ADENOSINE DEAMINASE DIAGNOSED T.B PLEURITIS-EMBRACING THE "NEW" MODALITY OF DIAGNOSIS IN RESOURCE LIMITED UGANDAN SETTINGS.

ELIAS NDIBAREMA[1], TIMOTHY MUGABI[2], and Ronald Olum[3]

[1]Uganda Martyrs University
[2]ST.FRANCIS HOSPITAL NSAMBYA
[3]Makerere University College of Health Sciences

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INTRODUCTION

Since its discovery by Robert Koch in 1882 (CDC, 1982), tuberculosis (TB) has and is still one of the leading causes of morbidity and mortality globally. In 2022 alone 10.6 million people were infected with TB and 1.3million died. It is the second leading infectious killer after COVID-19 (World Health Organization, 2023).

Tuberculous pleuritis or pleurisy (TBP) is the second most common form of extra-pulmonary tuberculosis. It affects the pleura, in both immunocompetent and immunocomprised persons (Cohen and Light, 2015) (Nanyoshi *et al.* , 2022) in as high as 25% of tuberculosis(TB) cases (Porcel, 2009). TBP usually presents as a unilateral pleural effusion, although about 10% of cases are bilateral (Wang *et al.* , 2015).

Effusions in TBP have long been postulated to be due to a delayed hypersensitivity reaction to mycobacterium antigens from raptured sub pleural caseous material; however recent advances have demonstrated a likelihood of paucibacillary bacterial infection within the pleural cavity (Morné J. Vorster, 2015).

In high TB endemic areas prevalence of TBP has been shown to be higher in young people (mean age 34) (Porcel, 2009) compared to elderly population(>65years) in low endemic areas (Baumann *et al.* , 2007). The most common symptoms for TBP are pleuritic chest pain which precedes a non-productive cough; then fevers, night sweats, weight loss and malaise (Wang *et al.* , 2015).

The gold standard for diagnosis of TBP is the demonstration of *Mycobacterium tuberculosis* in the pleural biopsy specimens, pleural fluid or sputum (Gopi *et al.* , 2007); the challenges to this include scarcity of thoracoscopy services, paucibacillary nature of effusion and lack of sputum (since the cough is usually non-productive). However, presumptive diagnosis can also be achieved with reasonable certainty, by showing parietal pleura granuloma (through biopsy and histology) or elevated levels of pleural fluid adenosine deaminase (ADA) or interferon-γ, considering the clinical context of the patient (Trajman *et al.* , 2008).

Since its discovery in 1978, adenosine deaminase(ADA) test on pleural fluid has become famous in diagnosis of TBP, more so in patients with exudative and lymphocytic pleural effusion in high TB endemic areas (Aggarwal *et al.* , 2019). The test is simple, affordable, rapid and minimally invasive (LIGHT, 2010). ADA levels greater or equal to 40 IU/L are associated with a sensitivity of 87.8 to 97.6% and specificity of 90.4 to 92.4 % (Huan *et al.* , 2021). However, in low and middle income settings the test hasn't been embraced because of laboratory inadequacies and lack of knowledge about it, and fear of false positives, majorly malignancy, empyema, Para-pneumonic, collagen diseases, (Valdés *et al.* , 1993) and rheumatoid pleuritis (Hooper, Lee and Maskell, 2010).

We present and discuss a case of TBP diagnosed by pleural ADA, in a 28-year-old woman who acquired symptoms during pregnancy through delivery. We also put forward the advantage and applicability of this test amidst lack of thoracoscopy services in Uganda and other low resource settings.

CASE PRESENTATION

History and Exam : We received a 28-year-old woman, no known chronic illnesses, HIV-seronegative, one month in puerperium having delivered by cesarean section, referred from the obstetrics unit because of persistent chest pain. The pain started when she was 7months pregnant, more on the right side, pleuritic in nature and had progressively increased over time and worsened after delivery. Post-delivery, she also started experiencing evening fevers, lost appetite, and got episodes of difficulty in breathing while lying down and/or sleeping. She had no cough. On examination she was in a fair general condition, not in respiratory distress but worried. Vital signs were: blood pressure 130/80mmHg, pulse 112 beats/minute, Temperature 37.8°C, peripheral saturation of oxygen(SpO2) 96%, respiratory rate 19breaths/minute. Significant findings on chest exam was stony dullness and reduced air entry in the right subscapular and basal lung zones**.**

Methods : A clinical diagnosis of right pleural effusion was made and patient sent for a chest x-ray (**figure1**) which confirmed an effusion. TB and malignancy were suspected as the cause of the effusion because of the long standing history and constitutional symptoms. Diagnostic thoracentesis was performed and fluid samples taken for ZN Stain, Gene expert, Gram-stain, Culture and cytology. We didn't do pleural biopsy due to lack of thoracoscopy. ZN Stain reported no acid fast bacilli, Gene expert reported no Mycobacterium tuberculosis detected and Gram-stain revealed no organisms. There was no growth on culture. Fluid cytology revealed scattered lymphocytes and polymorphs on a serous background(**figure2**), thus deducing as chronic lymphocytic pleuritis. There were no malignant cells, no TB on cytological exam. A second pleural fluid sample was taken off for ADA test, having seen the chronic lymphocytic picture of the first sample. ADA test result was 58.6 U/L (Biological Reference Interval 0-40). Based on these findings she was diagnosed with TBP five days after presentation and started on TB chemotherapy composed of rifampicin, isoniazid, ethambutol and pyrazinamide for 2months and Rifampicin and Isoniazid for 4months.

Outcome and follow-up: Treatment commenced on June 1st 2023 and completed on November 30th 2023 with 100% adherence as indicated on adherence monitoring tool (**figure3**). By two months of treatment (July 27th 2023), the effusion had reduced by almost 90%(**figure4**), and completely resolved by 1stDecember 2023 (**figure5**). She has since been fine, was last reviewed on 9th February 2024 with normal general and chest findings.

DISCUSSION

This was a case of TBP diagnosed by ADA, treated successfully with clinical and radiological improvement. The pointers to diagnosis were duration of symptoms, constitutional symptoms, pleuritic chest pain, age of the patient (Porcel, 2009) and presence of pleural effusion. Another pointer was cytological finding of chronic lymphocytic pleuritis (Cohen and Light, 2015). These features are key in the diagnosis of TBP and any laboratory finding must be interpreted in the context of the clinical picture of the patient (Trajman *et al.* , 2008) (Wang *et al.* , 2015).

ADA an enzyme produced by T-helper type1(TH-1) lymphocytes, involved in purine metabolism is significantly raised in patients with TBP(Aggarwal*et al.* , 2019).Other disease states (such as malignant lymphomas, other malignant effusions, autoimmune diseases, empyema, para-pneumonic effusions) can cause elevated ADA levels (Shimoda *et al.* , 2022) (LIGHT, 2010) (Liang *et al.* , 2008) (Valdes *et al.* , 1996). However, in a longitudinal comparative study by Shimoda et al.,(2022) ADA levels were highest in TBP and of all causes of raised ADA, TBP was the most common cause (Shimoda *et al.* , 2022).

ADA test is very resourceful in the diagnosis of TB due to the fact that most "preferred" microbiological methods for diagnosis of TBP are limited by the paucibacillary nature of the pleural fluid (Cohen and Light, 2015) and it being mostly a hypersensitivity reaction (Morné J. Vorster, 2015). In low resource settings with scarcity of thoracoscopy/pleuroscopy services, pleural biopsies and histology are rarely done. The latter has

demonstrated close to 100% sensitivity of the thoracoscopically obtained biopsies for histology and AFB stain (Diacon *et al.* , 2003) compared to 10% for pleural fluid AFB stain, about 45% for fluid MTB culture, and 38 to 75% for fluid Xpert ultra PCR (Lo Cascio *et al.* , 2021). The sensitivity of Abrams needle biopsies for histology and culture is estimated between 40 to 70% (Lo Cascio *et al.* , 2021).

No wonder, for our case pleural fluid ZN stain, Xpert ultra PCR, and culture were all negative for MTB. It is even more difficult to isolate the MTB in the fluid in HIV negative persons due to a robust immunity (lymphocytes, polymorphs and macrophage-monocyte system) which clear the organisms from the pleura (LUZZE, 2001).We didn't do pleural biopsy due to lack of thoracoscopy/pleuroscopy and Abrams needle. Thus pleural fluid ADA test was instrumental in the diagnosis of TBP putting in context the clinical presentation of the patient and chronic lymphocytosis on fluid cytology. No comparison case studies/reports have been published in Uganda. Other causes of a raised ADA notwithstanding, the diagnostic accuracy, sensitivity and specificity of pleural fluid ADA test have been studied and recommended as sufficient (Nanyoshi*et al.* , 2022) (Lo Cascio *et al.* , 2021) (Hooper, Lee and Maskell, 2010) (Liang *et al.* , 2008) (LIGHT, 2010) (Diacon*et al.* , 2003).

Conclusion and recommendation : As has been shown in this case study and discussion plus previous literature, pleural fluid ADA test should be seriously considered and is highly recommended in the diagnostic pathway of TBP putting the clinical presentation and fluid lymphocytosis at the fore front. More so, in resource limited settings with no thoracoscopy/pleuroscopy and no equipment for closed pleural biopsy. This solves the TBP diagnostic delay, reducing the risk for complications and mortality.

CONFLICT OF INTEREST STATEMENT: Authors have no conflict of interest to declare.

ETHICS STATEMENT: All information displayed in this report was obtained as part of routine patient care. No ethical approval was required.

CONSENT STATEMENT: Written informed consent was obtained from the patient to publish this report in accordance with the journal's patient consent policy.

DATA AVAILABILITY STATEMENT: The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

Aggarwal, A.N. *et al.* (2019) 'Adenosine deaminase for diagnosis of tuberculous pleural effusion: A systematic review and meta-analysis',*PLOS ONE* . Edited by G.A. Eapen, 14(3), p. e0213728. doi:10.1371/journal.pone.0213728.

Baumann, M.H. *et al.* (2007) 'Pleural Tuberculosis in the United States', *Chest* , 131(4), pp. 1125–1132. doi:10.1378/chest.06-2352.

Lo Cascio, C.M. *et al.* (2021) 'Diagnosis of tuberculous pleural effusions: A review', *Respiratory Medicine* , 188, p. 106607. doi:10.1016/j.rmed.2021.106607.

CDC (1982) *Historical Perspectives Centennial: Koch's Discovery of the Tubercle Bacillus* , *MMWR* . Available at: https://www.cdc.gov/mmwr/preview/mmwrhtml/00000222.htm#:˜:text=of e-mail.-,Historical Perspectives Centennial%3A Koch's Discovery of the Tubercle Bacillus,discovered the cause of tuberculosis. (Accessed: 7 February 2024).

Cohen, L.A. and Light, R.W. (2015) 'Tuberculous Pleural Effusion',*Turkish Thoracic Journal/Türk Toraks Dergisi* , 16(1), pp. 1–9. doi:10.5152/ttd.2014.001.

Diacon, A.H. *et al.* (2003) 'Diagnostic tools in tuberculous pleurisy: a direct comparative study: 1', *European Respiratory Journal* , 22(4), pp. 589–591. doi:10.1183/09031936.03.00017103a.

Gopi, A. *et al.* (2007) 'Diagnosis and Treatment of Tuberculous Pleural Effusion in 2006', *Chest* , 131(3), pp. 880–889. doi:10.1378/chest.06-2063.

Hooper, C., Lee, Y.C.G. and Maskell, N. (2010) 'Investigation of a unilateral pleural effusion in adults: British Thoracic Society pleural disease guideline 2010', *Thorax* , 65(Suppl 2), pp. ii4–ii17. doi:10.1136/thx.2010.136978.

Huan, N.-C. *et al.* (2021) 'Optimising the utility of pleural fluid adenosine deaminase for the diagnosis of tuberculous pleural effusion', *Proceedings of Singapore Healthcare* , 30(4), pp. 271–278. doi:10.1177/2010105820978998.

Liang, Q.-L. *et al.* (2008) 'Diagnostic accuracy of adenosine deaminase in tuberculous pleurisy: A meta-analysis', *Respiratory Medicine* , 102(5), pp. 744–754. doi:10.1016/j.rmed.2007.12.007.

LIGHT, R.W. (2010) 'Update on tuberculous pleural effusion',*Respirology* , 15(3), pp. 451–458. doi:10.1111/j.1440-1843.2010.01723.x.

LUZZE, H. (2001) 'Evaluation of suspected tuberculous pleurisy: clinical and diagnostic findings in HIV-1-positive and HIV-negative adults in Uganda', *International Journal of Tuberculosis and Lung Disease* , 5(8). Available at: http://hdl.handle.net/10570/1067.

Morné J. Vorster (2015) 'Tuberculous pleural effusions: advances and controversies', *JTD* , 7. doi:10.3978/j.issn.2072-1439.2015.02.18.

Nanyoshi, M. *et al.* (2022) 'Tuberculous Pleurisy Diagnosed From Massive Pleural Effusion in an Older Patient With No History of Tuberculosis', *Cureus* [Preprint]. doi:10.7759/cureus.32333.

Porcel, J.M. (2009) 'Tuberculous Pleural Effusion', *Lung* , 187(5), pp. 263–270. doi:10.1007/s00408-009-9165-3.

Shimoda, M. *et al.* (2022) 'Characteristics of pleural effusion with a high adenosine deaminase level: a case–control study', *BMC Pulmonary Medicine* , 22(1), p. 359. doi:10.1186/s12890-022-02150-4.

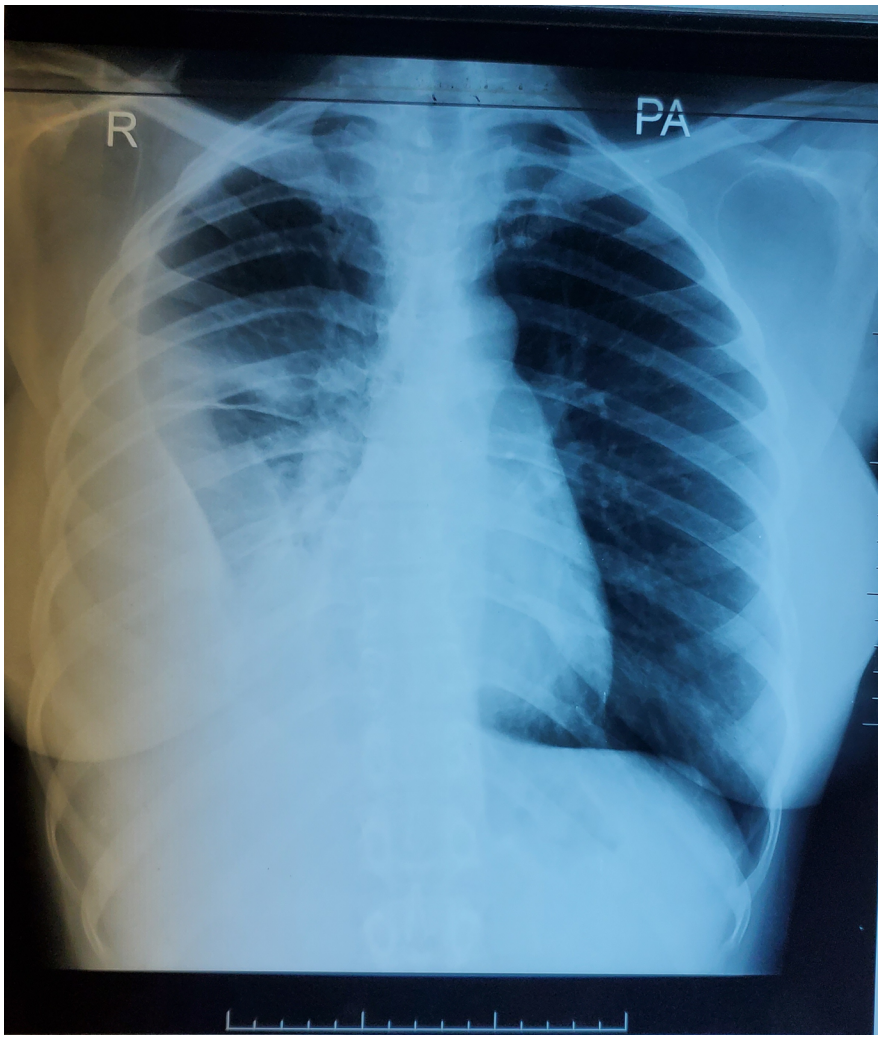
Trajman, A. *et al.* (2008) 'Novel tests for diagnosing tuberculous pleural effusion: what works and what does not?', *European Respiratory Journal* , 31(5), pp. 1098–1106. doi:10.1183/09031936.00147507.

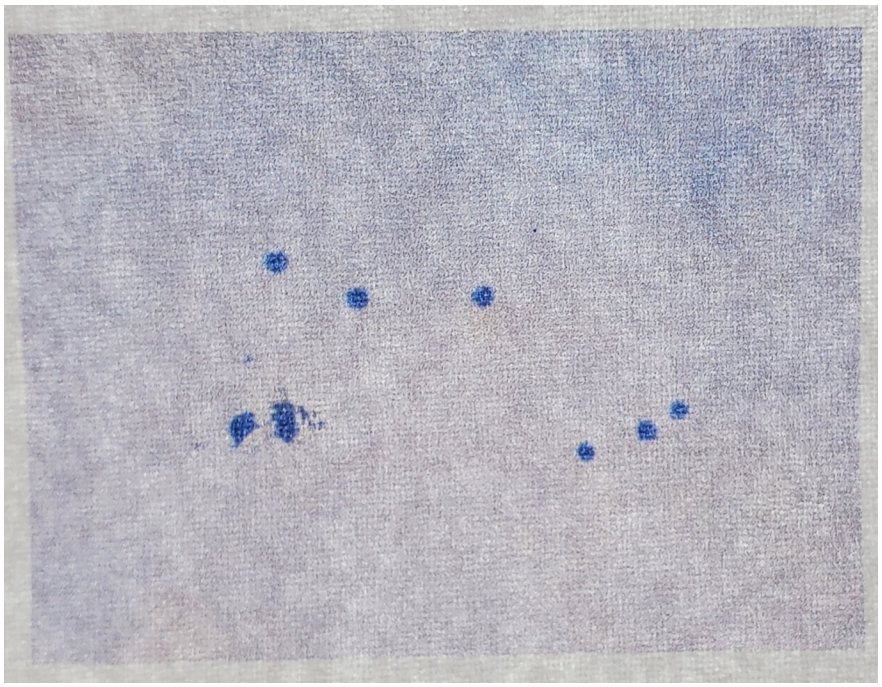
Valdes, L. *et al.* (1996) 'Adenosine deaminase (ADA) isoenzyme analysis in pleural effusions: diagnostic role, and relevance to the origin of increased ADA in tuberculous pleurisy', *European Respiratory Journal* , 9(4), pp. 747–751. doi:10.1183/09031936.96.09040747.

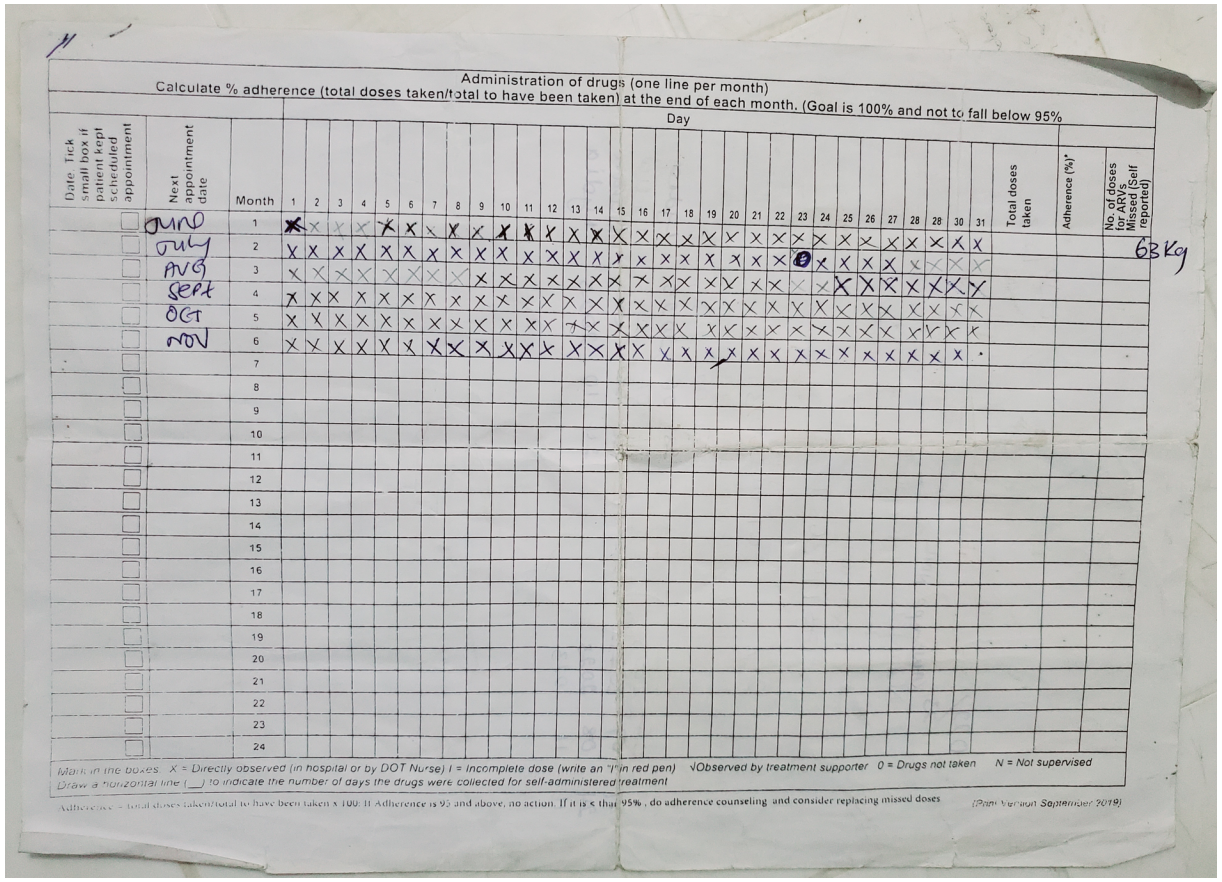
Valdés, L. *et al.* (1993) 'Diagnosis of Tuberculous Pleurisy Using the Biologic Parameters Adenosine Deaminase, Lysozyme, and Interferon Gamma', *Chest* , 103(2), pp. 458–465. doi:10.1378/chest.103.2.458.

Wang, Z. *et al.* (2015) 'Diagnostic value and safety of medical thoracoscopy in tuberculous pleural effusion', *Respiratory Medicine* , 109(9), pp. 1188–1192. doi:10.1016/j.rmed.2015.06.008.

World Health Organization (2023) *Tuberculosis* , *Fact sheets* . Available at: https://www.who.int/news-room/fact-sheets/detail/tuberculosis#:~:text=Globally in 2022%2C there were,and 0.37 million to diabetes. (Accessed: 7 February 2024).







Administration of drugs (one line per month)

Calculate % adherence (total doses taken/total to have been taken) at the end of each month. (Goal is 100% and not to fall below 95%

Date. Tick small box if patient kept scheduled appointment	Next appointment date	Month	Day 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	28	30	31	Total doses taken	Adherence (%)*	No. of doses for ARVs Missed (Self reported)
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Mark in the boxes: X = Directly observed (in hospital or by DOT Nurse) I = Incomplete dose (write an "I" in red pen) √Observed by treatment supporter 0 = Drugs not taken N = Not supervised

Draw a horizontal line (__) to indicate the number of days the drugs were collected for self-administered treatment

Adherence = total doses taken/total to have been taken x 100. If Adherence is 95 and above, no action. If it is < than 95% , do adherence counseling and consider replacing missed doses

(Print Version September 2019)

68 Kg