid was sent for cytological analysis where possible[1]. It is standard practice in our centre that after preparing slides from the centrifuged deposit, all pleural fluid cytology samples have a formalin fixed paraffin embedded cell block produced. All samples were reviewed by a consultant cytopathologist. Depending on the degree of clinical suspicion of malignancy and/or initial cytological assessment, immunostaining was requested. The panel of immunohistochemical stains often included EMA to distinguish between malignant cells and reactive

mesothelial cells. Markers to distinguish between adenocarcinoma cells (AUA1 or, in later years, BerEP4) and mesothelial cells (CK5/6 and Calretinin) were frequently used. In cases of adenocarcinoma, further immunostaining was undertaken to assess the most likely primary site. These markers included CK7, CK20, TTF1, ER (oestrogen receptor), PR (progesterone receptor) and Ca125. Overlap of staining patterns sometimes occurred, with variation in the exact panels used between patients, but generally this panel of immunohistochemical stains provided useful information for diagnosis. Flow cytometry for lymphoma was sent based on a previously published algorithm[12]. A full breakdown of positive immunohistochemical markers in malignant effusions is shown in Appendix 1. Samples that were 'non-diagnostic' for malignancy were those where a diagnosis of malignancy was not made based on the cytological specimen, with the patient requiring further investigations or interval radiological follow up. In this instance, and when malignancy was the most likely diagnosis, it was usual practice to proceed to definitive biopsy (e.g. thoracoscopy or CT guided biopsy), instead of repeating thoracentesis.

Diagnostic criteria

Predefined criteria were used to reach a 12-month diagnosis. Malignant effusions were diagnosed in the presence of any of the following criteria: (1) Malignant pleural fluid cytology or biopsy, (2) histologically confirmed pulmonary/extra-thoracic malignancy with radiographic evidence of metastasis to ipsilateral pleura on CT, (3) radiological changes meeting Leung's criteria which have progressed in keeping with malignancy on interval CT scan in the correct clinical context, or (4) autopsy confirming pleural malignancy. See Appendix 2 for full details of diagnostic criteria for non-malignant pathologies.

Statistical analysis

Descriptive statistics were used to summarise patient characteristics and clinical data. Sensitivity estimates with 95% C.Is were used to investigate the ability of pleural fluid cytology to detect malignancy. When comparing cytological sensitivity between two groups the classic Z-test was used with $p < 0.05$ used to define significance. Pleural fluid characteristics amongst the cohort were reported using descriptive statistics, with differences between cytology diagnostic and non-diagnostic effusions assessed using the independent samples T-test. Survival (from study entry) was censored at 20.12.17.

Results

Patient demographics

Between December 2008 and December 2016, 921 consecutive patients presenting with an undiagnosed unilateral pleural effusion were recruited. All had a diagnostic thoracentesis for standard pleural fluid investigations, with 40ml of fluid sent for cytological analysis in the majority (median 40ml, IQR 35-40ml). The cohort had a mean age of 70.2 (SD 13.8) and had a male predominance. The baseline characteristics of the cohort are shown in Table 1.

Table 1- Demographics

	All	Malignant	Non-malignant
Total	921	515	406
Mean Age	70	72	68
Sex (M:F)	601:320	317:198	284:122
Laterality (L:R)	385:536	222:293	163:243
Previous malignancy	212	176	36
Median Survival days (IQR)	474 (127-1632)	199 (74-465)	1700 (831-2522)
Asbestos exposure	274	166	108
PF analysis			
Transudate (%)	118 (13)	21 (4)	97 (24)
Predominant PF cell type			
Lymphocytic (%)	315 (34)	183 (35)	132 (33)
Neutrophilic (%)	87 (9)	13 (3)	74 (18)
Eosinophilic (%)	71 (8)	30 (6)	41 (10)
Non specific (%)	448 (49)	289 (56)	159 (39)

Effusion diagnoses

The majority of effusions had a malignant aetiology at 12-month consultant diagnosis (56%), see Table 1. There were 6 patients where the exact cause of the effusion could not be ascertained. In all 6, malignancy was excluded given resolution of effusion on follow-up imaging, so these cases have been placed in the non-malignant group for further analysis. Table 2 shows the breakdown of the malignant effusions by primary site. Lung was the most numerous cancer primary causing effusions within this cohort (32%, 166/515), with effusions secondary to mesothelioma accounting for 29% (148/515) of the malignant diagnoses.

Table 2- Cytological sensitivity by cancer type

	No. in cohort	PF cytology diagnostic	Sensitivity (95% C.I.)
All	515	239	46.4% (42.0-58.2)
Breast	58	41	70.7% (57.3-81.9)
ENT	7	1	14.3% (0.4-57.9)
Gastrointestinal	22	15	68.2% (45.1-86.1)
Haematological	30	12	40.0% (22.6-59.4)
Lung (all)	166	93	56.0% (48.1-63.7)
- Adenocarcinoma	100	82	82.0% (73.1-89.0)
- Squamous	28	4	14.3% (4.0-32.7)
- Small cell	16	7	43.8% (19.8-70.1)
- Other/unknown	22	0	0% (0-15.4)
Mesothelioma	148	9	6.1% (2.8-11.2)
Sarcoma/Melanoma	8	0	0% (0-36.9)
Ovarian	38	36	94.7% (82.2-99.4)
Urological	17	2	11.8% (1.5-36.4)
Unknown malignancy	21	7	33.3% (14.6-57.0)

Cytological sensitivity by cancer primary

The sensitivity of pleural fluid cytology for detecting different cancer types is shown in Table 2 and Figure 1 with 95% confidence intervals. Cytology has a higher sensitivity for detecting adenocarcinomas compared to other cancer types, even once mesothelioma is excluded ($p<0.01$). Within adenocarcinomas, there is a significant difference depending on cancer primary with ovarian cancer having a significantly higher diagnostic rate than breast, lung or GI malignancies, which all have similar sensitivities (p-0.013). Mesothelioma had a low sensitivity for detection on pleural fluid cytology alone with 94% of patients requiring a definitive biopsy before a diagnosis could be made. Of the 30 patients with a malignant effusion secondary to haematological malignancy (23 patients with lymphoma and 7 with leukaemia), less than half had clear evidence of malignancy on pleural fluid cytology. Flow cytometry was performed in 21 of these patients and assisted in the diagnosis of16. Malignant effusions from rarer primary sites such as urogenital, ENT or musculoskeletal had low diagnostic rates, but numbers were small. Of the 276 non-diagnostic malignant pleural effusions, 248 (90%) had a definitive histocytological diagnosis of malignancy (65% pleural biopsy, 24% biopsy from non-pleural tumour site with radiographic evidence of metastatic pleural disease, 1% post mortem). Pleural fluid cytology was repeated in 106 of these cases, often at the time of thoracoscopy or in patients unfit for more invasive investigations. Six of these samples were diagnostic for malignancy (5.6%). Thirty patients had a 3rd sample sent, all of which were non-diagnostic. There was no difference in overall cytological sensitivity if more pleural fluid was sent for analysis. Overall sensitivity was 48% in samples of 40ml or less, compared to 40% in fluid samples over 40ml (p=0.65)

Figure 1- Scatter plot of sensitivity of pleural fluid cytology by malignancy

(Error bars represent 95% C.I.)

Immunohistochemistry/Cytogenetic results

The full results of positive immunohistochemistry and cytogenetic markers are shown in Appendix 1. It is of note that cytogenetic practice has advanced significantly during the course of the 8 years of recruitment. Therefore, certain tests e.g. epidermal growth factor receptor (EGFR), have only become available towards the end of the study period, and were only requested if clinically indicated. There were 41 instances where further genetic information was request on the pleural fluid cell block. Two of the cell blocks had insufficient material for further analysis (5%).

Diagnostic flow chart; Risk of malignancy and sensitivity of pleural fluid cytology

Figure 2 is a flowchart demonstrating the variation in the risk of malignancy and sensitivity of pleural fluid cytology depending on basic patient characteristics and pleural fluid analysis. These factors have been chosen as they are easily obtainable and have the greatest discriminative value in malignancy risk and/or cytological sensitivity. Whether an effusion is an exudate or transudate has a considerable bearing on the risk of malignancy. Within this cohort the risk of malignancy was 15% (21/118) in transudative effusions, compared to 62% (495/803) in exudative effusions. Of the 21 patients with malignancy in the context of a transudative effusion, half (n=11) had a concurrent diagnosis of cardiac failure. The malignancies were 2 breast cancers, 5 lung cancers, 8 mesotheliomas, 6 other types.

The likelihood of malignancy in exudative effusions was over 60% and sensitivity of cytology remained over 40%. Amongst female patients with an exudative effusion, the likelihood of malignancy was high (67%), as was the sensitivity of cytology (66%). Male patients with a previous history of cancer (excluding prostate cancer) had a high risk of malignancy and cytological sensitivity remained over 40%.

Within the subgroup of asbestos exposed male patients without a history of cancer, the sensitivity of pleural fluid cytology fell to 11% (C.I. 6-17) which is significantly lower than other groups ($p<0.01$), despite a risk of malignancy of over 60%. The patients with malignancy in this subgroup had a high likelihood of a 'suspicion of malignancy' on their initial CT scan (117/132).

Figure 2- Diagnostic flow chart demonstrating risk of malignancy and sensitivity of pleural fluid cytology

*excluding prostate cancer. PFsens- Pleural fluid cytology sensitivity (presented with 95% C.I.s), Hx- History.

Survival: cytology diagnostic versus non-diagnostic malignant effusions

The median survival of all malignant effusions was 199 days (IQR 74-465). There was considerable variation depending on cancer type but there was no impact on survival between those with cytology diagnostic versus non-diagnostic effusions for individual cancers. For example, within lung

adenocarcinoma, survival for cytology diagnostic effusions was 114 days (47-281) compared with 97 days (IQR 32-201) (p= 0.13).

Characteristics of cytology diagnostic versus non-diagnostic adenocarcinomas

Cytology diagnostic malignant effusions secondary to adenocarcinoma were more likely to have serum or pleural markers of increased inflammation. This included a higher serum neutrophil/lymphocyte ratio, higher C reactive protein, and a higher pleural fluid LDH. There was no significant difference in survival between the two groups (p=0.57). See Table 3.

Table 3. Characteristics of cytology diagnostic versus non-diagnostic adenocarcinomas

	Diagnostic (n=173)	Non-diagnostic (n=45)	P value
Serum (SD)			
N/L ratio	7.19 (6.15)	5.05 (2.41)	**0.02**
C Reactive protein	48.8 (53.5)	30.4 (27.8)	**0.03**
Pleural fluid (SD)			
Protein	44.9 (9.2)	42.1 (8.55)	0.08
Glucose	6.15 (9.56)	5.86 (1.66)	0.85
LDH	919.1 (833.8)	644.9 (706.9)	**0.03**
pH	7.38 (0.17)	7.35 (0.49)	0.70
Median survival (IQR)	148 (56-425)	98 (40-241)	0.574

SD- Standard deviation, N/L ratio- Neutrophil/Lymphocyte ratio, LDH- Lactate dehydrogenase, IQR- Interquartile range.

Discussion

This is the largest ever prospective study examining the role of pleural fluid cytology in undiagnosed unilateral pleural effusions. With over 900 patients, we can give an accurate assessment of the strengths and limitations of cytological assessment. The size of this cohort has also allowed for analysis by cancer subtype and the construction of a diagnostic flowchart to demonstrate the likelihood of malignancy with the corresponding cytological sensitivity.

An unexplained pleural effusion is a common diagnostic challenge for the respiratory physician. In Europe and North America, a common cause is primary or secondary malignancy. Therefore, pleural fluid cytology is an essential aspect of pleural fluid analysis but one that is poorly understood. It is recognised that sensitivity is low, but estimates vary widely within international guidelines (40-87%)[1, 3]. This variation arises because estimates are based on retrospective analyses of hospital or outpatient data[4-7]. Porcel and colleagues published a series of 3077 undiagnosed pleural effusions, of which 840 had a malignant aetiology[13]. Overall, preliminary pleural fluid cytology was positive in 51% of malignant effusions, but due to geographical variation the prevalence of mesothelioma within the cohort was less than 1%, compared to 16% in our cohort. They also demonstrated that cytology was more accurate in adenocarcinoma of the lung (78%), breast (68%) and ovary (70%). The data was collected retrospectively from 1994 to 2013, which could explain why estimates for sensitivity were lower than in the current study, given the advancement in immunohistochemical analysis. Retrospective series of lab cytology samples have also been published, with very large numbers (>5000)[14-17]. These report the number of samples where malignant cells were seen, which, although epidemiologically useful, is not linked to clinical information or final diagnosis so does not reflect a measure of sensitivity.

Two studies have prospectively recruited and followed up patients to assess the accuracy of pleural fluid cytology. In 1979, Hirsch and colleagues recruited 300 patients who required diagnostic thoracentesis[18]. All patients were routinely followed up, but given the lack of modern diagnostics (i.e. CT scans) the final diagnosis was not identified in 20% of cases (compared to 0.6% in the current study). Malignancy was identified as a cause of the pleural effusion in 117 patients (39%). The sensitivity of pleural fluid cytology alone to identify malignancy was 54% (95% C.I. 44.4-63.1). Given the small numbers there was no subgroup analysis by cancer type or patient characteristics. A more recent study, from Thailand, prospectively recruited 353 patients who underwent a diagnostic thoracentesis[19]. There was a high prevalence of malignancy within the cohort (78%) with 1 case of mesothelioma. Pleural fluid cytology was diagnostic in 61% (95% C.I.55.5-66.9) of cases with a higher sensitivity in lung cancer (73.7%) compared to non-lung solid cancers (53.5%) and haematological

malignancy (35.5%). There was no further break down by cancer type or patient/fluid characteristics. However, the diagnostic criteria for malignancy were not robust with only 6% of cytology-negative malignant effusions having a definitive biopsy (compared to 90% in our cohort), with the remaining 94% being defined as cancerous following a 'response to chemotherapy'. This may account for the high prevalence of malignant effusions within this cohort and will significantly affect the estimate of sensitivity. Additionally, only 15ml of pleural fluid was sent for cytological analysis which is considerably less than recommended by guidelines[1, 20]. In the current study, 40ml of fluid was sent when possible. There was no significant difference in cytological sensitivity if less fluid was received, although numbers were small (44 samples less than 40ml).

We have demonstrated that the overall sensitivity of pleural fluid cytology is slightly lower than the above prospective studies at 45%. The most likely reason for this is the high proportion of mesothelioma diagnoses in our cohort (29%). This has a significant impact given the significant variability in sensitivity depending on cancer type. Mesothelioma was particularly low with only 6% of cases being diagnosed on cytology alone. If the prevalence of mesothelioma is artificially lowered to be more in keeping with a typical European centre (around 10% of all malignant effusions) the sensitivity of pleural fluid cytology rises to 55%.

Some centres from areas with very high mesothelioma incidence report higher predictive values from pleural fluid cytology, but these are not commonplace[21, 22]. In most UK and European centres patients will require definitive biopsy unless there is clear evidence of malignant mesothelial cells with corroborative immunohistochemical markers, especially given the medico-legal implications of the diagnosis.

Cytological sensitivity from other cancers varied considerably by primary site and cell type. Adenocarcinomas from the breast, lung, ovary or GI tract could be reliably detected on pleural fluid cytology alone (with a combined sensitivity of 80%). Sensitivity approached 95% in ovarian cancer, which was significantly higher than other adenocarcinomas ($p=0.013$). The pleura is the most common site for extra-abdominal spread in ovarian cancer[23].It is hypothesized that most malignant effusions from ovarian cancer result from direct pleural invasion of the diaphragm, or the migration of malignant ascitic fluid through diaphragmatic defects[24]. This mode of spread may result in more malignant cells being present in fluid, as opposed to the other malignancies which cause effusions due to disrupting normal pleural fluid recycling at the parietal membrane[25].

We have investigated the variation in cytological sensitivity within adenocarcinomas alone and found that cytology diagnostic effusions correlate with biochemical markers indicative of more advanced/inflammatory malignancy (higher serum NLR and CRP, and pleural fluid LDH). Several

previous smaller studies have correlated an increased cytological yield for other proxies of advanced tumours including lower pleural pH and glucose, macroscopic spread and survival[26-29]. It follows that more advanced tumours are likely to be cytology positive due to increased exfoliation of tumour cells into the effusion. However, we did not find the same relationship between survival and cytology positivity when assessing individual tumour types. This finding from previous studies is likely to be because adenocarcinomas with higher cytological sensitivity have slightly better overall survival e.g. breast and ovarian[30].

This variation in the utility of pleural fluid cytology has significant implications for planning further investigations. Guidelines recommend waiting for the pleural fluid cytology result before proceeding to other invasive and costly investigations (e.g. local anaesthetic thoracoscopy or CT guided biopsy)[1]. This can take between 5 to 7 days (or longer if additional immunohistochemistry is required), and the patient may still be symptomatic without definitive pleural drainage. This study has shown that in asbestos exposed male patients with no history of cancer, the likelihood of a diagnosing malignancy from an exudative effusion is just 6%, despite the risk of malignancy being over 60%. For this patient demographic we would support the approach of not waiting for the cytology result before performing a definitive biopsy. This is further supported by the finding that nearly 90% of the patients with a malignant effusion in this group had evidence of malignancy on their CT scan (117/132). In contrast, for patients not fulfilling these criteria, the higher sensitivity of pleural fluid cytology (>40%) justifies waiting for the result.

This study has weaknesses that may limit the generalisability of its findings. This was a single centre study, however, the cytological and immunohistochemical techniques are in use in most European centres. Secondly, the cytopathologists were not blinded to the clinical information, they had information from the requesting clinician as well as from the multi-disciplinary meeting (MDT). This may have influenced their interpretation of the cytology specimen, but this study is a pragmatic assessment of the value of pleural fluid cytology in day-to-day practice. Additionally, a concern when using pleural fluid cytology alone to diagnose malignancy is that there is insufficient material for further analysis. This is increasingly relevant given the continued development of targeted immunotherapy for malignancies that metastasise to the pleura. In our study, given the change in immunohistochemistry and cytogenetic practice over the 8-year recruitment period, the suitability of pleural fluid specimens for further analysis is difficult to quantify. In the 41 incidences where receptor status or genetic analysis was requested, the pleural fluid specimen was sufficient in 95% of cases (39/41). Other studies with a focus on this issue have found that pleural fluid samples can reliably provide genetic information that correlates with the primary malignancy [31-34].

In conclusion, this is the largest prospective study of pleural fluid cytology in the literature. We have shown considerable variation in the sensitivity of cytological assessment by primary cancer type with adenocarcinoma, especially ovarian, having especially high sensitivity. Haematological malignancy and mesothelioma were unlikely to be diagnosed with pleural cytology alone. This information can help to inform discussions with patients around the likelihood of needing further investigations for pleural effusions. In asbestos exposed male patients with an exudative effusion and no history of cancer, a strategy of not waiting for the cytology result before organising further tests is justifiable and would speed up the diagnostic and treatment pathway.

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