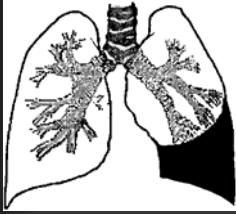


# International Pleural Newsletter



A Publication of the International Pleural Network

Volume 2 Issue 2  
April 2004

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## Adenosine Deaminase for Lymphocytic Pleural Effusions

Y C Gary Lee MBChB PhD FRACP  
Osler Chest Unit, Oxford &  
University College London, U.K.  
[ycgarylee@hotmail.com](mailto:ycgarylee@hotmail.com)

Richard W Light MD FCCP  
St Thomas Hospital & Vanderbilt University,  
Nashville, TN, USA  
[rlight98@yahoo.com](mailto:rlight98@yahoo.com)

Tuberculosis remains a global health problem, and is one of the commonest causes of exudative pleural effusions in many regions. Although TB pleural effusions usually resolve spontaneously, establishing the diagnosis is important. Untreated, up to 65% of patients with TB pleural effusion will eventually develop active TB in extra-pleural sites. However, diagnosing TB pleural effusion is not straightforward. TB pleural effusion often presents without active TB in other organs, and the pleuritis develops as a delayed hypersensitivity to the mycobacterial proteins. The mycobacterial load in the pleura is usually low; hence the poor yield of TB culture from pleural fluid or biopsy<sup>1</sup>.

Adenosine deaminase (ADA) has become increasing popular as a diagnostic test for TB pleuritis since 1978, and is considered a routine test for pleural fluid in countries where the prevalence of TB is high. Many reports have attested to the high sensitivity (usually >95%) of ADA<sup>2,3</sup>. Unlike PCR or interferon, ADA measurement is cheap (about \$15 in USA), easy and quick to perform (2 hours) and accurately reproducible even on stored samples. The latter point is an advantage because the diagnosis of TB is often overlooked during the initial investigations due to the non-specific clinical presentation. If the pleural fluid sample is stored properly, ADA determination can still be made several weeks later<sup>4</sup>.

The widespread use of ADA in pleural effusions was met with initial concern that false positives were frequent in early reports. Most of the false positive cases were parapneumonic effusions or empyemas<sup>3</sup>, which were neutrophilic predominant. On the other hand, most (>90%)

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TB effusions are lymphocyte-predominant with lymphocytes accounting for >50% of total WBC count in the pleural fluid<sup>5</sup>. TB is suspected in clinical practice if the pleural fluid is lymphocyte-predominant. Therefore, false positive diagnoses of TB effusion by ADA can be significantly reduced if ADA measurement is limited to lymphocytic pleural fluids.

Our study published in 2001<sup>4</sup> confirmed that ADA levels in a variety of non-TB lymphocytic pleural effusions seldom (<3%) exceeded the cut-off set for TB effusions. Two subsequent studies have also corroborated the results<sup>6,7</sup>. Since then Dr Miller, who measured the ADA in our study, has received many requests and has assisted setting up of ADA measurements in many hospital laboratories around the world.

We recommend that pleural fluid ADA be employed in the diagnostic workup for pleural effusions in countries where TB is endemic. Given its high sensitivity, ADA is an effective screening tool: a negative result nearly excludes the diagnosis of TB pleural effusion. A high ADA in a lymphocytic effusion indicates that the most likely diagnosis is TB, although clinicians must bear in mind that a small number of cases may have alternative etiologies. Occasionally, malignant effusions, especially lymphoma, can produce a lymphocytic effusion with elevated ADA, as can Q fever and brucellosis.

<sup>1</sup> Bothamley GH. Tuberculous pleurisy and adenosine deaminase. *Thorax* 1995;50:593-4.

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<sup>3</sup> Valdes L, Alvarez D, Valle JM, et al. The etiology of pleural effusions in an area with high incidence of tuberculosis. *Chest* 1996;109:158-62.

<sup>4</sup> Lee YCG, Rogers JT, Rodriguez RM, et al. Adenosine deaminase levels in nontuberculous lymphocytic pleural effusions. *Chest* 2001;120:356-61.

<sup>5</sup> Light RW, Erozan YS, Ball WC, Jr. Cells in pleural fluid. Their value in differential diagnosis. *Arch Intern Med* 1973;132:854-60.

<sup>6</sup> Jimenez Castro D, Diaz Nuevo G, Perez-Rodriguez E, et al. Diagnostic value of adenosine deaminase in nontuberculous lymphocytic pleural effusions. *Eur Respir J* 2003;21:220-4.

<sup>7</sup> Porcel JM, Vives M. Adenosine deaminase levels in nontuberculous lymphocytic pleural effusions. *Chest* 2002;121:1379-80.

## ***Measuring ADA in Pleural Fluids***

**Kent D Miller PhD MD**

Professor Emeritus, University of Miami and  
Diagnostic Ventures Inc., Daytona Beach, FL, USA

[sixfive65@worldnet.att.net](mailto:sixfive65@worldnet.att.net)

Worldwide studies have identified ADA as a highly sensitive marker for TB pleurisy. This originated from the seminal

report by Piras and Gakis<sup>1</sup>, who observed that patients with TB meningitis, but not those with bacterial, viral or other meningitides, had CSF levels of ADA >10 IU/L. Their observation also led to the eventual understanding that clinically significant quantities of ADA are secreted into adjacent fluids by sensitized macrophages at the infection sites.

Subsequently, many studies reported high pleural fluid ADA levels in TB pleurisy<sup>2-4</sup>, using the colorimetric ADA assay described by Guisti and Galanti<sup>5</sup>. The reproducible clinical correlations are explained by the accuracy inherent in that procedure. First, the method utilizes the time-tested Berthelot Reaction. Ammonia, released from adenosine by ADA action, is oxidized to chloramine, with formation of a stable indophenol in the presence of phenol. A second reason for the dependability of the procedure is the use of ammonium sulfate solutions as standards. Those stable solutions are easily and accurately prepared in any laboratory. Third, the Guisti procedure utilizes sample blanks analyzed simultaneously with the respective enzyme reactions, providing corrections for non-specific color contributed by the specimens. Thus, the test performs well even on moderately hemolyzed pleural fluids. ADA released by hemolyzed red cells is usually insignificant compared to that secreted by sensitized macrophage. Remarkable among those reports is the narrow range of cutoff values employed by each laboratory, 35-45 IU/L.

Although specific detection tests (eg PCR, ELISAs) for mycobacteria and their products have been advocated, those tests involve amplification by laboratory procedures *in vitro*. Such tests are unlikely to attain the sensitivity of the high ADA level. This is based on the well-known sequestration of mycobacteria within the phagosomes of macrophage, and the failure of those phagosomes to fuse with the lysosomes of the same cells<sup>6</sup>. Hence, organisms and organism products may not be available for detection in pleural or other body fluids. The advantage of the ADA measurement is the test amplification that is provided by the cells of origin at the infection sites, with the enzyme secreted into the adjacent fluids. Thus, the test detects the host responses to presence of the organisms, not the numbers or specific types of organisms present.

The specificity of the elevated ADA level for TB pleurisy is almost as high as its sensitivity. In most cases, false positives are associated with empyemas and rheumatoid arthritis<sup>4</sup>. ADA can be separated into the low- (ADA<sub>1</sub>) and high- (ADA<sub>2</sub>) molecular weight forms, of which ADA<sub>2</sub> is the dominant form in TB pleural effusions<sup>7</sup>. Thus, improved specificity and sensitivity for detection of TB pleurisy can be attained when convenient and inexpensive methods for specific ADA<sub>2</sub> measurement are established.

Improved specificity of the elevated ADA (to 95%) for mycobacterial infections can also be achieved through combined analyses for ADA and lysozyme<sup>8</sup>. In diseased lungs, lysozyme is produced not only by pulmonary

macrophage and epithelioid granuloma cells, prominent ADA sources, but also by tracheal serous glands and granulocytes in inflammatory reactions<sup>9</sup>. Thus, a substantial increase in lysozyme relative to ADA suggests processes other than TB.

This author's laboratory finds the elevated lysozyme level nearly as sensitive a marker for TB pleurisy as is an elevated ADA level. In a study of 100 TB pleural effusions, the ADA levels (40 IU/L cutoff) and lysozyme levels (17 ug/ml cutoff) were only falsely negative in 2 and 3 patients respectively. Only one specimen fell below both cutoff values for a combined sensitivity of 99%. My laboratory now routinely provides a lysozyme level with every ADA assay.

<sup>1</sup> Piras MA and Gakis C. Cerebrospinal fluid adenosine deaminase in tuberculous meningitis. *Enzyme* 1972;14:311.

<sup>2</sup> Piras MA, Gakis C, Budroni M, et al. Adenosine deaminase activity in pleural effusions: an aid to differential diagnosis. *Brit Med J* 1978;23:1751-2.

<sup>3</sup> Ocana I, Martinez-Vasquez, Segura RM, et al. Adenosine deaminase in pleural fluids. *Chest* 1983;84:51.

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<sup>5</sup> Guisti G and Galanti B. Adenosine deaminase. In: Bergmeyer HU, ed. *Methods in Enzymatic Analysis*, New York, Academic Press, 1974;1092-6.

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<sup>7</sup> Ungerer JPJ and Grobler SM. Molecular forms of adenosine deaminase in pleural effusions. *Enzyme* 1988; 40:7-13.

<sup>8</sup> San Jose E, Valdes L, Serandeses A, et al. Diagnostic value of adenosine deaminase and lysozyme in tuberculous pleurisy. *Clinica Chimica Acta* 1992; 209:73-81.

<sup>9</sup> Klockars T, Pettersson T, Riska M, et al. Pleural fluid lysozyme in human disease. *Arch Int Med* 1979; 139:73-7.



symptomatic. Repeat chest radiograph showed an increased left sided pleural effusion (see figure).

A 28F intercostal drain was inserted which drained 2.2L of stale blood over the next 24 hours and patient was transfused 3 units of blood. His coagulation profile and platelet count remained normal. The chest

drain was removed on the 4<sup>th</sup> day and he was discharged uneventfully after 18 days in hospital.

Standard contraindications to fibrinolytic therapy include: (i) known sensitivity to fibrinolytics, (ii) bleeding diatheses, (iii) recent hemorrhagic stroke, (iv) intracranial neoplasm, (v) recent surgery or (vi) recent head trauma. Life-threatening pleural hemorrhage has been reported in patients with mild to moderate disturbance of clotting indices and coexisting bronchopleural fistula and also in patients with recent rib fractures or cardiac valve surgery despite normal coagulation profiles<sup>2-4</sup>. The use of a large bore tube was proposed as the cause of pleural hemorrhage following intrapleural urokinase use in a child<sup>5</sup>. None of the above contributing factors were present in our patient and cannot account for bleeding in his case.

The optimal dose of SK per treatment is not known. Studies of simple clotting indices had not shown significant systemic fibrinolytic activity in humans even up to a cumulative instillation dose of 1.5 million units<sup>6</sup>. The dosage used in this case was no greater than that cited in the literature, but the concentration (diluted in only 20 ml saline) was higher and the duration of clamping the chest tube (4hr) was at the top of currently recommended range<sup>6</sup>.

According to our knowledge, this is the first reported case of delayed intrapleural bleeding following completion of the treatment regime and removal of the chest tube. Previous reports involved bleeding immediately after the first instillation or during treatment where blood loss is easily detected in the intercostal tube drainage. A high index of suspicion should be maintained for late-onset occurrences and patients should be closely monitored for symptoms. Clinicians should be aware that in cases of continuous slow occult bleeding, a drop in hemoglobin level may be the first and only clinical clue of intrapleural hemorrhage following fibrinolytics.

<sup>1</sup> Tillet WS, Sherr S, Read CT. *Thorac Surg* 1950; 21:275-97

<sup>2</sup> Godley PJ, Bell RC. *Chest* 1984;86:486-7

<sup>3</sup> Temes RT, Folles F, Kessler RM, et al. *Chest* 1996;110:102-6

<sup>4</sup> Porter J, Banning AP. *Thorax* 1998;53:720

<sup>5</sup> Blom D, van Aalderen WM, Alders JM, et al. *Pediatr Pulmonol.* 2000;30:493

<sup>6</sup> Davies CWH, Lok S, Davies RJO. *Am J Respir Crit Care Med* 1998; 157:328-30

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## ***Hemothorax Following Intrapleural Streptokinase - A Case Report***

**A.P.Chua & T.K.Lim Singapore**

Intrapleural streptokinase (SK) has been advocated as a safe and effective adjunctive treatment of empyema and hemothorax<sup>1</sup>. We present a patient who developed delayed hemothorax following intrapleural administration of SK.

A 42-year old Chinese male with left lower lobe pneumonia and a large, loculated parapneumonic effusion received intrapleural SK 250,000U (in 20 ml saline) bd over 2½ days (total 5 doses) after failing pleural drainage with an 8F pigtail tube. The chest tube was clamped for 4 hours after each dose. Four days after the last dose of SK, there was a 4 gram drop in his hemoglobin level and he became

## ***Eosinophilic pleural effusions: Clinical Approach***

**Ioannis Kalomenidis MD**

University of Athens, Athens, Greece

[jkalomenidis@hotmail.com](mailto:jkalomenidis@hotmail.com)

Pleural effusions containing  $\geq 10\%$  eosinophils are termed 'eosinophilic' and account for 5-16% of exudative effusions<sup>1,2</sup>. Most commonly, EPE develops secondary to the presence of *air* or/and *blood* in the pleural cavity (eg following trauma, pneumothorax or hemothorax), and may persist for weeks<sup>3</sup>. Pleural *malignancy* is the second most common etiology. A variety of bacterial, mycobacterial, parasitic, fungal, and viral *infections* may also cause EPEs. Eosinophils may appear late in the course of parapneumonic effusions, even after the resolution of the pneumonia. In places with a high prevalence of *TB*, 15% of EPEs were from patients with *TB* pleuritis<sup>2</sup>.

Many *drugs* cause EPE (see table)<sup>3</sup>. The latent time between the administration of a drug and the appearance of EPE varies from weeks to months. Symptoms attenuate during the first several days after drug withdrawal, but complete resolution of EPE may take months. *Pulmonary embolism* (PE) is accompanied by pleural effusions in 30-50% of cases; up to 18% of which are eosinophilic<sup>4</sup>. Up to 50% of *benign asbestos pleural effusions* (BAPes) are eosinophilic; conversely, 4% of EPEs are BAPes<sup>1</sup>. Rare causes of EPE include rheumatoid arthritis, SLE, sarcoidosis, eosinophilic pneumonias, Loeffler syndrome, hypereosinophilic syndrome, eosinophilic fasciitis, pancreatic pseudocyst, uremic pleuritis, abdominal infection, heart failure, and cirrhosis.

A significant proportion of EPEs remain undiagnosed. Indeed EPE that are undiagnosed are more common than EPE due to malignancy. The effusion usually resolves within the first year and rarely recurs. The prognosis is excellent.

**The diagnostic work-up:** Initial testing include direct examination of pleural fluid, sputum and stool specimens for parasites, ova, and fungi; cultures for fungi and mycobacteria; and cytologic examination for malignant cells. Serology may be helpful when certain parasitic infections are suspected. A pleural fluid pH  $< 7.1$  suggests paragonimiasis or Churg-Strauss syndrome<sup>5</sup>. Parapneumonic EPEs are usually sterile<sup>4</sup>. Bronchoscopy should be considered in the presence of clinical or radiologic evidence of endobronchial lesions, eosinophilic pneumonias or parasitic/fungal infections. In EPEs that remain undiagnosed after initial investigations, the underlying causes are likely to be viral infection, occult pulmonary emboli, BAPE or malignancy. Thus, spiral CT and/or pulmonary angiography should be performed to exclude PE.

No definitive data exist to support the need of invasive diagnostic procedures in every patient with EPE. If there is a

high clinical suspicion of malignancy, video-assisted thoracoscopic biopsy should be considered, during which, we recommend that both pleura and lung be biopsied for histology and for microbiologic studies. As many persistent EPEs are benign<sup>1</sup>, a conservative approach, with observation and repeated thoracentesis, when needed, may be followed in patients who have a low risk of malignancy.

**Table. Drugs associated with EPE**

Nitrofurantoin	Isotretinoin	Gliclazide
Vitamins B <sub>6</sub> /H	Mesalamine	Dantrolene
Bromocriptine	Fluoxetine	Warfarin
Propylthiouracil	Valproic acid	

<sup>1</sup> Adelman M, Albelda SM, Gottlieb J, et al. Diagnostic utility of pleural fluid eosinophilia. *Am J Med* 1984;77:915-20.

<sup>2</sup> Martinez-Garcia MA, Cases-Viedma E, Cordero-Rodriguez PJ, et al. Diagnostic utility of eosinophils in the pleural fluid. *Eur Respir J* 2000;15:166-9.

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<sup>4</sup> Light RW. *Pleural Diseases*. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001:271-2.

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### ***A BIT OF HISTORY***

In 1835 Dupuytren, Napoleon's surgeon, developed an empyema. When offered surgical drainage of his empyema, he refused and insisted that "*he would rather die at the hands of God than of surgeons*". Unfortunately, he died within two weeks.

Drainage of empyema by thoracentesis eventually became a standard approach to empyema in 1843.

Adopted from: P. Warren

<http://www.umanitoba.ca/faculties/medicine/units/history/notes/surgery/>

**If you have any interesting case of pleural disease to share, or any suggestion and comment on the Newsletter, please contact:**

**Mrs Emma Hedley**

**[emma.hedley@orh.nhs.uk](mailto:emma.hedley@orh.nhs.uk)**

**Fax: +44-1865-225205**

## THE MESOTHELIUM

**Steven E. Mutsaers PhD** [mutsaers@aari.uwa.edu.au](mailto:mutsaers@aari.uwa.edu.au)

*Asthma & Allergy Research Inst & Dept of Med, University of Western Australia, Australia*

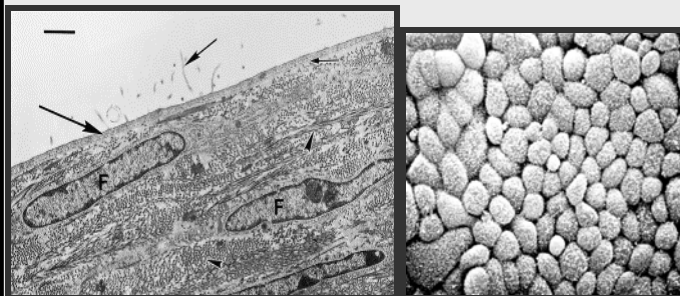
**Sarah E. Herrick PhD** [sarah.herrick@man.ac.uk](mailto:sarah.herrick@man.ac.uk)

*School of Biological Sciences, University of Manchester, UK*

The mesothelium was first described by Bichat in 1827. Using histological techniques he observed that the serous cavities were lined by a single layer of flattened cells similar to those of the lymphatics. In 1880, Minot, in a detailed study of the embryological origins of the mesothelium, initially referred to it as the 'epithelial lining of mammalian mesodermic cavities' and subsequently proposed the term 'mesothelium'. Embryologically, the mesothelium develops from the mesodermal tissue in humans around day fourteen of gestation, with cells gradually differentiating from round or cuboidal cells to elongated flattened cells which line the coelomic cavities.

The mesothelium is composed of an extensive monolayer of specialized mesothelial cells that line the body's serous (pleural, pericardial and peritoneal) cavities and most internal organs. Mesothelial cells rest on a thin basement membrane supported by connective tissue stroma that varies in quantity and quality depending on site and species. The cells are predominantly flattened, squamous-like, approximately 25µm in diameter, with the cytoplasm raised over a central round or oval nucleus. In addition to providing a slippery protective surface to facilitate intracoelomic movement, the mesothelium is now recognized as a dynamic cellular membrane with many important functions. These include transport and movement of fluid and particulate matter across the serosal cavities, leukocyte migration in response to inflammatory mediators, synthesis of pro-inflammatory cytokines, growth factors and extracellular matrix proteins to aid in serosal repair, release of factors to promote both deposition and clearance of fibrin, and antigen presentation. Furthermore, the secretion of molecules such as glycosaminoglycans and lubricants protect tissues from abrasion, infection and possibly tumor dissemination.

Transmission electron micrograph showing a mesothelial cell with a thin attenuated cytoplasm (large arrow) and surface microvilli (medium arrow) resting on a basement membrane (small arrow). Elongated fibroblast-like cells (F) beneath the mesothelium are surrounded by collagen (arrow heads) and other connective tissue.



Regenerating mesothelium 7 days after injury. The repopulated mesothelium has a cobblestone appearance with clearly defined cell borders. Reproduced with permission from Mutsaers 2002.

The mesothelium is a slowly renewing tissue with 0.16-0.5% of cells undergoing mitosis at any one time. Injury to the mesothelium triggers events leading to proliferation and migration of mesothelial cells from the edge of the lesion towards the wound center and accumulation of free-floating cells in the serosal fluid, which attach and incorporate into the regenerating mesothelium. The origin and nature of these free-floating cells is unclear. They may arise from desquamated surrounding mesothelial cells or daughter cells released into the serosal fluid following proliferation.

Although previous studies have suggested that regenerating mesothelial cells do not arise from bone marrow precursors, evidence from more recent studies suggest that a progenitor mesothelial cell may exist. For example, although mesothelial cells are of a mesodermal origin, they express characteristics of both epithelial and mesenchymal phenotypes. In addition, recent findings have shown that mesothelial cells can undergo an epithelial to mesenchymal transition and differentiate into myofibroblasts and possibly smooth muscle cells. Further evidence for a mesothelial progenitor comes from tissue engineering applications where mesothelial cells seeded on tubular constructs generate patent blood vessels and also graft transected nerve fibers. These findings suggest that mesothelial cell progenitors are able to switch between different cell phenotypes depending on the local environment. The existence of a mesothelial 'stem' cell needs further study.

If mesothelial healing is impaired, fibrous adhesions form between organs and the body wall, which impede vital intrathoracic and abdominal movement and can result in significant morbidity and mortality. Neoplastic transformation of mesothelial cells gives rise to malignant mesothelioma, an aggressive tumor predominantly of the pleura. Although closely associated with exposure to asbestos, recent studies have implicated other factors including simian virus 40 in its pathogenesis. For a more extensive review of the mesothelium see references below.

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