The impact of between analytical platform variability on the classification of pleural effusions into exudate or transudate using Light's criteria

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ABSTRACT

Background Light's criteria are ratios of pleural fluid to serum total protein (TP), pleural fluid to serum lactate dehydrogenase (LDH) and pleural fluid LDH to the upper reference limit for serum LDH. They are used to classify pleural effusions into an exudate or transudate when pleural fluid protein is 25–35 g/L. We evaluated the impact of between analytical platforms on the classification of pleural effusions using Light's criteria.

Methods Light's criteria were used to classify pleural effusions with fluid TP between 25 and 35 g/L into exudate and transudate. LDH and TP were analysed using an Abbott ARCHITECT c16000 analyser using a lactate to pyruvate method for LDH and two Roche Cobas 800 c702 analysers, one using a lactate to pyruvate method (laboratory B) and one a lactate to pyruvate method (laboratory C).

Results Eighty-three paired serum and pleural fluid samples were analysed. Of these, 44 samples had a pleural fluid TP between 25 and 35 g/L and were classified according to Light's criteria. Classification of pleural fluid into transudate or exudate using different analytical platforms was 82% concordant. The LDH ratio and TP ratio were similar in laboratory B and laboratory C, but these were respectively lower ($p<0.001$) and higher ($p<0.001$) than those at laboratory A.

Conclusions Although Light's criteria are ratios, which should minimise interassay variability, we report 18% discordance between different analytical platforms. The discordance was largely due to the performance of LDH and to a lesser extent protein assays in pleural fluid. Laboratories should be aware that assays may perform differently in serum and pleural fluid.

INTRODUCTION

Pleural effusions, the accumulation of fluid in the pleural space, are a common medical problem and may be due to several disease processes such as pleural disease, lung pathology, systemic illnesses and organ dysfunction. Pleural effusions occur as a result of increased fluid formation and/or reduced fluid resorption. Pleural effusions may be either transudates or exudates based on the mechanism of fluid formation. Transudates result from an imbalance in oncotic and hydrostatic pressures, whereas exudates are the result of inflammation of the pleura or decreased lymphatic drainage.[1 2]

Classification of pleural effusions into transudates and exudates is important to narrow down the differential diagnosis and direct further investigations and subsequent management. Classically, pleural fluid protein ≥30 g/L has indicated an exudate and <30 g/L a transudate.[3 4] However, although a pleural fluid protein >35 g/L reliably indicates an exudate and <25 g/L a transudate,[3–5] this classification is not accurate when the pleural fluid protein is close to 30 g/L. Therefore, in those where the pleural fluid protein is 25–35 g/L the application of Light's criteria is recommended[1 3–7] since they have a diagnostic sensitivity of 95%–99% and a diagnostic specificity of 65%–98%, which remains unsurpassed compared with other tests.[2] Using Light's criteria, a pleural effusion is an exudate if one or more of the following biochemical criteria are met:[1]

1. Ratio of pleural fluid to serum total protein (TP ratio) is >0.5.
2. Ratio of pleural fluid to serum lactate dehydrogenase (LDH; LDH ratio) is >0.6.
3. Ratio of pleural fluid LDH to the upper reference limit for serum LDH is >2/3.

Light's criteria, therefore, depend on the measurement of LDH and protein in serum and pleural fluid. There are different assays and platforms for LDH analysis. LDH may be analysed using either pyruvate to lactate reaction method or lactate to pyruvate reaction method and results differ depending on the assay (UK NEQAS for Clinical Chemistry. Report on Distribution 996 dated 12 June 2016. Personal communication). The impact, if any, of these differences on the classification of pleural effusions using Light's criteria is unknown. We therefore compared the classification of pleural effusion according to Light's criteria using LDH results generated by different analytical platforms and different assays.

METHODS

After routine analysis, left over, adult, paired pleural fluid and serum samples received over 3 months were collected. After exclusion of duplicate patient specimens, samples were anonymised and stored at −80°C until analyses. Samples were frozen at −80°C within 3 days of receipt in laboratory A. Samples were transported frozen to the other laboratories for analysis.

Serum and pleural fluid were analysed for TP and LDH on three different platforms in three different laboratories from the West Midlands of England. LDH was measured using an Abbott ARCHITECT c16000 analyser (Abbott Diagnostics, Abbott Park, Illinois, USA), which uses the lactate to pyruvate reaction (laboratory A), and on Roche Cobas 800 c702 analysers (Roche Diagnostics GmbH, Mannheim, Germany using either the

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lactate to pyruvate reaction (laboratory B) or pyruvate to lactate reaction (laboratory C). Protein was analysed using the biuret method on an Abbott ARCHITECT c16000 analyser (laboratory A), and on Roche Cobas 800 c702 analysers (laboratory B and laboratory C).

Those with pleural fluid TP levels >35 or <25 g/L were classified as exudate or transudate, respectively.[1] Light's criteria were then used to classify pleural effusions with fluid protein between 25 and 35 g/L into exudate and transudate.[1]

Table 1 The serum reference intervals for LDH and total protein

	LDH (U/L)	Total protein (g/L)
Laboratory A Abbott	125–264	60–80
Laboratory B Roche	Male: 135–225 Female: 135–241	60–80
Laboratory C Roche	200–500	60–80

LDH, lactate dehydrogenase.

Table 2 Discordant results

Laboratory A	Laboratory B	Laboratory C
Exudate†‡	Transudate	Transudate
Exudate†	Transudate	Transudate
Exudate†	**Transudate**	Exudate‡
Exudate‡	Exudate†	**Transudate**
Exudate†‡	Transudate	Transudate
Transudate	**Exudate***	Transudate
Exudate‡	Transudate	Transudate
Exudate‡	**Transudate**	Exudate*

Discordant results are in bold.
Classification of exudate was based on:
*Pleural fluid:serum protein is >0.5.
†Pleural fluid:serum LDH is >0.6.
‡Pleural fluid LDH is >2/3 the upper reference limit for serum LDH.
LDH, lactate dehydrogenase.

The Kolmogorov-Smirnov test assessed normality of pleural fluid and serum data. As data were not normally distributed, non-parametric repeated measures analysis of variance (Friedman test) with Dunn post-test comparison was used to measure the significance of difference of results between different analytical platforms. Data are expressed as medians with ranges in parentheses.

The Abbott LDH assay has a detection limit of 5 U/L with intra-assay and interassay coefficients of variation (CV) at levels of 125, 244 and 442 U/L of 5.0%–9.4%. The Roche LDH lactate-pyruvate and pyruvate-lactate assays have respective detection limits of 10 L and 40 U/L with intra-assay and interassay CVs of at levels of 89, 224 and 400 U/L of 1.8%–2.7% and at levels of 202 and 742 U/L of 1.5%–2%, respectively. The Abbott TP assay has a detection limit of 5 g/L with intra-assay and interassay CVs at levels of 45, 63 and 86 g/L of 4.9%–5.5%. The Roche TP assay has a detection limit of 2 g/L with intra-assay and interassay CVs at levels of 52, 74 and 88 g/L of 1.4%–2.4% for laboratory B and CVs at levels of 42 and 77 g/L of 1.2%–1.7% for laboratory C. The serum reference intervals in use for LDH and TP for the different platforms are shown in table 1. All serum assays performed appropriately for their methodology in the National External Quality Assurance Scheme (NEQAS).

RESULTS

Forty-four samples had a pleural fluid TP between 25 and 35 g/L in at least one laboratory and these were classified as either transudate or exudate according to Light's criteria.

Following the application of Light's criteria, there were eight discordant results (table 2). Four from laboratory A, three from laboratory B and one from laboratory C were discordant compared with the other two sites. Seven of the eight discrepancies were due to one or both LDH criteria and one also had a discrepant TP ratio. One was solely due to a difference in the TP ratio. The two discrepancies involving the TP ratio involved borderline results (table 2).

TP and LDH results in pleural fluid and serum from the three different analytical platforms are shown in table 3. Serum LDH

Table 3 Median (ranges) protein and LDH results in pleural fluid and serum and their ratios from the three different analytical platforms on 44 paired samples

	Laboratory A	Laboratory B	Laboratory C	p Value
Pleural fluid LDH (U/L)	160 (34–1398)	118 (21–781)	159 (28–741)	a=<0.001 b=<0.001 c=NS
Serum LDH (U/L)	285 (135–865)	240 (59–734)	364 (146–1291)	a=<0.001 b=<0.001 c=<0.001
Pleural fluid to serum LDH ratio	0.54 (0.13–5.50)	0.44 (0.09–3.90)	0.45 (0.10–2.20)	a=<0.001 b=NS c=<0.001
Pleural fluid total protein (g/L)	28 (9–47)	31 (10–57)	30 (11–49)	a=<0.001 b=NS c=<0.001
Serum total protein (g/L)	56 (39–65)	58 (43–70)	56 (38–67)	a=NS b=NS c=NS
Pleural fluid to serum total protein ratio	0.49 (0.20–0.80)	0.52 (0.23–0.83)	0.53 (0.22–0.83)	a=<0.001 b=NS c=<0.001

Where a is laboratory B compared with laboratory A, b is laboratory B compared with laboratory C and c is laboratory C compared with laboratory A. NS is p>0.05.
LDH, lactate dehydrogenase; NS, not significant.

Table 4 Classification of exudate by each Light's criterion

Laboratory	Pleural fluid to serum protein >0.5	Pleural fluid to serum LDH >0.6	Pleural fluid LDH is >2/3 the upper reference limit for serum LDH
A	21	18	26
B	24	14	15
C	25	12	9

LDH, lactate dehydrogenase.

results from laboratory B were lower ($p<0.001$) than those from laboratory A and both were lower ($p<0.001$) than those from laboratory C. Pleural fluid LDH results from laboratory A and laboratory C were similar and both were higher ($p<0.001$) than those from laboratory B. The LDH ratios were similar from laboratory B and laboratory C but these were lower ($p<0.001$) than those from laboratory A. Serum TP results were similar from all three sites. Pleural fluid TP results from laboratory B and laboratory C were similar but these were higher ($p<0.001$) than those from laboratory A. TP ratios from laboratory B and laboratory C were similar but these were higher ($p<0.001$) than those from laboratory A. The classification of exudate by criterion is shown in table 4.

DISCUSSION

The data from this multihospital service evaluation indicate that the classification of pleural effusions into transudate or exudates based on Light's criteria using three different analytical platforms was 18% discordant. The significantly different LDH and protein results between laboratories were expected but the relatively poor concordance is unexpected since each Light's criterion is based on ratios which should minimise the effect of interassay and between platform variability.

Discordant classification based on LDH criteria was all due to LDH results from laboratory A. This was investigated by comparing LDH data from the different laboratories (table 3). Serum LDH results from laboratory C were much higher than those from laboratory A and laboratory B, which is consistent with NEQAS data and show that the pyruvate to lactate (laboratory C) reaction method gives higher values compared with the lactate to pyruvate reaction method (laboratory A and laboratory B). In contrast, however, pleural fluid LDH results from laboratory A were unexpectedly similar to those from laboratory C and higher than those from laboratory B. The LDH ratio from laboratory A was, therefore, significantly higher than those from laboratory B and laboratory C (table 3) and at laboratory A more pleural fluid LDH values breached the >2/3 of the serum reference range criteria. Consequently, the two LDH criteria were more likely to result in a classification of exudate at laboratory A compared with laboratory B and laboratory C (tables 2 and 4). Since serum LDH results are consistent with NEQAS data, the discordance between platforms appears to be due to the LDH assay performing differently in pleural fluid compared with serum.

Although serum TP results were similar between platforms, comparative pleural fluid protein results and therefore TP ratios were lower from laboratory A compared with laboratory B and laboratory C. This explains the decreased classification of exudates at laboratory A compared with laboratory B and laboratory C based on this criterion (table 4). This also suggests that TP assays may also perform differently in serum and pleural fluid. The two discordant exudate results due to TP ratios were, however, just above the 0.5 cut-off but their respective LDH ratios were indicative of a transudate. We therefore suggest that at borderline TP ratios, consideration be given to the LDH criteria in further evaluating the type of pleural effusion appropriate to the clinical context.

In summary, classification of pleural fluid into transudate or exudate using Light's criteria using different analytical platforms and assay methodology was 18% discordant. Light's criteria implicitly assumes that the performance of LDH and TP assay in pleural fluid should mirror that in serum and in this study the variability between analytical platforms was largely due to the performance of LDH assays and to lesser extent protein assays in pleural fluid. Clinicians should be aware that classification of pleural effusions into exudate or transudate using Light's criteria is dependent on the analytical platform and they should review any classification inconsistent with the clinical picture. Laboratories should be aware that assays may perform differently in serum and pleural fluid. External quality assurance providers should consider providing schemes for fluids but perhaps less frequently.

Take home messages

- Serum assays do not always perform as expected when used to analyse other fluids and this needs to be verified.
- The application of lights criteria to the same samples across different analytical platforms can yeild discordant results.
- Clinicians should always query results that do not fit the clinical picture.
- Fluid assay performance should be part of the quality checking process in laboratories.

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