UNIVERSITY OF RWANDA

College of Medicine and Health Sciences
School of Medicin and Pharmacy

USE OF ADENOSINE DEAMINASE AS BIOMARKER IN DIAGNOSIS OF SUSPECTED TUBERCULOUS PLEURALEFFUSION AT TERTIARY REFERRAL HOSPITALS IN RWANDA

A Post Graduate Research Dissertation submitted to the College of Medicine and Health Sciences, School of Medicine and Pharmacy in Full Fulfillment of the Requirements for the Award of a Masters of Medicine in Internal Medicine of University of Rwanda

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DECLARATION

I, BARANSABIRAMarie Goretti, declare that this work entitled "Use of Adenosine Deaminase as biomarker in diagnosis of suspected tuberculous pleural effusion at tertiary referral hospitals in RWANDA" is my own work. Full acknowledgement is given where assistance has been sought most especially from my supervisors or where other views are quoted. It is a requirement by the College of Medicine and Health Sciences of University of Rwanda in partial fulfillment of the academic requirements for award of Master of Medicine Degree.

Signature .....

Approved for submission by

SUPERVISORS:

Dr CAMERON PAGE

……..Date……...

Dr DUSABEJAMBOVincent

..... Date.....

DrBITUNGUHARI Léopold

..... Date.....

DEDICATION

I dedicate this work to the following:

To my parent NYIRABAZIGAMartina

To my husband MILINDIAntoine

To my sisters

To my mentors

To my colleagues

ACKNOWLEDGEMENT

I thank all patients who accepted to participate in this study. I am grateful to the supervisors of this work, Assistant Prof CAMERON PAGE, Dr DUSABEJAMBOVincent and Dr BITUNGUHARI Leopold.

The great generous contribution and advice during this work are admirable. Their remarks and especially their scientific rigor have been of great importance to realize this work .This also goes to my training at University of Rwanda, all staff of College of Medicine and Health Sciences, and my treating team who cared of me at different hospitals. It would not end without thanking my family and all medical residents for their support and endless encouragement.

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LIST OF ABBREVIATIONS AND ACRONYMS

ADA	:Adenosine Deaminase
TB	:Tuberculosis
TPE	:Tuberculous Pleural Effusion
M.Tuberculosis	:Mycobacterium Tuberculosis
MDR	: Multi Drug Resistance
HIV	:Human Immunodeficiency Virus
WHO	:World Health Organization
IFN-g	: Interferon-gamma
BUTH	: Butare University Teaching Hospital
CHUB	: Centre HospitalierUniversitaire de Butare
CMHS	: College of Medicine and Health Science
ICU	: Intensive Care Unity
KUTH	: Kigali University Teaching Hospital
CHUK	: Centre Hospitalier Universitaire de Kigali
NPV	: Negative Predictive Value
PE	: Pulmonary Embolism
PPV	: Positive Predictive Value
PTP	: Pre-Test Probability
UR	: University of Rwanda

ABSTRACT

Background: Pleural effusion is a common problem and a number of diseases can cause it. The ineffectiveness of tests and procedures toconfirm pleural fluid etiologycan delay treatment. There is therefore a need for a sensitive and specific test that can differentiate tuberculosis from other causes of pleural effusion. Previous studies have shown that Adenosine Deaminase(ADA) is an accurate test forthe diagnosis of pleural TB, with sensitivity and specificity of up to 100%.[1][2]The use of Adenosine Deaminase as biomarker, with other parameters and tests were helpful in the diagnosis of tuberculous pleurisy.

Methods: Study participants were recruited through the Internal Medicine and Accident& Emergency Departments of the two tertiary referral hospitals in Rwanda (CHUK and CHUB). We included patients older than 16 years with pleural effusion who agreed to participate in the study. Patients with pleural effusion of a known cause, which had been previously diagnosed, and who came for pleural fluid evacuation to improve respiratory symptoms, were excluded.

Objective: This study tries to see the prevalence of pleural Tuberculosis base on use of AdenosisneDeaminase

Results: The majority of the study participants (N=90) were young (≤55years) at prevalence of 71.1%. Sex ratio (M: F) = 1: 0.83. Among 90 pleural fluid samples studied 58 (64.4%) were analyzed for Light's criteria and 44 (75.9%) were consistent with the exudative nature of disease, among them 34.1 % (15) had ADA >30 U/L. TB pleural effusion was diagnosed at prevalence of 32.2% (29) by ADA level >30U/l among 90 study participants.

Conclusions: In the area with high prevalence of Tuberculosis, the use of Adenosine Deaminase may help for diagnosis of pleural Tuberculosis with other causes of pleural effusion.

CHAPTER I.INTRODUCTION

1.1. Background

The tuberculosis is the one of oldest diseases in the world. It is caused by *Mycobacterium tuberculosis*. All organs of the human body can be involved, and the lungs are involved in up to 1/3 of cases. Tuberculous pleural effusion accounts for approximately 5% of tubercular disease[3].All humans are at risk for tuberculosis, but particular those who live in endemic regions.

In 2014-2015, according to WHO report, about 9.6 million people contracted tuberculosis, including 1 million children. In that year, one and a half million people died from the disease, among them 140,000 children. 95% of tuberculosis deaths occur in low- and middle-income countries, of which Rwanda is one. In 2014, the WHO reported that 1 in 3 deaths were due to tuberculosis, and 480,000 people developed multi drug resistant tuberculosis. However, the prevalence of tuberculosis is different in different countries.[4]

The TB program in Rwanda reported 5,828 cases of TB in 2015 (a positivity rate of 2.1%) and pulmonary localization represented 84.0%.[5] In 2016, the WHO Global Fund for TB reported that TB incidence for Rwanda was 56/100,000 people, and the mortality rate was 3.8/100,000.[4]

Due to the HIV pandemic, the incidence of TB pleural effusion in HIV/AIDS has been variably reported from 15-90%[6].Tuberculosis is a leading killer of HIV positive people. Tuberculous pleurisy is the second most common form of extra pulmonary TB after lymphatic involvement and the diagnosis remains a big problem.[7][8][9]**.**One study done by Dr BATUNGWNAYO J. using thoracentesis and pleural biopsy, 86% was diagnosed of pleural tuberculosis. Recent study of 2014, pleural Tuberculosis accounted 82% of all pleural effusion in Rwanda and 91% of pleural effusion is an exudate in Uganda[10]

Pleural fluid which is initially transudate, become later an exudates with predominant lymphocytes. The definitive diagnosis of TB pleural effusion is either M.tuberculosis in sputum,

pleural fluid (less 10% positive), pleural culture (sensitivity range from 12-70% ,the majority is less 30%) or pleural biopsy specimens(40-80%)[7][8][10][11][12]

Some biochemical markers are used for diagnosis of pleural Tuberculosis includingAdenosine Deaminase(ADA) most cost- effective, interferon gamma, lysozyme. In some countries where tuberculosis is endemic, ADA is routinely employed as a screening tool [12].
Measurement of ADA and interferon –gamma in the pleural fluid and polymerase chain reaction for M.Tuberculosis has gained wide acceptance in diagnosis of TB pleural effusion[7].

Many studies showed that ADA level more 70IU/L highly suggested TB while a level less 40IU/L virtually excluded the diagnosis. It was shown in a metanalysis of 40studies published from 1966 to 1999 which concluded on the test performance of ADA with sensitivity 95% and specificity 90% in diagnosis TB pleural effusion [7] .The accepted cut-off level of ADA for the diagnosis of TPE was 40 U /L[7][11][12]][13].

Different cut off values have been showed in different studies with different sensitivityand specificity from 30-100UI/L[2].Japanese patients withtuberculous pleural effusionhada lowerADA level,as itwasin India,where the ADA levelwas 28.8±7.8UI/L in tuberculous pleural effusions.[14][15]

Another study done in Dhaka in 2013 showed that ADA had 94% sensitivity and 88% specificity when the cut off value for diagnosis of TPE was set at 40 U/L.[3] Using ADA is most helpful in avoiding pleural biopsy among young patients who come from a region with high TB prevalence. The Dhaka study also found that despite the high sensitivity and specificity, ADA levels more than 40U/L were found in 12.2% of non-tuberculosis patients, and levels remained below 40U/L in 6.5% of tuberculosis patients.[3]

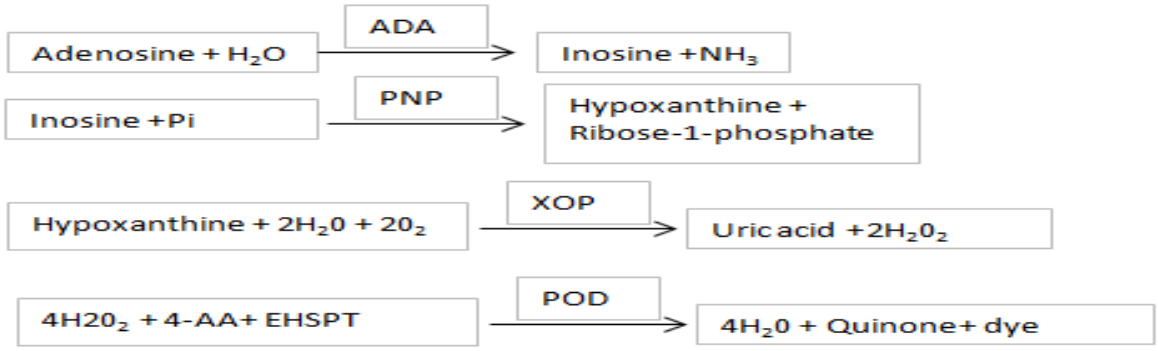
1.2. Adenosine Deaminase (ADA)

- **Definition, Pathophysiology.**

ADA is a protein produced by the cells of the body, and is associated with activation of white blood cells, especially lymphocyteswhich are in the process of proliferation and differentiation. [14] ADA plays a role in the immune response to infection, and is stimulated by the presence of *Mycobacterium tuberculosis*. There are2 isoenzymes, ADA1 and ADA2. Activity is highest in lymphoid tissues, where the isoenzymeADA2 is most active in TPE.[2][14] Testing for the presence of ADA in pleural fluid, in conjunction with other tests, helps make a diagnosis of pleural tuberculosis infection.[9] The ADA test may be positive even when the number of mycobacterium is very low in pleural fluid.[16]

ADA is an enzyme that catalyzesinosine from adenosine. In serum, this enzyme can be increased in a variety of situations, such as acute hepatitis, alcoholic hepatic fibrosis, chronic active hepatitis, and viral hepatitis. [17][18]

Figure 1: The summary of the enzymatic reaction of adenosine deaminase[19]



(λ max 556nm)

purine nucleoside phosphorylase(PNP). Hydrogen peroxide (H2O2), xanthine oxidase (XOD). N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (EHSPT), 4-aminoantipyrine (4-AA), peroxidase (POD)

One unit of ADA = the amount of ADA that generates one µmole of inosineper min at 37°C.

1.3. Clinical Presentation in pleural tuberculosis:

Symptoms most commonly associated with pleural TB are dry or productive cough (80%), pleuriticchest pain (75%), weight loss(< 75%),fatigue (70%),fever (60%),night sweats (55%),hemoptysis (35%), or no symptoms (20%)[7].

In addition to the clinical presentation, the authors proposed different models including ADA, globulins and the absence of malignant cells in the pleural effusion; or ADA, globulins and fluid appearance for the diagnosis of pleural effusions secondary to tuberculosis [20]

The physical findings are asymmetry expansion of the chest (74%), decreased vocal resonance (74%), reduced vocal fremitus (81%), decreased or absent breath sounds (88%),

dull percussion (90%), absent crackles(56%),pleural friction rub(5%), auscultatory

percussion(58%).Only asymmetric chest expansion and dull percussion have sensitivity and specificity up to 95%.[21]

CHAPTER II.MATERIALS AND METHODS

2.1.Objectives

2.1.1.General objective

The general objective of the study was to determine the added value of AdenosineDeaminase in suspected pleural tuberculosis at the two teaching hospitals [CHUB/CHUK] in Rwanda.

2.1.2. Specific objectives

The specific objectives of the study were:

- ✓ To compare ADA level in different diseases giving pleural effusion at tertiary referral hospitals in Rwanda.
- ✓ To compare ADA with other tests used and affordable for TB pleurisy's diagnosis in Rwanda (Microscopy, staining, chemistry and Expert).
- ✓ Compare ADA with the clinical presentation of pleural tuberculosis

2.2. Study design

It is a cross-sectional, descriptivestudy.

2.3. Site description

The research was conducted at CHUK (Centre Hospitalier-Universitaire de Kigali) and CHUB (Centre Hospitalier-Universitaire de Butare), which are referral and university teaching hospitals, and they are the main public health institutions in Rwanda. CHUK is a 524-bed hospital located in Kigali, the capital city of Rwanda. 70 participants were enrolled at CHUK during study period. CHUB is a 372-bed hospital located in Southern province of Rwanda, in the second-largest city in Rwanda. In six months, 20 participants were enrolled at CHUB. These hospitals were chosen because of the existing availability of TB diagnostic tools, such GenXpert, TBculture, and pleural fluid analysis of LDH and protein.

2.4. Study population

The study population was patients referred to or consultingatthe teaching hospitals with clinical features of tuberculous pleurisy. Those features included one or more of the following symptoms: pleuritic chest pain, non-productive cough, fever, dyspnea, fatigue, night sweats, weight loss, tachycardia, and lymphadenopathy. The presence of pleural effusion was confirmed clinically or with imaging. Some participants had been started on TB treatment prior to consulting at the referral hospitals, and others were started on TB treatment during the period of follow up at the referral hospital. All of these patients were eligible for inclusion in our study. Patients were recruited into the study at the Accident and Emergency Department or the Internal Medicine Department. The physician who received the patient informed the study investigatoras recruitment of participants occurred.

2.5. Inclusion criteria

- ✓ Age 16 years and above
- ✓ All patients with pleural effusion who came not for palliative pleural fluid evacuation
- ✓ All patients who agreed to participate in this study

2.6. Exclusion criteria

- ✓ Under 16years
- ✓ Patients with a known diagnosis who came for palliative pleural fluid evacuation
- ✓ Patients with post-traumatic pleural effusion
- ✓ Patients who refused to sign the consent form for participation in this study

2.7. Sample size

Sample size was calculated based on the following known data about TB prevalence:

About 1/3 of the world population has latent tuberculosis, and it is estimated that 10-25% of TB infections occur outside the pulmonary parenchyma. Tuberculous pleural effusion represents about 5% of all patients with *Mycobacterium tuberculosis.*[7].The incidence rate of tuberculosis

in Rwanda is 56/100,000.

Using this information, we calculated that we require a sample size (N) of

$N = Z^2 \times P \times Q / D^2$ N= Sample size

Prevalence (P) of pleural TB=5% Z=1.96

Q=1-P D = precision (0.05) with 95%CI

N = 72

We added 1/3 to the total number, to give an estimated sample size of 96 cases

2.8. Data collection and analysis

After the diagnosis of pleural effusion was made, the study was explained to each subject who was eligible. The subject was given the consent form, and they signed it if they agreed to participate. The thoracentesis was performed, and the pleural fluid sample was taken to the laboratory.

Demographic information and past medical history was obtained from patient interview. Physical findings were obtained by physical exam by the study investigator as well as the medical record. Imaging and laboratory data was obtained from the medical chart.

The study investigator gave no input or influence to the primary medical team caring for the patient. Two registers were kept, one containing the name of the patient, and another containing the patient's registration number. One register for the laboratory was kept by the technician, who recorded each new participant sample. The study investigator kept soft and hard copies of all the results.

To test for the level of ADA, we used the Adenosine Deaminase test kit DZ117-A-K Dual Vial Liquid Stable Format (Diazyme Corporation, Poway, CA, USA). The ADA kit was stored in the refrigerator at 2 to 8 degrees Celsius, until the day of pleural effusion analysis. All samples were stored in freezer at -80 degree Celsius until the day of analysis.

Analysis was performed at CHUK, and samples collected at CHUB were transported to CHUK one day before analysis. Results were written in both the register, where there was a patient ID number, and also written on the paper questionnaire in the data collection file.

Descriptive statistics such as frequencies were used for different variables. Frequencies and percentages were calculated for categorical variables. Distribution values of ADA level in all pleural fluid were measured and reported from the laboratory.

Basic characteristics with ADA levels at cut-off value were used to evaluate the influence. A p-value of < 0.05 was considered as evidence of a statistical significant difference.

Microsoft Excel was used to store the results. Data analysis was performed using SPSS version 16.

2.9. Quality control issues of the reagent

We used the Adenosine Deaminase test kit DZ117-A-K Dual Vial Liquid Stable Format (Diazyme Corporation, Poway, CA, USA). Reagents were conserved and stored at 2-8°C until their expiration date. For this test kit, either the manual or automated methodcould be used, or 5micro liters of the sample was needed. [18][22]

Table 1: Reference Range: The ADA levels

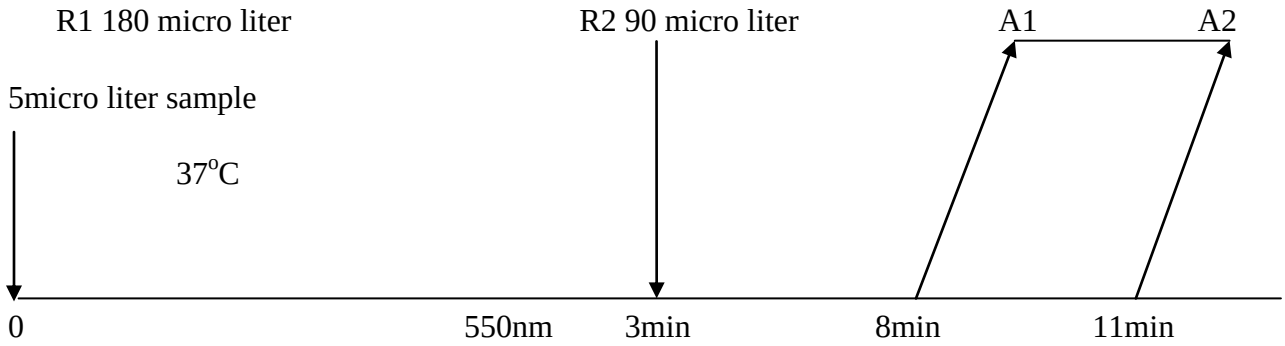
Reference Range: The ADA levels
Human serum: 0-15 U/L.
Pleural fluid: 0-24 U/L
C.S.F.:0-5 U/L.

It is recommended that each laboratory establish its own range of reference values.[19][22]

According to the Adenosine Deaminasekitwe used for our study, the reference range was 0-30 U/L.

For our study, we used manual technique with machine visualization. The ADA control and calibrator provided by the manufacturer were used for quality control before processing the samples.

Figure 2: Test Scheme for Chemistry Analyzers[18]



The test kit had an assay range from 0-200U/L. Manufacturer recommendations were to use sterile or heparinized tubes for pleural fluid collection,and to process samples within 2 hours at room temperature, or store samples at 4^0c. It is also acceptable to store samples over a long period of time, they can be stored at -20^0C for 2 days, orat -80^0C for up to 2.5years.[18][19]

Trained laboratory technicians using visual machine measured ADA levels. An independent statistician entered the data after ADA results were obtained and performed the data analysis.

2.10. Ethical considerations

The College of Medicine and Health Sciences (CMHS) Institutional Review Board (IRB), University of Rwanda approved this study as well as CHUB and CHUK scientific committees. All of the participants provided written consent to participate to the study, after explanation of the risks and benefits. For patients unable to provide written consent due to their medical condition or altered mental status, direct family members signed the consent form on their behalf. For participants under 20 years, the consent form was signed by the subject as well as

one of their family members. The participants were informed about their right to withdraw consent and exit the study at any time, without any repercussion to them. Data collection forms were kept under conditions of strict confidentiality.

CHAPTER III.RESULTS

From September 2016 to February 2017, 90 patients were enrolled who met inclusion criteria as described above. Informed consentwas obtained from all subjects. The great majority (71.1%) of patients were aged under55 years. Gender was evenly balanced, with 45.6% female and 54.4% male.

Table 2: Socio-demographic Characteristics of the Study Population

Socio demographic characteristic	Frequency [n=90]	Percent (%)
Age		
≤ 55	64	71.1
> 55	26	28.9
Minimum: 16		
Maximum: 87		
Gender		
Female	41	45.6
Male	49	54.4

N=total participants

Table 3: ADA Characteristics (U/L)

Adenosine Deaminase	Frequency	Percent (%)
≤30 U/L	61	67.8
> 30 U/L	29	32.2
Mean	27	
Std. Deviation	24	
Minimum	1	
Maximum	139	

Consistent with the manufacturer's recommendations for this assay, we divided the subjects into two groups based on the reference range (Adenosine Deaminase: ≤ 30U/L or >30U/L). The above table shows that ADA diagnostic level> 30U/L considered as pleural tuberculosis was found in 32.2% of study participants.

Table 4: Correlation between Clinical Features and ADA Level

Characteristic	ADA level		P-Value
	≤30 U/L	> 30 U/L	
Cough			
No	12 [85.7%]	2 [14.3%]	0.131
Yes	49 [65.3%]	26 [34.7%]	
Pleuritic chest pain			
No	16 [59.3%]	11 [40.7%]	0.213
Yes	45 [72.6%]	17 [27.4%]	
Fever			
No	20 [60.6%]	13 [39.4%]	0.267
Yes	41 [71.9%]	16 [28.1%]	
Dyspnea			
No	12 [57.1%]	9 [42.9%]	0.198
Yes	49 [72.1%]	19 [27.9%]	
Night sweat			
No	39 [67.2%]	19 [32.8%]	0.883
Yes	22 [68.8%]	10 [31.3%]	
Weight loss			
No	22 [59.5%]	15 [40.5%]	0.119
Yes	39 [75.0%]	13 [25.0%]	
Ascitis			
No	39 [67.2%]	19 [32.8%]	0.883
Yes	22 [68.8%]	10 [31.3%]	
LL edema			
No	41 [70.7%]	17 [29.3%]	0.426
Yes	20 [62.5%]	12 [37.5%]	

The Table above (Table 4) showsthat allpatients in this study had similar clinical features. We did not find that patients with high ADA had significantly different clinical presentation than patients with low ADA. The data analysis didn't show any statistically significant difference between the predominant symptoms of pleural effusion and the pleural fluid ADA level >30u/L (P-value>0.1)

Table 5: Relationship between Pleural Effusion Analysis and ADA Level

Laboratory characteristics	ADA level		P-Value
	≤ 30 U/L	> 30 U/L	
Pleural effusion WBC			
< 5000	34 [72.3%]	13 [27.7%]	0.889
≥ 5000	17 [73.3%]	6 [26.1%]	
Pleural effusion neutrophil			
< 5 %	14 [70.0%]	6 [30.0%]	0.713
≥5%	32 [74.4%]	11 [25.6%]	
Pleural effusion lymphocyte			
< 5 %	2 [66.7%]	1 [33.3%]	0.813
≥ 5%	43 [72.9%]	16 [27.1%]	
Light's criteria			
Transudate	11 [78.6%]	3 [21.4%]	0.372
Exudate	29 [65.9%]	15 [34.1%]	
GenExpert			
Negative	53 [67.1%]	26 [32.9%]	0.229
Positive	3 [100%]	0 [0%]	
Pleural effusion culture [ordinary]			
No growth	52 [67.5%]	25 [32.5%]	0.186
Growth	8 [88.9%]	1 [11.1%]	

As Table 5 above shows, we found no association between standard pleural effusion analysis and ADA level. The relationship between all types of pleural effusion analysis (cytology, macroscopic, GeneXpert, chemistry) and ADA level was not statistically significant.

Among the subjects who presented with exudate75.9 %(44), 34.1 % (15) also had ADA > 30U/L.Usingour standard methods without ADA, this group is generally treated for TB, so the presence of ADA would probably not change their treatment. 65.9% (29) had an exudative pleural effusion but were also found to have an ADA<30U/L. This group is often treated for TB, so in these cases the ADA is very useful, in helping not to give patients unnecessary TB treatment.

Among the patients who presented with transudate, 21.4 % (3) had a transudative effusion with an ADA level > 30. These patients would not generally be treated for TB, but because of their ADA level above 30U/L they could receive anti-TB treatment. In these cases, the ADA could be

very important to make the correct diagnosis of TPE. 78.6% (11) subjects had a transudative effusion with a low ADA < 30.

Of those 79 patients who were GeneXpert negative, 26 of them (32.9%)were found to have ADA > 30U/L. This group needs gold standard test to exclude tuberculosis and should have close follow up.

Table 6: Correlation between ADA Values in different diseases that give with pleural effusion in study participants

Charactestic	ADA Level		P-Value
	≤ 30 U/L	> 30 U/L	
Heart failure			
No	46 [71.9%]	18 [28.1%]	0.278
Yes	15 [60.0%]	10 [40.0%]	
Nephrotic syndrome			
No	60 [68.2%]	28 [31.8%]	0.495
Yes	1 [100%]	0 [0%]	
Renal disease			
No	49 [71.0%]	20 [29.0%]	0.233
Yes	12 [57.1%]	9 [42.9%]	
Cirrhosis			
No	55 [68.8%]	25 [31.3%]	0.576
Yes	6 [60%]	4 [40%]	
Acute hepatitis			
No	60 [68.2%]	28 [31.8%]	0.495
Yes	1 [100%]	0 [0%]	
Malignancy			
No	58 [67.4%]	28 [32.6%]	0.232
Yes	3 [100%]	0 [0%]	
Pulmonary embolism			
No	61 [69.3%]	27 [30.7%]	0.137
Yes	0[0%]	1 [100%]	
DM			
No	55 [70.5%]	23 [29.5%]	0.285
Yes	6 [54,5%]	5 [45.5%]	
HIV			
No	53 [67.1%]	26 [32.9%]	0.401
Yes	8 [80%]	2 [20%]	

The frequency of chronic disease was high: heart failure 27.8%, cirrhosis 11.1%, HIV 11.1%, renal failure 22.2%

No statistically significant difference was found between ADA levels in different diseases causing pleural effusions for study population.

CHAPTER IV.DISCUSSION

To our knowledge, this is the first study ever conducted in Rwanda using Adenosine Deaminase for the diagnosis of pleural tuberculosis. This research provides data on use of ADA as biomarker in diagnosis of suspected tuberculous pleural effusion at tertiary referral hospitals in Rwanda.

Tuberculous pleural effusion is a common and difficult problem in clinics and hospitals in Rwanda. Many times, reliable tests for making the diagnosis are missing, which delays proper treatment. On the other hand, the empirical strategy might lead to unnecessary treatment, which has heavy, long-term consequences, and also sometimes serious adverse events.

Using standard methods without ADA to diagnose pleural TB is often inaccurate. Acid-fast staining is less than 20% sensitive; GeneXpert is 22% sensitive; pleural fluid culture takes a long time (2-8weeks) with a sensitivity of less than 70%; interferon gamma levels and polymerase chain reaction are useful but expensive; pleural tissue biopsy is expensive and an invasive procedure that carries risks for the patient. Many previous studies have shown that increased level of ADA, combined with other investigations, is helpful to make a diagnosis of pleural tuberculosis[12][20][23]ADA is a rapid, safe, noninvasive and cheap test with high sensitivity and specificity.[23]

In our study, themajority of patients(71.1 %) were young, less than 55 yearsold. This is similar to the study done by J.Jasani, S.Mavadia et al where the study population was aged 18-50 years old. [24]As far as gender is concerned, our findings are similar to those ofJ.Jasani, S.Mavadia et al with sex ratio (M:F) 1:0.8 and 1:0.7 respectively [24]

The clinical features of patients in our study were similar to those found in previous research on ADA.About 84.3 % of subjects had cough;69.7% had pleuritic chest pain; 63.3%had fever;and 76.4% had dyspnea. A study done by ArunGopi et al in 2006foundpleuritic chest pain in 75% and nonproductive cough in 70%.[7] Patient may not have the all symptoms, but the suspicion of Tuberculosis pleurisy remains high which ADA level could be an added value for diagnosis.

Among clinical characteristics and ADA(Table 4) there was no statistically significant difference seen. This is important, because it means that there is no way of differentiating between tuberculous and non-tuberculous effusions using only clinical characteristics. Despite the different causes of pleural effusion, signs and symptoms of underlying infectious or inflammatory process are the same. The patients with ADA level above the cut off value need more tests, such as the gold standard of pleural biopsy and pleural fluid culture for exclusion of pleural tuberculosis.

The ADA, therefore, is a very valuable test, because it gives us information that cannot be found using our existing tools.

The same is true for our findings using standard pleural fluid analysis (Table 5). We found no statistically significant differences between low ADA and high ADA based on standard pleural fluid analysis. Again, this proves that the ADA test is important, because we cannot substitute any other investigation in the place of ADA. The ADA test gives us information about the pleural fluid that cannot be obtained using any of the tools that are currently available in Rwanda.

Forty-four patients were found to have an exudative effusion, 75.9% of total enrolled subjects. But only 15(34.1%) of these patients with exudates had ADA >30 U/L to confirm Tuberculosis. In other words, the other 29 patients with ADA < 30U/L (65.9% of patients who had exudates) may have beenreceiving unnecessary treatment.

It was shown in a study done by B.K.Gupta in 2013 that, in both tuberculousand non-tuberculous pleural exudates, the level of ADA can vary below or above the cut off value.[1]In our study, we had 27.1% exudates with lymphocytes predominance and also 25.6% of ADA level > 30U/L exudates fluid with neutrophils predominance. Our results were consistent with a study byHayoung Choi et al in South Korea on pleural tuberculosis withneutrophilic predominance.[25] In that study, 20% of HIV patients had ADA level >30 U/L which is close to the prevalence of Tuberculosis pleurisy in that HIV endemic area. [6].Thisis the main objective of national program of TB, which is to find all cases of TB in the area, and if ADA can help then this is an advantage.

Approximately one-third (32.9%) of pleural fluid GeneXpertsamples were negative (less sensitive 22%)[26], but with ADA levels greater than 30 U/L.This suggests that using our current methods we are missing some cases of pleural tuberculosis. We could reduce the rate of tuberculosis in the Rwandan population, and increase the general health of the country, if we add ADA to the tests we use now to diagnose pleural tuberculosis.

The cut-off level to differentiate between low ADA (non-tuberculous effusion) and high ADA (tuberculous effusion) is important. In previous research other studies have used different cut-off values. [2] Our cut-off value of 30U/L was determined by the laboratory and the product manufacturer, and based on this we found a prevalence of 32.3% of pleural tuberculosis. Other studies have found slightly lower ADA level, even with pleural TB, for example J.Jasani, S.Mavadia et al,inwhich the ADA level in pleural tuberculosis was 28.8±7.8IU/L. [24]

A number of factors are known to affect level of ADA. The time from sample collection to freezing the specimen at -80 degrees C should be as short as possible.[27][28] In our study, because of logistical constraints, we were not always able to immediately put the samples in deep freezer storage. In a few cases, this gave the sample time to continue to metabolize and alter the ADA value when we tested it later. This could mean that the prevalence of pleural tuberculosis is even higher than we found in our study.

CHAPTER V: CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion and recommendations

In our study, the prevalence of pleural tuberculosis,diagnosedby ADA> 30, was 32.9% among allpleural effusion screened; 34.1% among exudative pleural effusion based on Light's criteria; and 25.6% according to neutrophiliccellularity criteria of the effusion. Of the 90 patients in our study, only 20patients were started on anti TB drugs(22.2%), which is fewer than should have been treated for TB using ADA diagnosis(32.9%).

Thus,using the ADA testwill increase the accuracy of pleural TB diagnosis, while preventing unnecessary anti –TB treatment for a considerable numberof patients with pleural effusion.Furthermore, by using the ADA test, the pleural TB with neutrophils predominance diagnosis was improved with early start of treatment.

For some patients in our study, not all necessary investigations wererequested. Therefore,another important recommendation is that thetreating team must be aware of the importance of requesting all relevant tests done on pleural effusion.

Further research is needed to differentiate ADA value in tuberculous and non-tuberculous pleural effusion and compare ADA with the gold standard test of pleural biopsy.ADA is a safe, rapid, simple, noninvasive test, inexpensive test for diagnosis of tuberculouspleural effusion.

5.2. Limitation of the study

These study limitations were lack of gold standard to compare with ADA due to financial support and time.
Not all investigations were asked systematically for all 90 participants.

Poor documentation in the medical record was noted. This could be related to the laboratory if it doesn't do the test, treating health workers basic investigations (if all tests neededare not requested to make analysis or the tests are not available in place even if it is necessary).
These are important findings, because at tertiary referral hospitals in Internal Medicine and Accident & Emergency, many of the patients received are very sick. Many of the patients who we enrolled in our study are those who did not respond to treatment at the District Hospital.

Therefore if we continue to delay the diagnosis by not using ADA for pleural TB, we can have a poor prognosis and there may be great harm to the patient.

5.3. Recommendations

-Elucidate a minimum packet of investigations that should be performed on all pleural fluid samples for which TB is suspected, to increase the accuracy of pleural effusion diagnosis.

-Put mechanisms in place to ensure that when a pleural fluid sample is collected, it is taken immediately to the laboratory for analysis. If the amount of time that the fluid sits at room temperature is shortened, the accuracy of the ADA will be increased.

-In addition to added funds at MOH level for routine ADA testing, consider also adding funds for pleural tuberculosis gold standard tests, such as pleural biopsy and pleural fluid TB culture.

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APPENDICES

1. Questionnaire

Data collection form

Patient file number(code) ……
Patient contact and next of the kin: ……./…… ……/….……

1. Age: ……
2. Sex: 1.male
   2. Female

.Chronic disease

3. Congestive Heart Failure 1.yes 2.no
4. Renal failure 1.yes 2.no
5. Nephrotic syndrome 1.yes 2.no
6. Liver disease (cirrhosis) 1.yes 2.no
7. Active Hepatitis 1.yes 2.no
8. Malignancy 1.yes 2.no
9. Pulmonary embolism 1.yes 2.no
10. Autoimmune disease 1.yes 2.no
11. Diabetes Mellitus 1.yes 2.no
12. Retroviral disease 1.yes 2.no since……RecentCD4….
13. Acute infectious disease 1.yes 2.no .
14. Has contact with TB patient: 1.yes 2. no
15. AntiTB drug at admission or after: 1.yes 2. No Date of AntiTB drugs initiation……
16. Smoking: 1.yes 2. No

. Clinical features

17. Cough: 1.yes 2.no
18. Pleuritic chest pain: 1.yes 2.no
19. Fever: 1.yes 2.no
20. Dyspnea: 1.yes 2.no
21. Night sweat: 1.yes 2.no:
22. Weight loss: 1.yes 2.no
23. Ascitis: 1.yes 2.no
24. Lower limb edema: 1.yes 2.no
25. ADA level: …….
26. AFB
    1. Positive
    2. Negative
27. GeneXpert
    1. Positive
    2. Negative

28. Culture Pleural Mycobac.Tuberculosis:
1. Positive
2. Negative
29. Ordinary culture of pleural fluid
1. Growth
2. No growth
30. Pleural fluid (Light‘s criteria)
Protein pleural/protein serum 1.Exudate(more 0.5) 2.transudate(less 0.5) or
LDH pleural/LDH serum 1.exudate(more 0.6) 2.transudate(less 0.6)
31. Cytology
1. Malignancy cells
2. No malignancy cells
32. Pleural: WBC…….(N…..L…..)

33. Chest CT scan done
1. Yes 2.No
34. If yes, Chest CT Scan report……..
35. Radiological confirmation of pleural effusion
1. Yes 2.No
36. Pleural biopsy done
1.Yes 2.No
37. If yes, pleural biopsy report…….

Results from File

38. Blood pressure……..
39. Heart rate……
40. Body Temperature (Celsius)…….
41. Respiratory rate……..
42. WBC……
43. HB……
44. Platelets……
45. ALT……
46. AST…….
47. Albumin
1.<3.5mg/dl 2.>3.5mg/dl
48. Bilirubin……
49. Gamma GT…….
50. Creatinine …….
51. Proteinuria
1.>3.5g/dl 2.<3.5g/dl

Annex 2: Consent form

2.1.Subject Information and Consent Form

Study title: Use of Adenosine Deaminase as biomarker in diagnosis of suspected tuberculous pleural effusion at tertiary referral hospitals in Rwanda

Principal Investigator

Dr BARANSABIRA MarieGoretti

Co-Investigators: - DrCameron Page

- Dr DUSABEJAMBO Vincent

- DrBITUNGUHARI Leopold

Sites CHUK&CHUB

If you have any concerns about your rights as a research subject and/or your experiences while participating in this study, you may contact:

1) The UR-CMHS Institutional Review Board

Po Box:3286 Kigali Tel:+25078856331

Email:fsunday@khi.ac.rw

2) The Principal Investigator

Dr BARANSABIRA Marie Goretti

Telephone: +250788499514

Email: baransa3@yahoo.fr

Introduction

You are being invited to participate in this research study that aims to determine the Use of Adenosine Deaminase as biomarker in diagnosis of suspected tuberculous pleural effusion at tertiary referral hospitals in Rwanda.

The study will help to do have a rapid, safe and inexpensive test in diagnosis of suspected pleural Tuberculosis. This will help the outcome if the patient is treated early.

Participation

Your participation is entirely voluntary, so it is up to you to decide whether or not to take part in this study. Before you decide, it is important for you to understand what the research involves. This consent form will tell you about the study, why the research is being done, what will happen to you during the study and the possible benefits, risks and discomforts.

If you wish to take part in this study, you will be asked to sign this form. Neither your name, nor your photo, will be included in the data set or in any reports. If you do decide to take part in this study, you may refuse to answer any question or choose to end the interview at any time. You are also free to withdraw at any time and without giving any reasons for your decision; however, we hope you choose to participate in this study.

Who Can Participate? (Inclusion criteria)

1. Patients must be 16 years old and above

2. Patients must accept to sign a consent form

4. Patients with pleural effusion who need further investigations on it

Who Should Not Participate in This Study (Exclusion criteria)

Patients who do not meet the above criteria should not participate in the study.

Study procedures

We will ask you number of questions about your recent and past illness, how you feel, social life history and we will use your information recorded in your file. We shall need to take pleural fluid using simple needle, the way used take samples.

An overview of the study is provided below:

Study Questionnaire

We will review the study procedures and consent form .
It will take you about 10 minutes to answer to this study questions.

Risks

The pleural fluid collection procedure may cause some discomfort and slight pain or, very rarely, an infection at the site of the needle poke. After the fluid draw we will cover the spot where the fluid was taken.

Compensation

There will be no salary for participants of this study. Any alarming result shall be revealed to your attending clinician for management.

Confidentiality

In this study you will be identified by a study code and any identifying information will be kept behind locked doors. Your confidentiality will be respected. No information that discloses your identity will be released or published without your specific consent to the disclosure. No records which identify you by name or initials will be allowed to leave the Investigators' offices.

Consent

This study has been explained to you and you have been given the chance to ask all questions about taking part in this study. If you have questions you can ask DrBaransabira Marie Goretti

Participation and Withdrawal from this Study

Taking part in this study is voluntary. You may choose not to take part or may leave the study at any time and do not have to give a reason for your decision. If you decide not to take part or decide to leave the study, you may do so at any time without any consequences. All data collected about you up to the point of withdrawal will be retained for analysis. You will be given a copy of this signed and dated consent form.

Consent Form

•I have listened to or read and understood the subject information and consent form.

•I have had sufficient time to consider the information provided and to ask for advice.

•I have had the opportunity to ask questions and have had satisfactory response to my questions.

•I understand that all of the information collected will be kept confidential and that the result will only be used for scientific objectives.

•I understand that participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time without changing in any way the quality of care that I receive.

•I understand that I am not waiving any of my legal rights as a result of signing this consent form.

•I have read this form and I freely consent to participate in this study.

Name of Participant _____

Signature /Thumbprint _____

Name of the person explaining the consent _____

Signature of person explaining the consent _____

Place and Date _____

2.2. ASSENT FORM (for children aged 12 years to 20 years)

Project title: USE OF ADENOSISNE DEAMINASE AS BIOMARKER IN DIAGNOSIS OF SUSPECTED TUBERCULOUS PLEURAL EFFUSION AT TERTIARY HOSPTALS IN RWANDA

Investigator: Dr BARANSABIRA Marie Goretti

Tel: +250788499514

We are doing a research study about use of Adenosine Deaminase as biomarker in diagnosis of suspected tuberculous pleural effusion at tertiary hospitals in RWANDA.

If you decide that you want to be part of this study, the clinician will use your medical file to see more information needed.

You can ask questions any time, now or later. You can talk to the doctors, your family or someone else. You do not have to be in this study, no one will be mad at you if you don't want to do this. We will also ask your parents if they would like you to be in the study. Even if you say yes now, you can change your mind later and there is no impact on your treatment.

When we are finished with this study, we will write a report about what was learnt. This report will not include your name or that you were in the study.

ASSENT

I want to take part in this study. I know I can change my mind at any time.

Child's name: ……

Verbal assent given: Yes **Date**:…… …/ …..…/……..

I confirm that I have explained the study to the participant to the extent compatible with the participant understands, and that the participant has agreed to be in the study.

Name of person obtaining the assent and signature: ……

Date: ……../……/……