Diplomarbeit

Sensitivity and Specificity of the automatic determination of Adenosine Deaminase Activity (ADA) on the COBAS-8000 system to exclude tuberculous pleurisy in a low prevalence area

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Abstract

Aim

Globally, tuberculosis (TB) is one of the most important diseases, being the second leading cause of death caused by infectious diseases after the human immunodeficiency virus. Tuberculous pleurisy occurs in 3% to 25% of the TB patients and is the second most common form of extrapulmonary TB. It is therefore important to consider TB when a pleural effusion of unknown origin is diagnosed. The adenosine deaminase activity (ADA) is found to be a sensitive and specific marker for the diagnosis of extrapulmonary TB in pleural effusions and other biological fluids. Its usefulness is well studied in countries with a high incidence of TB but not in areas with low prevalence. We therefore evaluated the sensitivity, specificity, PPV and NPV of automated determination of ADA on the Cobas® 8000 system in Styria, Austria.

Methods

This study was performed as a retrospective manner including patients with undiagnosed pleural effusions between 30th November 2011 and 15th February 2013. Participating hospitals were the University Hospital of Graz and the State Hospital of Hörgas-Enzenbach. In this period, ADA was analysed with the Cobas® 8000 system at the University Hospital of Graz and from a total of 96 patients results were available. Patients with exudative pleural effusions with full records of laboratory and medical data were included in this study. Transudative and exudative effusions were discriminated by the Light's criteria. Finally 65 patients with exudative pleural effusions were included and sensitivity, specificity, PPV and NPV of ADA were calculated for TB.

Results

The main leading cause for exudative pleural effusions among our patients (n= 65) was an underlying malignant disease (n=24; 36.9%). Second leading cause was pleuropneumonia (n=9; 13.9%), followed by unspecific chronic pleuritis (n=8; 12.3%), pleural empyema (n=7; 10.8%), cardiac decompensation (n=5; 7.7%), tuberculosis (n=4; 6.2%) and pulmonary embolism (n=3; 4.6%). The mean age of

the patients with different entities of exudates was 66.8 years, in the group of confirmed TB cases it was 49.5 years. The laboratory analysis of pleural fluid showed that the group of empyema had the highest levels in: the total number of cells (mean: 38.2 G/L) with highest percentage of neutrophils (mean: 79.8%), pleural LDH (mean: 4134 U/l), pleural-to-blood LDH ratio (mean: 20.3), pleural total protein (mean: 5.1 g/dl), ADA (mean: 51.5 U/l), together with lowest levels of glucose (mean: 6 mg/dl) and pH (mean: 7.17). Tuberculous pleural effusions had the highest level of lymphocytes (mean: 80.5%) as well as of lymphocyte-to-neutrophil ratio (mean: 50.4) and they followed the group of empyema with some distance in the number of total cells (mean: 4.7 G/L), LDH ratio (mean: 2.14), the level of pleural total protein (mean: 4.7 g/dl) and ADA (mean: 43.5 U/l). The highest pleural-to-blood total protein ratio were reached in the groups of pulmonary embolism (mean: 0.68), TB pleurisy (mean: 0.67) and empyema (mean: 0.65). Using a cut-off level of 25 U/l, the sensitivity, specificity, PPV and NPV of ADA for tuberculous pleural effusions were 100%, 87%, 33% and 100%, respectively. ROC analysis revealed an area under the curve of 0.926 (95% CI 0.86-0.99) for differentiating between patients with TB pleuritis from those with other etiologies for exudates.

Summary

In countries with a high or moderate TB prevalence pleural ADA is a routinely used marker to rule out pleural TB. In Austria *M. tuberculosis* is a rare cause of pleural effusions but pleural TB has to be considered in unexplained pleural exudates. However, there is only little evidence for the value of ADA determination in a low prevalence setting like in Austria. Our study showed that the sensitivity of ADA determination for TB is very high but the PPV is low, which can be explained by false positive results mainly in the group of empyema and NHL. The NPV is very high which means that results below the used ADA cut-off (25 U/l) virtually exclude the diagnosis of TB pleurisy. The study demonstrates that in low prevalence areas the ADA determination is a useful tool to rule out tuberculous pleurisy. To reduce the false positive rate and increase the PPV, ADA results have to be interpreted in conjunction with the differential cell counts or the lymphocyte-to-neutrophil ratio.

Zusammenfassung

Zielsetzung

Die Tuberkulose (TBC) ist noch immer eine wichtige Infektionskrankheit, da sie weltweit nach HIV die zweithäufigste erregerbedingte Todesursache darstellt. 3% bis 25 % der Tuberkulosepatienten entwickeln eine tuberkulöse Pleuritis. Sie ist die zweithäufigste Form der extrapulmonalen TBC und sollte immer als Differentialdiagnose bei unklaren Pleuraergüssen berücksichtigt werden. Die Adenosindeaminase Aktivität (ADA) wird als sensitiver und spezifischer Marker für die Diagnose der extrapulmonalen TBC in Pleuraergüssen und anderen Flüssigkeitsansammlungen beschrieben. Ihr Nutzen wurde bisher vor allem in Ländern mit hoher TBC-Inzidenz untersucht, nicht aber in Gegenden mit geringem TBC-Vorkommen. Wir analysierten daher die Sensitivität, Spezifität, den PPV und NPV der automatisierten Bestimmung von ADA auf dem Cobas® 8000 System in der Steiermark, Österreich.

Methoden

In der Studie untersuchten wir retrospektiv Befunde von Patienten mit unklaren Pleuraergüssen zwischen dem 30. November 2011 und dem 15. Februar 2013. Teilnehmende Krankenhäuser waren die Universitätsklinik Graz und das Landeskrankenhaus Hörgas-Enzenbach. In dem angegebenen Zeitraum wurde die ADA-Aktivität mit dem Cobas® 8000-System an der Medizinischen Universität Graz bestimmt und von 96 Patienten waren daraufhin ADA Werte verfügbar. Eingeschlossen wurden Patienten mit exsudativen Pleuraergüssen sowie vollständigen laborchemischen und medizinischen Daten. Transsudative und exsudative Ergüsse differenzierten wir anhand der Light's Kriterien. Schließlich konnten wir 65 Patienten in die weiteren Analysen einschließen.

Resultate

Die Hauptursache für exsudative Pleuraergüsse bei unseren Patienten (n=65) war eine maligne Erkrankung (n=24; 36,9%), die zweithäufigste Ursache eine Pleuropneumonie (n=9; 13,9%), gefolgt von chronischer Pleuritis (n=8; 12,3%), Pleuraempyem (n=7; 10,8 %), Herzdekompensation (n=5; 7,7%), TBC (n=4; 6,2%)

und Lungenembolie (n=3; 4,6%). Das Durchschnittsalter der Patienten mit exsudativen Pleuraergüssen lag bei 66,8 Jahren, in der Gruppe der Tuberkulosefälle bei 49,5 Jahren. Die laborchemische Untersuchung der Pleuraergüsse zeigte, dass die Gruppe der Empyeme die höchsten Werte in der Zellzahl (Mittelwert: 38,2 g/l), dem Anteil der neutrophilen Granulozyten (Mittelwert: 79,8%), der Pleura/Blut LDH-Ratio (Mittelwert: 20,3), der Pleura LDH (Mittelwert: 4134 U/l), dem Pleura Protein (Mittelwert: 5,1 g/dl), der ADA (Mittelwert: 51,5 U/l) und die niedrigsten Glukose- (Mittelwert: 6 mg/dl) sowie pH-Werte (Mittelwert: 7,17) erreichte. Die tuberkulöse Pleuritis hatte den höchsten Anteil an Lymphozyten (Mittelwert: 80,5%) und das höchste Lymphozyten/Neutrophilen Verhältnis (Mittelwert: 50,4). An zweiter Stelle folgte die tuberkulöse Pleuritis den Empyemen mit großem Abstand in der Zellzahl (Mittelwert: 4,7 g/l), dem LDH-Verhältnis (Mittelwert: 2,14) und mit geringer Differenz bei Pleura Protein (Mittelwert: 4,7 g/dl) und ADA (Mittelwert: 43,5 U/l). Die höchste Pleura/Blut Protein Ratio wurde durch Lungenembolien erreicht (Mittelwert: 0,68), gefolgt von tuberkulösen Exsudaten (Mittelwert: 0,67) und Empyemen (Mittelwert: 0,65). Mit einem ADA cut-off von 25 U/l wurde für tuberkulöse Ergüsse eine Sensitivität, Spezifität, ein PPV und NPV von 100%, 87%, 33% und 100% erreicht. Die ROC-Kurve zeigt eine AUC von 0,926 (95% CI 0,86-0,99) für die Unterscheidung zwischen Patienten mit TB Pleuritis von denen mit anderen Ursachen für Exsudate.

Zusammenfassung

Tuberkulose stellt in Österreich eine seltene Ätiologie von Pleuraergüssen dar, muss aber immer berücksichtigt und ausgeschlossen werden. In Ländern mit mittlerer und hoher TBC-Prävalenz wird die ADA als Marker routinemäßig verwendet, aber es gibt kaum Evidenz für den Stellenwert der ADA-Bestimmung in Gegenden mit geringer TBC-Prävalenz. Unsere Studie hat gezeigt, dass die Sensitivität der ADA Bestimmung für TB sehr hoch ist, aber der PPV niedrig. Dies kann vor allem durch falsch-positive Ergebnisse in der Empyem- und NHL-Gruppe erklärt werden. Der NPV ist sehr hoch, was bedeutet, dass ADA Werte unter 25 U/l die Diagnose der tuberkulösen Pleuritis praktisch ausschließen. Für den klinischen Alltag zeigen diese Ergebnisse, dass die ADA Bestimmung in

Niedrigprävalenzländern eine hilfreiche Methode zum Ausschluss einer tuberkulösen Pleuritis darstellen kann. Um die Rate falsch positiver Ergebnisse zu reduzieren und den PPV der ADA Bestimmung zu erhöhen muss das Differentialzellbild oder die Lymphozyten/Neutrophilen Ratio bei der Ergebnisinterpretation berücksichtigt werden.

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Abbreviations

4AA	4-Aminoantipyrine
ADA	Adenosine Deaminase Activity
ANCA	Anti-neutrophil Cytoplasmic Antibodies
AUC	Area Under the Curve
BCG	Bacillus Calmette-Guerin
DST	Drug Susceptibility Testing
E	Ethambutol
EHSPT	N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-Methylaniline
EPTB	Extrapulmonary Tuberculosis
H	Isoniazid
H_2O_2	Hydrogen Peroxide
HIV	Human Immunodeficiency Virus
IFN-γ	Interferon-Gamma
IGRA	Interferon-Gamma Release Assay
LDH	Lactate Dehydrogenase
LTBI	Latent Tuberculosis Infection
MDR – TB	Multidrug-Resistant Tuberculosis
MTB	Mycobacterium Tuberculosis
MTC	Mycobacterium Tuberculosis Complex
NHL	Non-Hodgkin's Lymphoma
NPV	Negative Predictive Value
NTM	Nontuberculous Mycobacteria
PCR	Polymerase Chain Reaction
PNP	Purine Nucleoside Phosphorylase
POD	Peroxidase
PPD	Tuberculin-Purified Protein Derivate
PPV	Positive Predictive Value
QFT	Quantiferon-TB Gold Test
R	Rifampicin
ROC	Receiver Operator Characteristic

SLE	Systemic Lupus Erythematosus
TB	Tuberculosis
TBC	Tuberkulose
TGF-β	Transforming Growth Factor Beta
TNF-α	Tumor Necrosis Factor Alpha
TPE	Tuberculous Pleural Effusions
TST	Tuberculin Skin Test
WHO	World Health Organisation
XOD	Xanthine Oxidase
Z	Pyrazinamide

1 Tuberculosis

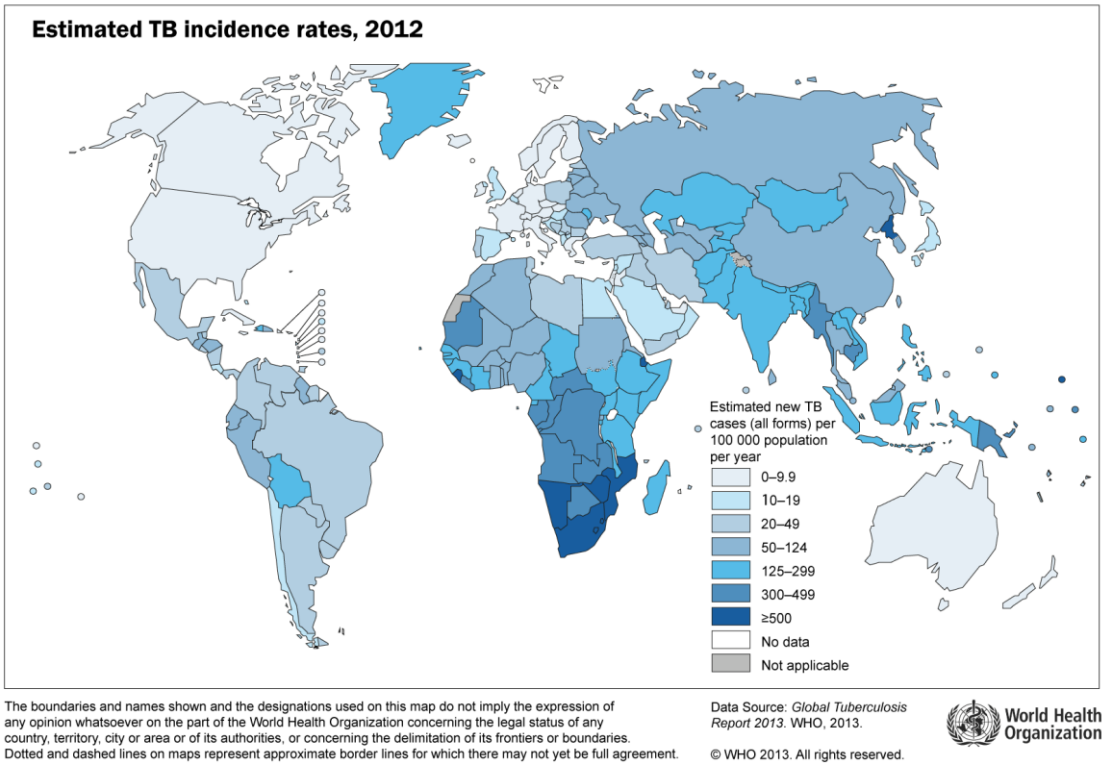
Tuberculosis (TB) is an infectious disease, mainly caused by *Mycobacterium tuberculosis* (MTB), with a wide range of possible clinical manifestations and a complex course of disease. In patients with suspect signs and symptoms active TB has to be diagnosed or excluded without significant delay (1).

1.1 Epidemiology and Etiology

1.1.1 Worldwide

Globally TB remains, after the human immunodeficiency virus (HIV), the second leading cause for death from a single infectious pathogen. Since 2001 the incidence rate but also the mortality rate are decreasing slowly.

Figure 1 Estimated TB incidence rates 2012 by the WHO (adapted from (2))

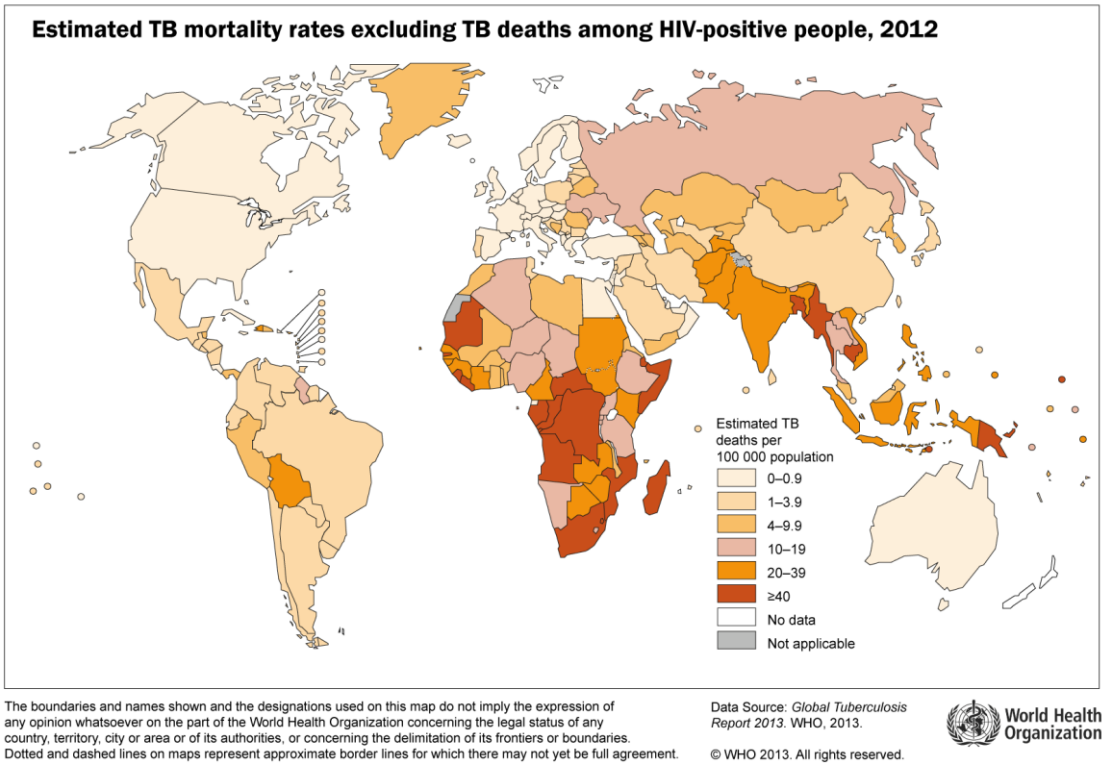


The WHO estimated that in 2012 8.6 million people fell ill with TB which equals 122 cases per 100 000 population. Most of the cases occurred in Asia and Africa,

with India and China facing the biggest problems in absolute numbers, followed by South Africa, Indonesia and Pakistan.

There were approximately 1.3 million people dying of TB in 2012, of these 75% in the African and South-East Asian Regions. India and South Africa account for one third of all the TB deaths.

Figure 2 Estimated TB mortality rates 2012 by the WHO (adapted from (2))



It should be stressed that 13% of the incident TB cases were co-infected with HIV, especially in countries in the African Region. Another genuine problem – particularly for the detection and treatment - poses the multi-drug resistance TB (MDR-TB) with 450 000 new cases in 2012, the highest levels in Eastern Europe and central Asia (2).

1.1.2 Austria

Over the last hundred years the TB rate in Western Europe declined continuously from 450 / 100,000 in 1900 to 8 / 100,000 in 2011.

Austria is nowadays a country with a low incidence of TB. In 2012 only 648 TB

cases were diagnosed (incidence 7.66 / 100,000). In the referred cases, the detection of mycobacteria by culture was possible in 406 cases, in 387 of them *M. tuberculosis* was identified and in one case *M. africanum*, *M. bovis* and *M. caprae*, respectively.

The incidence of TB among persons with non-Austrian citizenship is significantly higher than among Austrian citizens (33.5 vs. 4.4 / 100,000). Out of all non-Austrian citizens with TB, 69% migrated from other countries of the European Union, Balkan region or Turkey. There were 27 cases of MDR-TB reported, all of them being among non-Austrians (3).
Exact data about the prevalence of TB in Austria are not available. However, the WHO considers countries with a low incidence (less than 20 per 100,000 population) also as low prevalence countries. In this context, Austria is a typical non-endemic, low prevalence country.

1.2 Mycobacteria and Infection

1.2.1 Mycobacteria

The Mycobacteriaceae family involves aerobic, acid-fast rods that can be divided into non-tuberculous mycobacteria (NTM) and the *Mycobacterium tuberculosis* complex (MTC), which is responsible for causing the human TB and zoonotic disease (4). The MTC comprises the species *M. tuberculosis*, *M. africanum*, *M. bovis*, *M. canetti*, *M. microti*, *M. caprea* and *M. pinnipedi* (5). The majority of human TB is caused by the *M. tuberculosis* species whereas *M. africanum*, *M. bovis*, *M. canetti*, *M. microti, M. caprea* are seldomly found. Infecting mainly cattle, *M. bovis* was largely eradicated in industrialized countries by animal control and milk pasteurization (6). *M. caprea* is also linked to bovine TB but occurs even more rarely (7). *M. pinnipedi,* a pathogen for seals, was also reported to be transmittable to humans (8).

1.2.2 Transmission

M. tuberculosis is normally transmitted from person to person by inhalation of small aerosols which contain the mycobacterium. Generally, the contagious

person suffers from active pulmonary tuberculosis and the droplet nuclei are spread by coughing, sneezing or talking. The likelihood of transmissibility and infection is influenced by the amount of mycobacteria in the cough-generated aerosols, the distance between the persons, the ventilation in the rooms, the time of exposure and the status of the immune system. Due to vaccination of cattle, the infection by non-pasteurized cow milk with *M. bovis* is no longer relevant in middle Europe. Other possibilities of transmission (through damaged skin by smear infection of urine or pus) are possible but remain very unlikely (1,9).

1.2.3 Primary Infection

The infection might be completely prevented by early elimination of the MTB-containing droplet nuclei through mucociliary clearance or by phagocytosis and intracellular destruction of the mycobacteria by alveolar macrophages.

However, phagocytosis is a critical step in the pathogenesis of TB, as MTB is able to avoid intracellular elimination. In this scenario intracellular bacterial replication leads to apoptosis of the macrophages and progressive infection. After 2-6 weeks an adaptive immune response occurs, T-cells and monocytes release IFN-γ, TNF-α, TGF-β and will lead to the formation of caseating granulomas with giant cells that surround the mycobacteria. This initial focus is usually located in the basal upper or apical lower lobe of the lung. From there MTB spread through the lymphatics to the hilar and other regional lymph nodes. The primary complex (Ghon complex) is formed by this hilar and mediastinal lymphadenopathy and the primary focus. At that time, still being mainly asymptomatic, the TB infection can be detected only indirectly after 4-8 weeks with a tuberculin skin test (TST) or Interferon-γ-Release-Assay (IGRA) (4).

Leaving the primary complex MTB might also disseminate haematogenic leading to pulmonary lesions in other extrapulmonary locations. Inside the pulmonary system MTB spreads mainly to the apical and posterior part of the upper lobe and in most cases persists there asymptomatically (1). These pulmonary lesions in the upper lobe can calcify over the time, stay asymptomatic and do not progress over years but can cause later TB reactivation leading to a symptomatic post-primary TB disease (10).

Taken together, according to the individual situation the primary infection will progress or persist to one of the following three forms:

- Symptomatic (active) primary TB
- Asymptomatic (inactive) latent TB infection (LTBI)
- Symptomatic (active) post-primary TB (4)

1.2.4 Primary Tuberculosis

Up to 13% of the affected patients develop active TB in the following 2 years after infection (11). This form affects mainly the lungs (90%), is accompanied by fever and appears especially in children and young adults as typical primary TB. The progression leads to pulmonary infiltrates that are easily misinterpreted as community acquired pneumonia. In small children the enlargement of the hilar lymph nodes frequently produces bronchial collapse and furthermore distal atelectasis. Up to 30% will develop a pleural effusion (4). In children or immunosuppressed individuals a miliary TB may also occur which is rapidly progressive (12). An aggravation can lead to Landouzy septicemia (sepsis tuberculosa acutissima) (4).

1.2.5 Latent Tuberculosis Infection (LTBI)

It is estimated that one third of the world population are latently (asymptomatically) infected with *M. tuberculosis*, whereas in Austria and Germany the rate is presumably below 2% (2,4). In contrast to the symptomatic (active) primary TB persons with an effective cell-mediated immune function are able to arrest the MTB infection by forming intact granulomas. These encase effectively a small numbers of bacilli and prevent active TB (13). In the majority of cases, the bacteria stay dormant within the granulomas and the person remains asymptomatic for life. However, approximately 10% of the infected individuals with LTBI later develop active TB. In low-incidence nations (TB incidence <20 per 100,000 population) the majority of TB diseases occur as reactivation of LTBI rather than due to a recent person-to-person transmission (14-16).

There are a few indirect signs that indicate a LTBI, which include anamnestic, radiological and immunoreactive findings as shown in Table 1.

Table 1 Indicators for a Latent TB Infection (adapted from (4))

Indicators for LTBI		
Anamnestic Indicators	**Immunreactive Indicators**	**Radiological Indicators**
- Relevant TB exposition[a] - Originating from a high prevalence area - Incomplete treatment of active TB[a]	- IGRA positive - TST positive[b]	- Intrapulmonary nodules[c] - Scarred pleural residua[d] - Calcified lymph nodes - Calcified pericardium
[a] in the past. [b] with no BCG vaccination. [c] calcified or not calcified. [d] e.g. pleural fibrosis		

In low prevalence areas it is recommended to focus screening on groups with high risk for TB, so-called “targeted testing” and discouraged to screen among persons at lower risk (17).

1.2.6 Post-primary infection

A small percentage of individuals with LTBI, mainly adults, develop at a later date active, contagious TB (reactivated infection). Particularly endangered are immunosuppressed persons with the strongest risk factor being HIV-infected or patients after solid organ transplantation. Like primary active TB the reactivation occurs in most cases as pulmonary TB but differs in some characteristics (4,18):

- Post-primary TB begins with infiltrates that are mainly located in the upper lobe or in the apical region of the lower lobe.
- Development of extensive caseous areas, which tend to liquefy (cavitation) and drain into the bronchial tree, leading to a high infectivity.
- High concentration of bacteria stored in the resulting cavities leading to a bronchogenic spread and further development of foci, caseation, fibrosis or new cavities in other pulmonary lobes or the contralateral lung.
- Radiological images show small speckled, bronchiolar nodules and concomitant branching opacities called “tree in bud”.

- The hilar lymphadenitis plays only a minor role, whereas calcified lymph nodes or foci and pleural fibrosis can be detected as residues of the primary infection.

The symptoms may start nonspecific with chills, fever, weight loss, fatigue, night sweats and slowly progressive productive cough. In advanced disease, haemoptysis resulting from caseous sloughing or endobronchial erosion may occur (18). Untreated TB will lead to pulmonary destruction and further complications like pneumothorax, haemoptysis or bacterial superinfection. Without effective therapy the 10-year survival rate for post-primary TB is about 30% (4,19).

1.3 Diagnosis

The symptoms and radiographic pattern as shown in Table 2 can lead to a presumptive diagnosis for active TB, but it should be stressed that there are other differential diagnoses (e.g. cavities in the case of pulmonary emboli, lung cancer or granulomatosis with polyangiitis) for each of them, which should be considered especially in low-prevalence areas (4).

Table 2 Indicators for active Tuberculosis (adapted from (4))

Indicators for active TB
Coughing ≥ 2weeks if exposed to TB within the last 2 years
Coughing ≥ 2weeks with fever / night sweats or weight loss
Haemoptysis
Unexplained pulmonary complaints or drug-resistant pneumonia at TB risk patients
Spotty, inhomogeneous infiltrates in the apical, posterior upper lobe or apical lower lobe
Cavities
“tree-in-bud” sign
Small nodular opacities (Miliary TB)
Hilar or mediastinal lymphadenopathy
Exudative lymphocytic pleural effusion without sign of lung cancer

1.3.1 Clinical Features

1.3.2 Laboratory Diagnosis

If there is strong suspicion of active TB microscopic, cultural and polymerase chain reaction (PCR) analyses from clinical samples and specimen (e.g. sputum, pleural effusion, biopsies, cerebral spinal fluid) should be done.

To increase the sensitivity for pulmonary TB, three early morning sputum samples are recommended (4,18). If sputum cannot be produced, aspiration of gastric contents in the early morning is an alternative, particularly in children. Another way to induce sputum is by inhalation of hypertonic saline aerosols. This method has a comparable sensitivity as fiberoptic bronchoscopy and might be especially useful in ambulatory or resource-poor settings. (20)

1.3.2.1 Microscopic Examination

The first bacteriological evidence of mycobacteria in specimen is provided by detecting acid-fast bacilli in stained smears or samples. Mainly used for acid-fast staining are the Ziehl-Neelsen and Kinyoun methods (13). Staining is a fast and easy method but has little sensitivity due to the detection limit of 5,000 to 10,000 bacilli per millilitre of specimen (21). Consequently, negative staining results do not preclude TB as only in 50% to 80% of pulmonary TB patients acid-fast bacilli can be detected by primary microscopy (13).

1.3.2.2 Cultivation

All clinical specimens should be inoculated onto culture media because of its higher sensitivity compared to primary microscopy as well as for the possibility to identify the species, test the drug susceptibility and monitor the therapeutic success (4,13). After one week first positive results might be seen, but the final statement can only be made after 8 weeks. In pleural effusions the number of bacilli often remains too small to lead to cultural evidence (4).

1.3.2.3 PCR

This nucleic acid amplification method is sensitive and specific but quite complex and expensive. The detection level is between 1-10 bacilli per millilitre. Combined with the microscopy findings, the sensitivity of the early diagnosis can be doubled

as the results are normally available after 24 hours. Sensitivity in sputum smears is about 80%, in effusions around 60-70%, respectively. The distinction between NTM and MTB and the detection of MDR-TB can be quickly made with PCR. In regions with high prevalence of MDR-TB, real-time PCR is recommended to detect MDR and especially resistance against rifampicin within 2 hours (4).

1.3.2.4 Tuberculin skin test

The Tuberculin skin test (TST) is an indirect method for identifying a mycobacterial infection. Tuberculin is an extract of antigens. For the Mantoux method, tuberculin-purified protein derivate (PPD) is injected intracutaneously leading to a delayed-type hypersensitive reaction. The test should be read after 48h - 72h when the induration is at the maximum. This shall be done by inspection and palpation or ball-paint pen method (4,13).

However, being also positive among BCG vaccinated persons or individuals infected with NTM, the Mantoux-test is not specific for MTB infections (4). Moreover, in persons with active TB a false-negative rate of 25% has been described (13,22).

1.3.2.5 Interferon Gamma Release Assays (IGRAs)

These methods analyse the immune response by T-lymphocytes to MTB specific antigens in blood specimen. There is no cross-reaction to BCG vaccination or most NTM (except for *M. kansasii, M. marinum, M. flavescens,* and *M. szulgai*) but IGRAs are not able to distinguish between active TB and LTBI. Therefore, IGRAs are manly recommended for screening of LTBI in asymptomatic individuals and not recommended if active TB is suspected in symptomatic elderly persons or in symptomatic patients coming from high-prevalence areas (high prevalence of LTBI and low IGRA-specificity regarding active TB). However, IGRAs are useful to confirm the suspicion of active TB in children and young adults especially in a low endemic setting (low prevalence of LTBI and high IGRA-specificity regarding active TB).

Two approved IGRA systems exist: the T-SPOT-TB® and the QuantiFERON-TB Gold test® (QFT). Both IGRAs are more sensitive than the TST for immunosuppressed individuals but lack sensitivity (80%) and specificity (60%-80%) in patients with active TB. A negative IGRA result does therefore not exclude

active TB and direct detection methods (microscopy, culture, PCR) must always be performed (4).

1.3.2.6 Adenosine Deaminase Activity (ADA)

The determination of the ADA level is a useful marker in exudative effusions of unknown origin to rule out a TB infection. It is well studied for tuberculous pleurisy (see chapter 3), showing a sensitivity of 92% and a specificity of 90% in countries with high TB prevalence (23). It is also recommended if tuberculous pericarditis, tuberculous peritonitis or tuberculous meningitis is suspected (1).

1.3.2.7 Histology

Epithelioid necrotic granulomas are specific for TB disease but can also be caused by NTM infections, ANCA vasculitis, rheumatoid arthritis, endemic mycosis or sometimes even sarcoidosis. In 25% of all TB cases only non-caseating granulomas can be found (4).

1.4 Treatment

The current standard regimen for treating pulmonary TB recommends 6 months of therapy. The therapy starts with an intensive phase of 2 months with a combination of isoniazid (H), rifampicin (R), pyrazinamide (Z) and ethambutol (E). After this time period a sputum specimen should be taken to evaluate the therapeutic effect. The continuation phase follows with 4 months of HR therapy. Possible interactions especially of R and H and adverse reactions should be monitored closely. H, R and Z are hepatotoxic substances, so liver enzymes have to be controlled regularly. After 6 months, another cultivation of sputum specimen should be done. The therapeutic success rate is more than 95% (4).

As MDR-TB is becoming a constantly growing problem, for those patients with MDR risk factors (e.g. interruption of previous TB treatment, contact to drug resistant pathogens or originating from areas with high prevalence of MDR-TB) drug susceptibility testing (DST) is recommended (24). Different therapy regimens exist depending on the type of resistance but for MDR-TB at least 20 months of treatment is recommended. This complex therapy shows a lot more adverse effects and significantly higher costs. With cure rates of only 60-70% in some

cases even surgical interventions (resection of cavities or destroyed pulmonary areas) have to be considered (4).

2 Tuberculous pleurisy

Despite the anatomic proximity of pleura and pulmonary parenchyma, tuberculous pleuritis is counted among the group of extrapulmonary TB (EPTB). It is the second most common form of EPTB after lymph node tuberculosis (25).

2.1 Epidemiology

Globally, tuberculous pleurisy will occur in between 3% and 25% of patients with TB and should always be considered in patients with undiagnosed pleural effusions (26). In a Spanish study (between 1992 and 2002, high prevalence area) of large or massive effusions, TB was the third leading cause (12%) after malignancy (55%) and complicated parapneumonia or empyema (22%) (27).

2.2 Pathogenesis

Tuberculous pleural effusions (TPE) are either the consequence of a recent primary infection with MTB or a reactivated LTBI. In patients from industrialized countries TPE due to reactivated LTBI occur more frequently than due to primary infection (28).

The pleural effusion is thought to result from the rupture of a subpleural caseous focus, releasing infectious material into the pleural space (18). A hypersensitivity reaction follows, which increases the permeability of the pleural capillaries to protein, promoting increasing pleural fluid levels. As a secondary consequence of progressive pleural infection and inflammation, the lymphatics are obstructed and the clearance of pleural fluid decreases (26).

2.3 Clinical Features

The onset of TPE might be either abrupt or subtle and can be easily confused with a parapneumonic effusion. Usually, the pleural effusion in TPE is unilateral. Common symptoms are non-productive cough, pleuritic chest pain and fever. Night sweats, chilliness, weakness, weight loss and dyspnoea do appear but are less frequent (18,26,29).

2.4 Conventional Diagnostic Tests

TPE are usually lymphocyte rich exudates. However, biochemical pleural fluid analysis (determination of the pleural fluid protein, lactate dehydrogenase, glucose and pH) is unspecific (18,30-32). Especially lymphocytic malignant effusions can present with similar biochemical results and false negative cytological results occur in about 40% of malignant pleural effusions. Therefore, the definitive diagnosis of TPE depends on the direct demonstration of MTB in the pleural fluid, sputum, or pleural biopsy specimen, or the confirmation of typical granulomas in the pleura (26,33,34). Unfortunately, most of the available conventional tests have relevant limitations:

- Histopathological and bacteriological examination of pleural biopsy tissues reveals the highest diagnostic evidence (sensitivity of microscopic examination of pleural biopsy specimens 20%-26%; sensitivity of culture of biopsy specimens 51%-81%; time to diagnosis 11-24 days; observation of caseating granulomas in biopsy specimens 80%) (30,35). However, it needs invasive procedures like a thoracoscopy, which is not feasible for every patient and is in general only indicated in cases of persistent clinical suspicion if less invasive procedures (like simple thoracocentesis) did not show conclusive results.
- The culture of sputum or gastric content is reported positive in only 0-50% of the tuberculous pleurisy cases without pulmonary involvement (36,37)
- The sensitivity of cultures from pleural fluid specimens is very low (7-37%), the microscopic evaluation not helpful (sensitivity of staining and microscopic examination of pleural fluid 0-6%) and even PCR from pleural fluids show a insufficient sensitivity of only 62% (35,36,38).
- TST and IGRAs are supportive, but for TPE neither sensitive nor specific (26,39). The sensitivity and specificity are, respectively, 75% and 82% for pleural fluid IGRAs; 77-80% and 61-72% for blood IGRAs, 73% and 72% for TST (40,41).
- The chest radiography normally reveals only unspecific unilateral pleural effusion. Contrast enhanced computer tomography increases the diagnostic accuracy by detection of associated parenchymal lesions and lymphadenopathy but cannot confirm TB (42).

Regarding the aforementioned limitations of conventional diagnostic methods including microscopy, culture and PCR the determination of ADA seems to be promising. ADA is found to be a sensitive and specific marker for the diagnosis of extra-pulmonary TB in pleural effusions (23) and other biological fluids such as peritoneal, cerebrospinal or pericardiac fluids (43,44). For more detailed information about the clinical significance of ADA determination in pleural fluid see 3.2 (Clinical significance of ADA determination).

The measurement of gamma interferon levels is also a sensitive and specific method (sensitivity 77-98% and specificity 96-97%) to distinguish TPE from non-tuberculous effusions but more expensive than the ADA test (45). Table 3 shows the sensitivity and specificity of different laboratory diagnostic tools. According to the literature measurement of gamma interferon and ADA determination appear to be useful tools to detect tuberculous pleurisy (23,45).

Table 3 Sensitivity and Specificity of different diagnostic tools for TPE

	Sensitivity (%)	Specificity (%)
Culture from biopsy (35)	51-81	100
Culture from pleural fluid (30,35)	7-37	100
Histology from biopsy (30)	80	
Staining/Microscopy from pleural fluid (30,35)	0-6	
PCR from pleural fluid (38)	62	98
Gamma Interferon from pleural fluid (45)	77-98	96-97
ADA from pleural fluid (23)	86-92	88-90
IGRA from pleural fluid (41)	75	82
IGRA from blood (41)	80	72
TST (40)	73	72

2.5 Differential Diagnosis

Especially the increasing age of patients leads to diagnostic difficulties in the evaluation of pleural effusions since malignancy, congestive heart failure, pneumonia and pulmonary embolism are important and common problems in elderly individuals (29). In industrialized countries with a low prevalence of TB the most common cause of unilateral exudative pleural effusions is malignant disease followed by parapneumonic effusions. Other causes might be pulmonary embolism, rheumatoid arthritis, cirrhosis, coronary artery bypass graft surgery and others (46). In this context TPE remains a rare condition in Austria and other more common diseases are primarily suspected and TB frequently not considered.

However, the pleural fluid cell proportion is very helpful in narrowing down the differential diagnosis of a pleural effusion. In settings with a low TB prevalence unilateral exudative predominant lymphocytic (>50% cells are lymphocytes) pleural effusions are most likely associated with solid malignant diseases (e.g. lung cancer, mesothelioma, breast cancer) and less commonly caused by other conditions like coronary artery bypass graft surgery, lymphoma or TB. In contrast, unilateral exudative neutrophil-predominant pleural effusions are typical for parapneumonic causes (33).

3 Adenosine deaminase

3.1 Biological Function

Adenosine deaminase and its active enzymatic form (ADA) is part of the purine catabolism that catalyzes the pathway from adenosine to inosine (4). ADA is stored in most cell types but its physiological role is especially important for the differentiation and proliferation of T-lymphocytes (47). Several isoforms of ADA have been described, ADA1 has been found in lymphocytes and monocytes whereas ADA2 can be isolated mainly in monocytes or macrophages (48). Though lymphocytes, monocytes and macrophages play a major role in tuberculous pleuritis it was shown that ADA2 accounted for most of the total ADA elevation (49,50). ADA2 most likely reflects monocyte-macrophage turnover or activity and it is speculated that inflammatory changes caused by *M. tuberculosis* stimulates the production of ADA2 in the pleural cavity (49).

3.2 Clinical Significance of ADA Determination

Testing for pleural ADA with a cut-off level between 35-50 U/l is reported to be a sensitive and specific marker for TPE. The Method has been evaluated as diagnostic tool for TPE in many studies including a meta-analysis, which shows a sensitivity of 86-92% and a specificity of 88-90%. It can also be used for patients with suppressed immune system (23). For automated ADA assays on Cobas systems an adapted cut-off value between 25 U/l and 30 U/l should be used (43).
ADA determination is recommended as ‘rule out’ test in countries with low prevalence of TB in order to replace more invasive, expensive, sophisticated and often unavailable tests or procedures like biopsy, thoracoscopy and PCR (33,51).
However, ADA results should be interpreted together with the clinical findings and the results of the conventional tests as elevated ADA can also be observed in empyema, rheumatoid pleurisy and lymphoma (4). Moreover, the majority of studies evaluating the value of ADA testing were performed in Asia, Africa, South America or in European countries with an elevated TB incidence (>20 cases per

100,000 population) and the high sensitivity and specificity cannot automatically be presumed for a non-endemic country like Austria.

From a technical point of view ADA determination is simple, rapid (results available 2 hours after thoracocentesis), inexpensive and can be easily implemented in the clinical routine. The pleural effusion sample is collected by thoracocentesis, can be stored up to 28 days at 4°C and therefore allows for postponed testing in case of limited availability or shipping needs (51).

3.3 Methods of Measurement

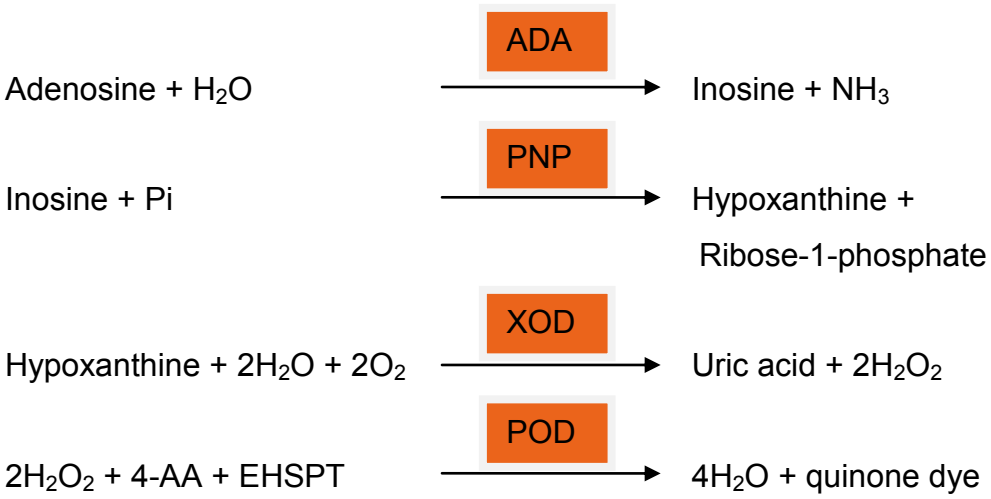
3.3.1 Galanti and Giusti methods

The principle of the method is the conversion of adenosine and water to inosine and ammonia catalyzed by adenosine desaminase. By adding sodium hypochlorite and phenol to ammonia, intense blue indophenol is formed. The catalyst here is sodium nitroprusside which at the same time stops the ADA reaction. The concentration of ammonia is directly proportional to the extinction of indophenol. The colour intensity is then measured spectrophotometrically at 620 – 650 nm (52).

3.3.2 Fully automated enzymatic / turbidimetric ADA assay

The fully automated determination of ADA requires the ADA test kit (Diazyme®) and its adaptation on a clinical chemistry laboratory analyzer. Such a test allows for measuring ADA in human serum, plasma, pleural fluid and cerebrospinal fluid (43). The Diazyme ADA Assay is based on a sequence of enzymatic reactions: Adenosine is converted to inosine, which is then converted to hypoxanthine by purine nucleoside phosphorylase (PNP). Hypoxanthine is remodelled to uric acid and hydrogen peroxide (H_2O_2) by xanthine oxidase (XOD). Hydrogen peroxide is further reacted with N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (EHSPT) and 4-aminoantipyrine (4-AA) by peroxidase (POD) to generate quinone dye (43). The entire enzymatic reaction is depicted in Figure 3.

Figure 3 Enzymatic reactions of the Diazyme ADA Assay

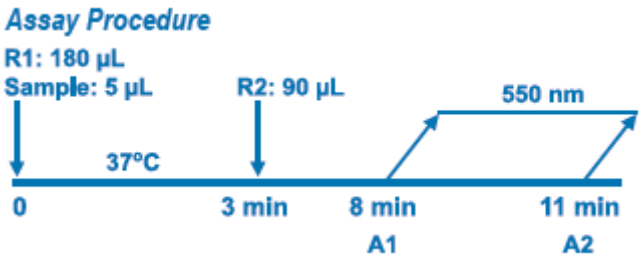


H_2O: water; ADA: adenosine deaminase; NH_3: ammonia; Pi: inorganic phosphate group; PNP: purine nucleoside phosphorylase; O_2: oxygen; XOD: xanthine oxidase; H_2O_2: hydrogen peroxide; 4-AA: 4-aminoantipyrine; EHSPT: N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline; POD: peroxidase;

One of the two reagents of the kit contains all the enzymes necessary for the reaction (R1), the other one contains adenosine and EHSPT (R2). In addition, the kit contains two internal run controls (low positive, high positive) to ensure quality control in every single run tested.

Figure 4 shows the test scheme for the assay, the amount of quinone dye is measured with spectrophotometry.

Figure 4 Test scheme for Chemistry Analysers (adapted from (53))



One unit of ADA is defined as the amount of ADA that generates one micromole of inosine from adenosine in one minute at 37°C (43).

Delacour et al. who analyzed the ADA determination with a Diazyme® ADA assay on a Cobas® 6000 in an area with a low prevalence of TB recommend using a cut-off value between 25 U/l and 30 U/l for the diagnosis of TB pleuritis (43).

4 Materials and Methods

4.1 Study Objectives

- Primary objective: To evaluate the sensitivity, specificity, PPV and NPV of the automated determination of ADA on the Cobas® 8000 system for the diagnosis of TPE in exudative pleural effusions from patients in a TB non-endemic setting
- Secondary objective: To characterise the biochemical markers of different diseases that lead to exudative pleural effusions

4.2 Study Design

This study was performed as a retrospective survey of patients with undiagnosed pleural effusions between 30th November 2011 and 15th February 2013. Participating hospitals were the University hospital of Graz and the State hospital of Hörgas-Enzenbach. In this period, ADA had been analysed with the Cobas® 8000 system at the University of Graz in 106 patients. The study was conducted at the Division of Pulmonology at the Medical University of Graz. The case report forms were completed by reviewing medical files with the use of the MEDOCS database. Data was collected anonymously.

Research ethic board approval was granted by the local ethic committee. Due to the non-interventional character of the study, a priori patient consent was not required.

4.3 Data Collection

We retrospectively studied data from 96 patients with undiagnosed pleural effusions by reviewing laboratory findings and medical charts. The data collection started in the end of November 2011 and ended in mid-February 2013.

Complete data included demographic data, clinical characteristics, final diagnosis and laboratory values. We had to exclude 12/96 (12.5%) of the patients because of missing data on LDH and protein concentration in blood and pleural specimen. We then identified 19/84 (23%) patients with transudative pleural effusions, which were classified by Light's criteria (see 4.4).
Finally 65 patients with exudative pleural effusions were included in the analysis.

We reviewed the laboratory findings and included laboratory values up to 5 days before and 5 days after the determination of ADA.
The following data were extracted from the medical files:

- Demographic data (birth date)
- Date of ADA determination
- Place of admission
- Laboratory values
- Indication for puncture of the pleura
- Final diagnosis (determined by reviewing medical results and diagnosis at least 6 month after ADA testing by a respiratory consultant physician)

The laboratory parameters determined in pleural fluid included lactate dehydrogenase (LDH), glucose, lactate, pH, total cell count, total protein, absolute counts and percentages of neutrophils, lymphocytes and other cells. The lymphocyte to neutrophil ratio was calculated. The corresponding serum LDH and serum total protein concentration were determined to calculate the ratios of pleural to blood LDH and total protein.

4.4 Definitions

Exudates were distinguished from transudates by Light's criteria. According to Light's traditional criteria, if at least one of the following three criteria is present, the fluid is defined as exudate (54):

- Pleural fluid-to-serum total protein ratio > 0.5
- Pleural fluid-to-serum LDH ratio > 0.6
- Pleural fluid LDH above two-thirds of the upper limits of the normal serum LDH

A case was defined TB positive if cultural evidence, PCR confirmation or high clinical suspicion with exclusion of other etiologies and fully recovery with TB specific treatment was administered.

4.5 Analysis

The statistics included the calculation of sensitivity, specificity, NPV and PPV. The ROC-curve was created with 'IBM SPSS Statistics 21.0'.

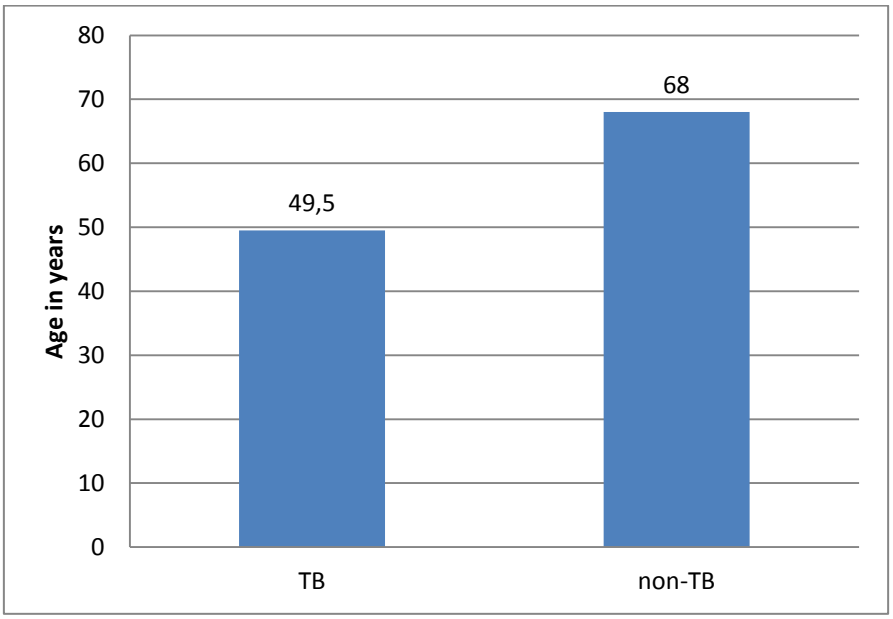
5 Results

5.1 Demographic data

During the study period, the ADA value was determined in a total number of 96 patients with undiagnosed pleural effusions. Complete data sets were available in 84 patients. After identification of all exudates, 65 patients could be included in the analysis.

The mean age of all patients with exudates was 67 years, with a range from 21 to 93 years. In the group of TB confirmed cases the mean age was 49.5 years with a range from 21 to 63 years. The non-TB cases had a mean age of 68 years, ranging from 36 to 93 years.

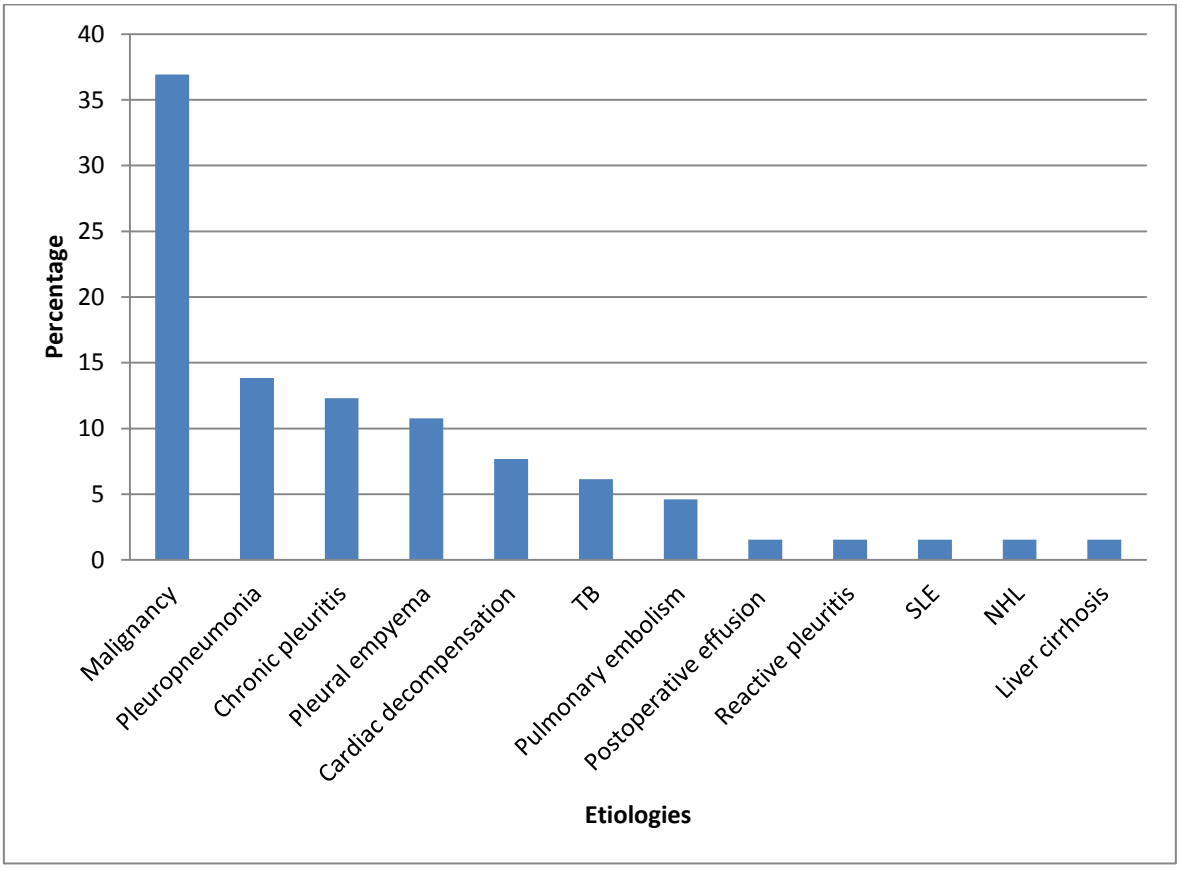
Figure 5 Mean Age in the group of TB and other etiologies



5.2 *Etiologies of exudative pleural effusions*

The main leading cause for the exudates among our patients was in 24 cases a malignant disease (36.9%). Second leading cause was pleuropneumonia with 9 patients (13.9%), followed by 8 cases with chronic idiopathic pleuritis (12.3%), 7 cases with pleural empyema (10.8%), 5 patients with cardiac decompensation (7.7%), 4 patients with TB (6.2%) and 3 cases with pulmonary embolism (4.6%). Postoperative effusions, reactive pleuritis (after a Yersinia infection), systemic lupus erythematosus (SLE), non-Hodgkin's lymphoma (NHL) and liver cirrhosis were represented in one case (1.5%).

Figure 6 Etiologies of exudative pleural effusions in 65 patients



5.3 Laboratory findings

5.3.1 Pleural cell count

The highest total cell counts were found in the group of empyema (mean: 38.23 G/l), followed by TB (mean: 4.66 G/l) and pleuropneumonia (mean: 2.80 G/l).

5.3.2 Neutrophil Granulocytes

The highest percentages of neutrophils in pleural fluid were obtained in empyemas (mean: 79.8%) followed by those in pleuropneumonia (mean: 53.2%) and reactive pleuritis (mean: 41%).

5.3.3 Lymphocytes

Lymphocytes in the pleural fluid analyses were distributed as followed: the highest percentages were found in the TB group (mean: 80.5%) followed by postoperative effusions (mean: 66%) and chronic idiopathic pleuritis (mean: 43.2%).

5.3.4 Lymphocyte-to-Neutrophil Ratio

The highest ratios of lymphocytes to neutrophils were found in TB cases (mean: 50.4) followed by chronic idiopathic pleuritis (mean: 12.8) and NHL (only one case with the ratio of 6.0).

5.3.5 Glucose

The glucose levels were highest in the group of cardiac decompensation (mean: 138.2 mg/dl) followed by liver cirrhosis (mean: 114 mg/dl) and chronic idiopathic pleuritis (mean: 112 mg/dl). The group of empyemas showed the lowest level with a mean value of only 6 mg/dl.

5.3.6 pH Value

As expected, the lowest pH values were measured in the group of empyemas (mean: 7.17), followed by TB (mean: 7.50) and malignant pleural effusions (mean: 7.53).

5.3.7 Pleural LDH

The highest LDH values in pleural fluid was found in the group of empyema (mean: 4134 U/l), followed by malignant pleural effusion (mean: 614 U/l) and TB (mean: 406 U/l). All the other groups reached lower levels.

5.3.8 LDH Ratio

The highest LDH ratio of pleural to blood LDH values were found in the group of empyema (mean: 20.32) followed by those obtained in the TB group (mean: 2.14) and malignant pleural effusions (mean: 2.09).

5.3.9 Pleural total protein

The highest total protein levels were observed in empyema (mean: 5.1 g/dl), TB (mean: 4.7 g/dl) and pulmonary embolism (mean: 4.4 g/dl).

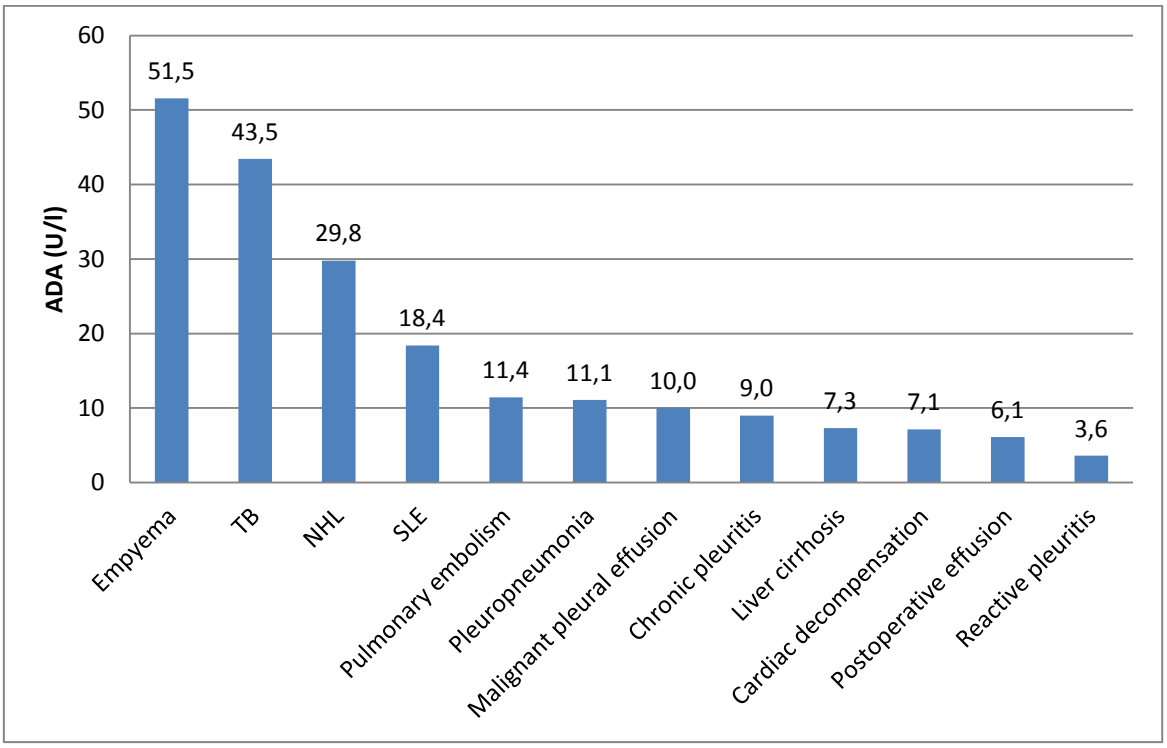
5.3.10 Protein ratio

The pleural to blood total protein ratio showed highest values in the group of pulmonary embolism (mean: 0.68), TB (mean: 0.67) and empyema (mean: 0.65).

5.3.11 Adenosine Deaminase Activity

Analysing the ADA in pleural fluid, empyema yielded in the highest levels (mean: 51.5 U/l), followed by TB (mean: 43.5 U/l) and NHL (mean: 29.8 U/l).

Figure 7 Mean ADA value in exudative pleural effusions



5.4 Tuberculosis

The mean age in the group of TB (n=4) was 49.5 years with a range from 21 to 63 years. Of the four cases two were confirmed by culture, the other two patients gave a high clinical suspicion of TB with exclusion of other etiologies and fully recovery after TB specific treatment.

The pleural effusions in the group of TB showed the following laboratory findings:

Table 4 Laboratory findings in tuberculous pleural effusions (n=4)

	Mean	(range)
Total cell count	4.66 G/l	(1.38 - 12.45)
Neutrophils	4%	(0-13)
Lymphocytes	81%	(77-84)
Ratio Lymphocytes/Neutrophils	50.4	(6.45 - 106)
Glucose	86 mg/dl	(72 - 99)
pH value	7.5	(7.3 - 7.7)
Pleural LDH	406 U/l	(223 - 618)
LDH ratio	2.14	(1.51 - 3.05)
Pleural protein	4.7 g/dl	(4.0 - 5.3)
Protein ratio	0.67	(0.59 - 0.70)
ADA	43.5 IU/l	(31.8 - 52.7)

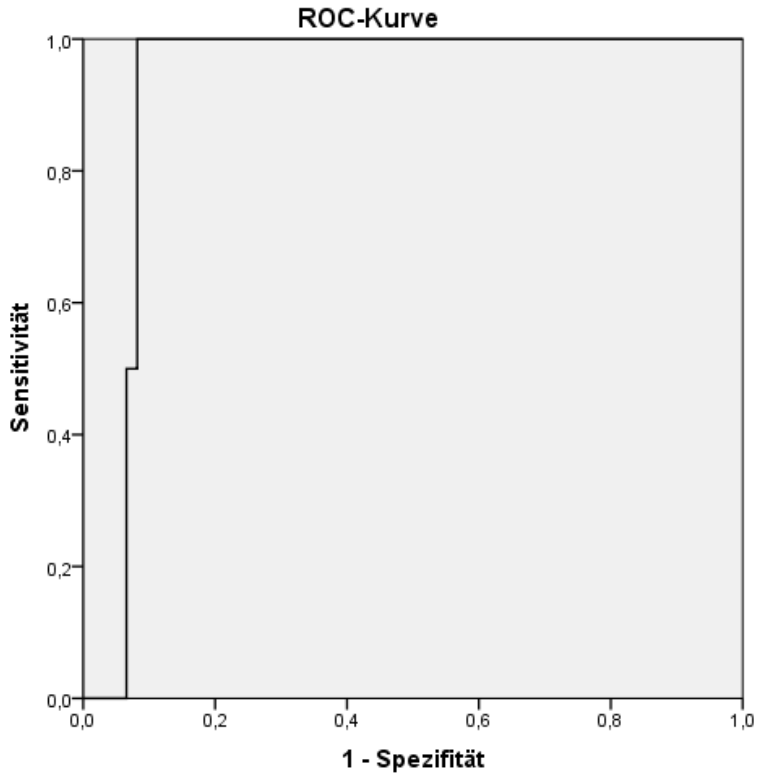
These results emphasize that the tuberculous exudates show high levels of lymphocytes, a high ratio of proteins, lymphocytes-to-neutrophil ratio and ADA values.

5.5 Sensitivity and Specificity

In four patients tuberculous pleuritis was diagnosed while 61 patients had non-tuberculous pleural effusions. Using a cut-off level of 25 U/l the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of ADA for TB pleuritis were 100%, 87%, 33% and 100%, respectively. ADA resulted positive in 6 patients (out of 7) with pleural empyema and one patient each with NHL and lung cancer (out of 24).

5.6 ROC Curve

Figure 8 ROC curve



ROC analysis with an ADA cut-off value of 25 U/l revealed an area under the curve of 0.926 (95% CI 0.86-0.99) for differentiating between patients with TB pleuritis, from those with other diseases.

6 Discussion

Pleural ADA measurement is routinely used in many countries with moderate or high prevalence of TB. It is an inexpensive, rapid and well evaluated test to exclude TPE. In endemic countries non-automated ADA determination with a cut-off level of 35-50 U/l reached high sensitivity and specificity of 86-92% and 88-90%, respectively (55).

In areas with a low TB prevalence the usefulness of ADA determination has been scarcely investigated (56) and for automated determination of ADA on a Cobas® system only one small study from France exists (43).

The aim of this study was to evaluate routine measurement of ADA using the Diazyme® assay on an automated laboratory system in a low endemic area. For this purpose, and as part of the routine biochemical work-up, pleural ADA was determined immediately after thoracocentesis. As proposed by Delacour et al. for the automated determination of ADA using the Diazyme® assay on a Cobas system a cut-off of 25 U/l was applied (43).

65 patients with unknown exudative pleural effusions were included in our study and the pleural ADA as biomarker for the diagnosis of TPE revealed a sensitivity, specificity, PPV and NPV of 100%, 87%, 33% and 100%, respectively. The excellent sensitivity and NPV verify the clinical value of pleural ADA determination as a reliable 'rule out' test for TPE. The specificity in our study (87%) is still in line with the known specificity from other studies using non-automated methods and higher cut-offs (35-50 U/l) (55).

The low PPV (33%) in our study is related to the known limitation of pleural ADA determination if neutrophilic pleural effusions (mainly related to complicated parapneumonic effusions or empyema) are not excluded from analysis (57). The group of empyemas showed the highest levels of ADA (mean: 51.5 U/l ranging from 18.4 U/l to 101 U/l; of the seven cases only one was below the 25 U/l cut-off) and explains, together with the low number of TB cases, the low PPV. Considering exclusively lymphocytic exudative pleural effusions with ADA levels > 25 U/l pleural lymphoma was the only relevant differential diagnosis to TPE in our study. This is consistent with the findings of Garcia-Zamalloa and Taboada-Gomez who suggest combining ADA and lymphocyte for the diagnosis of extrapulmonary TB (57). Sahn et al describe a specificity of 99.5% and sensitivity of 58.4% by

combining ADA values above 45 U/l and a percentage of > 80% of lymphocytes for diagnosing TPE in a moderate high prevalence area of TB (31). We also found a very high lymphocyte-to-neutrophil ratio in the tuberculous exudates. This ratio can also be used in cases of high ADA levels to distinguish between empyema and tuberculous pleurisy (58).

As relevant limitations of our study we admit the short study period (14 month), the resulting low number of patients and especially the low number of confirmed TB cases. To obtain statistical more robust results, the analysis should be repeated in one or two years with a significant higher number of patients. However, the rate of TB cases will not improve, as the location of the study site represents a TB low endemic area. Further investigations should include more pleural effusions caused by lymphoma or by autoimmune diseases, as these entities are also associated with lymphocytic exudative effusions, high levels of pleural ADA and are therefore difficult to distinguish from TPE. Finally, to prove the clinical benefit of routine ADA testing in a TB low endemic setting, a prospective interventional study should be performed, where ADA results are implemented in clinical decision making processes. Possible outcome parameters for such a study would be (A) time between first thoracocentesis and initiation of a specific therapy, (B) time between first thoracocentesis and discharge from hospital, (C) number of invasive procedures after initial thoracocentesis.

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Figures

Tables

Curriculum vitae

PERSONAL DETAILS

Name	Paula Schmidt
Date of Birth	8th of April 1986
Place of Birth	Leribe, Lesotho
Nationality	German
Gender	Female

EDUCATION

Aug 2011 - Jul 2012	Exchange year with the Erasmus Programme at Medical University Marseille, France
Since Oct 2008	Medical studies at the Medical University of Graz, Austria Passed the first diploma exam in September 2009 Passed the second diploma exam in February 2014
2006 – 2008	Studies of Psychology at the Karl-Franzens University of Graz, Austria
1996 – 2005	Secondary school at the Platen-Gymnasium, Ansbach, Germany

PROFESSIONAL EXPERIENCE

Apr 2013 – May 2014	Research assistant at the Clinical Research Centre, Graz

Clinical electives:

Sep 2013	Medical University Graz, Austria Department of Neurology
Sep 2012	Vivantes Klinikum am Urban, Berlin, Germany Department of Internal Medicine
Feb 2012	Hôpital Nord, Marseille, France Department of Infectious and tropical disease
Nov, Dec 2011	Hôpital Nord, Marseille, France Department of Internal Medicine
Oct 2011	Hôpital Timone, Marseille, France Department of Oncology
Sept 2011	Hôpital Timone, Marseille, France Intensive Care Unit

July 2011	Baylor Clinic, Maseru, Lesotho
	Hospital for HIV positive children
July 2010	Clinic Neuendettelsau, Germany
	Department of Orthopaedic Surgery

Practical year:

Mar/Apr 2014	Dr. Gudrun Zweiker, Straden, Austria
	General medicine
June/July 2014	Hôpital du Valais, Visp, Switzerland
	Department of Internal medicine
Sept 2014	Vivantes Klinikum Neukölln, Berlin, Germany
	Department of Dermatology and Venerology
Oct/Nov 2014	Schreiber Klinik, Munich, Germany
	Department of General-, Visceral-, Trauma- and Orthopaedic Surgery

SPECIAL ABILITIES

Basic Medical English I

Basic Medical English II

Basic course Medical Hypnotherapy

Basic course Ultrasonography

SKILLS

Fluent in German, English, French

Fundamentals in Norwegian