DIPLOMARBEIT

VALUE OF ADENOSIN DEAMINASE ACTIVITY (ADA) IN THE DIAGNOSIS OF INFECTIOUS NON-TUBERCULOUS PLEURAL EFFUSIONS WITH SPECIAL REGARDS TO COMPLICATED PARAPNEUMONIC EFFUSIONS INCLUDING EMPYEMA

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Nikolaus John

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Doktor der gesamten Heilkunde
(Dr. med. univ.)

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Klinische Abteilung für Lungenkrankheiten,
Universitätsklinik für Innere Medizin,
Medizinische Universität Graz

unter der Anleitung von

Dr. med. Holger Flick
Priv. Dozent Dr. med. univ. Martin Hönigl
Priv. Dozent Dr. med. univ. Reinhard Raggam

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1. ABSTRACT

1.1 AIM

Pneumonia is a very common disease affecting 5 to 11 per 1000 citizens per year in western countries. [1] [2] It is estimated that up to 57% of these patients develop pleural effusion. [3] Therefore pneumonia is the second most frequent cause of pleural effusion in the western world. [1] If antibiotic treatment is delayed a significant portion of these so called simple parapneumonic effusions (PPE) will progress into complicated parapneumonic effusions (CPE) including empyema (E). [4] In this case drainage and sometimes even surgical intervention is needed. In order to minimize risk of complications, early diagnosis of CPE is crucial. Adenosine Deaminase Activity (ADA) is a pleural fluid marker that is routinely used in the diagnosis of tuberculous pleurisy. It is highly effective in high and low prevalence tuberculosis settings. [5] [6] However, ADA is also known to be elevated in exudative effusions caused by non-tuberculous diseases like CPE including E, lymphoid malignancies and autoimmune diseases. [5] [7] [8] [9] [10] [11] The purpose of the study was to evaluate the benefit of ADA measurement for the detection of these non-tuberculous diseases in a TB low prevalence setting with special regards to CPE including E. To date complex algorithms and various pleural markers are needed to calculate risk of CPE/E. We proclaimed ADA to be an effective and reliable test that can improve current clinical algorithms.

1.2 METHODS

We retrospectively reviewed data from 400 patients with undiagnosed pleural effusion that underwent thoracentesis and subsequent ADA analysis between 30th November 2011 and 1th May 2015. ADA measurement was performed using the automated, standardized-turbidimetric ADA-analysis on the Cobas 8000 system at the Medical University of Graz. Hospitals that participated in this study were the University Hospital of Graz, the State Hospital Graz-West, the State Hospital of Hörgas-Enzenbach, the State Hospital of Feldbach and the State hospital Wagna. The cause of the pleural effusion was established by reviewing results of the patients' conventional pleural fluid analysis, microbiologic results and medical files by a consultant pulmonologist. Based on these end results sensitivity, specificity, PPV and NPV were calculated for CPE and E using different cut off levels for ADA.

1.3 RESULTS

Etiologies of pleural effusions included malignancy (118 of 400 cases), PPE (83 of 400 cases), CPE/E (19 of 400 cases), E (15 of 400 cases), PAE (10 of 400 cases), tuberculosis (14 of 400 cases), cardiac decompensation (90 of 400 cases) and 37 cases of chronic pleural effusion of unknown etiology. Highest ADA values were found in lymphoma, CPE including empyema, and tuberculosis. Mean ADA values were 100 U/l, 98 U/l, 26 U/l and 38 U/l respectively. In comparison, mean ADA values in patients suffering from cardiac decompensation and malignancy were 4.6 U/l and 9.3 U/l respectively. After examination of all currently used pleural fluid markers we concluded that ADA, LDH and neutrophil share are the most accurate laboratory values to diagnose non-TB pleural infection. In order to evaluate the diagnostic value of ADA we used ROC-analysis for the detection of CPE/E. We found that using a cut off level of 13 U/l yielded the most promising results. Sensitivity, specificity, PPV and NPV were 100%, 77%, 18% and 100% respectively. In addition we found that pleural pH and glucose, which currently used in most clinical algorithms for risk stratification of CPE/E, are far less reliable than supposed. Sensitivity, specificity, PPV and NPV for pleural pH using a cut off level of 7.2 were 38%, 98%, 41% and 97% respectively. Sensitivity, specificity, PPV and NPV for pleural glucose using a cut off level of 60mg/dl or less were 82%, 94%, 38% and 99% respectively. Both pleural pH and pleural glucose did not reach sufficiently high sensitivity in comparison to pleural ADA, LDH and neutrophil share. Best sensitivity and specificity for the diagnosis of CPE/E were found in pleural effusions that were negative for malignant cells if ADA cut off level of 13 U/l or above, LDH with a cut off level of 300 or above and neutrophil share of 60% or above were combined. Using these pleural markers in conjunction we found sensitivity, specificity, PPV and NPV for CPE/E of 88%, 98%, 68% and 99% respectively.

1.4 SUMMARY

ADA is a pleural marker that is recommended to detect pleural tuberculosis in low endemic countries. In addition, several studies stated that ADA levels are increased in CPE including empyema, lymphoma and autoimmune diseases. [5 7 8 9 10 11] Therefore we examined the value of pleural ADA in the diagnosis of pleural effusion caused by these diseases with special regards to non-tuberculous pleural infections.

Medical data of 400 patients suffering from undiagnosed pleural effusion that underwent thoracocentesis and subsequent ADA analysis were enrolled in this study. After reviewing all available medical files and defining final diagnosis for all cases we evaluated diagnostic value of ADA using ROC-analysis and evaluating different cut off levels. We found a cut off level of 13 U/l or above to be most promising for the diagnosis of CPE/E. Sensitivity, Specificity, PPV and NPV were 100%, 77%, 18% and 100% respectively. The combination of ADA, LDH and neutrophil share can further improve specificity and positive predictive value (PPV). Best results were found in pleural effusions that were negative for malignant cells. In these pleural effusions the combination of ADA with a cut off level of 13 U/l or above, LDH with a cut off level of 300 U/l or above and neutrophil share of 60% or above showed overall best results. Using these pleural markers we found sensitivity, specificity, PPV and NPV for CPE/E of 88%, 98%, 68% and 99% respectively. We compared this algorithm with currently used algorithms and found overall higher sensitivity, specificity and PPV for the new developed one. Therefore we recommend a prospective study in order to substantiate the benefit of ADA determination in clinical practice.

2. ZUSAMMENFASSUNG

2.1 ZIELSETZUNG

Pneumonie ist eine sehr häufige Erkrankung der unteren Atemwege an der jedes Jahr 5 bis 11 pro 1000 Menschen erkranken.[1] [2] Bis zu 57% dieser Patienten entwickeln im Laufe des Genesungsprozesses einen Pleuraerguss. [3] Aufgrund der hohen Inzidenz der Pneumonie geht man davon aus, dass Pneumonien die zweithäufigste Ursache von Pleuraergüssen sind.[1] Wird eine Antibiotische Therapie nicht umgehend eingeleitet, kommt es in einem signifikanten Teil dieser unkomplizierten Pleuraergüsse zur Infektion der Pleuraflüssigkeit und damit zur Entwicklung eines komplizierten Pleuraergusses welche sich unbehandelt häufig zu Empyemen weiterentwickeln. In diesem Fall sind eine Pleuradrainage und manchmal sogar eine chirurgische Intervention notwendig. Zur Vermeidung von Komplikationen, ist es wichtig die Diagnose des komplizierten Pleuraergusses bzw. des Empyems möglichst früh zu stellen.

Adenosindeaminase Aktivität (ADA) ist ein sensibler und spezifischer Marker für tuberkulöse Pleuritis. Heute wird pleurale ADA in Tuberkulose Hoch- und Niedrig-Prävalenzländern effektiv für die Diagnostik eingesetzt. [5] [6] Es hat sich allerdings gezeigt, dass einige pleurale Erkrankungen einen falsch positiven pleuralen ADA Wert verursachen. Zu diesen zählen komplizierte parapneumonische Ergüsse inklusive Empyeme, Lymphome und Bindegewebserkrankungen. [5] [7] [8] [9] [10] [11] Mit dieser Studie soll der Wert von ADA für die Diagnostik von komplizierten parapneumonischen Erüssen inclusive Empyemen in einem Land mit niedriger TB-Prävalenz untersucht werden. In der aktuellen klinischen Praxis sind komplizierte Algorithmen und eine Vielzahl von pleuralen Laborparametern notwendig um das Risiko eines CPE/E abzuschätzen. Wir hoffen in dieser Studie zeigen zu können, dass die ADA-Bestimmung diese Algorithmen deutlich vereinfachen kann.

2.2 METHODEN

Im Rahmen einer retrospektiven Erhebung von Datensätzen aus dem steirischen Patienten Informationssystem MEDOCS wurden medizinische Daten von 400 Patienten analysiert, bei denen zwischen 30. November 2011 und 1. Mai 2015 ein Pleuraerguss festgestellt und eine Pleurapunktion mit nachfolgender ADA-Wertbestimmung durchgeführt wurde. Die ADA-Wert Bestimmung erfolgte automatisiert, standardisiert-turbidimetrisch durch das Cobas 8000 Systems des Universitätsklinikum Graz. An der Studie teilnehmende Krankenhäuser waren das Universitätsklinikum LKH Graz, LKH Graz Standort West, LKH Feldbach, LKH Wagna und das LKH Hörgas-Enzenbach. Die klinische Enddiagnose wurde nach Durchsicht der im jeweiligen Fall vorliegenden medizinischen Befunde durch einen Lungenfacharzt gestellt. Auf Basis der klinischen Enddiagnose wurde die Sensitivität, Spezifität, NPV und PPV von ADA und anderer bereits in der klinischen Praxis verwendeten Marker mit jeweils unterschiedlichen Grenzwerten für die Diagnostik von CPE und E untersucht.

2.3 RESULTATE

Nach Durchsicht der vorliegenden 400 Fälle durch einen Facharzt der Pulmologie wurden in 118 Fällen die Diagnose Malignität, in 83 Fällen die Diagnose PPE, in 19 Fällen die Diagnose CPE/E (davon 15 Empyeme), in 10 Fällen die Diagnose PAE in 14 Fällen die Diagnose Tuberkulose, in 90 Fällen die Diagnose Kardiale Dekompensation und in 37 Fällen die Diagnose chronische Pleuritis unklarer Genese gestellt. Dabei wurden die höchsten ADA-Werte in den Subgruppen der Lymphome, Empyeme, CPE und Tuberkulose gefunden. Die durchschnittlichen ADA-Werten waren jeweils 100 U/l, 98 U/l, 26 U/l und 38 U/l. Die niedrigsten durchschnittlichen ADA-Werte wurden in der Gruppe der kardialen Dekompensation (ADA 4,6 U/l) und der malignen Pleuraergüsse (ADA 9,3 U/l) festgestellt. Nach Untersuchung aller aktuell verwendeten pleuralen Laborparameter kamen wir zu dem Schluss, dass insbesondere ADA, LDH und der prozentuale Neutrophilen Anteil die höchste Sensitivität für die Diagnose CPE/E aufweisen. Wir untersuchten insbesondere den diagnostischen Wert von ADA und nutzten ROC-Analysen um optimale Grenzwerte für ADA zu finden. Unsere Analyse zeigte, dass ein ADA Wert von mindestens 13 U/l die höchste Sensitivität bei noch ausreichender Spezifität erreichte. Dabei lag die Sensitivität bei 100%, die Spezifität bei 77%, der PPV bei 18% und der NPV bei 100%. Außerdem konnten wir mit unseren Daten zeigen, dass der pleurale pH-Wert und die pleurale Glukose weit weniger sensitiv und spezifisch für die Diagnose CPE/E sind als bisher angenommen, obwohl beide in den aktuell eingesetzten klinischen Algorithmen zur Diagnose von CPE/E zur Anwendung kommen. Der pleurale pH-Wert erreichte bei einem Grenzwert von 7.2 eine Sensitivität von 38% und eine Spezifität von 98%. Die pleurale Glucose erreichte bei einem Grenzwert von 60mg/dl eine Sensitivität von 82% und eine Spezifität von 94%. Unsere Ergebnisse zeigen, dass sowohl der pleurale pH-Wert als auch die pleurale Glucose eine zu niedrige Sensitivität erreichen. Wir kommen daher zu dem Schluss, dass die bisher in der Klinik verwendeten Algorithmen für die Diagnose CPE/E, in welchen pleuraler pH-Wert und pleurale Glukose eine nicht zu vernachlässigende Rolle spielen, in Frage gestellt werden sollten. Für Ergüsse ohne Nachweis von malignen Zellen entwarfen wir daher mit Hilfe der ROC-Analyse einen neuen Algorithmus. Für diesen nutzten wir ADA mit einem Grenzwert von mindestens 13 U/l, LDH mit einem Grenzwert von mindestens 300 U/l und einem prozentualen Neutrophilen Anteil von mindestens 60%.

Dieser Algorithmus erbrachte für die Detektion von CPE/E eine Sensitivität von 88%, eine Spezifität von 98%, ein PPV von 68% und ein NPV von 99%.

2.4 ZUSAMMENFASSUNG

ADA ist ein pleuraler Marker der heute in der klinischen Praxis für den Ausschluss von Tuberkulose bei Patienten mit suspekten Pleuraergüssen eingesetzt wird. Eine Vielzahl von klinischen Studien hat in der Vergangenheit zeigen können, dass auch komplizierte parapneumonische Ergüsse, Empyeme, Lymphome und Autoimmunerkrankungen erhöhte pleurale ADA Werte verursachen. [5 7 8 9 10 11] Daher untersuchten wir in der vorliegenden Studie den Wert von pleuraler ADA in der Diagnose dieser anderen non-tuberkulösen Ursachen von Pleuraergüssen mit speziellem Augenmerk auf komplizierte parapneumonische Pleuraergüsse und Empyeme. Hierfür verwendeten wir 400 Datensätze aus der steirischen Medizindatenbank MEDOCS von Patienten bei denen ein Pleuraerguss unklarer Ursache festgestellt und anschließend eine Pleurapunktion mit ADA-Wert Bestimmung durchgeführt worden war. Nachdem alle zur Verfügung stehenden medizinischen Unterlagen evaluiert worden waren stellte ein Pulmologe die wahrscheinlichste zugrundeliegende Enddiagnose des Pleuraergusses. Anhand dieser Enddiagnosen wurde anschließend der diagnostische Wert der ADA mit unterschiedlichen Grenzwerten untersucht. Mit Hilfe der ROC-Analyse konnte gezeigt werden, dass ein ADA Grenzwert von mindestens 13 U/l die vielversprechendsten Ergebnisse erzielte. Die Sensitivität, Spezifität, PPV und NPV erreichten jeweils 100%, 77%, 18%, und 100%. Neben der ADA erzielten LDH und der prozentuale Neutrophilen Anteil die besten Ergebnisse. Daher versuchten wir die aktuellen klinischen Algorithmen zur Diagnostik der CPE/E durch den Einsatz dieser Marker zu verbessern. Die Kombination von ADA mit einem cut off Wert von mindestens 13 U/l, LDH mit einem cut off Wert von mindestens 300 U/l und der prozentuale Neutrophilen Anteil von mindestens 60% in einem neuen Algorithmus erwies sich als sensitiv und spezifisch. Der neue Algorithmus erreichte eine Sensitivität von 88%, eine Spezifität von 98%, einen PPV von 68% und einen NPV von 99%. Diese Sensitivität und Spezifität ließ sich mittels keines anderen Algorithmus reproduzieren. Aus diesem Grund empfehlen wir eine prospektive Untersuchung zur ADA-Bestimmung als Teil des von uns vorgeschlagenen Algorithmus in Form einer prospektiven Studie um den Wert von ADA- Messungen in der klinischen Praxis zu erhärten.

Table of content

ABBREVIATIONS

PPE	Parapneumonic pleural effusion
CPE	Complicated parapneumonic effusion
E	Empyema
TB	Tuberculosis
CAP	Community acquired pneumonia
HAP	Hospital acquired pneumonia
HCAP	Health care associated pneumonie
CRP	C-reactive protein
PCT	Procalcitonin
NPV	Negativ predictive value
PPV	Positive predictive value
TNF	Tumor-necrosis factor
BAL	Bronchoalveolar Lavage
NAAT	Nucleic acid amplification test
LR	Likelihood ratio
PSI	Pneumonia severity Index
CT	Computed tomography
AFB	Acid-fast bacilli
t-PA	Tissue plasminogen activator
ESBL	Extended spectrum ß-lactamase
MRSA	Methicillinresistant *S. aureus*
LRTI	Lower respiratory tract infection
COPD	Chronic obstructive pulmonary disease
NICE	National Institute for health- and care-excellence
PCR	Polymerase Chain Reaction
LR	Likelihood Ratio
SaO_2	Oxygen saturation in arterial blood
PO_2	Oxygen partial pressure

3. INTRODUCTION

Pleural effusion is a very common medical condition affecting 3000 people per million inhabitants each year. [12] It is defined as pathological accumulation of pleural fluid in the pleural cavity. Small pleural effusions can be compensated easily. Yet if pleural effusion reach a critical level it will comprise lung tissue and thus impair respiratory function. Especially if complicated by other cardiorespiratory diseases pleural effusion may have grave impact on clinical course of affected patients. Therefore prompt evaluation is necessary in order to diagnose the underlying medical condition and establish proper therapy. [4]

3.1 PLEURAL CAVITY

3.1.1 ANATOMY

The pleura consist of two membranes that protect the lungs, allow them to move, contribute to their shape, and prevent the alveoli at the pleural surface from becoming over distended. The visceral pleura cover the lung while the parietal pleura cover the diaphragm, the mediastinum and the chest wall. Between these two pleural leaves the pleural cavity is formed. [13] Due to the anatomic features of the thorax and its adjoining anatomic compartments several recessus are formed. Of these the recessus costodiaphragmaticus, which originates between the lateral thorax wall and the diaphragm, is the clinically most important. Minor pleural effusion typically accumulates first in recessus costodiaphragmaticus leading to a blunting of the costophrenic angle on thoracal chest radiography. [13]

3.1.2 PHYSIOLOGY

In a healthy pleural space the pleural fluid is usually low in protein and lubricates the two layers of the pleura and thus allows them to slide smoothly over each other during respiration. Therefore pleural fluid is essential for the respiratory function of the lung. In a healthy adult individual the filtration and absorption of pleural fluid is constant and usually ranges between 5 and 10 mL. [14]

The physiological basis for pleural fluid movement was first described by Starling and Tubby (Figure 1). They proclaimed that pleural fluid transport is mainly driven by hydrostatic pressure, membrane permeability and oncotic pressure. In their believe hydrostatic pressure forces to filter water out of the vessels of the visceral pleura into the pleural cavity while oncotic pressure causes a reabsorption of water back into the vessels and therefore antagonises hydrostatic pressure. [15] Their hypothesis today forms the basis for understanding fluid accumulation in the pleural space. Filtrated pleural fluid is absorbed by the lymphatic system on the diaphragm and the mediastinal surfaces of the parietal pleura. [16]

As long as filtration and absorption are in balance the amount of pleural fluid is constant. Yet if this balance is impaired pathological pleural fluid accumulation is the consequence, which may ultimately inhibit proper respiratory function. As described by the Starling equation the reasons for accumulation of pleural fluid is an excessive filtration due to increased hydrostatic pressure, an increase in vessels membrane permeability, or a decrease in oncotic pressure. Furthermore diseases that restrict lymphatic reabsorption of pleural fluid may also cause pleural effusions. [15] [16]

Starling EH, **Tubby** A.
On absorption from and secretion into the serous cavities. J Physiol. 1894;16:140-155.

$$Q_F = L_P \times A[(P_{CAP} - P_{PL}) - \zeta_D(pi_{CAP} - pi_{PL})]$$

Hydrostatic pressure — membrane permeability — oncotic pressure

QF is fluid movement
LP is the filtration coefficient
A is the surface area of the pleura
ζD is the reflection coefficient for protein movement across the pleura (PL)
P is the hydrostatic pressure of the pulmonary capillary bed (CAP)
pi is the oncotic pressure of pleural space

FIGURE 1 FLUID MOVEMENT EQUATION IN SEROUS CAVITIES PROCLAIMED BY STARLING EH. AND

4. ETHIOLOGY OF PLEURAL EFFUSION

In developed countries most common cause of pleural effusion include heart failure, pneumonia, pulmonary embolism malignancy, viral disease, liver cirrhosis and renal failure. [1]

TABLE 2
Leading Causes of Pleural Effusion in the United States*

Cause	*Annual incidence*	*Transudate*	*Exudate*
Congestive heart failure	500,000	Yes	No
Pneumonia	300,000	No	Yes
Cancer	200,000	No	Yes
Pulmonary embolism	150,000	Sometimes	Sometimes
Viral disease	100,000	No	Yes
Coronary-artery bypass surgery	60,000	No	Yes
Cirrhosis	50,000	Yes	No

**–Based on analysis of patients subjected to thoracentesis.*

Reprinted with permission from Light RW. Clinical practice. Pleural effusion. N Engl J Med 2002;346:1971.

FIGURE 2 LEADING CAUSES OF PLEURAL EFFUSION IN THE UNITED STATES LIGHT RW. N ENGL J MED 2002; 346:1971 (1)

Yet if only unilateral exudative pleural effusions are considered one must acknowledge the possibility of more sinister causes of pleural effusion. Most common causes of unilateral exudative pleural effusion include PPE, pleural tuberculosis, malignancies including lymphoma, and autoimmune diseases. [1]

4.1 PNEUMONIA

4.1.1 INTRODUCTION

With an incidence of 300000 cases per year pneumonia is the second most frequent cause of a pleural effusion in the United States of America. [1] 30 to 57% of all patients suffering from pneumonia develop pleural effusion. [3] Early diagnosis and proper management are crucial to prevent possible complications of pleural effusion. Yet definite diagnosis of PPE and exclusion of other more sinister causes remains difficult.

4.1.2 DEFINITION AND ETHIOLOGY

Pneumonia is an infectious disease of the lung tissue that manifests in the lower respiratory tract. The infection affects the alveoli or the peribronchial interstitium. As soon as pathogens are recognised by the immune system, immune response leads to immigration of immune cells and inflammation of the lung tissue, which causes in most cases formation of a mucopurulent excretion that fills alveoli and impairs respiratory function of the infected lung. [17] Morbidity and mortality depend on the underlying pathogen's virulence and the host's immune status. Pneumonia usually is divided into community acquired and hospital acquired forms. This delimitation is important since both forms differ in causal pathogens, antibiotic resistance and clinical course. [18]

4.1.2.1 COMMUNITY ACQUIRED PNEUMONIA (CAP)

CAP is defined as acute microbiological infection of the lung tissue that develops in individuals that did not acquire this pathogen during or within 4 weeks after clinical hospitalisation. [18] Worldwide Community acquired pneumonia is the most frequent infectious cause of death. [19] In Germany it is estimated that 400000 to 600000 individuals sicken every year. [18] [20] In Europe the annual incidence of CAP ranges from 5 to 11 per 1000 persons, with most cases occurring in the winter. [21] [22]

4.1.2.1.1 MICROBIOLOGY

Studies concerning etiology of community acquired pneumonia often arrive at very different conclusions. This may be due to seasonal, local or technical differences in analysis.

However, there is broad consensus that the most relevant microbiological agent remains *S. pneumonia* with 25 to 45% of all diagnosed cases. [23] [24] [25] [26] [27] Another important pathogen is *H. influenza* which causes around 12% of documented cases (13) Infection with *H. influenza* is more likely in patients witch chronic lung disease like chronic bronchitis. [28] Globally so called atypical pathogen such as *M. pneumonia*, *Chlamydia,* and *Legionella spp*. account for an estimated 22% of instances. [26] Yet in Germany epidemiologic data gathered by CAPNETZ contradicts these findings. They could show that infections with atypical pathogen are much less common than stated previously. They stated that infections caused by chlamydia spp. account for less than 1% of diagnosed cases. [29] Incidence of legionella infections seems to be equally rare, accounting for less than 5% of infections. [26] In recent years advances in the molecular biology diagnosis and widespread availability of molecular biology tests has led to higher rates of viral detection in patients suffering from CAP. Therefore it should not surprise that in up to one third of CAP respiratory virus are found. Most common pathogens include *influenza*, *rhinovirus*, and *coronaviruses*. [30] Yet defining viral pathogen as primary causative agent of CAP remains difficult. [30] Other rare pathogens include *S. aureus*, *Pseudomonas aeruginosa* and *Enterobacteriaceae*. These pathogens may be resistant to standard empirical therapy regimen and therefore are associated with an increased risk of mortality. [31] [32]

4.1.2.1.2 STRECTOCOCCUS PNEUMONIAE

Streptococcus pneumonia is a gram-positive, alpha haemolytic, oval diplococcus, surrounded by a polysaccharide capsule. It is a facultative anaerobic and member of the genus Streptococcus that belongs to no Lancefield group. [33]

While *S. pneumoniae* colonises upper respiratory tract of 40-90 percent of children colonisation of the elderly is currently believed to be far lower. [34] If adults are concerned early epidemiologic studies in the beginning of the 20ths century showed that 45-60% of all healthy adults carried *S. pneumoniae* in their saliva. [35]

More recent studies found pneumococcal carriage in only 5% of adult individuals. [36] [37] This discrepancy may be caused by the introduction of vaccination and antibiotics or differences in test methods. [35] Infections with *S. pneumonia* can be divided in non-invasive respiratory infections such as sinusitis, pharyngitis and pneumonia and invasive pneumococcal disease (IPD) such as sepsis and pneumococcal meningitis. [38]

Disease occurs most likely in susceptible individuals, such as elderly, immunocompromised or children. [39] Transmission to uncolonised individuals occurs per droplet infection, such as coughing or sneezing.

4.1.2.1.3 Antibiotic Resistance

The annual AURES committee report on antibiotic resistance states that in 2013 the overall antibiotic resistance of *S. pneumoniae* in Austria is low. It seems that aside from tetracyclines there is no significant gain in antibiotic resistance. Fortunately resistance of S*. pneumoniae* against penicillin G has decreased and range between 0.3 and 0.7%. It has been stated that even in cases of intermediate susceptibility penicillin G, aminopenicillines and cephalosporins of group 3a are sufficient in therapy of LTRI caused by *S. pneumoniae*. In regard to macrolides level of resistance remains relatively high but stable. In 2013 80 percent of cultured *S. pneumoniae* were susceptible while 17.5 to 19.4 percent were resistant. Resistance against tetracyclines has increased and reached 15-20% of cases. Yet since tetracycline play no important role in the therapy of lower respiratory tract infections (LTRI) this fact is of little importance. *S. pneumoniae* is still fully susceptible to aminoglycosides. [40] [41]

4.1.2.2 Hospital acquired pneumonia (HAP)

HAP is defined as acute microbiological infection of the lung parenchyma that develops in adult individuals more than 48h after admission to hospital and therefore did acquire the causative pathogen inside hospital environment. [42] [43] If this infection occurs within first 96 hours of admission this condition is defined as early-onset HAP. For infections that take place beyond this time the term late-onset HAP is used. [44] [45] This terminology has been established to assess the rate of antibiotic resistance in a patient's endogenous flora. [42] [43]

HAP is one of the most common nosocomial infections with a pooled rate of 6.1 per 1000 discharges. [46] Due to higher rates of antibiotic resistance, poor immune status and comorbidities of most patients contracting HAP, mortality rate ranges from 20 to 50 percent. [42] [47] [48] [49]

4.1.2.2.1 MICROBIOLOGY

In most cases HAP is an endogenous infection that is caused by community acquired or hospital acquired pathogens that colonize the host. After initial colonization of the respiratory tract micro aspiration of oropharyngeal secretion frequently triggers the disease. [50] [51] Infection is more likely if hosts defences are impaired of if the causative pathogen is highly virulent. [50] The most frequent defined pathogens in patients with HAP include gram-negative bacilli and gram-positive cocci. Gram-negative bacilli were found in 35 to 80 % of patients suffering from HAP. Most important pathogens are *E. coli, Klebsiella pneumoniae, Enterobacter spp., Pseudomonas aeruginosa, Acinetobacter spp.* Gram-positive cocci were found in 9 to 46%. [52] [53] [54] Most important gram-positive pathogen are *Staphylococcus aureus*, including *MRSA* and *Strepptococcus spp*. Viral and fungal infections are far less common. [55] The most important pathogen, also referred to as core pathogen are *Streptococcus pneumoniae*, *Streptococcus species, Haemophilus influenzae*, *Enterobacteriaceae (e.g Klebsiella pneumoniae, E. colie, Enterobacter spp.*) as well as methicillin-susceptible *Staphylococcus aureus*. [52] [53] [54]. An empirical antibiotic therapy has to include at least all core pathogen and should only be administered after culture samples have been taken.

4.1.2.2.2 ANTIOBIOTIC RESISTANCE

The most important antibiotic resistance gene in gram-negative pathogen is ESBL. These enzymes are produced by gram-negative pathogen and effectively inactivate beta-lactamases including third-generation cephalosporin's, trimethoprim sulfamethoxazole and fluoroquinolones. ESBL is often found in *Klebsiella* and *E. coli spp*. yet it can easily be transmitted to other *Enterobacteriaceae*. [56]

4.1.2.3 HEALTHCARE-ASSOCIATED PNEUMONIA (HCAP)

Healthcare-associated pneumonia is defined as acute microbiological infection of the lung tissue that develops in non-hospitalized patients who came in extensive contact with healthcare facilities. This contact is defined as residence in a nursing home or other long–term care facility, hospitalization for at least two days within the prior 90 days or attendance at a hospital. [47] [57]

HCAP was first introduced in 2005 by the American thoracic society guidelines to highlight the risk of multidrug-resistant pathogen in patients coming from community settings with frequent health care contact. In general, clinical and microbiologic features of HCAP do deviate from CAP and tend to be more similar to HAP. Yet the incidence of HAP specific core pathogen and rates of antibiotic resistance in HCAP patients varies. [57] [58] A retrospective cohort study in the USA compared causative pathogen of HCAP, CAP and HAP and came to the conclusion, that the rate of *S. aureus* infection is significantly higher in HAP (47 %) and HCAP (49%) patients than in CAP (27%) patients. Furthermore *P. aeruginosa* was significantly more frequently detected in HCAP and HAP patients than in CAP patients. [57] These results contradict a prospective multicentre case-control study from Spain, which could show that the microbial etiology did not significantly differ. The most important pathogen detected in both CAP and HCAP patients was *S. pneumoniae* (55 vs 51 %). [59] Yet this study also showed that HCAP usually has a higher mortality rate than CAP.

4.1.3 DIAGNOSIS

4.1.3.1 PATIENT HISTORY, CLINICAL SIGNS AND PHYSICAL EXAMINATION

PATIENTS HISTORY: Since patient history is essential to guide initial empirical antibiotic therapy an in-depth performed patient history is an important first step in the diagnostic process of pneumonia. If a patient who suffers from chronic lung disease pathogen like *H. influenza* are more frequent. Furthermore, illnesses like COPD and mucoviscidosis are associated with usually rare pathogen like *S. aureus* and *P. aeruginosa*. [28] [60] *P. aeruginosa* is also more common in immunosuppressed individuals. A therapy of an equivalent of 10 mg prednisolon per day for more than 4 weeks is associated with a significant rise in *P. aeruginose* and *Legionella spp*. infections. [61] Infections caused by *Legionella spp*. more often affect individuals who travelled to countries of high *Legionella* burden and low hygiene standards or personal of special water processing crafts. [61] Patients from residential care homes have reportedly higher rates of resistant *Enterobacteriaceae* and *S. aureus*. [62] [63]

If a patient has received antibiotic therapy in the last 3 months causative pathogen may show increased resistance against the prescribed antibiotic group. [64] [65] In addition, fluoroquinolones are associated with emersion of multi drug resistant pathogen including *MRSA*, while cephalosporin intake seems to increase rates of ESBL positive gram negatives. [66] [67]

CLINICAL SIGNS: Typical clinical signs include productive cough, fever chills, tachycardia and tachypnea. [68] In severe cases patient's general condition rapidly declines and tachypnea is aggravated to dyspnoea which reflects the body's desperate struggle to maintain respiratory function. If inflammation affects the pleural cavity patients may suffer from pleuritic chest pain. [69] [68] The physician's judgment based on the clinical impression of a patient can be useful to rule out the diagnosis of pneumonia. The negative likelihood ratio [LR-] equals 0.24 in recent studies. [70] Therefore missing of typical clinical signs such as fever and mucopurulent sputum significantly reduce the probability of pneumonia. [71] However, classical symptoms may miss or be subtle in nature in older patients. Instead these patients often experience weakness, tachypnea, and a decline in general condition of acute onset and mental dysfunction. [2]

PHYSICAL EXAMINATION: Clinical signs and physical examination have an overall high positive likelihood-ratio for the diagnosis of pneumonia. If physical examinations are compared asymmetric breath sounds, pleural rubs, egophony and increased fremitus show the highest specificity for pneumonia (LR+ = 8.0). [71] Unfortunately these signs are far less common than rales or bronchial breath sounds yet if present pneumonia is very likely. Pathologic breath sounds occur frequently but are very unspecific in comparison to other clinical signs and chest radiography. [72]
However several studies stated that positive predictive value of physical examination does not exceed 50%. Therefore one has to conclude that physical examination alone is insufficient in making the diagnosis pneumonia. [73]

4.1.3.2 IMAGING

CHEST RADIOGRAPHY: Imaging of the chest is essential in the diagnosis of pneumonia. Historically chest radiograph is the most common used imaging technique. It is available at any hospital, easy to perform and can be applied at the bedside. Therefore chest radiography should be performed in all patients in whom pneumonia is suspected. [74]

Chest radiography has an overall diagnostic accuracy for pneumonia of 75 % if compared to CT as gold standard. [75] It can be used to estimate severity of illness and may allow conclusions on the causative pathogen. If lobar consolidation, cavitation and pleural effusion are present this suggests pneumonia of bacterial aetiology, while diffuse parenchymal shadowing is associated with legionella or viral pneumonia. [68]

COMPUTED TOMOGRAPHY (CT): It is generally agreed that CT is the most precise imaging technique for the diagnosis of thoracic pathologies. [75] Yet due to high costs, and lack in availability CT should be performed only to exclude other causes of lung consolidation such as pulmonary embolism or cancer. Furthermore CT can be applied in order to exclude complications of pneumonia such as lung abscesses or pleural effusion. [69]

LUNG ULTRASOUND: Lung ultrasound is a relatively new diagnostic procedure in the diagnosis of pneumonia. It does not use radiation and therefore can also be performed in pregnant women. Another advantage is the widespread availability of ultrasound and the possibility of dynamic evaluations. A recent meta-analysis stated that lung ultrasound proved to be a useful method in diagnosis of pneumonia in adults. [76] They found an overall sensitivity and specificity of 94 and 96% respectively. [77] If compared to chest radiography ultrasound examinations show higher diagnostic accuracy and consume less time. [78]

4.1.3.3 LABORATORY TESTING, MICROBIOLOGY AND DIAGNOSTICS FOR ATYPICAL PATHOGENS

LABORATORY TESTING: Blood testing may contribute to diagnosis and thus should be performed in all patients with clinically suspicion of LRTI. Inflammatory markers such as leucocyte cell count, C-reactive protein (CRP) and Procalcitonin (PCT) are of particular importance. A white blood cell count greater than 10400 per mm^3 and an elevated CRP value of more than 5 mg per dl show positive likelihood-ratios of 3.4 and 3.1 respectively. [79] Non-pathologic values do not exclude pneumonia. Negative likelihood-ratios were found to be 0.52 and 0.7 respectively. [79] It has to be concluded that while elevated leucocyte cell count and CRP value do substantiate suspicion of pneumonia these findings are not very specific and therefore are only modestly helpful in making the diagnosis. [79] Yet according to the 2014 NICE guidelines regarding CAP, CRP can be used to guide antibiotic therapy. They do not recommend offering antibiotics in CAP patients with a CRP below 20 mg/l in which clinical suspicion of pneumonia is low. [80] Another important inflammatory marker is PCT. Elevation in PCT indicates bacterial infection with high diagnostic accuracy. If PCT rises above 0.25 µg/l antibiotic therapy is indicated. Values below 0.1 µg/l discourage antibiotic therapy. In this case infection with nonbacterial pathogen is likely. [81] A recent meta-analysis showed that PCT is more reliable and accurate than CRP in the guidance of antibiotic therapy. They reported halved antibiotic exposure time and lower costs without changes in mortality or therapy failure for PCT guided antibiotic therapy. [82] [83] While most important items In order to evaluate severity of pneumonia are pO_2 and SaO_2 organ biomarkers such as NTproBNP may be helpful. [69]

MICROBIOLOGY: Gold standard in the detection of fast growing bacteria that can cause pneumonia such as *S. pneumoniae, H. influenzae, M. catarrhalis, S. aureus, P. aeruginose* and *Enterobacteriaceae* including *E. coli, Klebsiella spp*. etc. are microscopy and culture. However the sensitivity of microbiologic detection remains low. Several studies evaluated the value of blood cultures in CAP patients and found sensitivities of 0 to 16%. [84] [85] [86] In patients with suspected HAP blood cultures were positive in 0 to 40% of cases. [87] Sputum cultures which are much less specific than blood cultures detected pathogen in less than 20% of ambulant patients and 29 to 90% of hospitalised patients. [88]

Furthermore, since many pathogens may colonise a host without causing an infection detection of these microbes does not prove infection. [27] [89] [90] Therefore only detection of pathogen in primary sterile body compartments is evidentiary. Primary sterile body compartments include blood, lung tissue and pleural fluid. In general, appropriate samples include purulent sputum, material from low respiratory tract (BAL), pleural fluid or blood. [91] If samples are collected after administration of antibiotics the rate of false-negative results increases. Thus, samples should be taken before antibiotic therapy is started. [91] Although diagnostic value of microbiology is limited sample collection for gram stain and culture should be performed in hospitalised patients with purulent sputum. [92] [80] The primary value of positive culture lies in the detection of rare pathogen that are not included in empirical antibiotic therapy. Furthermore, positive culture enables execution susceptibility testing which may guide antibiotic therapy. [18]

DIAGNOSTICS FOR ATYPICAL PATHOGENS: Most atypical pathogens of pneumonia are obligatory intracellular pathogens. Therefore, culture and microbiology are not useful in the diagnosis. To detect atypical agents serology tests have been developed, but their clinical use is limited due to their restricted reliability. PCR tests which are done on bronchoalveolar lavage (BAL) fluid or nasopharyngeal swabs are the most accurate test in the detection of *Mycoplasma*, *Chlamydia* and *Legionella spp*. In times of real-time and multiplex-panel PCR results are available within a few hours. [93]

***Legionella spp.*:** Infection with *Legionella spp.* has to be suspected in returning travellers from countries with high Legionella burden and low hygiene standard. Furthermore severe clinical course with high fever (>40°C), multilobulary shadowing in chest radiography and gastrointestinal and neurological signs should rise suspicion of legionella pneumonia. In cases when an infection with Legionella is suspected current guidelines recommend the use of urinary antigen tests. These tests hold reasonable accuracy and achieve results within 15 minutes. [80] Sensitivity for *L. pneumophila* (serogroup 1) were reported to range from 74 and 100% respectively. [94] [95] Due to the very high specificity (99 to 100%), positive test defines *Legionella spp.* as causative pathogen. [94] [95] Yet negative test results do not exclude the diagnosis. Thus, repetition of the test is recommended in cases of strong clinical suspicion. [80]

Mycoplasma spp.: *Mycoplasma* can be cultivated in special cultures. However, cultivation remains difficult and therefore unworkable in a routine clinical setting. [96] Antibody detection kits (EIA) are available yet diagnostic accuracy is variable. In general, elevated IgM levels, which would indicate active infection, are rare and most commonly seen in children who first came in contact with *M. pneumoniae*. (8)

Most adults do have elevated IgG levels and negative IgM levels even in cases of active *Mycoplasma pneumonia*. Therefore, negative IgM levels do not exclude *Mycoplasma* infection. Yet if IgM levels are elevated in a patient with clinical suspicion of pneumonia sensitivity and specificity of 90 and 100% respectively were demonstrated. [97] IgA antibody levels are far more often elevated than IgM and therefore more sensitive and specific for *Mycoplasma* infections. [97]

NAAT's are more practical than culture and more sensitive and specific than serology. [98] [99] [100] Therefore PCR-assays have been established as international standard in the diagnosis of *Mycoplasma*. PCR is usually performed on sputum or BAL samples. Test results should be interpreted with caution since *M. pneumonia* is able to colonise the respiratory tract without causing an LTRI. In cases of CAP with prolonged clinical course (>12d), and initial antibiotic therapy fail NAAT's may be indicated. Yet current guidelines discourage the routine performance of tests to detect M. pneumonia. [80]

Chlamydia spp.: *Chlamydia* is very difficult there is no real gold standard. The best detection seems to be possible with NAAT's. Yet actual sensitivity and specificity of NAAT's are unknown. Studies trying to establish accurate figures of accuracy come to very different conclusions. [96] [101] Very similar to *M. pneumonia, Chlamydia spp.* can colonise the respiratory tract. Therefore, a differentiation between colonisation and infection is difficult. Up to this date a routine diagnostic of *Chlamydia spp*. is discouraged by current guidelines.

Influenza: There are bed side tests for detection of Influenza virus. Results are viable within 15 to 20 minutes. Several studies show an overall sensitivity of 70 to 90 %. [102] In recent years NAAT's did gain in importance in the diagnosis of influenza virus. NAAT's do show higher rates of diagnostic accuracy than other test procedures. Detection of influenza is encouraged because pathogen detection does have clinical consequence in CAP. However, use of NAAT's should be restricted to hospitalised patients who are at high clinical risk. [103] [104]

4.1.4 Therapy

In patients with suspected community acquired pneumonia clinical impression and basic physical and laboratory tests are combined to evaluate the severity of pneumonia. Several severity scores have been established yet the PSI (pneumonia severity index) and the CURB-65 score are the most important. [80]

CRB-65 / CURB-65 RISK EVALUATION: CRB-65 is a clinical score that calculates risk of mortality in patients suffering from CAP. It can be applied in clinical and primary care settings using different items. In either case CRB65 is calculated by giving 1 point for each of the following prognostic features: Confusion (new disorientation in person, place or time), raised respiratory rate (30 breaths per minute or more), low blood pressure (systolic <90mmHg or diastolic less than 60mmHg), age of 65 or more. [105] A Score of 1 equals a low mortality risk (less than 1% of mortality), score of 1 to 2 equal an intermediate risk (1 to 10% mortality) and scores of 3 to 4 equal a high risk (more than 10% mortality). [105] In hospitalised patients CURB-65 score is applied which adds raised blood urea nitrogen over 7mmol/l to the previous test items. CURB-65 score of 0 to 1 equals a low risk (less than 3% mortality risk), a score of 2 equals an intermediate risk of 3 to 15% mortality and a score of 3 to 5 equals a high risk of more than 15% mortality. [106] Patients with low clinical risk profile should not be hospitalised. Patients with intermediate risk profile should be hospitalised. A microbiological examination should be performed. Patients with high risk profile should be transferred to ICU and microbiological examination should be performed. [69]

RESPIRATORY SUPPORT: Another important test to evaluate severity of pneumonia is arterial gas analysis and oxygen saturation of arterial blood. New decrease of SaO_2 <92 % should indicate hospital admission and further monitoring. [107] It has to be stressed that oxygen saturation is an important marker for outcomes [107] Respiratory support is needed in case of respiratory failure due to pneumonia. In this case delay of respiratory support increases mortality. [108]

ANTIBIOTIC THERAPY OF CAP: Antibiotic therapy should be administered as soon as possible in patients with clinical suspicion of CAP. Preferably, first dose should not be delayed more than 4 to 8 hours of hospital arrival. [109] In unstable patients suffering from septic shock administration should be performed within the first hour after diagnosis. Delay of antibiotic therapy is associated with significantly higher mortality. [109110] The coverage of S. pneumoniae and atypical pathogens in intermediate and high severity cases of community acquired pneumonia is recommended.[2 80 90] While dual coverage in low and intermediate severity patients is still debated there is evidence that coverage of typical and atypical pathogens is beneficial in high severity patients.[2 90 80] Commonly administered antibiotics include β-lactam-antibiotics, macrolides or respiratory fluoroquinolone. Antibiotic regimen depends on clinical severity of pneumonia. In cases of low severity administration of a single β-lactam-antibiotic is sufficient. Based on current resistance reports European guidelines suggest administration of amoxicillin for 5 days. If clinical course of patient does not improve within 3 days extending therapy should be considered. [80] In patients with allergy against β-lactam-antibiotics macrolides or doxycycline can be administered. [80] In cases of moderate and high severity administration of a beta-lactamase stable beta-lactam such as amoxicillin plus clavulanic acid in combination with a macrolide such as clarythromycin for 7 to 10 days is recommended. [80] If therapy fails administration of respiratory fluoroquinolones should be considered. [80] As already stated biomarkers should be used to guide antibiotic therapy duration. In this context PCT or CRP may be applied. To date PCT value seems to be more reliable. PCT values lower than 0.25 µg/l or a decrease of 80 to 90% is strong indicators that antibiotic therapy may be abandoned. [82]

ANTIBIOTIC THERAPY OF HAP: If HAP is suspected in a patient it is of vital importance to assess severity of disease and potential risk factors for resistant pathogen as fast as possible. Antibiotic therapy should be offered as soon as possible and should not be delayed for more than 4 hours. It is recommended that the duration of antibiotic therapy should range from 5 to 10 days based on clinical course and microbiologic findings. In general resistant pathogen are more likely in HAP patients than in CAP or HCAP patients therefore other more aggressive antibiotics are needed. Furthermore, therapy should only be initialised after cultures are obtained.

Cultures and subsequently performed susceptibility tests are essential in targeted therapy of HAP. [80] In basic evaluation of risk factors, it is important to differentiate between early and late onset hospital acquired pneumonia. Late onset of hospital acquired pneumonia is defined as onset of symptoms after at least 96 hours of hospital stay. If pneumonia emerges after this course of time it has to be assumed that resistant or multi drug resistant pathogens are the causative pathogen of pneumonia. [91] Typical resistant pathogens include *MRSA*, *P. aeruginosa* and ESBL positive *Enterobacteroceae*. Therapy of these resistant pathogen demands therapy that is much more aggressive.

Commonly administered antibiotics in empirical therapy of early onset pneumonia with no risk factor for resistant pathogens include third-generation (ceftriaxone or cefotaxime) or fourth-generation chephalosporins (cefepime), beta-lactam/beta lactamase inhibitor combinations (piperacillin-tazobactam) or fluoroquinolones such as levofloxacin or moxifloxacin. Antibiotic agents should be selected in accordance with local hospital microbial pathogens and resistance patterns. [80] After initialisation, therapy should be guided by microbiological findings and inflammatory biomarkers. [87]

Late onset HAP or early onset pneumonia with risk factors for resistant organisms: If there is suspicion of microbial antibiotic resistance or a late HAP more potent therapy is recommended. In this case empirical antibiotic therapy has to target all core pathogen plus *MRSA* and *P. aeruginosa.* Therefore, suggested therapy regimens consist of beta-lactam/betalactamase inhibitor combination (piperacillin-tazobactam) or a third- or fourth-generation cephalosporin or carbapenem (imipenem or meropenem) combined with a fluoroquinolone (levofloxacin or moxifloxacin). If *MRSA* is suspected fluoroquinolones (levofloxacin or moxifloxacin) should be combined with either vancomycin or linezolid. Especially in ventilated hospitalised patients infection with *P. aeruginosa* has to be suspected. In cases of clinical doubt *P. aeruginosa* has to be targeted in antibiotic therapy regimen. Usually an antipseudomonal cephalosporin like ceftazidime or cefepime combined with antipseudomonal fluoroquinolone like levofloxacin is applied. Instead of an antipseudomonal cephalosporin meropenem may be combined with levofloxacin [87]

Signs of severe presentation include hypotension, need for intubation, rapid progression, sepsis or septic shock. If one or more of these signs emerge, aggressive combination therapy is indicated. These aggressive regimens may include a carbapenem plus fluorocquinolone like levofloxacin. If MRSA is suspected the combination of either a carbapenem plus a fluoroquinolone or an aminoglycoside plus vancomycin or linezolid may be applied. [87]

5. PNEUMONIA AND PLEURAL EFFUSION

5.1 EPIDEMIOLOGY

Pneumonia is the major cause of exudative pleural effusion in the western world. It is estimated that 20 to 57 % of patients suffering from pneumonia will develop PPE. [3] [111] [112] These pleural effusions usually remain small and do subside under efficient antibiotic therapy. Yet in 10 to 40 % of cases immigration of microbes into the pleural cavity causes CPE or E. [111] [112] These conditions are associated with increased mortality and morbidity. [113] Therefore prompt diagnosis and accurate therapy is of vital importance.

5.2 PATHOPHYSIOLOGY

If pathogens reach the lower respiratory tract they may cause local infection depending on the pathogens virulence and the immune-competence of the patient. If immune defence is overwhelmed pathogen begin to proliferate within the alveoli. The immune system responds by releasing inflammatory cytokines causing immune cell immigration and local inflammation. In acute phase of the disease mainly neutrophils and monocytes are involved. Leaving the blood stream and entering the infected lung tissue these immune cells are activated by inflammatory cytokines and bacterial proteins. These activated neutrophils and monocytes start phagocytosing pathogens and fuel inflammatory process by releasing further inflammatory cytokines like IL-8 and TNF- α thus increasing vascular permeability. [114] [115] If pleural wall is affected by the inflammatory process, increase in capillary permeability will lead to rapid formation of a primary sterile pleural effusion. Furthermore, local inflammation of the pleura causes characteristic pleuritic chest pain. [112]

This early stage of parapneumonic pleural effusion is called exudative stage. If thoracentesis is performed at this stage of disease pleural fluid is usually clear, with glucose level greater than 60 mg/dL and a pH above 7.20. Cytology shows mainly neutrophils and an overall low white blood cell count. [112] [116] Untreated this stage can progress into the fibrinopurulent stage. In this case migration of microbes into the pleural cavity leads to formation of fibrin clots and membranes that cause septations in the pleural cavity. Since bacterial invasion and subsequent immune response interfere with local fibrinolysis these septations persist and if found in ultrasound or CT are highly suspicious of pleural infection. When pathogen proliferate in the pleural cavity they consume glucose and produce lactic acid and CO_2, which leads to a decrease in pleural glucose and pH. This condition is usually referred to as CPE. [117] [118] If infection continues immigration of immune cells combating proliferation of pathogen will lead to formation of frank pus within the pleural cavity. This condition is referred to as empyema and is considered a severe form of CPE. In CPE and Empyema (CPE/E) antibiotic therapy alone is not sufficient in curing the infection and chest tube drainage is indicated. If invasive intervention is not performed immigration of fibroblasts leads to development of a solid fibrous pleural peel that may restrict lung expansion. This final stage of parapneumonic effusion is called organizing stage. [113]

5.3 MICROBIOLOGY

In general it is believed that the pathogens that cause pneumonia are also causative pathogen in complicated pleural effusion and empyema in most cases. Yet there is a certain degree of discrepancy between the pathogen that cause pneumonia and the pathogen that are usually found in complicated pleural effusion and empyema. This discrepancy is believed to be caused by the hypoxic, acidic environment of the pleural cavity and certain bacterial virulence factors that allow some pathogen to survive these conditions [113] [119]. In general anaerobic and polymicrobial infections are far more frequent in complicated pleural effusion than in CAP or HAP. [112]

5.4 Community-Acquired Complicated Effusions and Empyema

Complicated pleura effusions in cases of CAP are most frequently associated with gram positive aerobic bacteria. The most important gram positive bacteria include *Strepptococcus pneumoniae* which is the causative pathogen in 21% of cases, *Streptococcus milleri* that caused up to 24% of cases and *Staphylococcus aureus* that accounts for up to 14% of cases. [120] interestingly anaerobic organisms are a very frequent finding in complicated pleural effusion account for 12 to 34% of cases. [121] [122] Yet cultivation of anaerobic microbes remains difficult in cultures. The vaccination of sterile blood cultures may increase the sensitivity of microbiology in the diagnosis of anaerobic pathogen.[123] Frequently detected anaerobic pathogens include *Fusobacterium nucleatum*, *Prevotella spp.* and *Peptostreptococcus*. [124] Based on results of studies that examined NAAT's in the diagnosis of pathogen of complicated pleural effusion, it is estimated that anaerobic microbes may be involved in up to 70% of cases (34, 35). [124] Infections with gram negative microbes such as *H. influenzae*, *E. coli*, *Pseudomonas spp*. and *Klebsiella spp*. occur in around 10 % of complicated pleural effusions. [120] [125]

5.5 Health care associated CPE/E

In HAP associated complicated pleural effusion and empyema in more than 50% of cases the detected pathogen was S. aureus. [87] [113] Pleural infections caused by gram negative microbes such as *E. coli, Enterobacter* spp., *Pseudomonas* spp., *Klebsiella* spp. are frequent and often associated with severe clinical course and ICU transmission. [126] Other pathogens (e.g. fungal pathogen) are rare and are most frequently seen in patients with immunosuppression. [127]

5.6 Diagnosis

5.6.1 Patient history

If diagnosis of pneumonia is likely parapneumonic effusion is the most probable cause of pleural effusion as 20 to 57% of patients suffering from pneumonia will develop parapneumonic effusion. [3]

Yet if the underlying disease is unknown and other diseases are just as likely as pneumonia clinical history can be very helpful in indicating appropriate investigation and may even narrow down differential diagnosis. For example, significant weight loss and cachexia could be due to cancer or tuberculosis, joint, skin, or eye symptoms could be caused by connective tissue disorder, known or suspected asbestos exposure may have caused pleural mesothelioma. [128] Furthermore patients history should always include an accurate drug history as a number of medications are able to cause exudative pleural effusions. Most important drugs include MTX, amiodarone, phenytoin, nitrofurantoin and ß-Blockers. [12] [128]

5.6.2 Chest radiography

Chest radiography is a sensitive and specific tool in the investigation of pleural effusion. Hence, it is recommended to perform erect posterior-anterior and lateral CXR in any patient with typical clinical signs. [12] Blunting of the costophrenic angle may be the only sign of an effusion. It is usually caused by pleural effusions that exceed 200 mL of fluid volume but can also be caused by pleural thickening. Small effusions less than 200mL fluid may be missed, especially in recumbent patients and posterior-anterior chest radiographs. Therefore lateral chest radiography and additional views such as the lateral decubitus film and the shoot through lateral supine film can be used in order to detect even small pleural effusions with minimal amount of pleural fluid and pneumothoraxes [129] [130]. In addition CT and sonography can be applied in order to establish the diagnosis. As the effusion size increases a characteristic meniscus may become apparent. [131]

5.6.2.1 Unilateral pleural effusion

Any unilateral pleural effusion evident on chest radiographs is suspect for an exudative pleural effusion and should be investigated by thoracentesis, pleural fluid analysis and if necessary contrast enhanced CT, thoracoscopy and or VATS. [12]

5.6.2.2 Bilateral pleural effusion

The differential diagnosis for bilateral pleural effusions includes causes of transudates. Congestive heart failure is the most common cause of bilateral transudates in patients with clinical or radiological evidence of congestive heart failure, investigation of the effusion need not go any further if there are no conflicting test results or clinical features. [129]

Bilateral effusions usually improve quite quickly once diuretic therapy is started. Diagnostic thoracentesis is required only if a patient has bilateral effusions that are unequal in size, has an effusion that does not respond to therapy with diuretics, has pleuritic chest pain, is febrile, suffers from severe dyspnoea or is hemodynamic instable. [112]

5.6.3 THORACIC ULTRASOUND

Thoracic ultrasound is superior to plain radiography in diagnosing pleural effusion especially in recumbent patients with small effusions. Ultrasound can be used in order to quantify pleural effusions and distinguish pleural fluid from thickening of the pleura with high specificity. If pleural effusion is detected ultrasound may indicate whether or not thoracentesis is needed. In patients suffering from complex, septated or echogenic pleural effusion chances are high that the underlying effusion is exudative. In this case diagnostic thoracentesis should be performed. Yet anechogenic pleural effusion may be of transudative or exudative nature. [132] In the detection of septations within pleural fluid ultrasound is more sensitive than CT-scanning. [133]

A septated appearance of pleural fluid is highly suspicious of pleural infection thus indicating parapneumonic effusion. [129134] Yet septations are also frequently seen in malignancy which is an important differential diagnosis. [134] If thickening of the parietal pleura or the diaphragma or forming of nodules is present pleural malignancy is almost certain. Detection of these alterations shows specificity for pleural malignancy between 95 and 100%. [135] It has to be concluded that ultrasound can be an effective tool in diagnosis and differential diagnosis of pleural effusion.

5.6.4 CT

CT scans can be used to visualize alteration of the thorax wall, the pleura and the lungs themselves. They can provide crucial information for the differential diagnosis of pleural effusion. Especially if pleural thickening is suspected and there is a history of asbestos exposure computed tomography can be vital in order to distinguish benign from malignant pleural pathology. That's why computed tomography should be applied in any undiagnosed exudative pleural effusion. [129] [103] Computed tomography is not as sensitive and accurate for the assessment of pleural fluid as sonography [133] yet there are several signs in pleural effusions that may narrow down differential diagnosis. Homogeneous echogenic effusions in CT scans are most often caused by haemorrhagic effusion or empyema. [136] Air bubbles in pleural fluid may indicate septations witch occur most often in malignancy and complicated parapneumonic pleural infection or empyema's. [134] If an empyema is suspected intense enhancement of the pleura around the pleural effusion this sign does substantiate this suspicion. [137] Even if computed tomography is not as accurate as sonography in detection of pleural fluid alterations it is very effective in differentiation between benign and malignant pleural effusion. Lueng et al. described several typical features of malignancy in pleural alteration using contrast enhanced CT scans of the thorax. They found that nodular pleural thickening , mediastinal pleural thickening, parietal pleural thickening greater than 1 cm, and circumferential pleural thickening are highly specific (94, 94, 88 and 100%, respectively) features of malignant pleural pathology. Sensitivity of these features where 51, 36, 56 and 41%, respectively. [138] These findings where confirmed by several other studies who came to very similar conclusions. [139] If pleural thickening is diagnosed the most likely cause is metastatic disease.

5.6.5 THORACENTESIS

Thoracentesis is an invasive procedure to remove fluid or air from the pleural space for diagnostic or therapeutic purposes. Therefore a cannula, or hollow fine-bore (21G) needle, is carefully introduced into the thorax, generally after administration of local anaesthesia. Removed fluid is used for diagnostic purposes and thereby narrows the differential diagnosis of an effusion. Evacuation of uncalled-for pleural fluid will relieve patient's dyspnoea and better his clinical condition. [129]

5.6.5.1 INDICATION

Thoracentesis is not required if pleural effusion is bilateral, the patient has clinical evidence of cardiac failure or other typical signs of a transudate. Yet if diuretic therapy fails, pleural effusions are unequal in size or associated with other abnormal features like pleuritic chest pain or fever pleural aspiration is recommended. For all unilateral pleural effusions that are larger than 1 cm in height on lateral decubitus x-ray, ultrasound or CT thorax should be aspirated. [140] [141]

5.6.5.2 FLUID APPEARANCE

The typical aspirate consists of a straw-yellow fluid. This finding alone is nonspecific since it occurs in many types of pleural effusion. However, other appearances may be useful in differential diagnostic of pleural effusion. Therefore, any deviation of appearances should be recorded and concerned in process of diagnosis. In general, pleural fluid may appear serous, blood-tinged, frankly bloody, milky or purulent. It is supposed that transudates have a clear appearance. This opinion may be flawed. Villena V. et al [142] conducted a prospective study of 766 consecutive patients with pleural effusion. Only 11 of 82 (13%) transudates had a watery appearance. [142] [143] Most transudate effusions were straw-coloured (67%). Yet a significant proportion was bloody (11%) or turbid (9%). These qualities are often found in exudative effusions. [143]

5.6.5.2.1 BLOOD STAINED FLUID

A uniformly blood-stained fluid with a haematocrit higher than 1 percent is most likely caused by trauma (including recent cardiac surgery), malignancy, pleural embolism, pneumonia, TB or benign asbestos-related effusion. [143] [144] [145] A fluid haematocrit below 1 percent is non-significant. [146] If pleural haematocrit is higher than 50 percent of the patient's peripheral blood haematocrit the patient is diagnosed of a haematothorax. [147]

5.6.5.2.2 TURBID PLEURAL FLUID

A milky turbid appearance is associated with Empyema or Chylothorax. If a turbid pleural fluid sample is centrifuged empyema will form a serous supernatant pellet. In this case milky appearance was due to cell debris. Yet if the pleural fluid sample remains turbid after centrifugation chylothorax or pseudochylothorax are likely. [12] [141] [142]

5.6.5.2.3 Rare pleural fluid appearance

A typically unpleasant smell can indicate anaerobic infection of the pleura. Smell of ammonia suggests urinothorax. If Pleural fluid is black this fact is most likely associated with an Aspergillus infection. [141]

5.6.6 Pleural fluid analysis

Once aspirated, the fluid is sent for biochemical, microbiological, and cytological analyses. Biochemical analyses include determination of protein content, albumin, pH, LDH, glucose, pleural fluid cholesterol and pleural fluid amylase. [12]

5.6.6.1 Transudate versus Exudate

Determining whether a patient has a transudate or an exudate is an important first step in the analysis of pleural fluid and profoundly affects further investigation and treatment. Transudates are more commonly caused by congestive heart failure (80%), Liver cirrhosis and renal failure. [148] When it comes to exudative effusions 75% of exudative effusions are caused by malignancy, pneumonia and tuberculosis. [140] [149]

5.6.6.2 Light's criteria

According to Light's criteria, if at least one of the following three criteria is present, the fluid is defined as an exudate. Pleural fluid protein/serum protein ratio greater than 0.5, pleural fluid LDH/serum LDH ratio greater than 0.6 or pleural fluid LDH greater than two-thirds the upper limits of the laboratory's normal serum LDH. [150] Light's criteria have a sensitivity and specificity for exudate of 97 % and 73 %, respectively. [151] Several studies stated that diagnostic accuracy of light's criteria may be increased by usage of two instead of one positive light criterion. [152] It has to be stressed that in patients with congestive heart failure that undergo diuretic therapy the concentration of protein, lactate dehydrogenase (LDH) and lipids in pleural fluid is increased and, in this context, Light's criteria may misclassify a significant proportion of effusions as exudates. [153] [154] In order to adjust for this flaw pleural effusions in patients with CHF and diuretic therapy with a difference between serum and pleural levels of protein > 31 g/L, should be classified as a transudate instead of exudate. [155] Albumin levels can also be used in this manner: a difference of more than 12 g/L between serum and fluid levels indicates a transudate. [156]

More recent studies conclude that the evaluation of NT-pro BNP is the most effective test in order to correctly identify cardiac transudates that are mislabelled as exudates by Light's criteria. [154] [157] Nevertheless Light's criteria remain robust with diagnostic accuracy of 93 to 96%. [152] [158]

5.6.6.3 TRANSUDATE

If none of the underlined Light's criteria are fulfilled the pleural effusion is regarded as transudate. Transudates are caused by an imbalance in hydrostatic and oncotic forces. [140] The most common causes of transudate pleural effusions are congestive heart failure, liver cirrhosis and renal failure (common cause of hypalbuminaemia). [12] [140] [143] [159] Congestive heart failure is the single most important cause of all pleural effusions and it accounts for the majority of transudate pleural effusion. [160] These transudates occur bilateral and are usually equal in size. [159] Hepatic transudates develop in 6 percent of patients with liver cirrhosis. Hepatic hydrothorax is typically unilateral and right-sided, but may occur bilaterally in up to 16 percent of cases. [161] [162] [159]

5.6.6.4 EXUDATE

If more than one light's criteria are met the underlying pleural fluid is very likely to be an exudate. Yet as already outlined light's criteria may misclassify a significant proportion of transudates as exudate in patients with CHF and diuretic therapy. [153] [154] When it comes to exudates most common causes of pleural exudate are malignancy, pneumonia and tuberculosis. [140] [149] Followed by pulmonary embolism, adverse drug effects, rheumatoid arthritis and other connective tissue diseases. [12]

5.6.6.5 DIFFERENTIAL CELL COUNT

Differential cell analysis is a useful tool in the differential diagnosis of pleural effusion. Fluid lymphocytosis occurs in conditions such as tuberculosis, sarcoidosis, chylothorax, rheumatoid arthritis, and malignant neoplasm, including lymphoma. [145] However any effusion that persist for a longer duration of time is infiltrated with lymphocytes. [12] Pleural fluid predominated by neutrophils is associated with acute inflammation such as parapneumonic effusion and acute pulmonary embolism. [145] Eosinophilic pleural effusions are usually defined as 10% eosinophils of the white blood cells [163] [164].

After years of observation and discussion clinical significance of eosinophil pleural effusion remains controversial. [165] Certain benign conditions are known to be frequently associated with pleural fluid eosinophilia. These include chest trauma and consecutive haematothorax, pneumothorax, repeated thoracentesis [143] [144]. Yet overall diagnostic value of eosinophilia in pleural effusion seems to be low. [163] [164]

5.6.6.6 PLEURAL PH

The pH of normal pleural fluid is around 7.6 if pleural fluid pH level falls below 7.30 this condition is called pleural acidosis.[12] Pleural acidosis occurs in CPE and E, tuberculous pleural effusion, malignancy, connective tissue diseases and oesophageal rupture. [166] [12] It is caused by an increase of metabolic activity of cells or bacteria in the pleural fluid which leads to a consumption of glucose and an accumulation of lactic acid and carbon dioxide. In this case pleural fluid often has a low pH and low glucose concentration [167] In most cases complicated pleural infection or malignancy cause this constellation. Pleural fluid pH levels of 7.20 or below in a patient with suspected PPE is regarded as the most specific discriminator of complicated pleural infection. [168] In this case, chest tube drainage is recommended. [168] A meta-analyses compared the value of pH as indicator of chest tube drainage and concluded that pH alone is the most powerful indicator for chest tube drainage. In comparison pleural fluid LDH and glucose did not improve diagnostic accuracy. [168] However, pleural pH may also be lower than 7.2 in malignancies, tuberculosis and connective tissue diseases. [169]

5.6.6.7 GLUCOSE

In a healthy pleural space glucose diffuses freely across the pleural membrane. Thus pleural fluid glucose concentration equals blood glucose. [12] [144] Pleural glucose concentration below 3.4 mmol/l or 60 mg/ dl is considered pathologic. [12]

This condition is caused by parapneumonic effusion and empyema, malignancy, tuberculosis, and other rare causes. [144] Very low levels of pleural glucose (<1.6 mmol) are found in empyema and connective tissue diseases. [168] [170]

5.6.6.8 LDH

Elevated levels of lactate dehydrogenase occur in lymphoma, tuberculosis, complicated parapneumonic effusions and empyema. Levels greater than 1000 U/L are typically associated with empyema. [12] Furthermore pleural fluid LDH levels are an important part of the light's criteria. Which is gold standard in separation of transudates and exudates as already described. [152] [158]

5.6.6.9 LDH AND GLUCOSE

Although LDH levels alone do not yield a high diagnostic accuracy it is often used in combination with pH and glucose levels in order to predict if pleural drainage or other chirurgical procedures are needed. [168] [171]

Sahn and Light have proposed a cut off level for pH lower than 7.2, a LDH level of at least 3 times normal serum levels or 1000 IU/l and a glucose level of below 60 mg/ dl for surgical drainage. [117] [171] [172]

5.6.7 DIAGNOSTIC ALGORITHM FOR RISK STRATIFICATION OF CPE/E

In currently applied algorithms to assess risk of CPE or Empyema at least 6 parameters (pleural and serum LDH, protein, pleural pH, glucose, neutrophil count) are evaluated. Initially neutrophil count is the most important parameter that is analysed. A Neutrophil share of above 30% makes an acute pleural disease or at least affection very likely. Almost all cases of complicated parapneumonic effusion and empyema are associated with high neutrophil count. If neutrophil count is high, LDH levels above 160 U/l, Protein levels higher than 2.9 G/l, pH below 7.2 or Glucose levels below 60 mg/dl substantiate clinical suspicion of CPE/E. [4] If microbiology shows growth of pathogen a pleural infection and therefore empyema is certain. Histological evidence of malignant cells within a unilateral pleural effusion confirms malignancy and should be excluded previous to evaluation of CPE/E.

5.6.8 MICROBIOLOGY

In patients with an exudative pleural effusion with typical clinical signs of pneumonia or sepsis an infection in the pleural space must be suspected. Thus microbiological analysis of pleural fluid is crucial. Traditionally microbiological examinations of pleural fluid include Gram and Ziehl-Neelson stains and conventional as well as unconventional cultures to detect pathogens.

In recent times PCR was introduced in the clinical diagnostic process. This technique may be highly effective but remains limited to special clinical question. [12 4] The British Thoracic Society recommends to obtain gram stain and micorbiologic culture both aerobically and anaerobically, in all patients with a pleural effusion of unknown aetiology. [12 4] Evaluation of culture sensitivity remains difficult since there is no gold standard to compare results other than cultures. However, the detection of causative pathogen in pleural effusion by culture is rare. Maskell et al. stated gram stain and culture are negative in about 40% of cases of suspected pleural infections. [113]Barnes et al. published even less reassuring data. They examined 525 patients that underwent thoracentesis and pleural fluid analysis. In 476 of these 525 patients cultures were performed (91%) and only 39 culture results were positive. Moreover, 20 of these 39 cultures were contaminants and did not add to diagnosis. Of the 1320 performed pleural fluid cultures only 19 were true positive. [173] In addition, from 357 gram stains, 109 fungal smears and 232 smears for acid-fast bacilli only 2.5% gram stains yielded positive results. Both fungal and the acid fast bacilli smears were never positive at all. Only 1.3% of all microbiologic smears performed turned out to be positive. They concluded that smears as well as cultures yield very low diagnostic value and have almost no impact on clinical process or the patient's outcome. [173] Similar results have been stated by Davies et al [174], Poe et al [175] and Ferrer et al [176] In summary these data suggest to limit the use of cultures and smears especially in patients with clinical suspicion for pleural infection and free flowing non loculated pleural effusions. [173]

5.7 Therapy

5.7.1 Antibiotic Therapy in parapneumonic pleural effusion (PPE)

Empiric antibiotic therapy has to target the most frequent causative pathogen of underlying pneumonia and must be able to penetrate into the pleural cavity. Fortunately almost all antibiotics do penetrate the pleural wall and are affective in the pleural cavity.

Aminoglycosides are impaired by the low pleural fluid pH and therefore should not be administered in PPE. [177] Since anaerobic infections are common in PPE these pathogens have to be targeted by antibiotic therapy. Antibiotics that are effective against anaerobes include beta-lactam antibiotics plus beta-lactamase inhibitors, fluoroquinolones, metronidazole, clindamycin and carbapenems. Therefore, ß-lactam antibiotics in combination with beta-lactamase inhibitors are a reasonable first choice in therapy of community acquired PPE. Even though atypical pathogens are frequent in CAP patients, cases of PPE caused by Legionella spp. or Mycoplasma are very rare. [113] [123] Therefore macrolides are generally not indicated in therapy of PPE. [171] If nosocomial infection is likely presence of resistant pathogen such as MRSA, resistant anaerobes and gram negatives including pseudomonas have to be taken into account. Antipseudomonal antibiotics like ß-lactam/ ß-lactamase-inhibitor combinations (piperacillin-tazobactam) or carbapenem should be therapy of first choice. If MRSA is confirmed by culture, therapy has to include vancomycin or linezolid. Very similar to pneumonia therapy of PPE should be guided by inflammatory markers such as CRP and PCT. In any case antibiotics should be administered for at least 5 days depending on clinical course. [171] As soon as culture results are available, antibiotic therapy should be re-evaluated according to pathogens susceptibility. [61] PPE does usually resolve with antibiotic therapy alone and in most cases no drainage is needed. However, observation should ensure that signs of an evolving CPE are not overlooked. In this case more invasive procedures should not be delayed [178].

5.7.2 Invasive management of PPE

Invasive measures are indicated in patients that suffer from CPE. Yet since definition of complicated pleural effusion is based on imaging techniques and pleural fluid analysis, a diagnostic thoracentesis is a reasonable first step in clinically evident pleural effusion. In general, CPE is defined as a parapneumonic effusion that shows glucose levels below 60 mg/dl, a pleural pH below 7.20 or an elevated LDH over 3 times the upper normal serum limit. [171] If these findings are accompanied by positive culture results or frank pus in the pleural cavity this condition is referred as empyema. CPE including require tube drainage or other invasive procedures. [12] [172] Furthermore, invasive therapy should be considered in CPE if conservative therapy fails and clinical progress is slow. [171]

5.7.2.1 THORACENTESIS

Thoracentesis is defined as single puncture of the pleural cavity without placement of a tube or administration of chemical agent in order to aspirate pleural fluid and ease patients' respiratory discomfort. Thoracentesis is a possible option in the therapy of PPE in absence of septations and in free flowing pleural effusion. In this case it may be possible to evacuate the pleural effusion by a single aspiration. [4] In any case, aspirated pleural fluid should be analysed to evaluate current stage of disease. If pleural effusion reoccurs repeated pleural aspirations are not recommended due to higher complication and mortality rates. [179] [180] Therefore implantation of a chest tube in order to continuously drain pleural effusion should be preferred.

5.7.2.2 TUBE THORACOTOMY

Tube thoracotomy also referred to as chest tube drainage is a technique to drain air or fluid from pleural cavity. This is performed by puncture of the pleural cavity and subsequent insertion of a tube in the pleural space. This tube may then be used to evacuate pleural effusion or air. Tube thoracotomy may also be used to administer medications directly into the pleural cavity. Chest tube drainage is a reasonable escalation of therapy if primary pleural aspiration shows signs of CPE.

It is most effective to drain free-flowing fluid in uniloculated pleural effusions but may also be applied in multicloculated empyema. To date there is conflicting evidence whether large or small tube size is superior in chest tube drainage. Smaller size chest drainage is accompanied with lower complication rate and pain but a higher rate of occlusion. [181] [182] Several studies found no difference in effectiveness of large, medium and small size chest tube drainage. [182] [183] Therefore British thoracic society recommends small-bore drainage with regular flushing and continuous suction in order to prevent occlusion. [4] Chest tubes stay within the pleural cavity until the rate of drained fluid falls beneath 50 ml/day or the pleural cavity is closed.

5.7.2.3 FIBRINOLYTICS

Several randomized controlled trials and reviews evaluated the benefit of pleural administration of fibrinolytic agents such as streptokinase and t-PA. [171] [184] [185] [183] [181] None of these trials could show reduction of mortality in patients treated with fibrinolytics. Several studies found at least improved drainage of loculated parapneumonic effusions and empyemas [186] [187], radiographic appearance, reduction in surgical referral and hospital stay. [184] [185] Unfortunately the administration of fibrinolytics into the pleural cavity is associated with severe complications depending on the applied agent such as haemorrhage [188] [189], fever, leucocytosis and malaise [188] [190]. Therefore, fibrinolytics are not indicated for routine use in complicated parapneumonic effusion. [4] However in selected cases fibrinolytics may be beneficial in the therapy of multiloculary parapneumonic pleural effusion. [4]

5.7.2.4 THORACOSCOPY

Thoracoscopy is the insertion of a small and flexible endoscope inside the pleural cavity via a small intercostal cut. This technique can also be performed in local anaesthesia. Therefore, it is especially useful in multimorbid patients that are unfit for general anaesthesia. [171] In the therapy of PPE, thoracoscopy can be applied to open loculations in order to form a single pleural space that can be drained by chest tube. Therefore at least one author recommends performing thoracoscopy before chest drain insertion. [191] While other state the procedure should be applied after initial fail of chest drainage or when loculations need to be resolved. [192] [193]

Indication of thoracoscopy in therapy of PPE are chronic empyema, multiloculary pleural effusion, and decortication. [194] [195]Success rates of thoracoscopy in these indications range from 60 to 90% [196]. Several studies showed that thoracoscopy is superior to intrapleural fibrinolysis. [185]

5.7.2.5 VATS

Video-assisted thoracoscopic surgery (VATS) is an advancement of thoracoscopy and is usually performed in general anaesthetic. VATS is indicated for diagnostic purposes, management of multiloculated parapneumonic effusion and decortication of pleural fibrosis. Yet its use is limited to patients that are fit for general anaesthesia. A recent study found VATS to be highly effective in therapy of multiloculated pleural effusion and empyema. [197] Furthermore it is stated that VATS is superior to intrapleural fibrinolysis and equal to thoracostomy. [198] [199]

If compared to thoracoscopy VATS seems to be equally effective [196] [193] yet VATS is superior in case of severe loculation and pleural decortication. [200]

In chronic empyema pleural fibrosis is common. In this case, pleural decortication is needed to re-expand so called “trapped” lung and eliminate infection. While some authors state that decortication can be performed in thoracoscopy, VATS and thoracostomy most surgeons perform decortication in VATS or thoracostomy. [201] [202]

6. ADA (ADENOSINE DEAMINASE ACTIVITY)

Adenosine deaminase is an enzyme that catalyses the conversion of adenosine into inosine as part of purine metabolism. [203] It occurs in any human cell that contains a cell core. Highest Adenosine Deaminase concentrations are found in lymphocytes and monocytes. [204] Adenosine Deaminase plays a central role in differentiation of T-lymphoid cells and reaches highest concentration in these immune cells. [205] There are two isoforms of ADA that are associated with immune cells. ADA1 is predominantly found in lymphocytes and monocytes while ADA 2 is mainly produced in monocytes and macrophages. [206] ADA levels are usually measured in pleural fluid. Elevated levels are found in tuberculous pleural effusion. Therefore, ADA measurement with a cut off level between 35- 40 U/l is routinely used as tool in the differential diagnosis of pleural effusions to confirm or to exclude pleural tuberculosis. [12] The test is highly cost efficient [207], quick to perform and if fluid is stored at 4 °C within 1 hour after collection, determination of ADA can be performed up to 28 days later without distortion of results. [208]

6.1 CLINICAL SIGNIFICANCE

6.1.1 TUBERCULOSIS

A recent meta-analysis examined the diagnostic value of ADA in tuberculous pleural effusion on basis of 63 studies with an ADA cut-off level of ≥ 30 U/l. It stated a pooled sensitivity of 92%, specificity 90% and positive and negative likelihood ratios of 9.0 and 0.1 respectively. [6] In countries with a low TB prevalence positive predictive value (PPV) diminishes and ADA is less accurate in confirming diagnosis but the negative predictive value (NPV) remains high. Hence in countries with low TB burden ADA can be used to exclude TB with high accuracy. [7] [209]

If ADA isoenzymes analysis is applied ADA accuracy can be further improved. Several studies concluded that ADA-1 is increased in bacterial non TB empyema while ADA-2 is increased only in TB effusions. [210] ADA-2 isoenzyme analysis increases specificity of ADA for diagnosing TB up to 96% [211], or 98.6% [212] in a different study. Several ratios proposed by Pe'rez-Rodr'iguez E. et al. could even improve these figures.

Their findings include the ratio of ADA 2 / ADA which reached a sensitivity of 100%, a specificity of 98.6% and an efficiency of 99% for tuberculous pleural effusion. [210]

False-negative results are often found in early stages of pleural TB. In many cases repeated pleural fluid aspiration several days after first aspiration found elevated ADA levels in the same patient. [204] Lee SJ et al. found that ADA levels may also be impaired in current smokers, and elderly TB patients. In these patients ADA results should be interpreted with caution. [8]

In contrast to other more recent found biomarker, ADA has been demonstrated to be a reliable marker of pleural TB in immunocompromised patients. Baba et al. Examined ADA in HIV positive TB patients and found that ADA levels are elevated even when CD4 cell count was very low. [212]

6.1.2 NON-TUBERCULOUS DISEASES

Soon after introduction of ADA it became evident that false positive results can be caused by parapneumonic effusion and empyema, rheumatoid pleurisy and in some cases malignancy such as mesothelioma and lung cancer. [7 8 9 10 11 213 214 215] All these diseases cause unilateral pleural effusion that in almost all cases turns out to be an exudate. There are few studies that examine the value of ADA for the diagnosis of parapneumonic effusion. Petterson et al. found moderately elevated pleural ADA levels in parapneumonic pleural effusion and high ADA levels in empyema. [216] Porcel et al. analysed 2100 cases of pleural effusion and found that ADA was elevated above 35 U/l in 40% of PPE. [11] However, the clinical use of ADA determination for non-tuberculous pleural diseases especially for neutrophilic pleural effusions and for the differentiation between CPE and PPE was never explored.

7. MATERIALS AND METHODS

7.1 STUDY OBJECTIVES

- Evaluation of sensitivity, specificity, PPV, and NPV of ADA and other pleural markers in the diagnosis of complicated parapneumonic effusion and empyema.
- Can elevated ADA levels indicate invasive pleural drainage more effectively than pleural pH or glucose levels?

7.2 STUDY DESIGN

This study was performed as a retrospective survey of patients that suffered from undiagnosed pleural effusions between 30th November 2011 and 1th May 2015 that underwent thoracentesis. Within this period of time 400 patients were included. In these patients' pleural fluid analysis and ADA level measurement was performed. ADA levels were determined by using the automated, standardized-turbidimetric analyses of ADA on the Cobas® 8000 system at the University of Graz. Participating hospitals were the University Hospital of Graz, the State Hospital Graz-West, the State Hospital of Hörgas-Enzenbach, the State Hospital of Feldbach and the State Hospital Wagna. This survey was performed at the Division of Pulmonology at the Medical University of Graz. In order to define the most likely cause of pleural effusion, all available medical files were reviewed by using the Styrian database MEDOCS. All patients' data were evaluated anonymously. Ethic board approval was granted by the local ethic committee. Due to the retrospective non-interventional character of the study a priori patient consent was not required.

7.3 DATA COLLECTION

We retrospectively reviewed data from 400 patients with undiagnosed pleural effusion that underwent thoracentesis and subsequent ADA analysis between 30th November 2011 and 1th May 2015. Collected data included demographic data, final diagnosis, laboratory values from serum and pleural fluid, microbiological and histological findings. Laboratory results 5 days before and after ADA determination were included. Final diagnosis was determined by reviewing all available medical files at least 6 month after ADA testing by a respiratory consultant physician. Laboratory values were obtained from patient's serum and pleural fluid.

Pleural fluid values included total cell count, absolute neutrophil and lymphocyte cell count, absolute count of other non-defined cells, LDH, protein, glucose, pH, lactate and amylase. In the patient's serum levels of LDH and protein were determined. Serum and pleural fluid total protein and LDH concentration were used to calculate ratios of pleural to blood LDH and total protein in order to apply Light's criteria.

7.4 Definitions

In order to distinguish transudates from exudates Light's criteria were applied. According to Light's criteria, if at least one of the following three criteria is present, the fluid is defined as an exudate. Pleural fluid protein/serum protein ratio greater than 0.5, pleural fluid LDH/serum LDH ratio greater than 0.6 or pleural fluid LDH greater than two-thirds the upper limits of the laboratory's normal serum LDH. [150] A pleural effusion was defined as parapneumonic effusion in patients with high level of clinical suspicion of pneumonia if other aetiologies were excluded and the patient recovered under antibiotic pneumonia therapy. Both simple and complicated parapneumonic effusion were defined by results of pleural fluid analysis. The term complicated parapneumonic pleural effusion was used for parapneumonic effusions that showed a neutrophil share higher than 40%, a glucose level below 60 mg/dl or pleural pH below 7.20. (157) Empyema was defined as complicated parapneumonic effusion with either frank pus or the detection of relevant non-tuberculous pathogens in the pleural effusion. If none of these criteria was met the underlying parapneumonic pleural effusion was defined to be a simple parapneumonic effusion.

7.5 Analysis

All results were statistically analysed using IBM SPSS Statistics 21.0. Data were used to calculate sensitivity, specificity, NPV and PPV and ROC-curves.

8. RESULTS

8.1 DEMOGRAPHIC DATA

Demographic data were available for all 400 patients with undiagnosed pleural effusions. We evaluated mean age of all relevant diseases. (Figure 3) After final evaluation pleural effusions were divided into uncomplicated PPE, CPE and Empyema, TB, lymphoma, malignancy, SLE, PAE, cardiac decompensation and chronic pleurisy of unknown etiology.

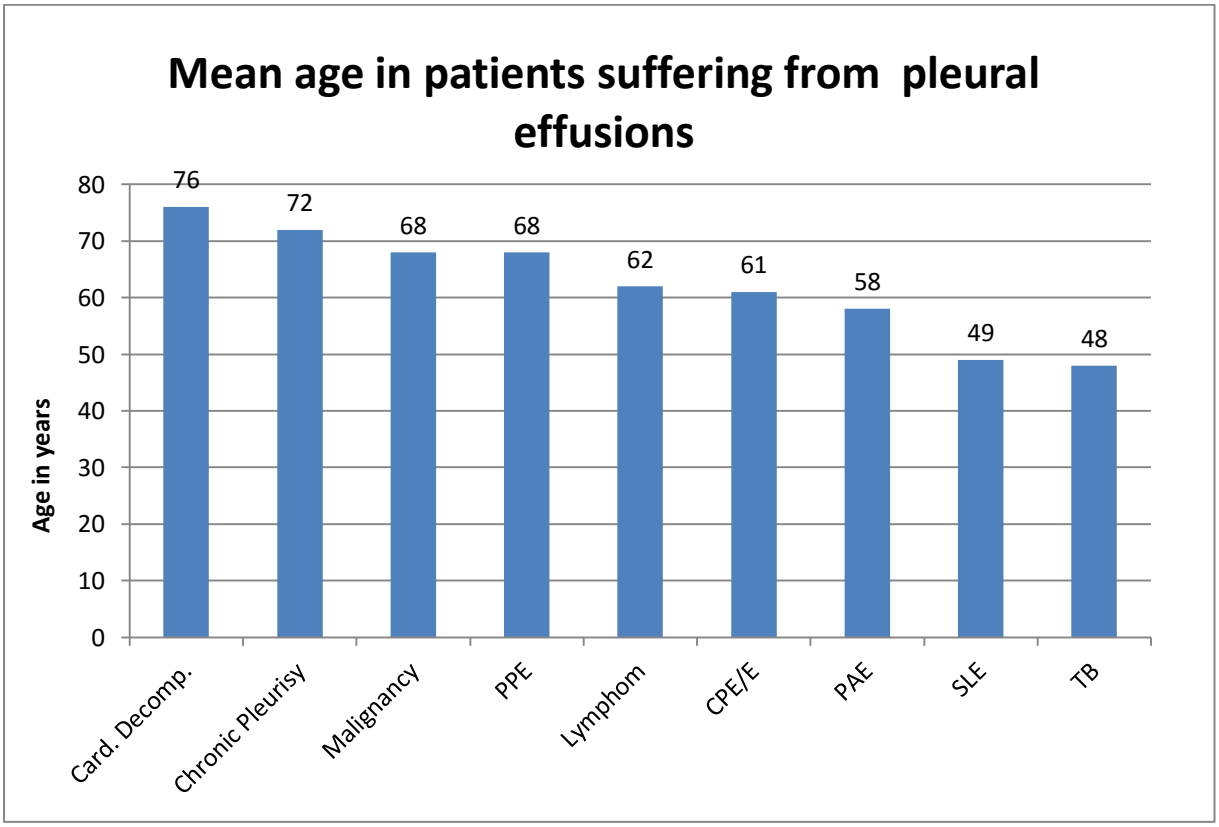


FIGURE 3 MEAN AGE IN PATIENTS SUFFERING FROM PLEURAL EFFUSION

Highest mean age was found in the cardiac decompensation group. Mean age in patients suffering from cardiac decompensation was 76 years with a standard deviation of 11.3 years. Mean age was lowest in patients suffering from TB (mean age: 48; S.D +/- 22.9). If non-tuberculous pleural infections are considered we found that mean age in the uncomplicated PPE group was 68 years. (S.D +/- 15.2). The mean ages in the CPE and empyema subclass was 61 years (S.D +/- 15.2). When it comes to malignant pleural effusion mean age in with exclusion of lymphoma was 68 years. (S.D +/- 11.7). Mean age in the lymphoma subgroup was 62 years. (S.D +/- 19.2)

8.2 ETIOLOGIES OF PLEURAL EFFUSIONS

Etiologies of pleural effusions included malignancy (118 of 400 cases), uncomplicated PPE (43 of 400 cases), CPE/E (19 of 400 cases), PAE (10 of 400 cases), tuberculous pleurisy (14 of 400 cases), cardiac decompensation (90 of 400 cases), lymphoma (12 of 400 cases) and 37 cases of chronic pleurisy of unknown etiology. (Figure 4)

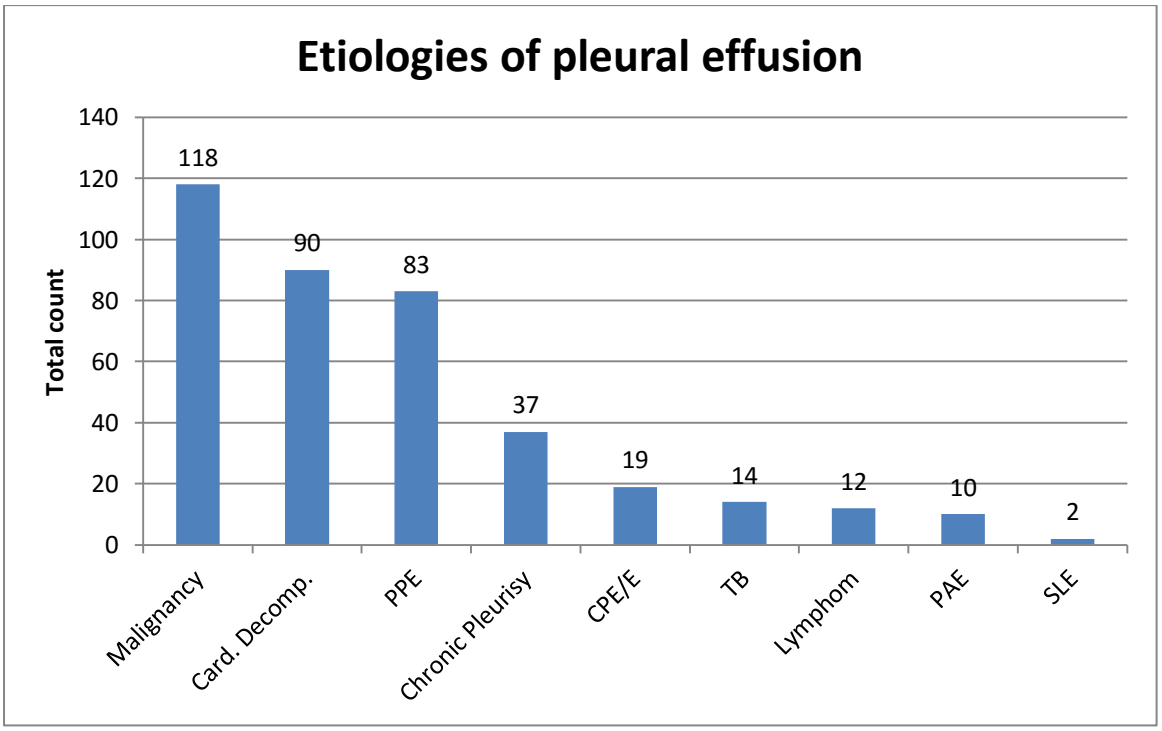


FIGURE 4 ETIOLOGIES OF EXUDATIVE PLEURAL EFFUSIONS

8.3 LABORATORY FINDINGS

Laboratory findings of 400 patients with pleural effusions were analysed. Evaluated laboratory findings included total cell count (G/l), glucose (mg/dl), LDH (U/l) in pleural fluid and serum, lymphocyte share (%), neutrophil share (%), total protein (G/l), pleural pH and ADA (U/l). (Table 1-3)

TABLE 1 MEAN LABORATORY FINDINGS IN INFECTIOUS PLEURAL EFFUSIONS

Laboratory Items	PPE (n=83)	CPE/E (n=19)	E (n=15)	TB (n=14)
ADA (U/l)	11,7 (S.D +/- 8,3)	85,1 (S.D +/- 139)	98,4 (S.D +/- 153,5)	36,9 (S.D +/- 14,6)
Cellcount (G/l)	2,3 (S.D +/- 3,0)	21,5 (S.D +/- 29,5)	22,4 (S.D +/- 33,4)	3,3 (S.D +/- 2,9)
Glucose (mg/dl)	111 (S.D +/- 30,3)	39,8 (S.D +/- 58,7)	41 (S.D +/- 63,7)	93,7 (S.D +/- 38,8)
L/N Ratio	4,2 (S.D +/-12,4)	0,1 (S.D +/- 0,2)	0,1 (S.D +/- 0,2)	42,6 (S.D +/- 79,0)
LDH (U/l)	311 (S.D +/- 315)	8047,4 (S.D +/- 20821)	9596 (S.D +/- 23147)	344 (S.D +/- 173,2)
Lymphocytes (%)	24,7 (S.D +/- 23,6)	5,2 (S.D +/- 5,1)	4 (S.D +/- 2,8)	64,5 (S.D +/- 19,0)
Neutrophils (%)	36,5 (S.D +/-26,7)	77,4 (S.D +/- 21,6)	77 (S.D +/- 24,1)	8,6 (S.D +/- 15,0)
Protein (G/l)	3,7 (S.D +/- 1,2)	3,9 (S.D +/- 2,4)	3,1 (S.D +/-1,1)	4,8 (S.D +/- 1,0)
Pleural pH	7,59 (S.D +/- 0,1)	7,27 (S.D +/- 0,32)	7,2 (S.D +/- 0,3)	7,55 (S.D +/- 0,2)

TABLE 2 MEAN LABORATORY FINDINGS IN MALIGNANT PLEURAL EFFUSIONS

Laboratory Items	Malignancy (n=118)	Lymphoma (n=12)
ADA (U/l)	9,3(S.D +/- 5,4)	100,8 (S.D +/- 241,5)
Cellcount (G/l)	1,7 (S.D +/- 2,5)	12,5(S.D +/- 21,4)
Glucose (mg/dl)	102,4 (S.D +/- 41,1)	75,3 (S.D +/-47,9)
L/N Ratio	7,4 (S.D +/- 13,3)	5,4(S.D +/- 6,1)
LDH (U/l)	397 (S.D +/- 420)	1539 (S.D +/- 1743,8)
Lymphocytes (%)	33,9 (S.D +/- 27,3)	43,2 (S.D +/- 30)
Neutrophils (%)	17,6 (S.D +/-19,4)	13,1 (S.D +/- 21,2)
Protein (G/l)	3,9 (S.D +/- 0,8)	3,6 (S.D +/- 0,9)
Pleural pH	7,54 (S.D +/- 0,18)	7,41 (S.D +/- 0,2)

TABLE 3 MEAN LABORATORY FINDINGS IN BENIGN PLEURAL EFFUSIONS

Laboratory Items	SLE (n=2)	PAE (n=10)	Card. Decomp. (n=90)	Chronic Pleuritis (n=37)
ADA (U/l)	13,7 (S.D +/- 4,7)	9,4(S.D +/- 3,4)	4,6 (S.D +/- 2,6)	11,2 (S.D +/- 9,1)
Cellcount (G/l)	0,7 (S.D +/- 0,1)	1,9(S.D +/- 1,4)	0,5(S.D +/- 0,7)	1,5 (S.D +/- 1,3)
Glucose (mg/dl)	103,5 (S.D +/- 0,5)	107 (S.D +/- 25,7)	142,3 (S.D +/- 49,5)	108,4 (S.D +/- 34,4)
L/N Ratio	17,2 (S.D +/- 16,8)	6,1 (S.D +/- 10,8)	5 (S.D +/- 11,3)	8,9 (S.D +/- 15,1)
LDH (U/l)	189 (S.D +/- 116,5)	264 (S.D +/- 150,9)	96,4 (S.D +/- 49,6)	214 (S.D +/- 186,6)
Lymphocytes (%)	31,5 (S.D +/- 26,5)	24,4 (S.D +/- 26,4)	30,3 (S.D +/- 24,6)	38,0 (S.D +/- 29,7)
Neutrophils (%)	8(S.D +/- 6)	22,9 (S.D +/- 26,3)	18,8 (S.D +/- 16,3)	22,5 (S.D +/- 25,1)
Protein (G/l)	3,9 (S.D +/- 0,2)	3,9 (S.D +/-1,0)	2,3 (S.D +/-1,0)	3,8 (S.D +/- 0,8)
Pleural pH	7,59 (S.D +/- 0,2)	7,6(S.D +/- 0,1)	7,65(S.D +/- 0,2)	7,59 (S.D +/- 0,2)

8.3.1 PLEURAL CELL COUNT

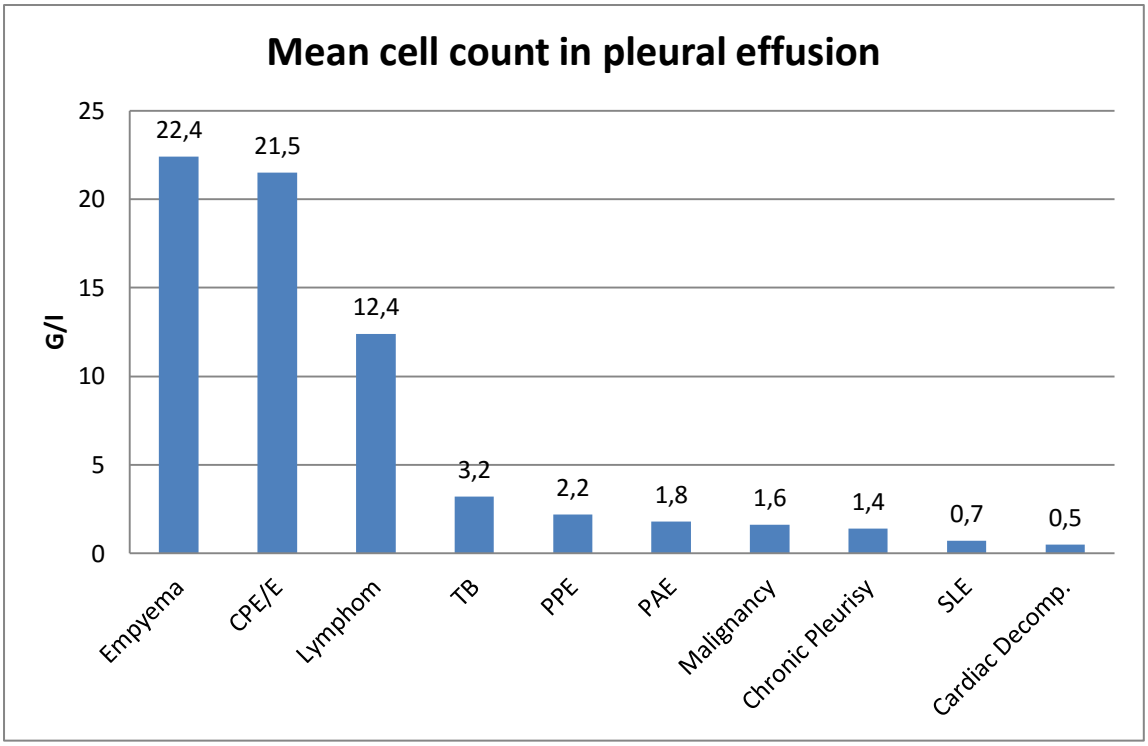


FIGURE 5 MEAN CELL COUNT IN PLEURAL EFFUSIONS

As expected overall pleural cell count was highest in CPE/E (mean: 21.5 G/l; S.D +/- 33.4) and the empyema subgroup (mean: 22.4 G/l; S.D +/- 29.0) followed by lymphoma (mean: 12.5 G/l; S.D +/- 10.8). Intermediate pleural cell count was obtained in TB (mean 3.2 G/l; S.D +/- 2.9), uncomplicated PPE (mean 2.2 G/l; S.D +/- 3.0), PAE (mean 1.8 G/l; S.D +/- 1.4), malignancy (mean 1.6 G/l; S.D +/- 2.5), chronic pleurisy (mean 1.4 G/l; S.D +/- 1.3) and SLE (mean 0.7 G/l; S.D +/- 0.1). Lowest pleural cell count was found in pleural effusions caused by cardiac decompensation (mean: 0.5 G/l; S.D +/- 0.7) and SLE (mean: 0.7 G/l; S.D +/-0.1). (Figure 5)

8.3.2 NEUTROPHIL GRANULOCYTES

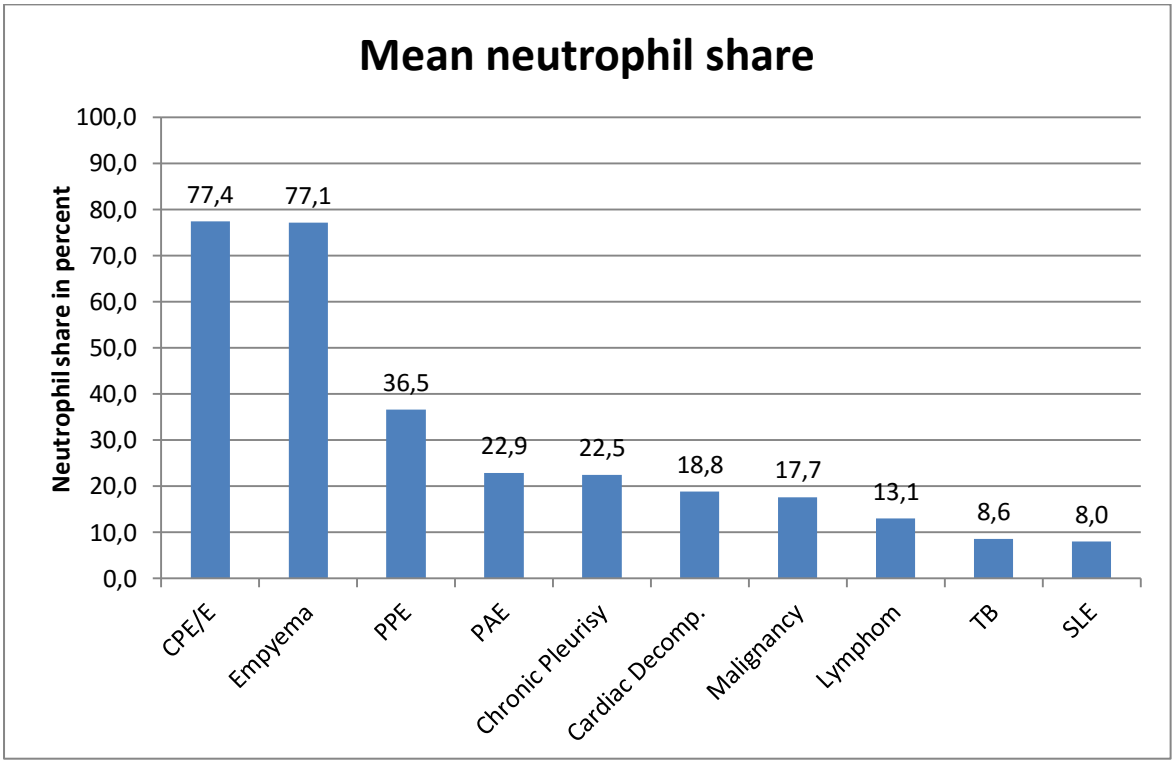


FIGURE 6 MEAN NEUTROPHIL SHARE IN PLEURAL EFFUSIONS

Neutrophil granulocyte share was highest in CPE/E (mean: 77.4% neutrophils of total cell count; S.D +/- 21.6) followed by the subgroup of empyema (mean: 77.1% neutrophils of total cell count; S.D +/- 24.1) and uncomplicated PPE (mean: 36.5% neutrophils; S.D +/- 26.7)). In PAE neutrophil granulocyte share was found to be also relatively high (22.9% neutrophils; S.D +/- 26.3). Intermediate neutrophil share was obtained in chronic pleurisy (mean: 22.9% of total cell count; S.D +/- 26.3), cardiac decompensation (mean: 18.8% of total cell count; S.D +/- 16.3) and malignancy (mean: 17.7% of total cell count; S.D +/- 19.4). Lowest neutrophil share was found in lymphoma (mean: 13.1% neutrophils; S.D +/- 21.2), TB (mean: 8.6% neutrophils; S.D +/- 15.0) and SLE (mean: 8.0% neutrophils; S.D +/- 6.0). (Figure 6)

8.3.3 LYMPHOCYTES

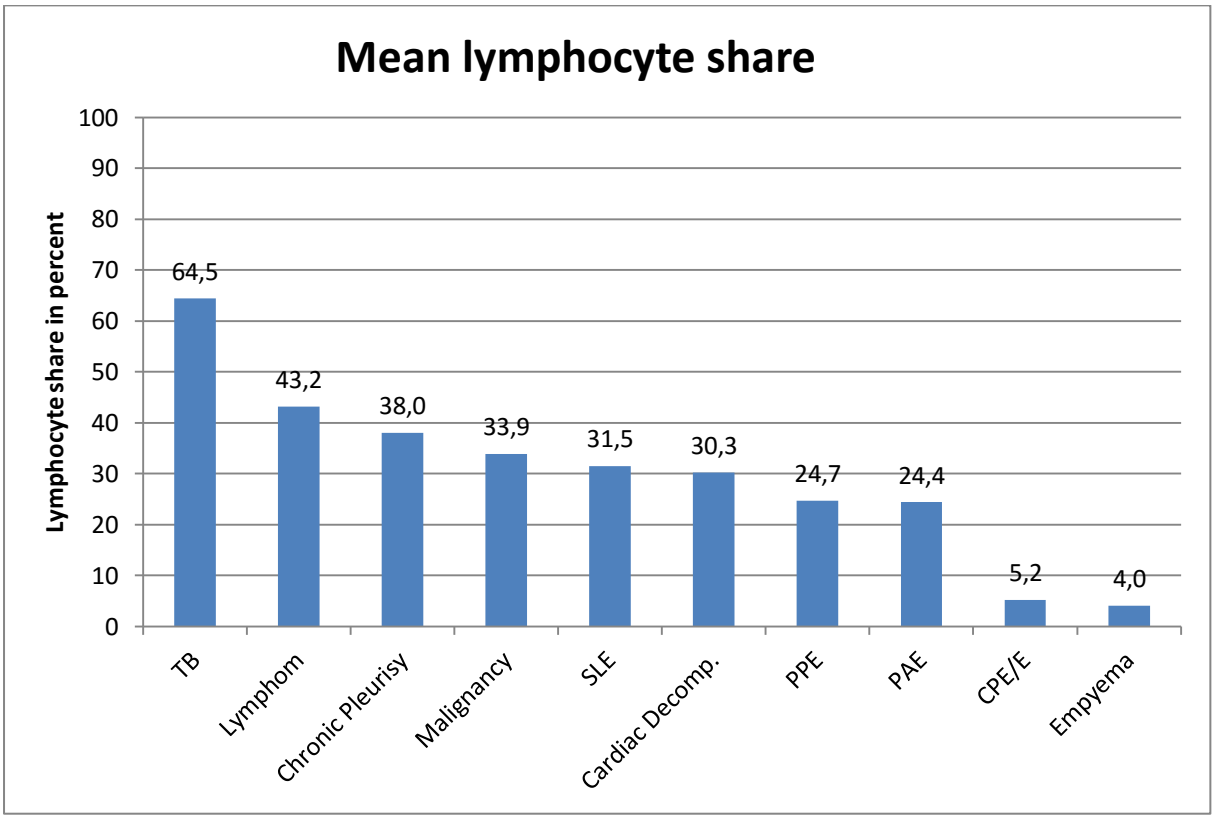


FIGURE 7 MEAN LYMPHOCYTE SHARE IN PLEURAL EFFUSIONS

The percentage of lymphocytes in pleural fluid was highest in tuberculous pleurisy (mean 64.9% lymphocytes; S.D +/- 19.2) and lymphomas (mean 43.2% lymphocytes; S.D +/- 30). Followed by chronic pleurisy (mean: 38% lymphocytes; S.D +/-29.7), malignancy (mean: 33.9% lymphocytes; S.D +/- 27.3) and SLE (mean: 31.5% lymphocytes; S.D +/- 26.5). Lowest lymphocyte shares were found in CPE/E (mean: 5.2% lymphocytes; S.D +/- 5.1) and the empyema subgroup (mean: 4.0% lymphocytes; S.D +/- 2.8). (Figure 7)

8.3.4 LYMPHOCYTE-TO-NEUTROPHIL RATIO

The lymphocyte to neutrophil ration in pleural fluid is high in pleural effusions that are lymphocyte rich and or poor in neutrophils. As expected tuberculous pleurisy (mean: 42.6; S.D +/- 79), and SLE (mean: 17.2; S.D +/- 16.8) are associated with a high lymphocyte to neutrophil ratio. Intermediate ratios were found in pleural effusions caused by lymphomas (mean: 5.4; S.D +/- 6.1), cardiac decompensation (mean: 5.0; S.D +/- 11.3) and PPE (mean: 4.2; S.D +/- 12.4). Low ratios were found in CPE/E (mean: 0.1; S.D +/- 0.2) and the empyema subgroup (mean: 0.1; S.D +/- 0.3).

8.3.5 GLUCOSE

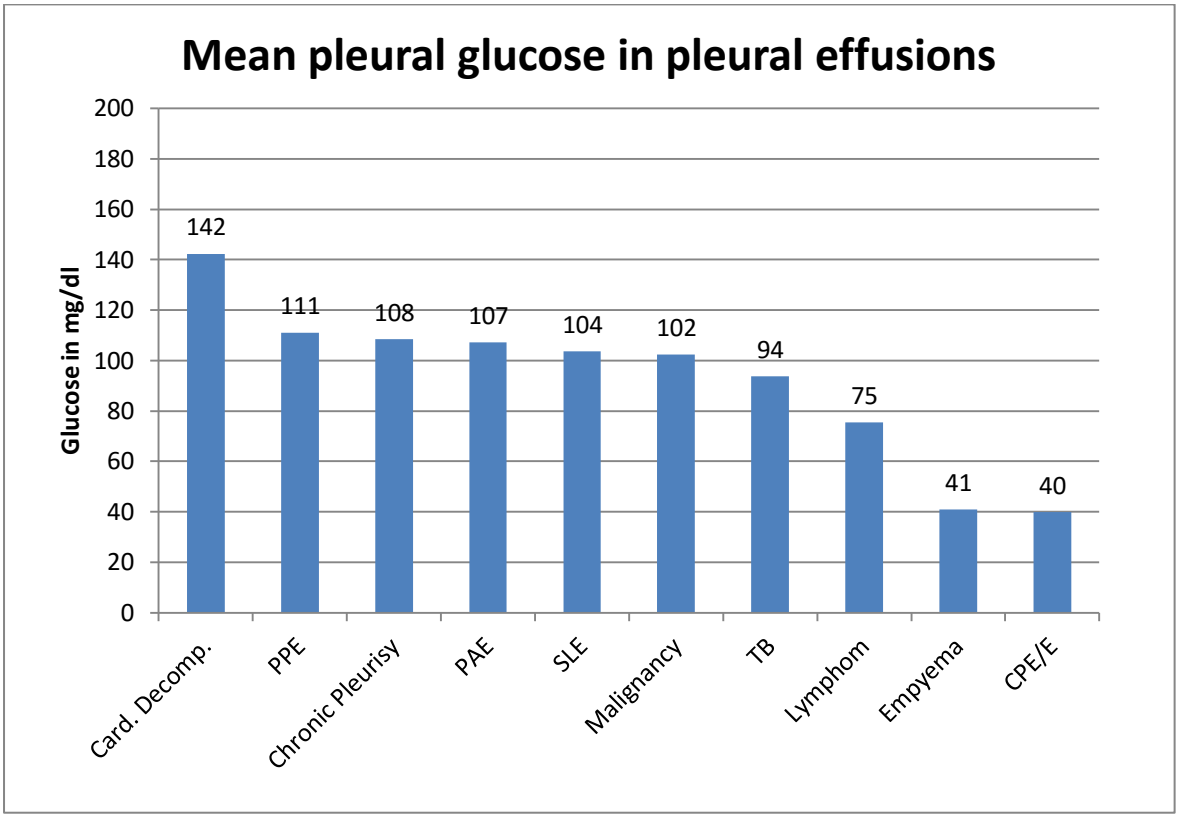


FIGURE 8 MEAN GLUCOSE IN PLEURAL EFFUSIONS

Mean glucose levels were lowest in CPE/E (mean: 39.8 mg/dl; S.D +/- 58.7) and the empyema subgroup (mean 41 mg/dl; S.D +/- 63.7). Glucose was also decreased in pleural effusions caused by lymphoma (mean glucose 75.3 mg/dl; S.D +/- 47.9), tuberculous pleurisy (mean glucose 93.7 mg/dl; S.D +/- 38.8) and malignancy (mean glucose 102.4 mg/dl; S.D +/- 41.1). Marginal normal glucose levels were found in uncomplicated PPE (mean glucose 111.0 mg/dl; S.D +/- 30.3), chronic pleurisy (mean glucose 108.4 mg/dl; S.D +/- 34.4) and PAE (mean glucose 107.1 mg/dl; S.D +/- 25.7). Highest pleural glucose was found in cardiac decompensation (mean glucose 142 mg/dl; S.D +/- 49.5).

8.3.6 PH VALUE

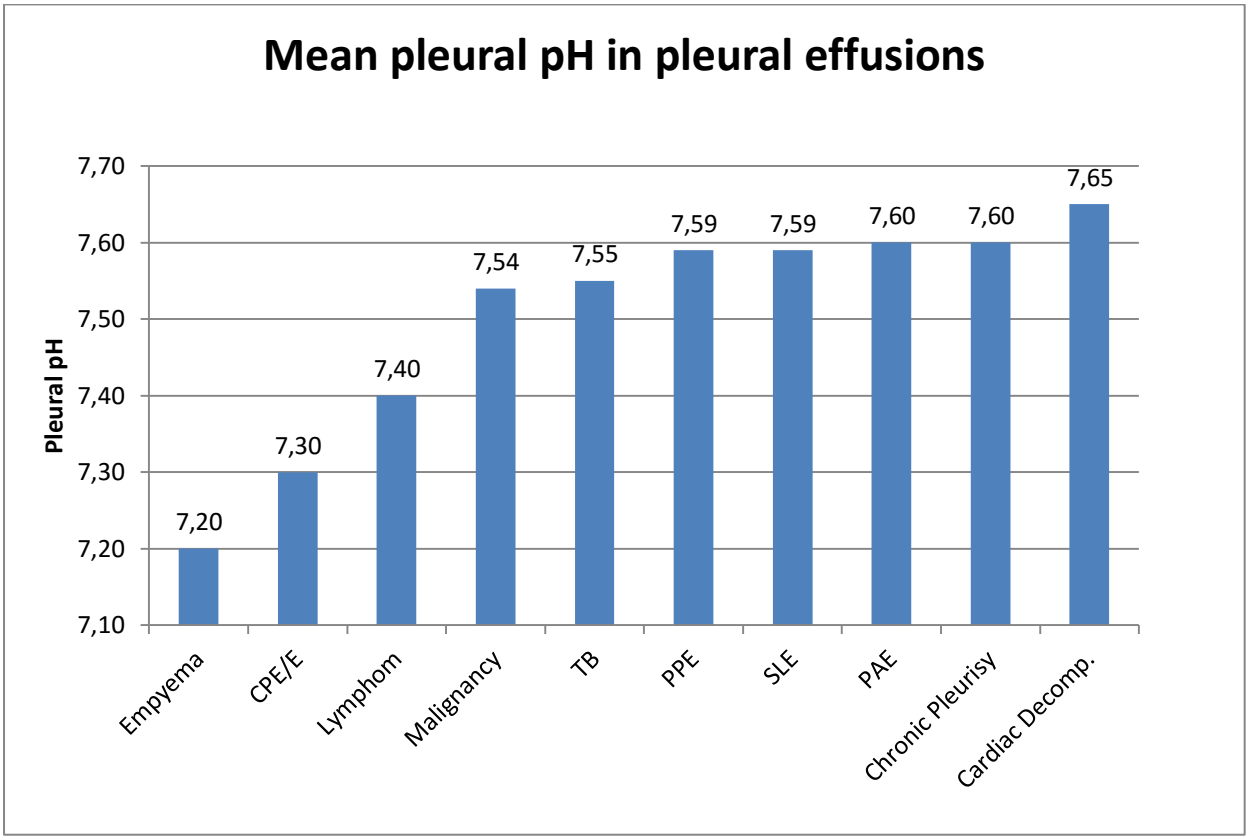


FIGURE 9 MEAN PLEURAL PH IN PLEURAL EFFUSIONS

As presumed lowest pH values were measured in the CPE/E (mean pH: 7.30; S.D +/- 0.3) and the empyema subgroup (mean pH: 7.20; S.D +/- 0.32). Followed by lymphoma (mean pH: 7.40; S.D +/- 0.2), malignancy (mean pH: 7.54; S.D +/- 0.2), TB (mean pH: 7.55; S.D +/- 0.2), SLE (mean pH: 7.59; S.D +/- 0.1) and uncomplicated PPE (mean pH: 7.59; S.D +/- 0.1). High pH values were found in chronic pleurisy (mean pH: 7.61; S.D +/- 0.2), PAE (mean pH: 7.63; S.D +/- 0.1) and cardiac decompensation (mean pH: 7.65; S.D +/- 0.2)

8.3.7 PLEURAL LDH

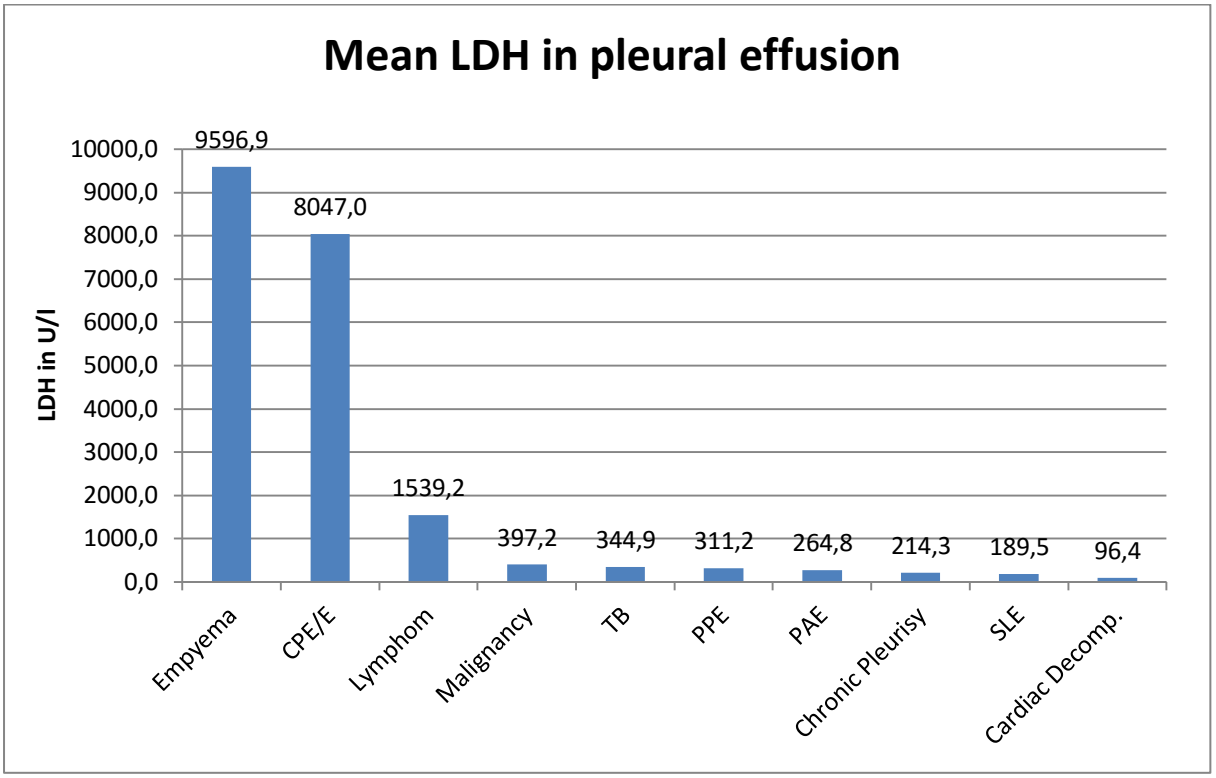


FIGURE 10 MEAN LDH IN PLEURAL EFFUSIONS

Highest Pleural LDH values were found in CPE/E (mean LDH 8047 U/l; S.D +/- 20821), the empyema subgroup (mean 9596 U/l; S.D +/- 23147), and lymphoma (mean LDH 1539 U/l; S.D +/- 1743). Followed by malignancy (mean LDH 397 U/l; S.D +/- 420), TB (mean LDH 344 U/l; S.D +/- 173.2) and uncomplicated PPE (mean LDH 311 U/l; S.D +/- 315.8). Lowest pleural LDH values were found in SLE (mean LDH 189 U/l; S.D +/- 116.5) and cardiac decompensation (mean 96 U/l; S.D +/- 49.6).

8.3.8 LDH IN PLEURAL FLUID TO LDH SERUM RATIO

This ratio is elevated in patients with pleural effusion in which high number of cells perish. Therefore one would expect a high ratio in empyema and malignancy. The analysed data confirm this hypothesis. We found highest ratios in CPE/E (mean: 39.2; S.D +/- 67.7) and the empyema subgroup (mean: 45.9; S.D +/- 74.6). Intermediate LDH ratios were obtained in lymphoma (mean: 2.5 S.D +/- 3.4), TBC (mean: 1.7; S.D +/- 0.59) and uncomplicated PPE (mean: 1.6; S.D +/- 1.6). Lowest LDH ratios were found in PAE (mean: 1.28 S.D +/- 0.97), SLE (mean: 0.46 S.D +/- 0.03) and cardiac decompensation (mean: 0.43 S.D +/- 0.3).

8.3.9 PLEURAL TOTAL PROTEIN

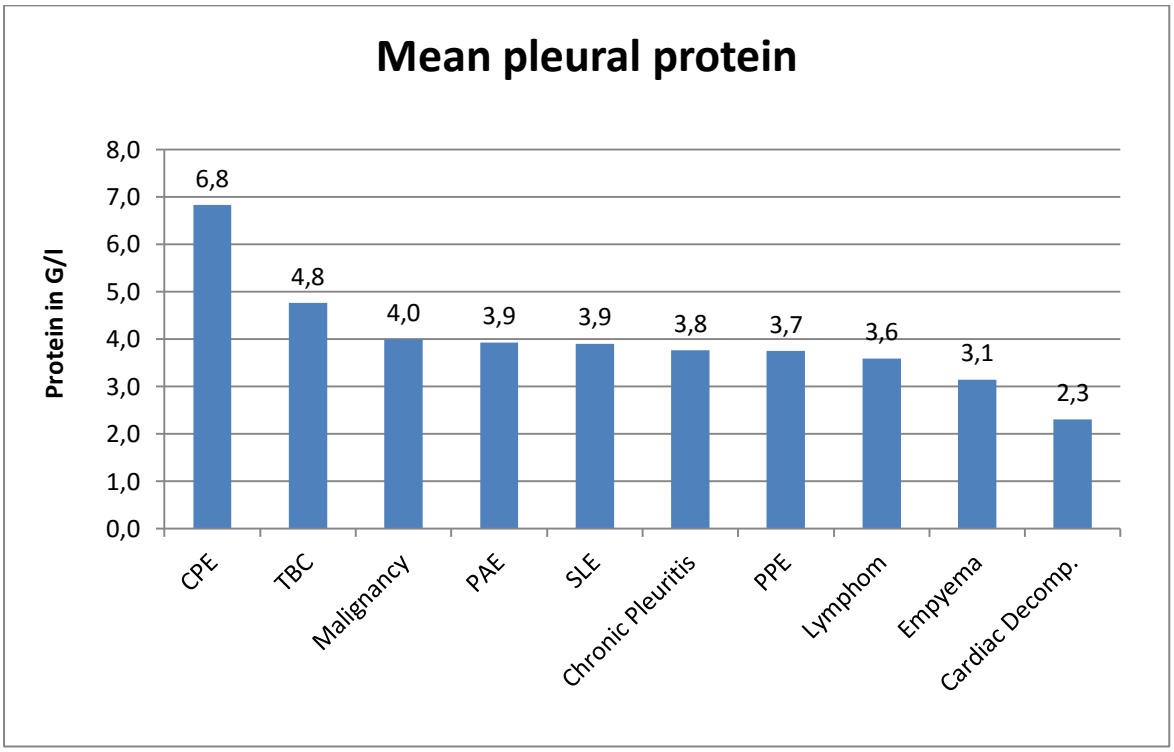


FIGURE 11 MEAN PLEURAL PROTEIN IN PLEURAL EFFUSIONS

Total protein levels were highest in the tuberculous pleurisy group (mean: 4.8 G/l; S.D +/- 1.0), malignancy (mean: 4.0 G/l; S.D +/- 0.8) and CPE/E (mean: 3.9 G/l; S.D +/- 1.1). Followed by PAE (mean: 3.9 G/l; S.D +/- 1.0), SLE (mean: 3.9 G/l; S.D +/- 0.2), chronic pleurisy (mean: 3.8 G/l; S.D +/- 0.8) and uncomplicated PPE (mean: 3.7 G/l; S.D +/- 1.2). Lowest values were found in lymphoma (mean: 3.6 G/l; S.D +/- 0.9), empyema subgroup (mean: 3.1 G/l; S.D +/- 1.1) and cardiac decompensation (mean: 2.3 G/l; S.D +/- 1.0).

8.3.10 PROTEIN RATIO

The pleural to blood total protein ratio is one of three criteria of light's criteria and is crucial to distinguish transudates and exudates. Highest levels of pleural to blood total protein ratio were found in TB (mean: 0.68; S.D +/- 0.04), SLE (mean: 0.61; S.D +/- 0.01) and malignancy (mean: 0.6; S.D +/- 0.12) and CPE/E (mean: 0.59; S.D +/- 0.17). Intermediate ratios were obtained in lymphoma (mean: 0.0.56; S.D +/- 0.12), uncomplicated PPE (mean: 0.56; S.D +/- 0.13) and chronic pleurisy (mean: 0.55; S.D +/- 0.1). Lowest ratios were found in PAE (mean: 0.54; S.D +/- 0.16), the empyema subgroup (mean: 0.53; S.D +/- 0.14) and cardiac decompensation (mean: 0.35; S.D +/- 0.14).

8.4 Adenosine Deaminase Activity (ADA)

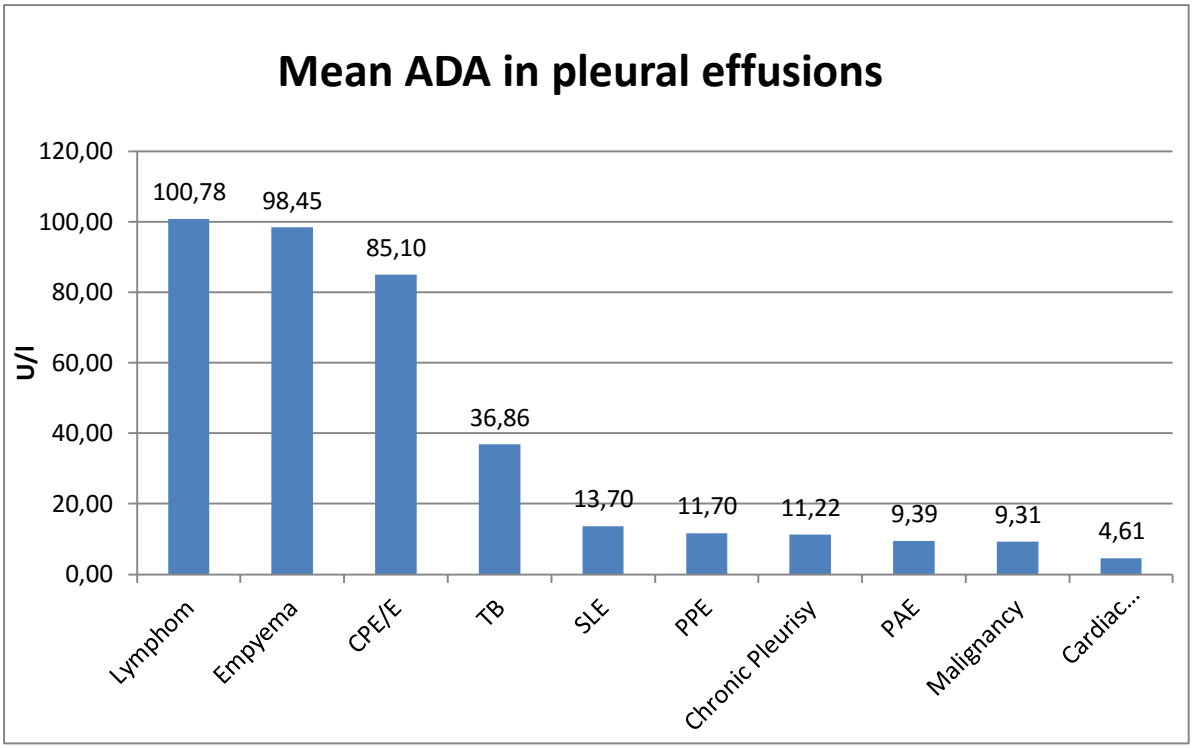


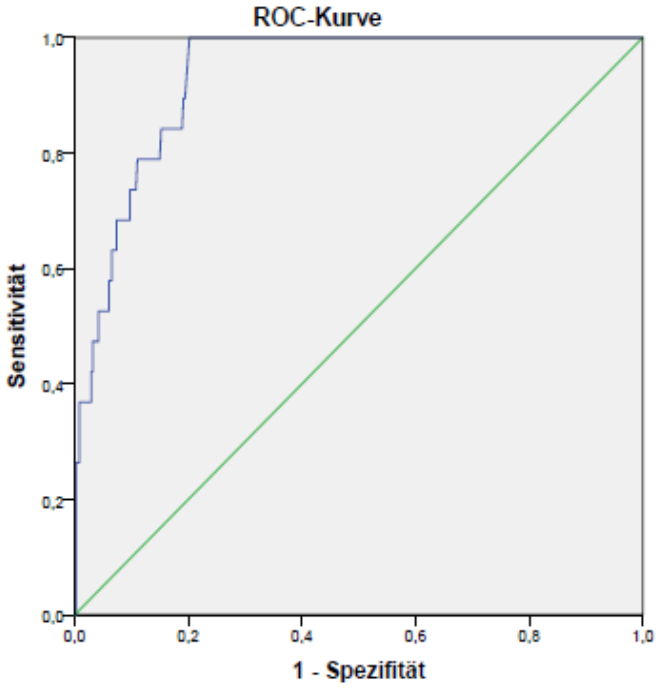
FIGURE 12 MEAN ADA IN PLEURAL EFFUSIONS

Highest ADA levels were found in lymphoma (mean ADA: 100.7 U/l; S.D +/- 241.4), CPE/E (mean ADA: 85.1 U/l; S.D +/- 139) and the empyema subgroup (mean ADA: 98.4 U/l; S.D +/- 153). Intermediate ADA levels were found in TB (mean ADA: 36.8 U/l; S.D +/- 14.5), SLE (mean ADA: 13.7 U/l; S.D +/- 8.3), uncomplicated PPE (mean ADA: 11.7 U/l; S.D +/- 8.32) and chronic pleurisy (mean ADA: 11.2 U/l; S.D +/- 9). Lowest ADA levels were obtained in PAE (mean ADA levels: 9.3; SD +/- 3.4), malignancy (mean ADA: 9.3 U/l; S.D +/- 5.3) and cardiac decompensation (mean ADA: 4.6 U/l; S.D +/- 2.6).

8.5 Diagnostic value of laboratory parameters for the diagnosis of CPE/E

We evaluated the sensitivity, specificity, PPV and NPV for every single pleural parameter as well as several different combinations of these pleural markers to find best diagnostic accuracy for pleural infection (CPE/E). Using different cut off levels we found neutrophil share, LDH and ADA to be the most accurate pleural fluid markers for CPE/E. Using ROC-curve analysis we searched for optimal cut off levels for each parameter. As optimal we regarded a sensitivity of at least ≥ 90% and a specificity of at least ≥ 70%.

8.5.1 ADA IN THE DIAGNOSIS OF CPE/E



ROC-curve analysis for ADA found highest diagnostic accuracy using a cut off level of 13 U/l or above and revealed an area under the curve of 0.933 (95% confidential interval: 0.898-0.968) for the diagnosis of CPE/E. (Figure 13)

FIGURE 13 ROC-CURVE ANALYSIS OF ADA IN THE DIAGNOSIS OF CPE/E

We evaluated ADA in the diagnosis of CPE/E using different cut offs. ADA yielded most promising diagnostic value if using a cut off level of 13 U/l and above. In this case we found sensitivity, specificity, PPV and NPV of 100, 77, 18 and 100 respectively. We conclude that pleural ADA is a sensitive and specific diagnostic tool in the diagnosis of CPE/E however several other pleural markers as for example LDH and neutrophil share showed comparable diagnostic accuracy. (Figure 14)

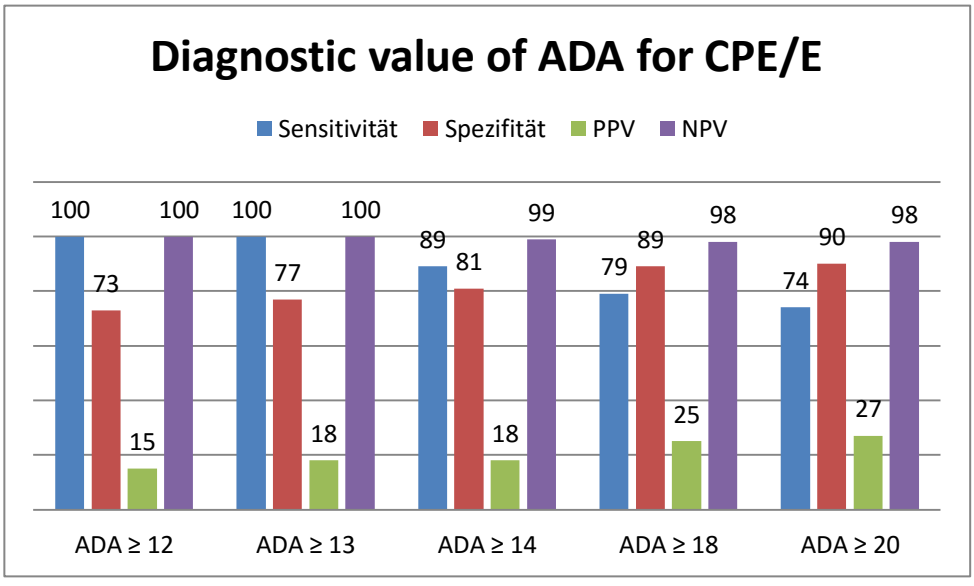
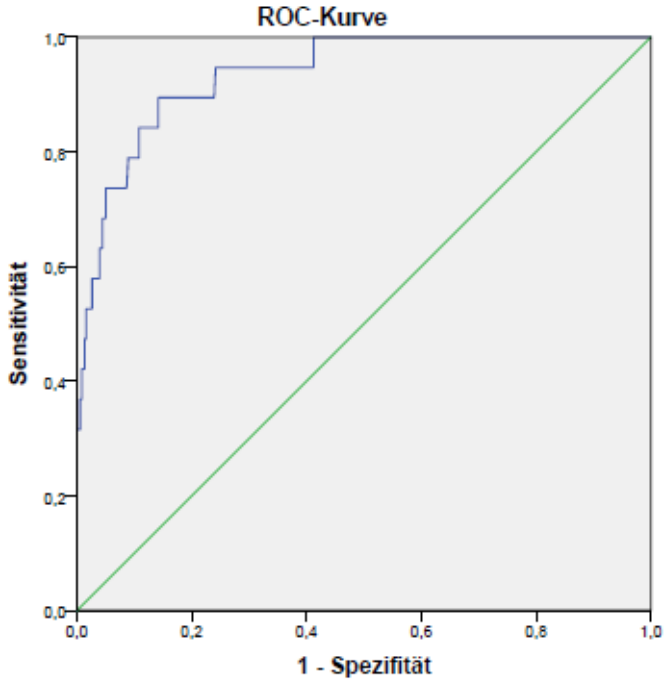


FIGURE 14 SENSITIVITY, SPECIFICITY, PPV AND NPV OF ADA FOR THE DIAGNOSIS OF CPE/E

8.5.3 LDH IN THE DIAGNOSIS OF CPE/E



ROC-curve analysis for LDH using a cut off level of 300 U/l or above found highest diagnostic accuracy and revealed an area under the curve of 0.937 (95% confidential interval: 0.890-0.985) for the diagnosis of CPE/E. (Figure 15)

FIGURE 15 ROC-CURVE ANALYSIS OF LDH IN THE DIAGNOSIS OF CPE/E

We found that LDH had high potential in the diagnosis of CPE/E and therefore evaluated several cut off levels for LDH. Furthermore, we compared LDH to other pleural fluid markers including ADA. Best results were found using a cut off level of 300 U/l. Sensitivity, specificity, PPV and NPV reached 0.90, 0.70, 0.13 and 0.99 respectively. These findings suggest that these criteria are robust in the diagnosis of pleural infection. (Figure 16)

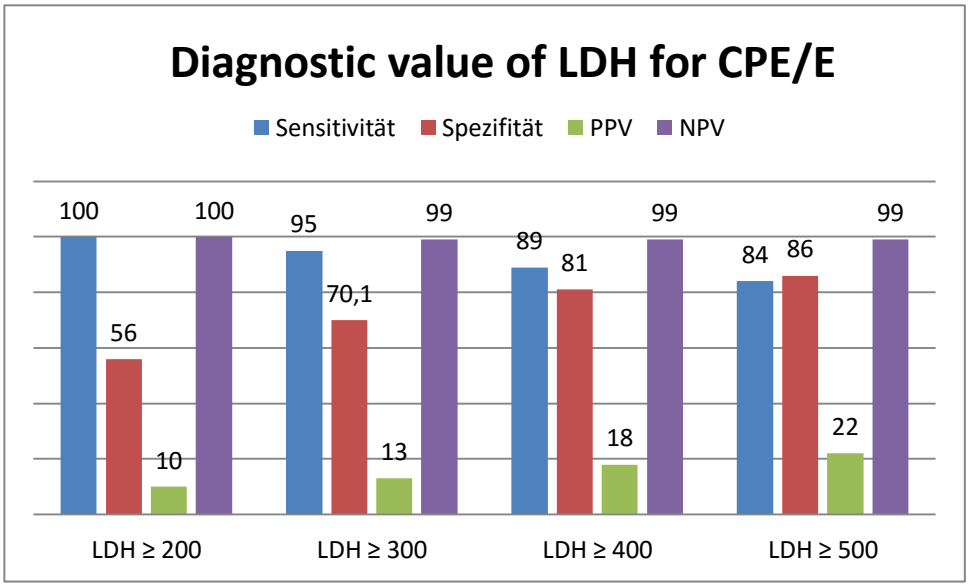
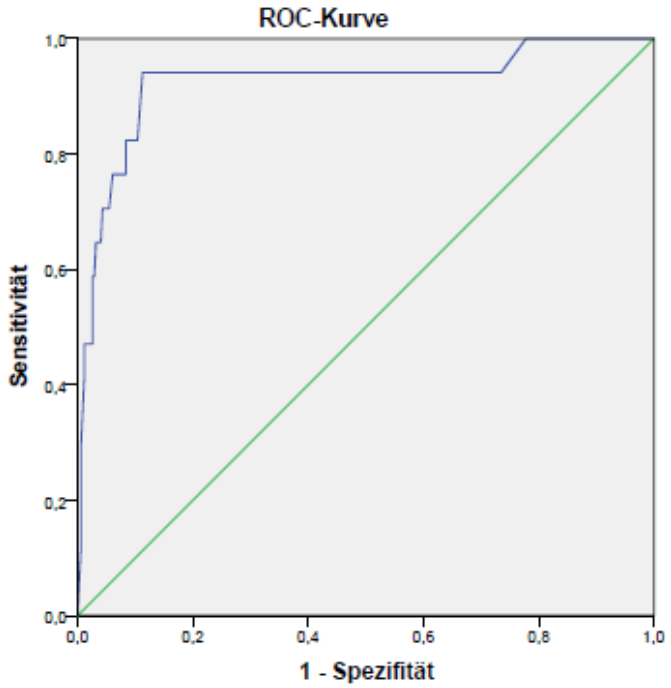


FIGURE 16 SENSITIVITY, SPECIFICITY, PPV AND NPV OF LDH FOR THE DIAGNOSIS OF CPE/E

8.5.4 NEUTROPHIL SHARE IN THE DIAGNOSIS OF CPE/E



ROC-curve analysis for Neutrophil share found highest diagnostic accuracy using a cut off level of 60% U/l or above and revealed an area under the curve of 0.924 (95% confidential interval: 0.841-1.0) for the diagnosis of CPE/E. (Figure 17)

FIGURE 17 ROC-CURVE ANALYSIS OF NEUTROPHIL SHARE IN THE DIAGNOSIS OF CPE/E

We calculated sensitivity, specificity, PPV and NPV for several neutrophil share cut offs and found that neutrophil share has an overall high diagnostic value for die diagnostic of CPE/E. Best results were found by using a cut off level of 60%. In this case sensitivity, specificity, PPV and NPV was found to be 94, 89, 29 and 99 respectively. (Figure 18)

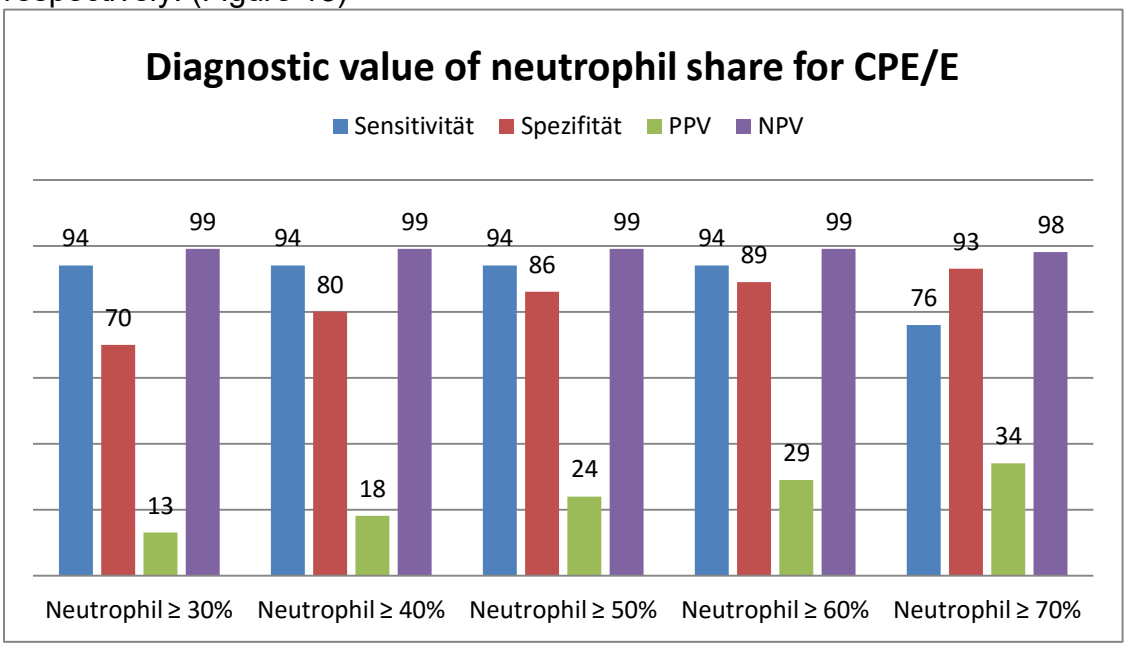
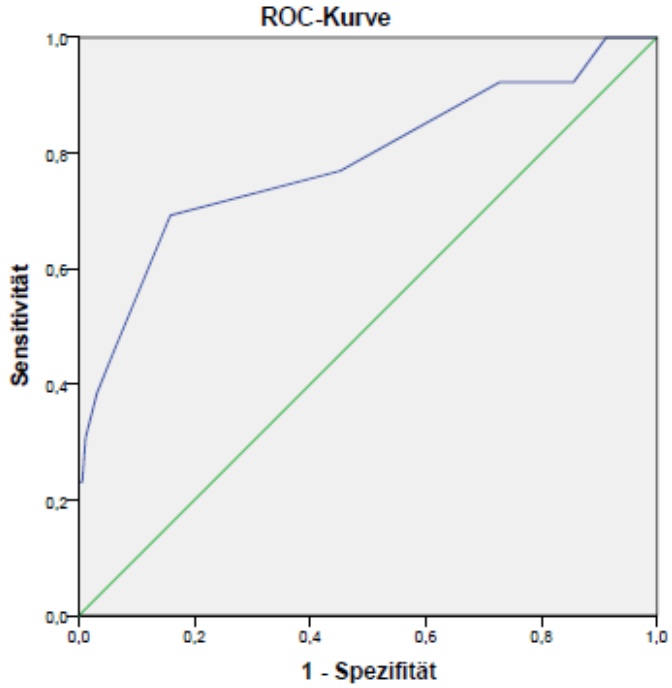


FIGURE 18 SENSITIVITY, SPECIFICITY, PPV AND NPV OF NEUTROPHIL SHARE FOR CPE/E

8.5.5 Pleural pH in the diagnosis of CPE/E



Using ROC-curve analysis for pleural pH we found highest sensitivity for a cut off level of 7.5 U/l or below. Area under the curve was 0.78 (95% confidential interval: 0.632-0.941) for the diagnosis of CPE/E. (Figure 19)

FIGURE 19 ROC-CURVE ANALYSIS OF PLEURAL PH IN THE DIAGNOSIS OF CPE/E

We evaluated the diagnostic value of pleural pH for the diagnosis of CPE/E. While specificity for a pH of 7.20 or below was very high (99% for CPE/E), sensitivity and PPV were too low for clinical practise (33% and 56% respectively). (Figure 20) Although several cut off levels were evaluated none of them was comparable to the accuracy of other pleural fluid markers like LDH, neutrophil share or ADA. (Figure 14, 16, 18, 20)

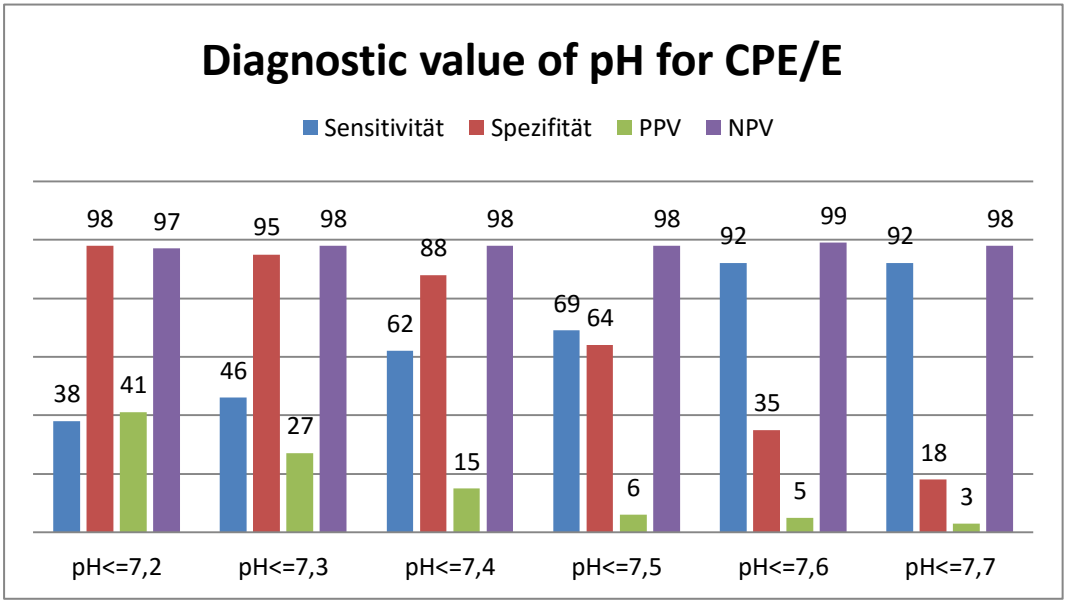
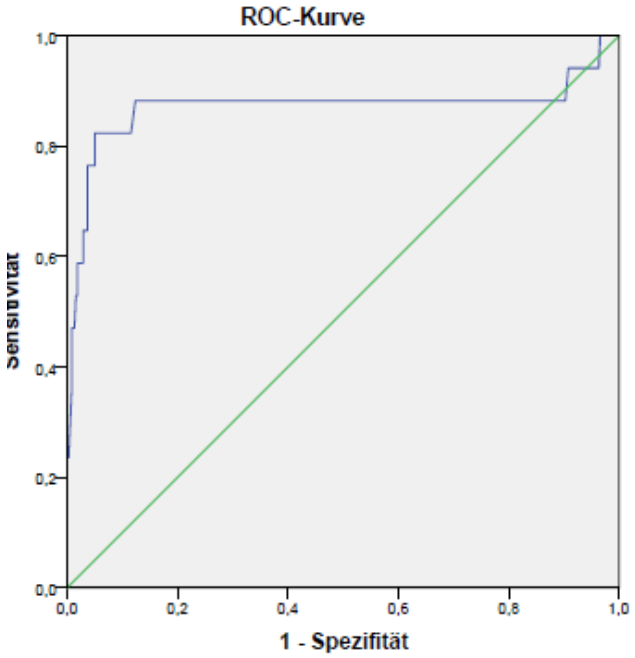


FIGURE 20 SENSITIVITY, SPECIFICITY, PPV AND NPV OF P-PH FOR CPE/E

8.5.6 Pleural Glucose in the diagnosis of CPE/E



ROC-curve analysis for pleural glucose found highest diagnostic accuracy using a cut off level of 60 mg/ dl or below and revealed an area under the curve of 0.871 (95% confidential interval: 0.730-1.0) for the diagnosis of CPE/E. (Figure 21)

FIGURE 21 ROC-CURVE ANALYSIS OF PLEURAL GLUCOSE IN THE DIAGNOSIS OF CPE/E

We evaluated several cut off levels of pleural glucose for the diagnostic of CPE/E. Best results were generated by using a cut off level of 60 to 80mg/dl. (Figure 22) However, although initial results were promising and several cut off levels were analysed, sensitivity of pleural glucose did not reach 90%. (Figure 22) Therefore, pleural glucose was not introduced in our proposed clinical algorithm for the diagnosis of CPE/E.

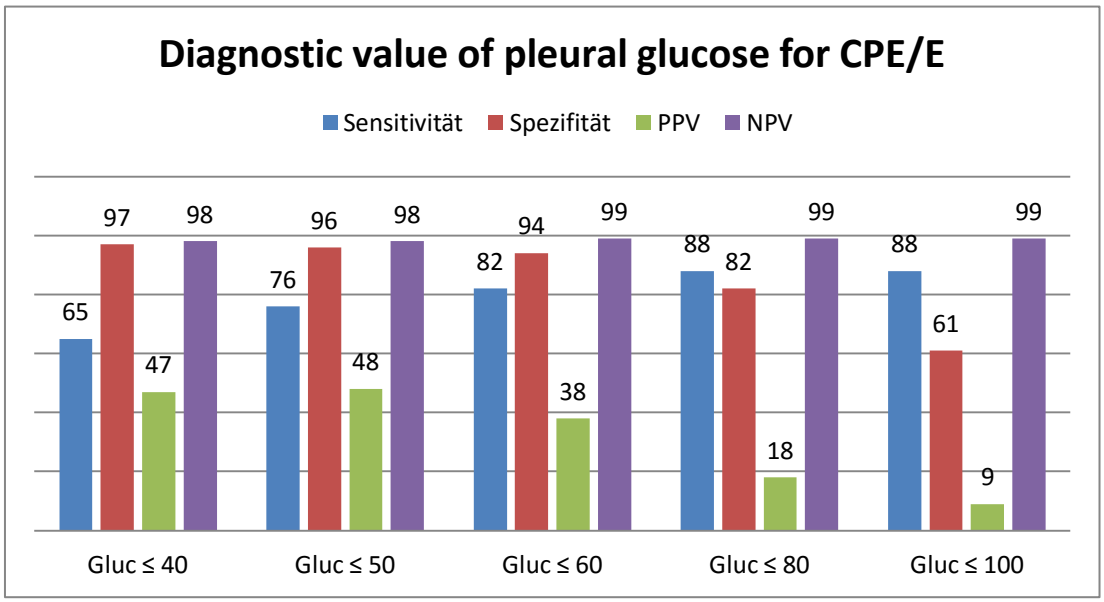


FIGURE 22 SENSITIVITY, SPECIFICITY, PPV AND NPV OF PLEURAL GLUCOSE FOR CPE/E

8.6 PROPOSAL OF A NEW ALGORITHM FOR THE DIAGNOSIS OF CPE/E

Based on our results we propose ADA, LDH and pleural neutrophil share to be combined in a new and more practical clinical algorithm for the diagnosis of CPE/E in non-malignant effusions. Using these pleural markers overall highest diagnostic accuracy was reached using ADA with a cut off level of ≥ 13 U/l combined with neutrophil share of ≥ 60% and LDH ≥ 300 U/l after exclusion of malignant cells in pleural fluid sample. These cut off levels were defined using ROC -curve analysis in order to optimize test efficiency.

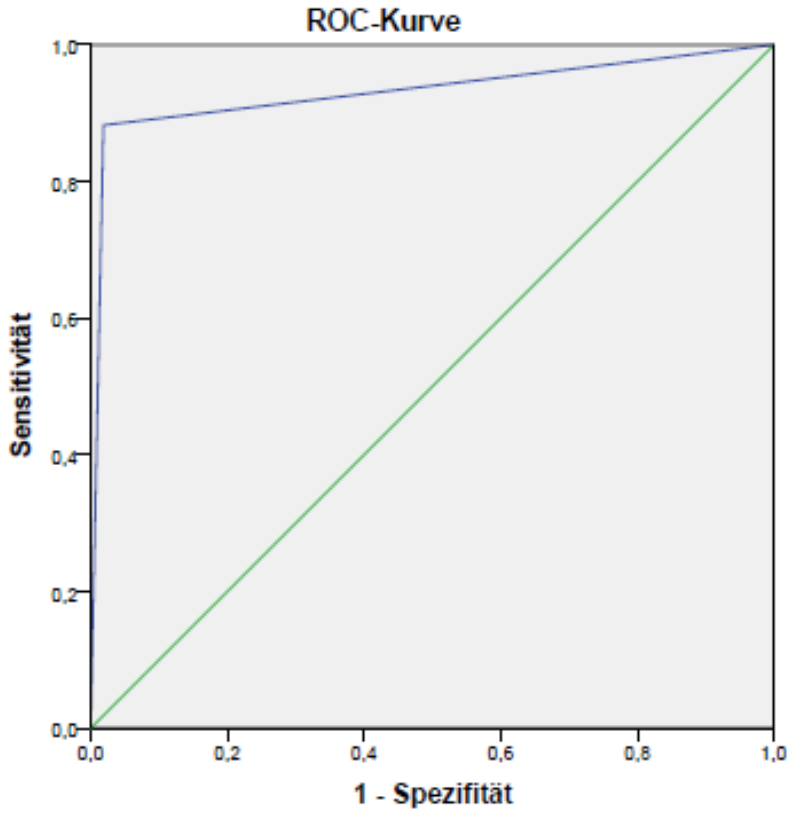
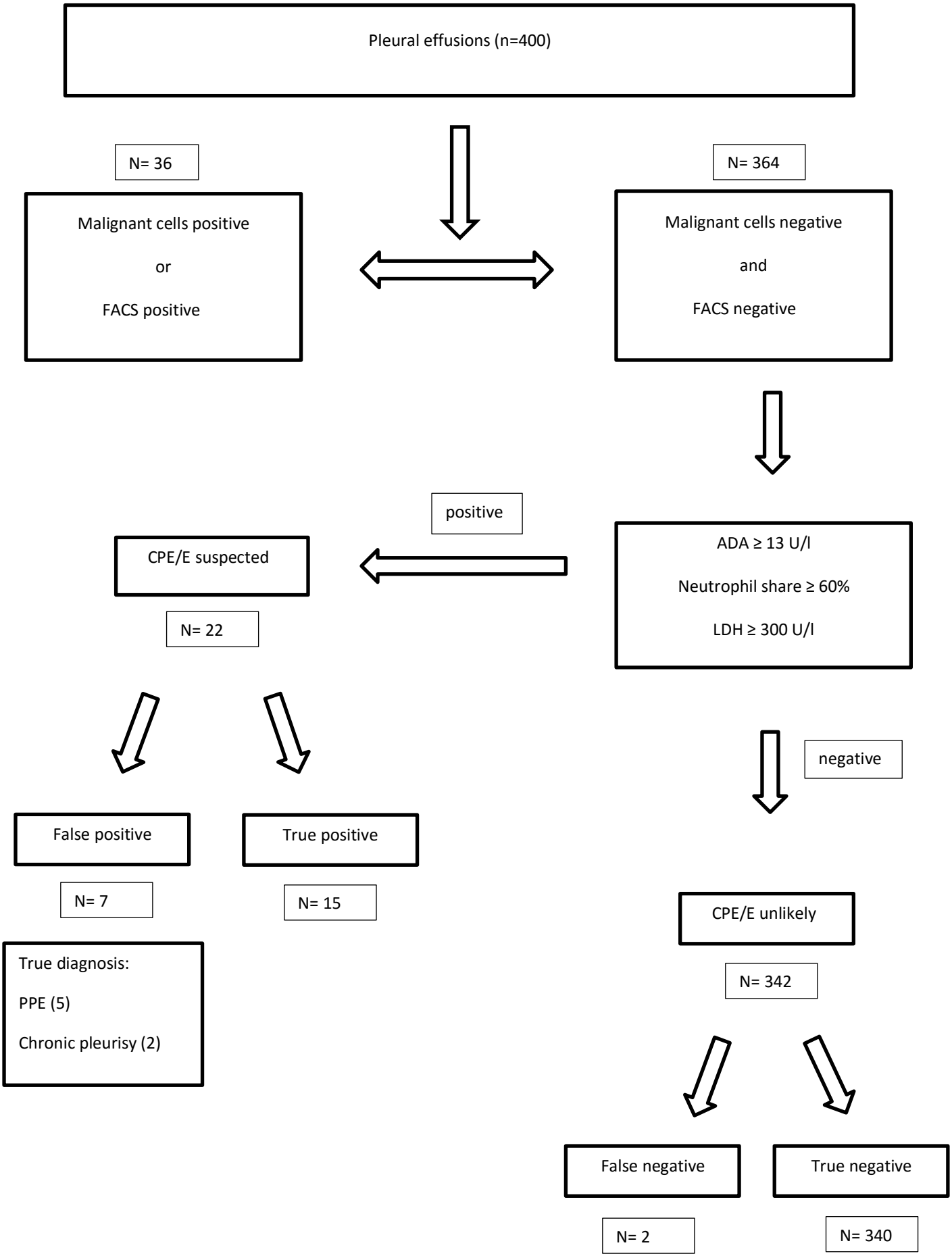


FIGURE 23 ROC-CURVE ANALYSIS FOR ADA ≥13, LDH ≥300 AND NEUTROPHIL SHARE ≥ 60% AFTER EXCLUSION OF MALIGNANT CELLS

ROC-curve analysis for this new algorithm using an ADA (≥ 13 U/l), LDH (≥300 U/l), neutrophil share (≥ 60%) and exclusion of pleural effusions that showed malignant cells found an area under the curve of 0.932 (95% confidential interval: 0.842-1.0) for the diagnosis of CPE/E. Using these laboratory values we found sensitivity, specificity, PPV and NPV of 88, 98, 68 and 99 respectively.


Pleural effusions (n=400)
N= 36
Malignant cells positive
or
FACS positive
N= 364
Malignant cells negative
and
FACS negative
ADA ≥ 13 U/l
Neutrophil share ≥ 60%
LDH ≥ 300 U/l
positive
CPE/E suspected
N= 22
negative
False positive
N= 7
True positive
N= 15
True diagnosis:
PPE (5)
Chronic pleurisy (2)
CPE/E unlikely
N= 342
False negative
N= 2
True negative
N= 340

9. DISCUSSION

Pneumonia is a common disease, often associated with PPE. With bacterial invasion into the pleural space, PPE can progress into a highly inflammatory, severely neutrophilic, exudative pleural effusion, called complicated PPE or empyema (CPE/E). CPE/E has to be diagnosed early and require immediately specific therapeutic measures (placement of pleural drainage and prolonged antibiotic treatment) to prevent treatment failure and complications like chronic pleural thickening.

The diagnosis of CPE/E is based on clinical signs (pure putrid pleural effusion), biochemical parameters (neutrophilic exudate with low glucose and/or pH) or microbiological results (detection of relevant bacteria in pleural fluid). However, in CPE/E putrid effusion is not always present and bacterial growth is frequently suppressed due to early antibiotic treatment. But even in case of a positive culture, results of microbiological tests are only available with a relevant timely delay of some days.

Therefore, a structured approach for the interpretation of immediately after thoracentesis available biochemical results is crucial. The recommended standard approach for the diagnosis for suspected CPE/E is not simple and based on the Light's criteria and additional criteria for CPP/E (neutrophilic effusion with low pH). For that purpose, at least 6 parameters have to be determine (pleural fluid protein, serum protein, pleural fluid LDH, serum LDH, pleural fluid pH, cell differentiation) and 2 ratios calculated (pleural fluid protein/serum protein ratio, pleural fluid LDH/serum LDH ratio). In neutrophilic effusion the detection of a pH lower than 7.2 is regarded as the most specific discriminator for CPE/E and as indicator for urgent chest tube drainage. [168] However, pleural pH may also be higher than 7.2 in CPE/E and therefore not sensitive enough, to detect all relevant CPE/E.

Pleural ADA determination is routinely used in the diagnosis of tuberculous pleurisy. However, ADA is also known to be elevated in exudative effusions caused by non-tuberculous diseases like CPE/E, lymphoid malignancies and rheumatoid pleurisy. [5] [7] [8] [9] [10] [11] The purpose of our study was to evaluate the benefit of pleural ADA measurement for the detection or exclusion of non-tuberculous diseases in a TB low prevalence setting with special regards to CPE/E. We proclaimed ADA to be a

sensitive and specific marker for the diagnosis of CPE/E in neutrophilic pleural effusion that might substitute pH measurement for the diagnosis of CPE/E.

Medical files of 400 patients with performed thoracentesis were enrolled in this study. After reviewing all available medical data and defining final diagnosis for all cases by an experienced specialist for pulmonary and infectious diseases we evaluated the diagnostic value of pleural ADA measurement using multiple cut off levels.

In general and as already shown by other investigators, in our study pleural ADA was also elevated in exudative effusions caused by non-tuberculous diseases like CPE/E and lymphoid malignancies (Table 1, 2, 3 and Figure 12) . [5 7 8 9 10 11] Interestingly, we found higher ADA levels in neutrophilic effusions caused by CPE/E than in lymphocytic tuberculous pleurisy (mean ADA: 85,1 U/l vs 36,9 U/l) and share of non-neutrophilic cells in pleural effusions associated with CPE/E was very low (mean share of non-neutrophilic cells: 15.2%). This finding (higher ADA-levels in neutrophilic effusions) is not in line with previous results from other studies, where highest ADA concentrations were found in lymphocytes, monocytes and macrophages (ADA1 predominantly in lymphocytes and monocytes, ADA 2 mainly in monocytes and macrophages). [204 205 206] Our data suggests, that activated neutrophilic cells are able to provide high levels of ADA activity in case of CPE/E. As a limitation of our study, ADA isotype differentiation was not performed, which would be highly interesting to answer the question if activated neutrophils produce more ADA1 or ADA2 subtypes. Moreover, the determination of ADA subtype might be useful for the differentiation between CPE/E, tuberculosis or lymphoma, as mentioned by Valdes et al. [211]

Based on our data, the best value of ADA level for the diagnosis of CPE/E was found at a cut off level of 13 U/l or above. This cut off reached a high sensitivity, specificity and NPV (100%, 77% and 100%, respectively). As expected the low PPV (18%) for CPE/E was mainly caused by cases of tuberculous pleurisy (13 ADA positive cases), lymphoid malignancies (11 ADA positive cases) and other malignant pleural effusions (13 ADA positive cases). However, using the same ADA cut off but considering only non-malignant effusions with a neutrophil share ≥ 60% and a pleural LDH ≥ 300 U/l, the PPV for CPE/E can be improved to 68% with maintaining high values for sensitivity, specificity and NPV (88%, 98% and 99%, respectively; Figure 23).

Furthermore we examined the diagnostic value of neutrophil share, pleural LDH, pleural pH and glucose levels for die diagnosis of CPE/E. Comparable diagnostic

accuracy was reached by pleural LDH using a cut off level of ≥ 300 U/l and neutrophil share using a cut off level of ≥ 60%. Sensitivity, specificity, PPV and NPV for neutrophil share using a cut off level of ≥ 60% were 94%, 89%, 29% and 99% respectively. Sensitivity, specificity, PPV and NPV for LDH using a cut off level of ≥ 300 U/l were 95%, 70%, 13% and 99% respectively. Our findings suggest that pleural neutrophil share is the most sensitive and specific pleural fluid marker for the diagnosis of CPE/E. By contrast, pleural pH and pleural glucose did not fulfilled the predetermined optimal cut off criteria (cut off with a sensitivity ≥ 90% and specificity ≥70%, Figure 20 and 22) and were overruled by the high sensitivity and specificity of pleural ADA, neutrophil share and pleural LDH (Figure 14, 18, 16). The pleural pH of ≤ 7.2, as the actual standard for the diagnosis of CPE/E, showed even in neutrophilic effusions (neutrophil share ≥ 60%) a disappointing sensitivity of only 42% (specificity, PPV and NPV: 99%, 71% and 98%, respectively). In six microbiologically confirmed CPE/E cases with positive cultures for *Streptococcus constellatus* (1x, ADA=181 U/l), *Streptococcus intermedius* (1x, ADA=18 U/l), *Enterococcus spp.* (2x, ADA=26 and 16 U/l), *Staphylococcus aureus* (1x, ADA=14 U/l), *E. coli* + *Candida albicans* + *Streptococcus mitis* (1x Boerhaave syndrome, ADA=14 U/l) the pH was > 7.2. In all of these six cases ADA was > 13 U/l. Although a low pH is very specific for CPE/E and has a high NPV, based on our data and the insufficient sensitivity of pH ≤ 7.2, we discourage the use of pH as parameter for the diagnosis of CPE/E. In order to improve currently used clinical algorithms for risk stratification of CPE/E we proposed a new clinical algorithm based on the pleural fluid analysis results. This new algorithm was based on ROC analysis of the most promising pleural fluid markers. In non-malignant effusions most promising results were found combining ADA ≥ 13 U/l, LDH ≥ 300 U/l and neutrophil share ≥ 60%. This new algorithm showed sensitivity, specificity, PPV and NPV of 89%, 98%, 68% and 99%, respectively (Figure 23). Currently used algorithms are mainly based on neutrophil share, LDH, glucose levels and pH.

If compared to our proposed algorithm we found much lower accuracy for the currently used pleural markers (Figure 20 and 22). As mentioned above, pleural pH and pleural glucose appear to be far less reliable than currently believed. A limitation of our study might the limited number of CPE/E cases. From the 400 reviewed cases only 19 cases of CPE/E could be diagnosed. A higher number of pleural effusion cases would be necessary to substantiate the value of routine measurement of

pleural ADA. However, the data collection continues and the same analytic evaluation can be repeated in 1 or 2 years. Another limitation of our study was the determination of ADA activity using the automated, standardized-turbidimetric analyses of ADA on the Cobas 8000 system. As the range of ADA levels for tuberculous pleurisy in our cohort (mean 36,9 U/l) was lower than in other ADA studies (mean ADA 127.5 +/- 2.9 IU (Valdes et al. 1996), mean ADA 54.7 +/- 23.5 IU (Pérez-Rodríguez et al 1999), we hypothesize, that COBAS-systems measured ADA-values cannot be compared with non-automatic analysis. [210] [211] Therefore re-evaluation of currently clinically applied ADA cut off levels for the diagnosis of tuberculous pleurisy and CPE/E should be considered if automated, standardized-turbidimetric analyses of ADA on the Cobas 8000 system is performed.

In summary, we believe that the results of this study should be further investigated by performing a prospective interventional study using our proposed ADA-based algorithm for the diagnosis of CPE/E.

10. FIGURES

11. TABLES

11. REFERENCES

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