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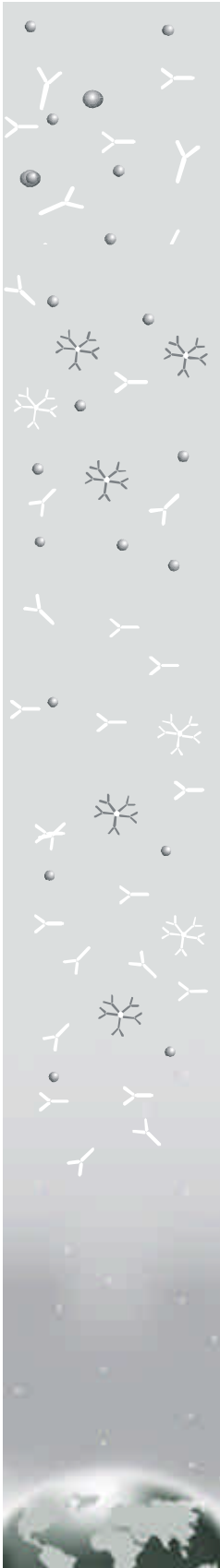
Preface

Tulip Group of companies believes in offering our valued customers the technical support and scientific information to keep updated with the latest international standards and trends in diagnostic testing.

Laboratory results play a pivotal role in providing the clinician the scientific data in diagnosing, monitoring and prophylaxis of deserving patients. Keeping in mind our valuable customers, Tulip Group will offer periodically a series of Tech Notes presented with a short, summarized overview pertaining to a specific technique/product/disease related information.

We hope that the Tech Notes will assist and benefit the laboratorians in enhancing the standards of reporting results thereby helping the clinician for better diagnosis and patient management.

Yours faithfully,
Micropress.



Adenosine Deaminase

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What is Adenosine Deaminase (ADA)?

Adenosine Deaminase (ADA) is an enzyme widely distributed in animal and human tissues.

Adenosine Deaminase is found in most cells, but its chief role concerns the proliferation and differentiation of lymphocytes and has been looked on as a marker for cell-mediated hypersensitive reactions.

Adenosine Deaminase enzyme is involved in the purine catabolism. It is capable of catalyzing the deamination of adenosine to inosine and of deoxyadenosine to deoxyinosine.

Adenosine Deaminase exists in several isoforms, the prominent ones being ADA₁ and ADA₂. ADA₁ isoenzyme is found in all cells, with the highest concentration in lymphocytes and monocytes, whereas ADA₂ isoenzyme appears to be found only in monocytes. ADA₂ is the predominant isoform in tuberculous pleural effusion suggesting that ADA₂ is the most efficient marker in tuberculosis. However, in clinical practice, the difference in the use of total ADA and isoform ADA₂ is not significant.

What is the role of Adenosine Deaminase in infectious diseases?

Increased levels of Adenosine Deaminase have been observed and documented in certain infectious diseases with an active participation of cell-mediated immune responses.

Increased serum activity of this enzyme have been found in many infectious diseases caused by microorganisms infecting the macrophages, in leprosy, brucellosis, HIV infections, viral hepatitis, infectious mononucleosis, liver cirrhosis and tuberculosis.

Patients with other infectious or non-infectious diseases in which high fever occurred also showed slight to moderate increase in serum Adenosine Deaminase levels.

What is the role of Adenosine Deaminase in tuberculosis?

The diagnosis of pulmonary tuberculosis is confirmed mainly by sputum examination for acid fast bacilli. However, diagnosis of extrapulmonary tuberculosis requires investigation of pleural aspiration, fluid biochemistry, cytology and biopsy. Further, it has been observed that cultures for acid fast bacilli are positive in 20-30% of pleural fluid samples and in 50-80 % of pleural biopsy specimens, making pleural tuberculosis often difficult to diagnose. This is because *Mycobacterium tuberculosis* in pleural fluid is scanty and rarely observed on direct examination. Relatively new techniques such as Adenosine Deaminase, Interferon Gamma and Polymerase Chain Reaction have been reported to help in diagnosis of tuberculosis. However, the sensitivity of Polymerase Chain Reaction (PCR) for tuberculosis is relatively low (0.42-0.81) and the test is expensive. Interferon Gamma appears to exhibit a better sensitivity (0.89-0.99) but there are relatively few studies of its use.

Adenosine Deaminase, has been proposed to be a useful surrogate marker for tuberculosis in pleural, pericardial and peritoneal. Studies have confirmed the high sensitivity and specificity of Adenosine Deaminase for early diagnosis of extrapulmonary tuberculosis and meningitis.

Adenosine Deaminase activity in tuberculous pleuritis.

Several studies have suggested that an elevated pleural fluid Adenosine Deaminase level predicts tuberculous pleuritis with a sensitivity of 90-100% and a specificity of 89-100%. The reported cut-off value for Adenosine Deaminase varies from 47 - 60 U/L .

Study¹: 221 patients subdivided into 6 groups; Group I–48 cases of tuberculosis; Group II–46 cases of malignancies; Group III–30 postpneumonic effusions; Group IV–19 cases of several diseases; Group V–18 pleural effusion of unknown origin and ; Group VI–60 acellular transudates/control groups were studied to map the specificity of ADA determination in diagnosis of tuberculous pleural effusions.

Conclusion: The study confirms that measurement of Adenosine Deaminase activity is a very good parameter for diagnosis of tuberculous effusions. Sensitivity (100%) and specificity (97%) of the test in diagnosing tuberculosis is very high. Further, none of the patients with tuberculous effusion had Adenosine Deaminase vales less than 45U/L.

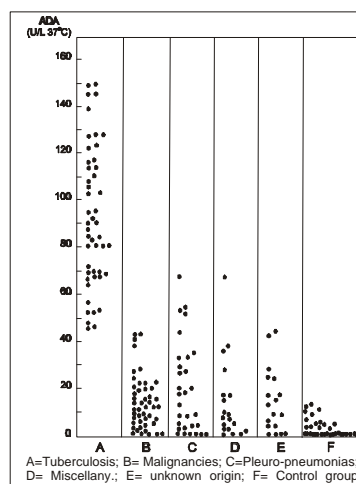


Fig.1: Levels of ADA activity in different groups of pleuroperitoneal effusions.

Adenosine Deaminase activity in tuberculous pericarditis.

Study²: Adenosine Deaminase activity in pericardial fluid of 56 patients with various etiologies were studied. Determination of Adenosine Deaminase was carried out by Giusti and Galanti method.

Conclusion: 3 patients with tuberculous pericarditis showed specific increase in Adenosine Deaminase activity as compared to the remaining 53 non-tuberculous patients. The Adenosine Deaminase activity of patients with tuberculous pericarditis is similar to the mean values found in other studies of tuberculous pleural or peritoneal effusions.

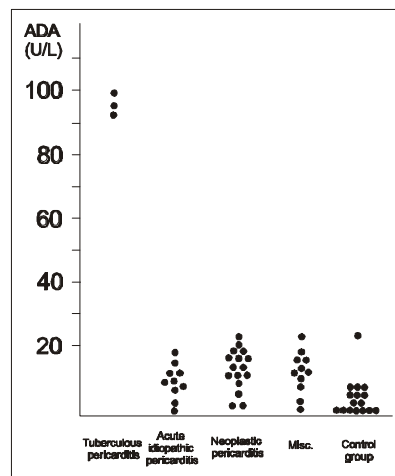


Fig.2: ADA activity in pericardial fluid of individual patients in various groups.

Adenosine Deaminase activity in tuberculous peritonitis.

Study³: 66 patients with various causes of ascites were studied.

Group	Cases	ADA	Range
Tuberculous peritonitis	10	108.5	72.5-148
Non-tuberculous septic peritonitis	8	1.7	0 - 6
Neoplasms	17	6.3	0 - 44
Miscellaneous	6	6.9	3 - 19
Control subjects	25	0.01	0 - 5

Table.1: Results of Adenosine Deaminase test in the peritoneal fluid (median values).

Conclusion: The study indicated that, in patients with tuberculous peritonitis, Adenosine Deaminase value was significantly higher than for the other non-tuberculous groups suggesting that Adenosine Deaminase activity is of great value in identifying tuberculous etiology of ascites.

Adenosine Deaminase activity in CSF for diagnosis of tuberculous meningitis.

Study⁴: Activity of Adenosine Deaminase in CSF of 40 normal controls and 205 patients grouped according to various diseases were measured.

Group	Cases	ADA Mean (\pm SD) levels	Range
Tuberculous meningitis	21	15.7 \pm 4.3	9.1- 22.7
Viral meningitis	40	1.4 \pm 1.3	0 - 6.8
Purulent meningitis	35	1.2 \pm 1.9	0 - 10.4
Neoplasms	28	2.4 \pm 1.8	0 - 11.2
Stroke	40	1.3 \pm 1.2	0 - 4.1
Miscellaneous	30	1.5 \pm 1.7	0 - 8.2
Control subjects	40	0.4 \pm 0.6	0 - 2.8

Table.2: Levels of Adenosine Deaminase in CSF.

Test	Sensitivity	Specificity
Tuberculin Skin Test	0.67	0.82
CSF cytology (>50% lymphocytes)	0.81	0.47
Glucose content of CSF (<40 mg/dl)	0.76	0.8
Culture of <i>M. tuberculosis</i> from CSF	0.76	1
Adenosine Deaminase in CSF (>9 U/L)	1	0.99

Table.3: Sensitivity and specificity of the Adenosine Deaminase test in comparison with other methods of diagnosing tuberculous meningitis.

Conclusion: Mean Adenosine Deaminase values were higher for patients with tuberculous meningitis than for other patient groups. The sensitivity of the test for diagnosis of tuberculous meningitis was 100% and specificity was 99% with a cutoff of 9 U/L when compared to other tests.

Can mapping of Adenosine Deaminase activity indicate response to therapy ?

Adenosine Deaminase can be employed for monitoring response to therapy. This is done by taking the base Adenosine Deaminase values of the patient prior to commencing drug therapy and then mapping Adenosine Deaminase levels after a few weeks/days. A decrease in Adenosine Deaminase levels indicate response to therapy whereas resistance to the drugs may be suspected if Adenosine Deaminase levels remain unchanged or increases. Drug resistance can then be confirmed by drug susceptibility testing.

Study⁴: Adenosine Deaminase activity and progression of the disease was studied in 32 patients with tuberculous meningitis.

Conclusion: Adenosine Deaminase showed an increase during the first 10 days of antituberculous therapy. After this initial phase, there is a progressive fall in Adenosine Deaminase activity, until it normalizes about three to four months later. The test proved to be a simple and reliable method for early diagnosis and follow-up of tuberculous meningitis.

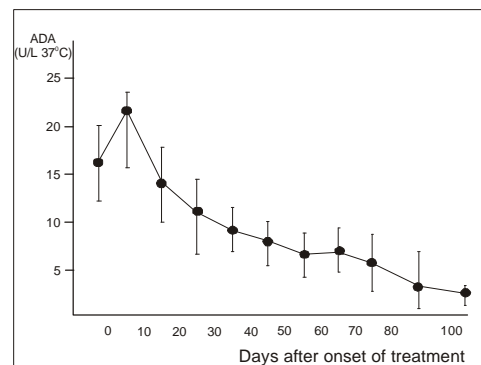


Fig.3: The mean ADA activity at 10-day intervals after beginning chemotherapy in patients with tuberculous meningitis.

What is the correlation of Adenosine Deaminase levels in body fluids?

When evaluating Adenosine Deaminase for diagnosis of tuberculosis, an important point to be borne in mind is that levels of Adenosine Deaminase during an infection may vary, due to individual variations in immune response.

In the above studies, it is well documented that the sensitivity and specificity of Adenosine Deaminase for pleural effusions, pericardial and peritoneal fluids as well as CSF is approximately 90% and thus is strongly recommended for diagnosis of extrapulmonary tuberculosis, in conjunction with other clinical tests.

In case of tuberculous pleurisy, the infection is characterized by the accumulation of activated T-lymphocytes and macrophages in the pleural spaces. The infection being localized and restricted to a certain area results in T-lymphocyte rich pleural effusion, leading to high Adenosine Deaminase concentration in pleural effusions. It was also observed that levels of Adenosine Deaminase in pleural fluid was significantly higher than those observed in serum in both tuberculosis and non tuberculosis patients, suggesting a localized intrapleural production of Adenosine Deaminase.

Although serum Adenosine Deaminase levels increase during infection with tuberculosis, some researchers suggest that serum Adenosine Deaminase measurement should not be used in tuberculosis for diagnostic purposes. However, it can serve as a useful parameter for the purpose of monitoring therapy provided the base line values before initiation of therapy are determined.

Does Adenosine Deaminase levels increase in an immunocompromised individual infected with tuberculosis?

The deficiency of Adenosine Deaminase has been documented in many studies and is observed in severe combined immune deficiency disease. Thus resulting in low production of Adenosine Deaminase or absence of Adenosine Deaminase synthesis during an infection, inspite of clear, convincing and definite clinical symptoms and laboratory tests results.

Other studies mention that Adenosine Deaminase levels among the HIV - positive patients did not differ from Adenosine Deaminase levels among the HIV-negative patients.

However, interpreting Adenosine Deaminase levels in immunocompromised patients should be done with caution.

How is Adenosine Deaminase determined in the laboratory?

The colorimetric method for measurement of total Adenosine Deaminase described by Giusti and Galanti has an advantage over other methods due to its low cost, simplicity of technique and rapid turnover.

With this method, the sensitivity and specificity of elevated level of Adenosine Deaminase in tuberculosis ranges from 91-100 % and 81-94% respectively. The PPV and NPV range from 84 – 93% and 89 – 100% respectively.

Further, the reported diagnostic cutoff value for Adenosine Deaminase varies from 40-60U/L. Lowering the cutoff value will indeed increase sensitivity but at the expense of specificity.

What is the diagnostic relevance of determining Adenosine Deaminase Activity?

Determination of Adenosine Deaminase activity is useful for

- (A) Diagnosis of extrapulmonary tuberculosis in:
- Serum / Plasma
 - Peritoneal fluid
 - Ascitic fluid
 - Pleural fluid
 - Pericardial fluid
 - CSF
- (B) Monitoring response to anti-tubercular therapy.

What are the diagnostically significant reference values for Adenosine Deaminase?

Specimen	Interpretation	Values
Serum, Plasma, Pleural Pericardial & Ascitic fluids	Normal Suspect Positive	<40 U/L >40U/L to <60 U/L > 60 U/L
CSF	Normal Positive	<10 U/L >10 U/L

What do researchers and articles on Adenosine Deaminase quote about the test?

- Assessment of Adenosine Deaminase in pathologic fluids is of great value in the diagnosis of tuberculosis of the pleura¹.
- The specific increase in Adenosine Deaminase activity suggest that assay of this enzyme in pericardial fluid could be of great value in the early diagnosis of tuberculous pericarditis².
- Adenosine Deaminase activity in the peritoneal fluid has proved to be a simple and reliable method for early diagnosis of tuberculous peritonitis³.
- It is apparent that determining the activity of Adenosine Deaminase in the CSF is a simple and very useful test for the early diagnosis of tuberculous meningitis even in those patients already receiving tuberculostatic drugs⁴.
- Several studies have suggested that an elevated pleural fluid ADA level predicts tuberculous pleuritis with a sensitivity of 90-100% and a specificity of 89-100% when the Giusti method is used⁶.
- The ROC curve identified 60 U/L as the best cutoff value. The sensitivity of ADA in patients with TB was 0.95, specificity was 0.96, PPV was 0.96, and 1-NPV was 0.95¹¹.
- Adenosine Deaminase determination is a quick and inexpensive technique which has been shown to be an accurate method for identifying tuberculous peritonitis¹².
- Rapid confirmation of tuberculous meningitis has always been difficult for the microbiologist. Recently, adenosine deaminase, a host enzyme produced by activated T cells and easily detected by a colorimetric procedure, was shown to increase in concentration during the active stage of tuberculous meningitis and to decrease to normal levels after effective antituberculosis therapy¹³.

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