CASE REPORT

High pleural ammonia negatively interferes with the measurement of adenosine deaminase activity

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SUMMARY

Pleural adenosine deaminase activity (ADA) is a sensitive and specific test for tuberculous pleurisy. Here, we report a case of undetectable ADA in the pleural fluid of a man presenting with chronic cough, fever and night sweats. Subsequent laboratory investigations and review revealed that the presence of high concentrations of ammonia in pleural fluid, commonly seen in empyema, negatively interferes with ADA results when measured by the Guisti and Galanti method. The source of ammonia may come from deamination of amino acids, ammonia-producing microbes and/or leucocytes. This interference invalidates ADA results and is present in ~2% of our laboratory requests. It is important to keep this interference in mind when tuberculosis/ammonia-producing bacteria coinfections are suspected and during early phases of tuberculous pleurisy, when neutrophils predominate in the pleural fluids.

BACKGROUND

Adenosine deaminase (ADA, EC 3.5.4.4) is an enzyme that catalyses the deamination of adenosine (or deoxyadenosine) to inosine (or deoxyinosine) and ammonia. ADA activity in the pleural fluid, when elevated above a defined threshold, is a rapid and sensitive marker for tuberculous pleurisy.¹⁻³ Falsely low ADA results can cause missed diagnosis and delay treatment. Here, we report a case of undetectable ADA activity in the pleural fluid of a man presenting with chronic cough, fever and night sweats.

CASE PRESENTATION

An elderly man presented to the emergency medicine department with 3 weeks of fever, chills, cough, night sweats and loss of appetite. His thoracic CT showed a large loculated calcified collection around the posterior aspect of the left lower lobe and collapse consolidation of the left lower lobe, most likely due to mass effect. A sample of the loculated fluid was collected and sent to the laboratory for measurement of ADA and bacterial and mycobacterial cultures.

INVESTIGATIONS

The ADA was measured by the Guisti and Galanti method.⁴ Briefly, the patient sample was incubated with phosphate-buffered saline adenosine solution for an hour at 37°C. The reaction was stopped by adding phenol and sodium nitroprusside in hypochlorite solution, which formed intensely blue indophenols with ammonia. The concentration of indophenol was measured using spectrophotometry.

The ADA activity was derived by subtracting the absorbance of the sample blank (no substrate added) from the absorbance of the patient sample, multiplied by a conversion factor.

The initial ADA result returned as 0 U/l and assay interference was suspected due to a high clinical index of suspicion. A review of the spectrophotometer output revealed very high absorbance of 3.4 in the sample blank. The sample of the patient was diluted 5 and 10 times and the repeat ADA were 182 and 289 U/l, respectively. The increased recovery after dilution suggested the presence of assay interference. The concentration of ammonia in the pleural fluid was >1000 µmol/l (Advia 2400, Siemens, Germany). The relationship between pleural ammonia and the sample blank is shown in figure 1. Differential cell counts of the sample revealed leukocytosis of 21 000/µl, 95% neutrophils. Significant numbers of neither erythrocyte nor haemolysis were detected. *Streptococcus constellatus*, *Fusobacterium nucleatum* and *Peptostreptococcus* species were grown from the sample. The patient was diagnosed with and treated for empyema. *Mycobacterium tuberculosis* was not isolated after 8-week incubation of cultures.

To examine the extent of this interference in our laboratory, all spectrophotometer output (n=994) for ADA performed between 4 January 2011 and 12 March 2012 was reviewed. This study received institutional approval (Ref: DSRB/C/2012/00200).

OUTCOME AND FOLLOW-UP

In all, 18 samples (1.8% of total requests), including this case, had sample blank absorbance of more than 0.2. Of these, 15 samples were diluted at the time of analysis and all showed markedly increased recoveries (125 to >10 000%) after 2–10 times dilution, except one (sample blank absorbance of 0.36) that recovered 68% after 2 times dilution. In contrast, five samples with high ADA (>150 U/l) and sample blank absorbance below 0.2 showed acceptable recovery (100±20%) after dilution.

To determine the source of ammonia in the pleural fluids, the medical records of patients with high sample blank absorbances were reviewed. Thirteen of the 18 patients had differential cell counts performed, which showed leukocytosis that ranged from 2400 to >500 000/µl, with predominant neutrophils (>90% in nine cases). Of the 17 patients who had microbiology cultures performed, 8 had *S constellatus*, *F nucleatum*, *P micros*, *Proteus mirabilis* or *Klebsiella pneumoniae* isolated from their sample, all of which produce ammonia. *Bacteroides caccae* and *Streptococcus dysgalactiae*

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Figure 1 Relationship between pleural ammonia and sample blank absorbance.

Arcanobacterium haemolyticum were isolated in another two cases. No microorganism was isolated from the pleural fluids of the remaining patients. All these cases had a diagnosis of empyema. None of them were positive for *M tuberculosis*.

DISCUSSION

ADA in pleural fluids can be falsely low due to early sampling, sample deterioration with time and spectral interference from haemoglobin (when >500 mg/l).⁴ These problems can be minimised by repeat sampling,² addition of enzyme stabilisers glycerol and ethylene glycol, which increase the enzyme activities to more than 21 days at 37°C,⁵ and early centrifugation of pleural fluid samples to separate cellular components.⁴ On the other hand, empyema can produce falsely elevated ADA.¹ Here, we highlight the negative interference of endogenous ammonia, present in empyemic patients, on the measurement of ADA in pleural fluids by the Guisti method.

This interference cannot be eliminated by dilution, and when present at high concentrations (sample blank >0.2), invalidate the ADA results. The cut-off for blank sample absorbance should be determined by an individual laboratory. High concentrations of ammonia in a patient sample may come from the deamination of amino acids, ammonia-producing microbes and/or leucocytes.⁶ The leucocytes may elevate the ammonia concentration by releasing ammonia or ADA during cellular lysis.⁶

The presence of high concentrations of ammonia in pleural fluid, commonly seen in empyema, may result in falsely low ADA results. While this phenomenon appears to be relatively rare, clinicians and laboratory practitioners should be aware of it. This is particularly important in tuberculosis/ammonia-producing bacteria co-infections and during early phases of

tuberculous pleurisy when neutrophils predominate in the pleural fluids.²

Possible solutions to overcome this interference include simple desalting on a gel-filtration column or Sephadex G-10 spin column (GE Healthcare, Buckinghamshire, UK) prior to analysis. Unfortunately, these solutions were not explored in our laboratory due to lack of resources. In doubtful cases, γ -interferon, C reactive protein (<4 mg/dl) and lymphocyte/neutrophil ratio (>0.75) can improve diagnostic accuracy.⁷ Pleural fluid or pleural biopsy subjected to PCR and culture for *M tuberculosis* remains the gold standard.

Learning points

- High ammonia in pleural fluid negatively interferes and invalidates adenosine deaminase (ADA) measurements.
- It is important to interpret ADA results in the clinical context.
- It is important to keep this interference in mind when tuberculosis/ammonia-producing bacteria coinfections are suspected and during early phases of tuberculous pleurisy, when neutrophils predominate in the pleural fluids.
- Pleural fluid or pleural biopsy subjected to PCR and culture for *Mycobacteria tuberculosis* remains the gold standard.

Competing interests None.

Patient consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

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