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The diagnostic value of biochemical and tumour markers in the differential diagnosis of pleural effusions

Summary of the Doctoral thesis
for obtaining a doctoral degree (*Ph.D.*)

Sector – Medicine
Sub-sector – Pneumology

Rīga, 2020

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Defence of the Doctoral Thesis will take place on 29 May 2020 at 15.00 online on Zoom meetings platform

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ABBREVIATIONS

BNP	Brain natriuretic peptide
CA 125	Cancer carbohydrate antigen 125
CA 19-9	Cancer carbohydrate antigen 19-9
CA 15-3	Cancer carbohydrate antigen 15-3
CEA	Carcinoembryonic antigen
CT	Computed tomography
LDH	Lactate Dehydrogenase
MPE	Malignant pleural effusion
MNC	Monomorphonuclear cells
NT-proBNP	N-terminal pro-brain natriuretic peptide
PAI-1	Plasminogen activator inhibitor 1
PATE	Pulmonary artery thromboembolism
PMC	Polymorphonuclear cells
REUH	Riga East University Hospital
ROC curve	Receiving operating curve
US	Ultrasound
VATS	Videoassisted thoracoscopy

INTRODUCTION

Pleural effusion – content in pleural space that appears due to inflammation, proliferation of malignant cells and/or pathologically modified capillary permeability – is a frequent complication of different diseases; its main causes are malignant tumours, pneumonia and tuberculosis. Although wide epidemiologic studies are not available, the most frequent causes of pleural effusion are cardiac hydrothorax and malignant pleural effusion, but the third most common cause is parapneumonic pleurisy (*Marel et al.*, 1993; *Zablockis et al.*, 2002; *Valdes et al.*, 1996; *Broadus et al.*, 2016). Treatment and prognosis of pleural effusion of different etiology is significantly different so precise diagnosis is very important. Diagnostics of cardiac hydrothorax usually does not present any difficulties in clinical practice, but it can be complicated to diagnose MPE, especially in conditions when thoracoscopy is not available to obtain the material of pathological tissue and perform further histological analysis. So, it is important to search for other available, relatively easily accessible markers for the diagnosis of these pleural effusions.

Malignant pleural effusion is a common issue that cannot always be easily managed in the clinical practice. Currently there are not many recommendations in the world regarding pleural effusion – the latest are the guidelines for malignant pleural effusions issued by American Thoracic Society in 2018 (*Feller-Kopman*, 2018), Spanish guidelines 2014 (*Villena Garrido et al.*, 2014) and Guidelines of British Thoracic Society 2010 developed by Pleural Diseases Guidelines Group (*Du Rand I., Hooper C., MuccDuff A., Roberts M. E., Davies H. E., Rahman N. M., Havelock T. et al.*, 2010). Conventional methods – clinical, biochemical and cytological examination of pleural effusion is not always informative enough to specify the etiology of the effusion quickly and reliably. That is why the 2001 Report on malignant pleural effusion by the European Respiratory Society and American Thoracic Society recommends the

identification of new markers of malignant pleural effusion as one of future research directions in this field (*Antony et al., 2001*). This Doctoral Thesis is a summary of research efforts conducted to improve diagnosis of MPE in Latvia, using additional biochemical and tumour markers.

Aim of the study

The aim of the study is to find out whether it is possible to improve the diagnostic tools available in the detection of malignant pleural effusion by means of additional biochemical and tumour markers.

Objectives of the study

1. To analyse the available literature of pleural effusion diagnosis and differential diagnosis.
2. To investigate the proportion of patients with pleural effusion of different underlying etiology, including malignant pleural effusion, in the departments of internal diseases in REUH “Gaiļezers” within a year.
3. To assess the current approach of pleural effusion diagnostics thods used in clinical practice.
4. To study whether the use of additional biochemical (PAI-1, BNP) and tumour (CEA and CA 125) markers increases sensitivity of laboratory tests in the diagnosis of malignant pleural effusion.
5. To formulate diagnostic recommendations of pleural effusion

Hypothesis of the study

Addition of biochemical and tumour markers in pleural effusion improves diagnostic yield and the differential diagnosis of malignant pleural effusion.

Scientific novelty

There is no analysis of pleural effusion prevalence among inpatients in Latvia so far. This Doctoral Thesis studies the currently unclear role of fibrinolytic parameters (PAI-1), as well as concentrations of tumour markers in serum and pleural effusion, to be implemented the diagnostic and therapeutic algorithm of pleural pathology.

1. MATERIAL AND METHODS

The study protocol was approved by the RSU Ethics Committee. The study was performed in Riga East Clinical University Hospital “Gaiļezers”.

To evaluate the current situation and significance of the problem, patients diagnosed with pleural effusion from 01.01.2010 to 31.12.2010 at Internal Diseases Departments of Riga East University Hospital were retrospectively reviewed. Medical records of patients with pleural effusion diagnosis by discharge were analysed according to an originally created scheme (see Table 1.1).

Table 1.1

Evaluation scheme of medical records

No	Parameters
1	Department
2	Number of bed days
3	Month of the year
4	Sex
5	Age
6	Alive/dead by discharging
7	Side of pleural effusion
8	Presence of chest X-ray
9	Chest X-ray results
10	Presence of chest CT
11	Presence of pleural US
12	Presence of thoracentesis
13	Day of thoracentesis
14	Number of thoracentesis
15	Presence of white cell differential in pleural effusion
16	If yes, white blood cell differential
17	Presence of LDH analysis in pleural effusion
18	If yes, level of LDH
19	Presence of protein analyse in pleural effusion
20	If yes, protein level
21	Presence of cytological analyse of pleural effusion
22	If yes, is there malignancy?
23	Presence of bacteriological tests
24	If yes, tests results?

Table 1.1. Continued

No	Parameters
25	Additional investigations
26	Discharge diagnosis
27	Etiology of pleural effusion according to discharge diagnosis
28	Primary tumour localisation among the patients with MPE

To evaluate the importance of additional biochemical and tumour markers in the diagnostics of MPE, patients with pleural effusion sequentially admitted in the Pulmonology Department of Internal Diseases Clinic of Riga East University Hospital “Gaiļezers” from 08.08.2011 to 13.06.2014 were prospectively analysed.

Inclusion criteria:

- pleural effusion detected with X-ray and ultrasound;
- diagnostic and/or therapeutic indications of thoracentesis;
- signed informed consent.

Indications of thoracentesis were considered to be:

1. Pleural effusion of unclear etiology, incl. suspected MPE;
2. Parapneumonic pleurisy, incl. suspected empyema in patients with an acute disease, febrile temperature, elevated inflammatory indicators (C reactive protein levels) and consolidation on X-ray;
3. Large amount of pleural effusion causing breathlessness (*Huggins et al.*, 2011; *Roberts et al.*, 2010, *Havelock et al.*, 2010)

Exclusion criteria:

- re-hospitalised patients with pleural effusion;
- patients with decompensated heart failure clinic (known pre-existing cardiac disease, progressive shortness of breath, peripheral oedema, signs of cardiac failure in echocardiography (ehoCG), congestion in small circulation circle in thorax X-ray) and cardiac hydrothorax, which has neither diagnostic, nor therapeutic indication of thoracentesis.

Thoracentesis was made under local anaesthesia with Sol. *Lidocaini* 2 %, using *Pleurocan* (*Braun*) pleural catheters. Samples of pleural effusion of all patients were sent to the laboratory to analyse the following parameters:

- White blood cell differential and cytological analysis:
 - White blood cells differential (mononuclear and polymorphonuclear percentage, haematocrit);
 - cytological analysis (presence of malignant cells);
- Clinical chemistry:
 - LDH;
 - Protein;
 - BNP;
 - PAI 1.
- Analysis of tumour markers;
 - CEA;
 - CA 125;

Puncture of peripheral vein was performed concurrently with thoracentesis in all patients and venous blood samples were taken to determine the following parameters:

- LDH;
- Protein;
- BNP;
- PAI-1.
- Analysis of tumour markers;
 - CEA;
 - CA 125;

To determine LDH and protein, pleural effusion and venous blood sample were collected in tubes without anticoagulant and sent to REUH Laboratory Medicine Centre. Protein is determined by colorimetric method. LDH is

determined by kinetic UV method. Light's criteria were calculated after receiving the results with the aim to divide pleural effusions in transudates and exudates (*Light et al, 2007*):

1. Pleural effusion/serum protein ratio > 0.5;
2. Pleural effusion/serum LDH ratio > 0.6;
3. Pleural LDH > upper limit of two-thirds of normal LDH upper limit (normal LDH upper limit in REUH is 240 U/L).

For white blood cell differentials pleural effusion was collected in EDTA tube and sent to REUH Laboratory Medicine Centre for clinical analysis (manual cells counting under the microscope and the automated method):

- counting 200 cells under light microscope and determining the percentage polymorphonuclear and mononuclear white blood cells;
- determining the percentage of polymorphonuclear and mononuclear white blood cells and the level of haematocrit and haemoglobin using automated methods.

30 ml of pleural effusion in sterile container was sent for cytologic analysis to REUH Laboratory Medicine Centre.

Bacteriology examination of pleural effusion was performed for patients with an acute disease, febrile temperature, elevated inflammatory indicators (C reactive protein levels) and consolidation on X-ray.

To determine tumour markers, pleural effusion and venous blood samples were collected in tubes without anticoagulant, 35 minutes settled and 10 minutes centrifugated at 3000 rpm, supernatant was separated from pleural effusion and serum from blood. Samples were frozen at -80° till the analysis was performed. CA 125 and CEA were determined by ELISA (*enzyme-linked immunosorbent assay*) method with Abbott Architect analyser in accordance with the manufacturer's protocol.

To assess BNP, pleural effusion and blood sample were collected in EDTA tube and sent to REUH Laboratory Medicine Centre. BNP level was established by Chemiluminescent Microparticle Immuno Assay (CMIA).

To determine PAI-1, venous blood and pleural effusion samples were collected in tubes without anticoagulant, 45 minutes settled and centrifugated for 10 min at 1000 rpm. Supernatant and serum were separated and frozen at -80° till the analysis is performed. PAI-1 was determined by cytometric xMAP technology (Luminex equipment) in the Department of Human Biochemistry and Physiology of Rīga Stradiņš University.

The diagnosis of cardiac hydrothorax was established in patients with previously proven cardiac failure, existing symptoms of cardiac failure – peripheral oedemas, symptoms of cardiac failure in echocardiography or venous congestion in thoracic X-ray and hypoxaemia below 93 % SpO₂, which did not improve significantly after additional supply of oxygen.

The diagnosis of parapneumonic pleurisy was established according to the following criteria: acute illness with cough, fever, elevated inflammatory indicators (C reactive protein) and appropriate radiographic finding.

MPE was diagnosed on the basis of cytologic examination of pleural effusion. If cytologic examination was negative, exudate was regarded as MPE also in cases, if the malignant disease already has been proven and another cause of the exudate was not established (*Sahn*, 1997).

Closed pleural biopsy was performed for patients, who had > 95 % MNC in pleural effusion. The diagnosis of tuberculous pleurisy was proven by histological examination of pleural biopsy, by finding specific changes – epithelioid cell granulomas with caseous necrosis and PCR confirmation of *Mycobacterium tuberculosis* DNA from the effusion. Pancreatic pleural effusion associated was diagnosed by elevated lipase levels in pleural effusion.

Talc slurry pleurodesis with 4 g talc suspension and 40 ml 0.9 % NaCl solution by adding 10 ml 2 % lidocaine solution was performed for patients with MPE who agreed to talc pleurodesis. Drain was closed for two hours and evacuated when less than 100 ml of liquid excreted for two days.

1.1. Methods of statistical analysis

Statistical calculations of all data were made by SPSS (*Statistical Package for the Social Sciences*) for Windows software version 23.0 and MS Excel 2007. In accordance with generally accepted principles in medical statistics, p-value of 0.05 was considered as the statistical reliability threshold of bilateral test results. Generally accepted statistical methods were used to characterise the group of persons (Teibe, 2007; Dawson, 2001; Altman, 1997). Conformity of distribution of quantitative data to the norm is verified by means of histograms and Kolmogorov-Smirnov test. Since the data distribution did not conform to normal distribution, median 25 and 75 percentile was used to characterise the average indicators. Categorical or qualitative variables are characterised by percentage proportion. 95 % reliability interval limits were calculated, so that the obtained results could be generalised for the population to be studied. Mann-Whitney test was used to compare two independent groups because the variables were not in line with the normal distribution. Pearson chi χ^2 test was used to compare the categorical variables, performing also the continuity correction according to Yates method and odds ratio (OR) was also calculated. To determine sensitivity and specificity of different tests, ROC (*receiver operating characteristic*) curve was used when calculating the area under the curve.

2. RESULTS

2.1. Analysis of retrospectively obtained data

2.1.1. Characterisation of patients

By analysing medical records, it was established that between 01.01.2010 and 31.12.2010 from Departments of Internal Diseases of REUH “Gaiļezers”, 14,838 patients were discharged alive and 741 patients died (in total 15,588 patients).

There were 716 (4.6 %) medical records of patients with pleural effusion of any etiology mentioned in the discharge diagnosis. 615 (85.9 %) of these patients were discharged alive, 101 (14.1 %) died. Overall, in-hospital mortality was 4.4 %.

Among the analysed patients, 337 (47 %) were male and 379 (53 %) – female aged from 18 to 98 years. Distribution of the inspected patients by age is unimodal, modal age 71 to 80 years. The number of patients increases at the age of over 40 – in the age groups up to 41, there were only 4.2 % of the analysed patients, 95.8 % of the patients were older than 41 years.

2.1.2. Investigation results and diagnoses

By analysing the investigations documented in the medical records of patients with pleural effusion, chest X-ray was performed in 651 patients (90.9 %) in inpatient setting and in 11 (1.5 %) patients in previous outpatient setting. Chest X ray was not performed in 54 patients (7.5 %). 167 (23.3 %) patients had undergone chest computed tomography. By analysing both these investigations, 155 patients (21.6 %) had undergone both X-ray and computed tomography. 494 patients (68.9 %) had undergone only X-ray, 11 patients (1.5 %) – only computed tomography, 42 patients (5.9 %) had undergone neither X-ray nor computed

tomography. In 141 (19.7 %) patients pleural ultrasound was recorded (Figure 2.1). 104 of these patients had undergone thoracentesis. In 407 (56.8 %) patients' medical records there were no pleural ultrasound and thoracentesis recorded. 168 patients (23.5 %) had undergone thoracentesis and had no records of pleural ultrasound.

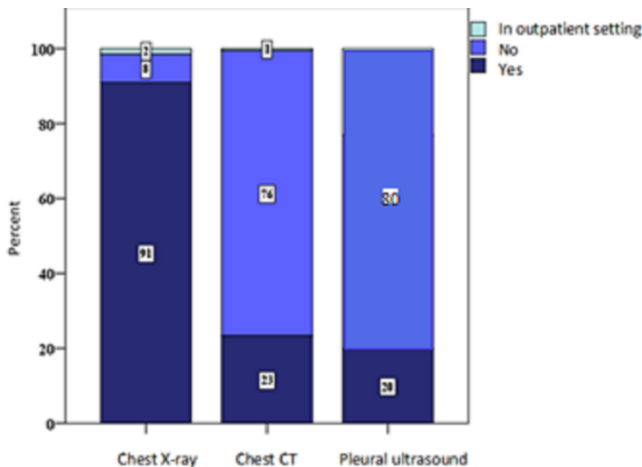


Fig. 2.1. **Proportion of patients who underwent diagnostic imaging**

By summarising the documented laboratory examination of pleural effusion, white blood cells differential analysis, LDH, protein and cytology was determined most frequently – in 130 cases (47.8 % of all pleural effusions samples). No analysis of pleural effusion was made in 29 cases (10.7 %) (Figure 2.2).

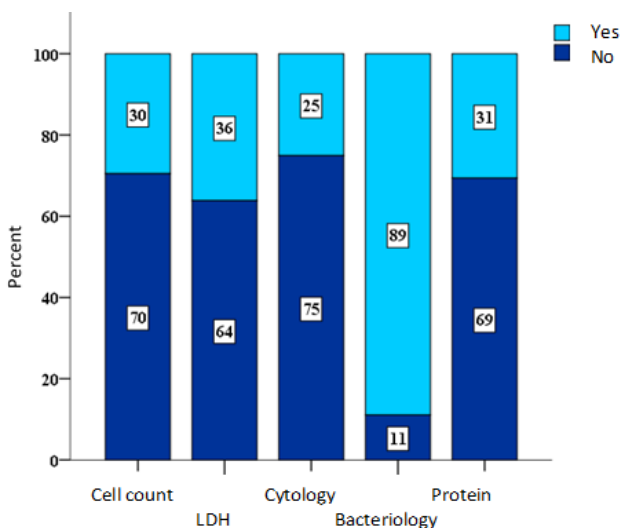


Fig. 2.2. Proportion of performed and unperformed laboratory analysis of pleural effusion

When assessing discharge diagnosis, in 426 (59.5 %) patient's pleural effusion was considered as cardiac hydrothorax, in 103 (14.4 %) as MPE, in 71 (9.9 %) – parapneumonic pleurisy, in 30 (4.2 %) as pleural empyema, in 11 (1.5 %) – a haemothorax, and in 6 (0.8 %) – tuberculous pleurisy. Effusion of other etiology was mentioned in 36 patients (5 %): nephrotic syndrome in 9 cases, hepatic cirrhosis – 6, thromboembolism of pulmonary artery – 7, hyperhydration in patients with chronic renal failure – 6, post-traumatic pleurisy – 2, Meig's syndrome – 1, Lupus erythematosus – 1, iatrogenic hydropneumothorax – 1, unclear diagnosis – in 3 cases. A final diagnosis of pleural effusion was not made in 33 patients (4.6 %) (Figure 2.3).

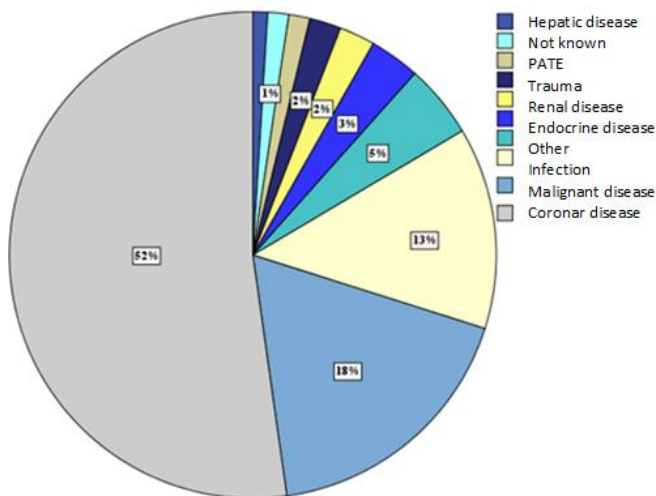


Fig. 2.3. **Distribution of patients (n = 716) according to discharge diagnoses**

When analysing discharge diagnoses and pleural effusion examinations more thoroughly, it was found that in discharge diagnosis of 426 (59.5 %) patients' pleural effusion was associated with cardiac failure, thoracentesis was performed for 98 (23 % of cardiac hydrothorax) of them. Diagnosis of 51 (11.9 %) patients was also confirmed in laboratory – LDH level in pleural effusion through LDH levels confirmed an exudate in 13 (3.1 %) patients. Laboratory tests of 34 (23 %) patients were insufficient to specify the type of the effusion.

MPE was the discharge diagnosis of 103 – 14.4 % of all patients, thoracentesis was performed for 62 (60.2 %) of these patients and only for 37 (36 %) the diagnosis was cytologically confirmed. LDH level was determined for 8 (7.8 %) of these patients, for 5 (4.9 %) of them it corresponded to a transudate. Laboratory examinations were insufficient to determine the type of the pleural effusion for 17 (16.5 %) of the patients.

Parapneumonic pleurisy was the discharge diagnosis of 71 – 9.9 % of all patients, 37 (52.1 %) of them underwent thoracentesis. An exudate was confirmed in 23 (32.3 %) of the patients, LDH level of 7 (9.9 %) patients were indicative of a transudate and laboratory examinations of 7 (9.9 %) have not been sufficient to specify the type of the pleural effusion. In 30 patients – 4.2 %) discharge diagnosis was pleural empyema. In 12 (40 %) patients from this group, empyema was confirmed bacteriologically, 15 (50 %) of the patients had an exudate and in 2 cases laboratory tests were incomplete.

2.2. Analysis of prospectively obtained data

2.2.1. Characterisation of patients

From 08.08.2011 to 06.13.2014 in Pulmonology Department of Clinical of Internal Diseases of REUH “Gaiļezers”, 144 patients with pleural effusion were consecutively admitted. In all patients X-ray and ultrasound confirmed pleural effusion and there were indications of thoracentesis for diagnostic and/or therapeutic reasons. All patients signed an informed consent for the study and thoracentesis. 69 (47.9 %) of them were male. Age of the patients was from 22 to 97 years. 136 (94.4 %) of patients were discharged from hospital, 8 – died (5.5 %).

2.2.2. Characterisation of pleural effusion etiology

All patients were divided into three groups: cardiac hydrothorax group – 42 patients (29.2 %), 22 (52.3 %) of them were men; MPE group – 67 (46.5 %) patients, 21 (31.3 %) of them were men; parapneumonic pleurisy group – 27 (18.8 %) patients, 19 (70.3 %) of them were men. Six (4.1 %) patients were diagnosed with tuberculous pleurisy, one (0.7 %) – with pancreatic pleurisy and one (0.7 %) – PATE with pleurisy (Figure 2.4). Considering the small number

of patients with tuberculous pleurisy, pancreatic pleurisy and PATE, these patients were not analysed further.

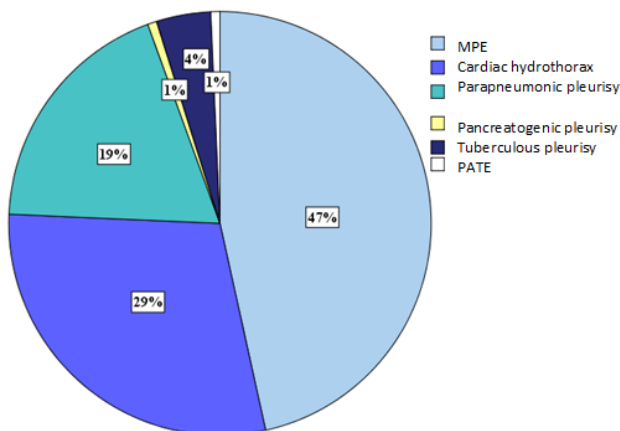
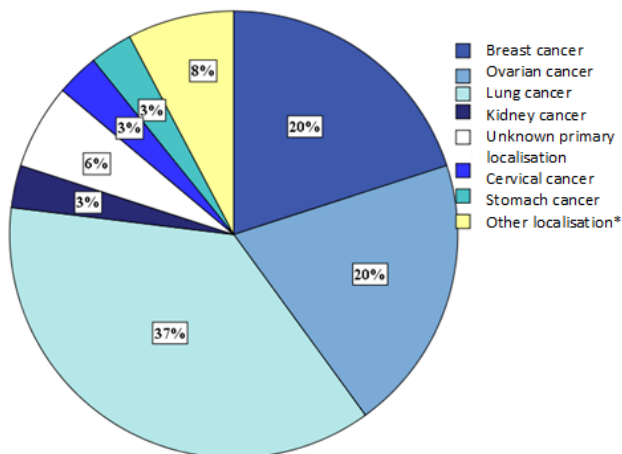


Fig. 2.4. **Pleural effusion etiology (n = 144)**

24 (35.8 %) of the patients in MPE group had lung cancer, 14 (20.9 %) female patients – ovarian cancer, 13 (19.4 %) female patients – breast cancer, 2 patients – kidney cancer, 2 female patients – cervical cancer, 2 patients – gastric cancer, 1 patient – cancer of the pancreas, 1 patient – sarcoma, 1 patient – mesothelioma, 1 patient – melanoma, 1 patient – prostate cancer. The primary localisation of tumour could not be clarified for 5 patients (Figure 2.5).



*Mesothelioma, prostata cancer, pancreatic cancer, melanoma, sarcoma

Fig. 2.5. Distribution of MPE depending on primary localisation of tumour (n = 67)

2.2.3. Light's criteria

Group of cardiac hydrothorax

In the group of cardiac hydrothorax 42 (29.2 %) patients with previously proven cardiac failure were included, aged from 50 to 92 years, in whom symptoms of chronic heart failure decompensation were clinically observed.

Light's criteria of 34 (81 %) patients confirmed transudate, but Light's criteria of 8 (19 %) patients indicated an exudate (in 2 patients, protein effusion/serum ratio was > 0.5 ; in 3 patients LDH effusion/serum ratio was > 0.6 , 1 patient had LDH > 160 U/L (2/3 of the upper normal LDH serum level); all Light's criteria were a bit elevated in 3 patients. The protein gradient was evaluated in all of these patients. In 7 patients it was > 31 g/l and in 1 patient it was < 31 g/l, but BNP serum for this patient was 1631 pg/ml. 39 (92.9 %) patients

from the group of cardiac hydrothorax were discharged, but 3 (7.1 %) patients had died 2, 4 and 11 days after hospitalisation, accordingly.

Parapneumonic pleurisy

In the group of parapneumonic pleurisy 27 (18.6 %) patients between the age of 38 to 97 years were included, that fulfilled the diagnostic criteria of pneumonia: acute illness with cough, febrile temperature, elevated inflammatory indicators (C reactive protein level) and appropriate radiographic finding. All of the patients had at least one positive Light's criterion. All patients (100%) had positive Light's 3rd criterion – LDH level in effusion > 2/3 of laboratory standard (168–1006 U/L), 26 (96.3 %) of patients had a positive Light's 2nd criterion – LDH level in effusion against LDH level in serum > 0.6 (0.51–54.67) and 20 (74 %) of patients had a positive 1st Light's criterion, i.e. protein level in effusion against protein level in serum > 0.5 (0.29–2.28). Thus, 20 (74%) patients had positive all Light's criteria, 26 (96,3%) patients – two (the 2nd and the 3rd) Light's criteria and 1 patient has positive only one – the 2nd Light's criterion.

Effusion in pleural space was examined microbiologically for all patients, but was positive only in two (7.4 %) of them: one patient had *Klebsiella pneumoniae*, one patient – *Staphylococcus sacchrolyticus*. 3 patients required drainage of pleural space administering fibrinolytic enzymes. 26 (96.3 %) patients were discharged, but 1 (3.7 %) died on the 9th day after hospitalisation.

Malignant pleural effusion

The MPE group contained 67 (46.5 %) patients at the age 40–90 years. Light's criteria were negative for 2 patients (2.9 %).

Light's 1st criterion – protein effusion/serum ratio (0.51–4.25) > 0.5 – was positive in 59 (88 %) of all patients; 2nd criterion – LDH effusion/serum ratio > 0.6 (0.64–9.78) – was positive in 50 (74.6 %) patients and 3rd Lights criterion –

LDH level in pleural effusion $> 2/3$ of the upper laboratory limit (163–1908 U/L) – was positive in 52 patients (77.6 %).

42 (62.6%) of patients had all three criteria positive, 8 (11.9%) – only 1st criterion positive, 5 (7.5%) – the 1st and the 3rd criteria positive, 4 (6%) – the 1st and the 2nd criteria positive, 4 (6%) – the 2nd and the 3rd criteria positive, 1 patient (1.5%) has only 2nd criterion positive and 1 (1.5%) patient has only 3rd criterion positive.

Pleural effusion was examined cytologically for all patients, malignant cells were identified in 45 (67.2 %) patients. Talc slurry pleurodesis was performed in 18 (26.9 %) patients. 63 (94 %) patients were discharged, but 4 (6 %) patients died (on day 3, 9, 14 and 22, accordingly), including 1 patient died after talc pleurodesis.

2.2.4. Cytological examination

Malignant cells in pleural effusion were found in 44 (65.7 %) of 67 patients with MPE. Malignant cells for 38 patients (83.4 %) were detected during the first cytological examination, in 6 patients (13.6 %) in the second cytological assessment.

2.2.5. Bacteriological analysis

Pleural effusion of 27 patients was sent for microbiological examination, it was positive only for 2 patients – *Klebsiella pneumoniae* and *Staphylococcus saprophyticus* were identified.

2.2.6. Tumour markers

CA 125 and CEA

To identify whether there is a statistically significant difference in CA 125 and CEA concentrations of pleural effusion with different underlying etiology, the groups of parapneumonic pleurisy, malignant pleural exudates and transudates were analysed further. Median values of 25th and 75th percentile of tumour markers in serum and pleural effusion as well as the effusion/serum ratio were determined (Table 2.1). CA 125 values for patients with MPE statistically significantly differ both in pleural effusion and serum between patients with transudate and parapneumonic pleurisy, with the effusion/serum ratio reliably distinguishing a MPE from a transudate. CEA values statistically significant differ among the groups of MPE, transudate and parapneumonic pleurisy only in pleural effusion. CA 125 level in cardiac hydrothorax did not differ significantly from the level in the parapneumonic pleurisy group neither in pleural effusion ($p = 0.850$), nor in serum ($p = 0.694$).

Table 2.1.

The median CA-125 and CEA value in serum and pleural effusion in patients with cardiac hydrothorax, MPE and parapneumonic pleurisy

Tumour marker	Fluid	Median value (25 th and 75 th percentile)			Statistical significance p1	Statistical significance p2
		Cardiac hydro- thorax	Para- pneumoni c pleurisy	MPE		
CA 125	Effusion U/ml	686 (379–1029)	412 (245–695)	1644 (813–2982)	0.001	0.002
	Serum, U/ml	182.0 (89–316)	62 (41–164)	267 (107–597)	0.013	0.010
	Effusion /serum	3.64 (2.45–5.75)	5.5 (3.0–11.0)	5.53 (3.82–10.61)	0.001	0.216
CEA	Effusion ng/ml	0.75 (0.51–1.60)	1.91 (1.09–3.63)	6.23 (0.92–57.3)	0.005	0.020
	Serum, ng/ml	2.34 (1.40–3.67)	2.57 (1.40–4.48)	3.07 (1.30–12.79)	0.218	0.284
	Effusion /serum	0.38 (0.26–0.66)	0.70 (0.37–1.48)	1.23 (0.65–4.78)	0.061	0.116

p1 – statistical significance transudate vs MPE

p2 – statistical significance parapneumonic pleurisy vs MPE

To assess the clinical relevance of the 2 tumour markers, sensitivity and specificity of concentrations in serum and effusion at various cut-offs, and effusion/serum ratio were calculated (Table 2.2).

Table 2.2

CA 125 and CEA sensitivity and specificity for different values in MPE

Tumour marker	Fluid	25th percentile; Median value; 75th percentile	Sensitivity (%)	Specificity (%)
CA 125	Pleural effusion U/ml	≥ 813	79.1	70.1
		≥ 1644	53.7	96.1
		≥ 2982	28.4	98.7
	Serum U/ml	≥ 108	76.2	69.3
		≥ 268	50.8	77.9
		≥ 597	25.4	98.7
	Effusion/serum	≥ 3.82	76.1	49.4
		≥ 5.53	50.8	66.2
		≥ 10.61	25.4	87.0
CEA	Pleural effusion ng/ml	≥ 0.92	76.1	44.1
		≥ 6.23	50.7	94.8
		≥ 57.3	25.4	98.7
	Serum ng/ml	≥ 1.30	76.1	18.2
		≥ 3.07	50.8	66.2
		≥ 12.8	25.4	96.1
	Effusion/serum	≥ 0.60	82.0	61.0
		≥ 1.23	50.6	81.8
		≥ 4.78	25.4	97.4

ROC curve analysis showed that the highest area under the curve is for CA 125 level in pleural effusion – 0.751; 0.706 – for serum and 0.606 – for effusion/serum ratio –, which indicates highest diagnostic sensitivity of CA 125 in pleural effusion – see Figure 2.6 and Table 2.3.

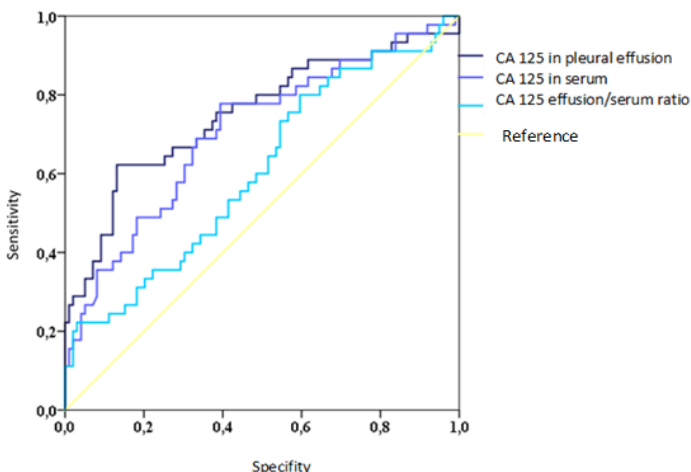


Fig. 2.6. CA 125 ROC curve and the area under the curve

Table 2.3

Calculation of AUC (*area under the curve*) of tumour marker CA 125

Tumour marker	Area under the curve	SE	Statistical significance	95% CI	
				Lower	Higher
CA 125 in pleural effusion	0.751	0.048	<0.001	0.658	0.844
CA 125 in serum	0.706	0.048	<0.001	0.612	0.801
CA 125 effusion/serum ratio	0.606	0.051	=0.041	0.506	0.707

SE – standard error; 95% CI – Confidence interval within the limits of 95%.

Analysis of ROC curve of CEA showed that the area under the curve for pleural effusion was 0.720, 0.575 – for serum and 0.715 – for effusion/serum ratio, which also points to the highest diagnostic sensitivity of CEA in effusion, but unlike CA 125, the CEA effusion/serum ratio is a diagnostically sensitive test, while CEA levels in serum appear not to be sensitive enough for a diagnostic test – see Figure 2.7 and Table 2.4.

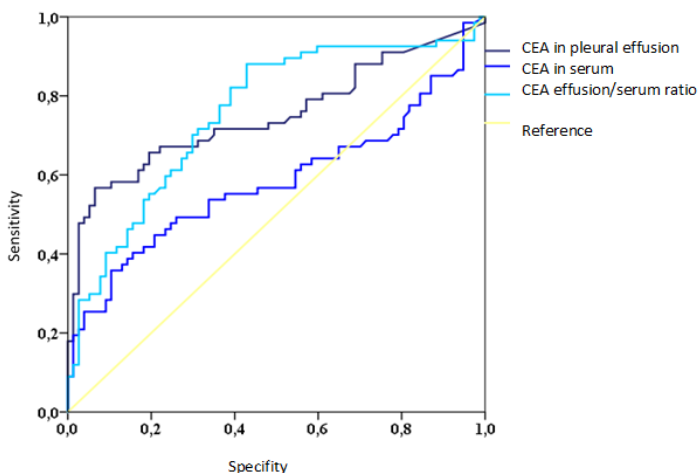


Fig. 2.7. ROC curve and the area under the curve of CEA

Table 2.4

Calculation of AUC (*area under the curve*) of tumour marker CEA

Tumour marker	Area under the curve	SE	Statistical significance	95% CI	
				Lower	Higher
CEA in pleural effusion	0.720	0.050	<0.001	0.621	0.819
CEA in serum	0.575	0.056	=0.152	0.465	0.684
CEA effusion/serum ratio	0.715	0.49	<0.001	0.618	0.811

SE – standard error; 95 % CI – Confidence interval within the limits of 95 %.

Neither CEA levels in pleural effusion, serum and effusion/serum ratio, nor CA 125 level in pleural effusion, serum and pleural effusion/serum ratio statistically significantly differed between MPE, with positive and negative cytology – in all cases $p > 0.05$.

When combining the best indicators of both markers with the highest sensitivity and specificity (CA $125 \geq 813$ U/ml and CEA effusion/serum ratio ≥ 0.6) we obtained a 56.4 % sensitivity and 93.3 % specificity.

2.2.7. BNP

BNP in serum and pleural effusion was analysed as an additional marker to differentiate cardiac hydrothorax from MPE. BNP median values and 25th and 75th percentiles in serum and pleural effusion can be seen in Table 2.5. BNP levels distinguish reliably between a transudate, a MPE, and parapneumonic pleurisy, but do not differentiate between parapneumonic pleurisy and MPE specifically.

Table 2.5

BNP median values in pleural effusion and serum in patients with transudate, parapneumonic pleurisy and MPE

Marker	Material	Median value (25 th and 75 th percentiles)			Signi-ficance p1	Signi-ficance p2	Signi-ficance p3
		Cardiac transudate	Para-pneumonic pleurisy	MPE			
BNP pg/ ml	Pleural effusion	1097 (494–1582)	129 (53–295)	97 (61–159)	0.010	0.504	0.006
	Serum	1631 (071–3386)	124 (59–289)	109 (46–170)	0.001	0.932	0.001

p1 – Transudate vs. MPE

p2 – parapneumonic pleurisy vs. MPE

p3 – parapneumonic pleurisy vs. transudate

The analysis of ROC curve of BNP showed that AUC is the same for pleural effusion and serum – 0.92 and 0.92 respectively, which points to equal

and sufficiently high diagnostic value of the method, regardless of the source where BNP is determined – see Figure 2.8 and Table 2.6.

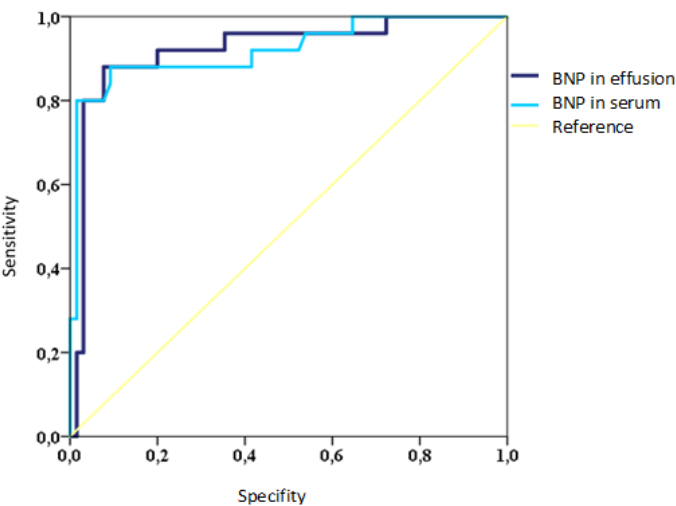


Fig. 2.8. ROC curve and area under the curve of BNP

Table 2.6

Calculation of the *area under the curve* for BNP

Marker	Area under the curve	SE	Statistical significance	95% CI	
				Lower	Higher
BNP in effusion	0.921	0.036	<0.001	0.851	0.991
BNP in serum	0.921	0.037	< 0.001	0.849	0.993

SE – standard error; 95 % CI – Confidence interval within the limits of 95 %.

To assess the clinical relevance of tumour markers, sensitivity and specificity of marker levels in serum and effusion and effusion/serum ration were calculated (Table 2.7). The table shows that the highest sensitivity is at the lowest values both in effusion and serum. If BNP in effusion is ≥ 494 pg/ml, the

sensitivity is 76.9 %, but specificity – 96.8 %; but if BNP of serum is ≥ 971 pg/ml, the sensitivity is 73.1 %, but specificity – 95.9 %.

Table 2.7

Sensitivity and specificity of BNP in cardiac transudate group

Marker	Material	Value	Sensitivity (%)	Specificity (%)
BNP (pg/ml)	Effusion	≥ 494	76.9	96.8
		≥ 1097	50.0	97.3
		≥ 1582	24.0	97.3
	Serum	≥ 971	73.1	95.9
		≥ 1631	50.0	95.9
		≥ 3386	23.1	97.3

2.2.8. PAI-1

Median PAI-1 level in pleural effusion (ng/ml) was: in transudates group 135 (20–236); in group of malignant effusion 188 (73–287); in group of parapneumonic pleurisy 291 (213–499). Difference of PAI-1 levels between parapneumonic pleurisy and malignant pleural effusion was statistically significant – $p < 0.001$, but there was no statistically significant difference between PAI 1 values of transudate and malignant effusion – $p = 0.07$. Also, concentrations were statistically significant different between transudate and parapneumonic effusion ($p < 0.001$) – Figure 2.9.

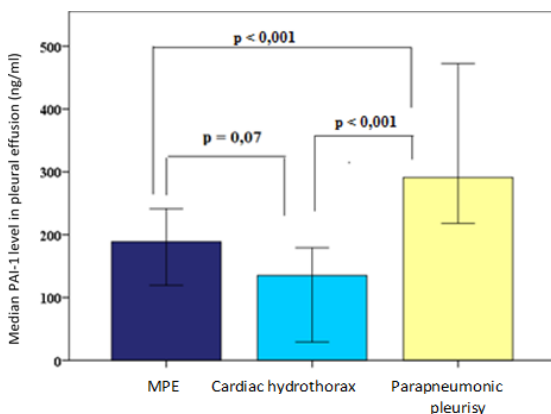


Fig. 2.9. PAI-1 level in pleural effusion

PAI-1 level (ng/ml) in serum of patients with MPE was 144 (77–207), in patients with cardiac hydrothorax – 69 (34–166), but in the group of parapneumonic pleurisy – 204 (151–412). In serum samples the difference was statistically significant between the groups of parapneumonic pleurisy and MPE ($p = 0.003$) and groups of parapneumonic pleurisy and cardiac hydrothorax – < 0.001 , but groups of MPE and transudate were not statistically significantly different – $p = 0.052$) – Figure 2.10.

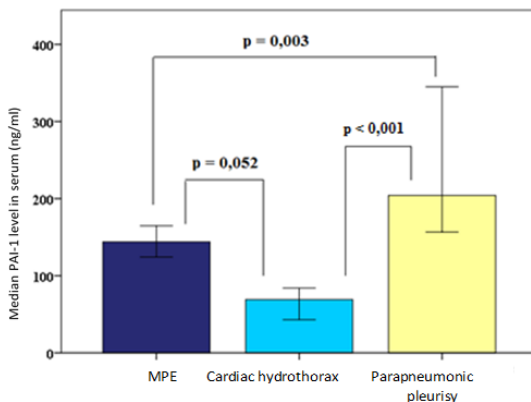


Fig. 2.10. PAI-1 level in serum

3. DISCUSSION

The main objective of the study was to identify whether it is possible to use additional biochemical markers in order to diagnose MPE in a situation where the golden standard of MPE diagnostic – video assisted thoracoscopy is not available, as in most hospitals in Latvia. To understand the clinical impact and diagnostic tools used for MPE in a hospital setting, first a retrospective analysis of data was performed. Then, a prospective analysis of patients with pleural effusion who were admitted in the Pulmonology Department of our institution was performed, with assessment of the relevance of additional biochemical markers (CA 125, CEA, PAI-1 and BNP) in the differential diagnosis of MPE were assessed. It has to be concluded that there is a lack of consistency in the selection of examination methods during the retrospective analysis period, although the necessary examination methods and algorithms are well defined and described in medical literature. It was established that 4.6 % of all patients treated during the period of interest had a pleural effusion, with mortality of patients with pleural effusion being 3 times higher compared with patients without pleural effusion. The most common cause of pleural effusion in this report was cardiac hydrothorax, which was differentiated reliably by determining BNP levels in blood samples. CA 125 in pleural effusion can serve the best as additional marker in MPE diagnostics, but PAI-1 levels probably indicate of activated fibrinolysis in MPE compared with parapneumonic pleurisy.

3.1. Epidemiology and etiology of pleural effusion

The data obtained during this study showed that pleural effusion was detected more frequently in persons over 40, with 95.8 % of the patients older than 41 years. It should be considered that the risk of cardiovascular and

malignant diseases, which is the most common cause of pleural effusion, increases with age.

Considering the fact that pleural effusion usually is a complication of another disease, there are very few epidemiological studies regarding incidence and prevalence of pleural effusion in different populations. One of the best organised pleural effusion epidemiological studies is the study by *Miloslav Marel* conducted in Bohemia in 1988 (*Marel et al.*, 1993). The objective of that study was to identify patients with pleural effusion both during their lifetime and post mortem, in order to assess incidence and etiology of pleural effusion. The patients were selected according predefined criteria. In total, pleural effusion was detected in 142 patients in area of 44 000 inhabitants within a year. In 45.8 % of all patient's effusion was of cardiac etiology, 21.8 % of all patients had MPE, 17 % had parapneumonic pleurisy, 5.6 % had pleural effusion due to thromboembolism of pulmonary artery, and 4.2 % had a haemothorax. Incidence was 32 pleural effusion cases per 100,000 inhabitants.

Our study retrospectively analysed 716 hospital medical records of patients with pleural effusion according to discharge diagnosis, but it was impossible to precisely define the region which these patients were hospitalised from; thus, the incidence in this study cannot be calculated; however, the data obtained are comparable to the data obtained by *Marel*. The most frequent cause of pleural effusion was cardiac failure – according to discharge diagnosis, 426 (59.5 %) of the patients had cardiac hydrothorax. The second most common cause, just like in the study by *Marel*, was MPE 103 (14.4 %) of patients, but the third cause – parapneumonic pleurisy in 71 (9.9 %) patients and pleural empyema – 30 (4.2 %) patients.

Colleagues in Kauna (*Zablockis et al.*, 2002) analysed 220 pleural effusion cases within a year. Transudates were detected in 24 % of cases, but exudates – in 76 % of cases. Cardiac hydrothorax was detected in 14.5 %,

nephrotic syndrome – in 5.5 % of cases, transudate caused by hepatic cirrhosis – 2.5 % of cases, parapneumonic pleurisy – 13 %, pleural empyema – 9 %, tuberculosis – 6 %, and MPE – 16.5 % of cases. PATE with exudate in pleural cavity was detected in 5.5 % of cases (*Zablockis et al.*, 2002). In the current retrospective study, there were significantly more cardiac hydrothoraxes – 59.5 %, which was explained by the fact that the study also included Cardiology Departments, while prospective study analysed only patients from Pulmonology Department, cardiac hydrothoraxes, similarly as in case of Kauna, were 29.2 %. In the retrospective study, 14.4 % of the patients had MPE like colleagues in Kauna, but MPE among the patients analysed prospectively was considerably higher – 46 %, which is also explained with the specialised hospitalisation department. The parapneumonic pleurisy and pleural empyema was similar in a number of cases – in retrospective study 9.9 % and 4.2 % respectively. The tuberculous pleurisy in the retrospectively analysed data was only 0.8 % of cases; but in the prospectively analysed group of patients – in 3.5 % of cases, which is also explained with hospitalisation in the specialised department. Pleural effusion caused by PATE in the retrospectively analysed group was only in 1 % of cases, but in the prospective study – only in 1 patient. Considering the above-mentioned study data, where PATE pleural effusions were detected in approximately 5 % of cases, as well as the calculation of *Broadus* (2016) regarding the frequency of PATE effusions, it can be considered that PATE could be diagnosed too rarely in RAKUS “Gaiļezers” during the analysed period.

Valdes with his colleagues performed a prospective study within hospitals in Spain in a specific region in 1996. Over the 5-year period, they discovered 642 patients with pleural effusion with an average age of 57 years, 401 of them were male. In this area the most common cause of pleural effusion was tuberculosis (25 %), malignancy (22.9 %) and chronic cardiac failure (17.9 %). In the malignancy group, the most common cause of MPE was lung cancer – 32 % of

cases, breast cancer – 11.5 % of cases, lymphoma – 10.8 % of cases, and ovarian cancer – 7.5 % of cases. The primary tumour could not be identified in 14.3 % of cases. 69.4 % of patients with tuberculous pleurisy were patients under the age of 40, but 83 % of patients with MPE were over 50 years (*Valdes et al.*, 1996). In our country, the incidence of tuberculosis is significantly higher than in Spain – the cases of new and already treated cases of tuberculosis per 100,000 population 2010 was 20 – 50, but in Spain – 10 – 19 (*Dara et al.*, 2013), however the tuberculous pleurisy was found less frequently in the data processed for the current study. Significant age differences of patients are also observed – only 4.2 % of patients were younger than 41 in the retrospectively analysed selection of patients, but the modal age of patients was 71–80 years, which can be explained with the fact that tuberculous pleurisy mainly occurs in young people, while cardiac failure and malignant diseases – in older ones. In the prospectively analysed group, distribution of MPE per groups of primary tumours was similar – in 37 % of cases it was lung cancer, 20 % – breast cancer and 20 % – ovarian cancer. Patients with MPE due to lymphoma were not observed, it could be because patients with haematological diseases in Latvia are mainly hospitalised in specialised department.

In a study with 1,000 patients who had undergone thoracentesis, *Villena Garrido and colleagues* detected MPE in 36 % (364 patients) of cases (*Villena López Garrido et al.*, 2002). In our retrospective study, MPE was detected only in 14.7 % of 272 patients who had undergone thoracentesis, which is considerably less, but in the prospective study 46.5 % had MPE in the specialised department, which perhaps could suggest of MPE under-diagnostics in the entire hospital and better MPE diagnostics in the specialised department.

A study was conducted in the Czech Republic where only those patients were included who were hospitalised in the lung disease hospital in Prague within the last 4 years. MPE was detected in 44.6 % of cases, 11.7 % of patients had

parapneumonic effusion, 6.4 % – empyema, 6.4 % tuberculous pleurisy (Loddenkemper *et al.*, 2002), which is comparable to the data of the current prospective study in Pulmonology Department, where MPE was detected in 46.5 % of patients and parapneumonic pleurisy – in 18.8 %, and tuberculous pleurisy – in 3.5 % of patients.

It is important to note that the overall mortality in therapeutic departments of hospital “Gaiļezers” was 4.4 % during a year, but mortality among patients with pleural effusion in the discharge diagnosis was more than three times higher – 14.1 %. That points statistically significantly ($p < 0.001$) to the considerably higher risk among pleural pathology patients. Such data cannot currently be found in the world literature.

3.2. Diagnostics methods of pleural effusion

Examinations that were performed in patients with pleural effusion were retrospectively analysed. It was found that the most frequently performed examinations were thoracic X-ray – 90.9 %, but ultrasound of pleural space was documented in medical records only in 19.7 % of all cases. In 5.9 % of the patients, neither thoracic X-ray, nor computed tomography had been performed.

Theoretically, thoracentesis should be performed in all patients who are diagnosed with pleural effusion for the first time and who have no clinically convincing data about transudate (cardiac or hepatic failure decompensation or pre-existing renal disease) (Havelock *et al.*, 2010, Villena López Garrido *et al.*, 2014). According to the data of medical records analysed during the study, thoracentesis was performed in 272 (38 %) of 716 patients. Cardiac hydrothorax was referred to in the discharge diagnosis of 74 % of patients of those who had not undergone thoracentesis, but for 116 patients (26 %) pleural effusion was not related to cardiac pathology; however, thoracentesis was not performed. It should also be noted that only 22.8 % of the dead patients with pleural effusion had undergone thoracentesis, which should, however, show a too conservative

management. After having evaluated the results, it was established that among patients with malignant exudate in discharge diagnosis, thoracentesis was performed most frequently (60.2 %), but most rarely among patients with cardiac hydrothorax – (23 %). This tendency is recognised as correct; however, the number of not performed thoracentesis suggests on insufficient analysis of clinical data, including pleural effusion biochemistry and, therefore, of potential diagnostic errors.

In cases where there are indications of thoracentesis, it should be done as soon as possible or immediately if the patient has respiratory insufficiency or suspicion of pleural empyema, but in all other cases, thoracentesis has to be performed by trained expert (*Havelock et al.*, 2010). The current approach provides that thoracentesis should be performed by an experienced physician under appropriate conditions (ultrasound facilities, complications prevention), so usually during the working day. Invasive procedures which without strong indications are performed after midnight, usually have more complications (*Havelock et al.*, 2010; *Feller-Kopman et al.*, 2018); however, a long delay, especially in case of parapneumonic pleurisy is not recommended, considering the fact that parapneumonic pleurisy may be complicated with fibrin septa and pleural empyema even within 12 hours (*Villena Garrido et al.*, 2014, *Davies et al.*, 2010).

Over the last decades, it is increasingly emphasised that thoracentesis needs ultrasound control – either before or during thoracentesis; besides, it is not recommended to mark the potential place of thoracentesis in another room or department. The highest risk of complications is for physicians who are not trained in thoracentesis and who do not use ultrasound – pneumothorax develops in 15 % of cases, 4.7 % of them need drainage of pleural space and fluid cannot be obtained in 12.9 % of cases (*Havelock et al.*, 2010, *Feller-Kopman et al.*, 2018). In comparison, relevant risks of a trained doctor who uses ultrasound are

3.6 %, 0.9 % and 2.7 %. In the retrospective analysis, ultrasound was performed only in 104 cases of 272 performed thoracentesis. In total, ultrasound was performed on 141 patients (19.7 %), which indicates of insufficient application of ultrasound during the analysed period. Data on complications after thoracentesis were not gathered in the study.

The following data is a sign of the lack of algorithm during the evaluated period in hospital “Gaiļezers”.

Publications about the pleural effusion diagnostics and treatment in a particular hospital analysed in this way were not found in literature, perhaps because even if such data are analysed, they serve more for internal quality control system of the hospital. Also, the task to retrospectively analyse the medical records of patients was to determine the existing situation in a hospital and understand how it can be improved.

Pleural effusion laboratory examination methods

3.2.1. Clinical chemistry

The minimum pleural effusion examinations should be guided by clinical symptoms. In accordance with the instructions of Light and other authors (*Sahn*, 2003, *Havelock et al.*, 2010, *Broaddus et al.*, 2016), in cases of high probability of transudate, it is permissible to determine only LDH and albumin in pleural effusion and serum, the so-called Light's criteria. If pleural effusion/serum protein ratio < 0.5 ; pleural effusion/serum LDH ratio < 0.6 and LDH in pleural effusion $< 2/3$ of the upper limit of laboratory norm in serum, a transudate is confirmed and no further examination is required.

Light's criteria are currently the main method used for differentiation of transudates and exudates. Since further management of transudates and exudates is radically different, the importance of these criteria cannot be overestimated. Light's criteria in both available guidelines regarding pleural effusion are

considered as compulsory, and require concentrations of protein and LDH not only in pleural effusion, but also in serum in order to complete the Light's criteria (Hooper *et al.*, 2010, Villena Girrargo *et al.*, 2014).

If the clinical picture suggests an exudate (no signs of cardiac, hepatic or renal failure, suspicion of malignancy, pneumonia or thromboembolism of pulmonary artery), cell differential and pH of the effusion should be determined additionally. If pH of the pleural effusion is < 7.3 , but the pH of blood is normal, then, in case of an exudate, it further differential diagnosis need to be considered, such as complicated parapneumonic pleurisy or empyema, malignancies, oesophageal rupture, rheumatoid pleurisy, *lupus* pleurisy and tuberculous pleurisy (Sahn, 2003, Havelock *et al.*, 2010, Villena Girrardo *et al.*, 2014). If pH cannot be analysed due to technical reasons, one may be guided by the clinical picture or determination of the glucose level. If the pleural surface is intact, glucose levels in pleural effusion are equal to glucose levels in serum. Low glucose levels are indicative of a bacterial infection, rheumatoid arthritis or tuberculous pleurisy, malignancy or oesophageal rupture. Glucose < 1.5 mmol/l is usually in case of pleural empyema and rheumatoid arthritis. If glucose is < 3.4 mmol/l, it is an indication to drainage of pleural space (Hooper *et al.*, 2010).

In the study, having analysed the data retrospectively, it was established that combinations of pleural effusion analysis are very different. Most often – in 130 (47 %) cases – clinical analysis, LDH, protein and cytology were determined simultaneously. Other analysis and combinations of analysis are determined significantly less frequently. In 29 (10.7 %) cases, pleural effusion analysis was not performed at all. The effusion/serum protein ratio and effusion/serum LDH ratio was not assessed in any of the cases, which attests the weak understanding about the significance of differential diagnostics of transudate and exudate. It is important to note that LDH was not determined in 98 cases (36 % of thoracentesis), which is the most important parameter in differentiation of

transudate and exudate (*Light*, 2013). It should also be noted that pH was not determined for any of the patients, which perhaps can be explained with technical difficulties to use the gas analyser. It has been estimated when the determination of pH in a gas analyser is technically difficult (large distance to the laboratory and lack of staff, to deliver the sample quickly enough to the laboratory), it is completely permissible to determine glucose in pleural effusion instead. However, according to the analysed data, glucose was determined in only one case of 272, lipase was determined in six cases. Parietal pleural biopsy was performed in nine (1.3 %) patients, tuberculous pleurisy was identified in five of them. Thoracoscopy was performed only on two (0.3 %) patients, which can be explained by the fact that no thoracic surgery department is available in the hospital. Data in the literature regarding availability of testing methods are scarce, so it is difficult to compare the diagnostic yield of hospital “Gaižezers” significantly with other hospitals; however, the data appears to suggest insufficient adherence to recommendations in clinical practice.

In the prospective study, Light’s criteria were determined for all patients. It was established that 42 patients had cardiac hydrothorax, Light’s criteria of 34 (81 %) patients corresponded to transudate, and Light’s criteria of 8 (19 %) patients corresponded to an exudate which coincided precisely with the mentioned conclusions described in literature (*Porcel et al.*, 2004; *Bielsa et al.*, 2012; *Light R.W.*, 2013). When making additional calculations, protein gradient was determined in these patients. For seven patients it was > 31 g/l, but for one patient it was < 31 g/l, and BNP in serum for this patient was 1631 pg/ml. All patients in the group of parapneumonic pleurisy (29 patients) had at least one positive Light’s criterion. All patients in the group of parapneumonic pleurisy had the 3rd Light’s criterion positive – LDH levels in effusion (168–1006 U/L), suggest that in case of parapneumonic pleurisy only the 3rd Light’s criterion could be used, not determining additionally LDH and protein in serum. In MPE group

(67 patients), 65 (97.1 %) patients had at least one positive Light's criterion, which corresponds to the data described in literature. Only 52 (77.6 %) patients had a positive 3rd Light's criterion, which shows that in case of uncertain diagnosis, especially in case of suspected MPE, LDH and protein should anyway be determined in serum as well.

3.2.2. Cytology

Cytological examination is a widely used method in MPE diagnostics; however, malignant cells in pleural effusion are found on average in only 60 % of all cases; besides, repeated examination of pleural effusion sample do not increase the sensitivity of the method (*Hooper et al.*, 2010). In the current study, cytological analysis of pleural effusion was performed prospectively for all patients, malignant cells were detected in 44 (65.7 %) patients, for 38 (83.4 %) of them, they were found already in the first sample, but for 6 (13.6 %) – after sending repeated pleural effusion for examination. These results are broadly in line with the published data on sensitivity of cytological examination.

3.2.3. Bacteriology

Bacteriological examination must be performed in all cases where the possible cause of exudate has infectious nature, but sensitivity is generally low. Bacteria are identified in cultures in between 15 % to 20 % of pleural effusion samples (*Mohanty et al.*, 2007; *Broaddus et al.*, 2016). In the analysed case, bacteria (*Klebsiella pneumoniae* and *Staphylococcus saccrolyticus*) were identified in in only 2 (7 %) of the examined 27 samples. The very low sensitivity of the method in the studied case can be explained with the fact that patients have already received antimicrobial therapy or that samples are not delivered to the microbiology laboratory fast enough.

3.2.4. Tumour markers

Considering the fact that cytological examination of pleural effusion has quite low sensitivity, as well as the fact that, just like in hospital “Gai̇lezers”, thoracoscopy is not always available also in many other hospitals in the world to approve MPE or another diagnosis, alternative methods are studied that could help to differentiate MPE from exudates with other etiology. The level of tumour markers in pleural effusion is one of the study directions, although lately further studies in this field are no longer recommended (*Feller-Kopman et al.*, 2018).

Comparison of CEA and CA 125 threshold, AUC, sensitivity and specificity

Feng and colleagues have analysed CEA in pleural effusion of 156 patients, 114 of them with MPE, but 42 with tuberculous pleurisy. At the value of 4.5 the method sensitivity was 75 %, and specificity – 96 % (*Feng et al.*, 2016). *Xu* and colleagues determined CEA level in 60 malignant and 58 non-malignant pleural effusions, establishing that CEA level of pleural effusion of 54 patients with malignant pleural effusion was greater than 5.5 ng/ml and at such threshold, combining CEA with a tumour marker sRCAS, the sensitivity was 98.3 and specificity – 91.4 %. (*Xu et al.*, 2014), which shows that sensitivity increases when combining different diagnostic markers. According to the data of the current study, at the value of 6.23 ng/ml ng/ml, CEA sensitivity was only 50.7 % and specificity 94.8 %, but after combining CA 125 and CEA, their sensitivity was 56.4 % and specificity – 93.3 %.

Son and co-authors have analysed 47 non-malignant and 52 malignant pleural exudates, comparing the diagnostic values of tumour markers CD66c, CEA, CA 19-9 and CYFRA 21-1 in pleural effusion, establishing that CEA has the highest diagnostic value at the value 2.5 ng/ml – sensitivity 87.2 % and specificity 92.3 % (*Son SM*, 2015). *Sharma* with colleagues also analysed CEA

in pleural effusion. In the study with 30 lung cancer patients having MPE and 18 patients having tuberculous pleurisy, the serum CEA sensitivity at value 4.8 ng/ml was 78.3 %, but CEA sensitivity of pleural effusion – 82.6 % (*Sharma et al.*, 2015). In the study with 601 MPE and 595 other etiology pleural effusions, CEA was established in pleural effusion and at the value 0.69 ng/ml the sensitivity was 69 %, and specificity – 82 % (*Li et al.*, 2015). *Bunjhoo* with colleagues analysed 28 MPE and 28 malignant pleural exudates and established that CEA sensitivity in effusion at the value 3.48 ng/ml was 75 %, and specificity – 86 % (*Bunjhoo et al.*, 2012). According to the data obtained in the currently performed study, sensitivity at the higher value (≥ 6.23) was significantly lower (50.7 %), but specificity was similar – 94.8 %. Perhaps these differences can be explained with the fact that the current study comprised a smaller number of patients.

Both CEA and CA 125 in pleural effusion and serum were determined in 95 patients with MPE and in 35 patients with tuberculous pleurisy. The greatest CEA area under the curve, sensitivity and specificity was in the effusion at value of 3.35 ng/ml (0.86, 75 % and 94 %, respectively), and the best results of CA 125 were in the effusion at value 644 U/ml – 0.78; 61 % and 83 % respectively (*Gu et al.*, 2016). CEA and CA 125 pleural effusion was determined also by *Antonangelo* with colleagues in 114 patients with MPE and 42 with tuberculous pleurisy. CEA at the value 5.2 ng/ml had 65 % sensitivity and 97.5 % specificity, but CA 125 at value 345.65 U/ml – 68 % sensitivity and 83 % specificity (*Antonangelo et al.*, 2015). Like the current study, the sensitivity and specificity of both markers was the highest in effusion, but the value at which sensitivity and specificity were the highest (53.7 % and 96.1 %, respectively) was significantly higher – 1644 U/ml.

In these studies, with different number of patients, CEA in effusion was analysed in parallel with other markers. Only one study analysed CA 125 and

CEA both in serum and effusion and the effusion/serum ratio. In all of the analysed studies, the value of both markers for the best test sensitivity and specificity was lower than in the current study: CEA 2.9 – 8.0 ng/ml (current results – 6.23 ng/ml), CA 125 medians were 345 – 644 U/ml (current – 1644 U/ml), also the sensitivity according to the current data was lower than all of the indicators, but the specificity – similar. These studies have shown that the area under the curve in CEA effusion is 0.74 – 0.92 (according to the current study data – 0.72), but CA 125 in effusion – 0.78 – 0.85 (according to the current study data – 0.75). The area under the curve in CEA serum – 0.79 (according to the current study data – 0.57), but for CA 125 in serum – 0.78 – 0.85 (according to current study data – 0.706), which in general are comparable indicators.

In the calculations of the study, the greatest area under the curve was for CA 125 level in effusion (0.751), not in serum (0.706) or effusion/serum ratio (0.606), which suggest on CA 125 local synthesis both in the damaged cells of mesothelium and malignant cells. The high Ca 125 of pleural effusion/serum ratio (≥ 10.61) in malignant exudates when compared with effusion of other etiology in pleural space could suggest of limited systemic diffusion.

The area under the curve CEA, just like CA 125 was the highest in pleural effusion – 0.720, but unlike CA 125, also effusion/serum ratio had relatively high AUC – 0.715, which could also suggest a more local production than systemic diffusion.

Nguyen with colleagues (2015) carried out a metanalysis of 49 studies. 37 case control studies were included, 33 studies of them were prospective, but in 25 studies samples of pleural effusion were taken from consecutively hospitalised patients. The average number of patients in this study was 140 patients (25–654). MPE in all studies was confirmed with a positive cytology, pleural biopsy or autopsy. The united sensitivity and specificity in MPE diagnostics were respectively: CEA 54.9 and 96.2 %; CA 15-3 50.7 and 98.3 %;

CA 19-9 37.6 and 98.0 %; CA 125 – 57.5 and 92.8 %; CYFRA 62.5 and 93.2 %. When compared to this metanalysis, the calculated values were very similar – CEA sensitivity at the median values was 50.7 %, but specificity – 94.8 %, while CA 125 – 53.7 % and 96.1 %, respectively. The authors concluded that, although all markers have high specificity, the low sensitivity limits the routine use of these markers in clinical practice. Combining markers improves the sensitivity.

Data about 2115 patients (85 years of age and older) were analysed retrospectively – cardiac failure of these hospitalised patients was confirmed clinically and by echocardiography, tumour markers, including CA 125 and NT-proBNP were determined in serum, and presence of peripheral oedema and transudates were marked. Patients were followed up for 180 days. CA 125 and NT-proBNP levels elevated statistically significantly with increase of the level of cardiac failure, besides, linear correlation between these two markers was established ($r = 5103$, $p = 0.05$). The average level of CA 125 in serum was statistically significantly higher in patients with transudate in pleural cavity when compared with patients without transudate (108.5 U/L vs 12.1 U/L) and in patients with peripheral oedema when compared with patients without peripheral oedema (78.4 U/L vs 11.9 U/L). Within 180 days, cardiac death was detected in 305 patients, but 461 patients were re-hospitalised. CA 125 of these patients was statistically significantly higher than of the other patients – 78.2 U/L vs 11.7 U/L. Kaplan–Meier curves demonstrated a significant difference in patients with normal and in patients with elevated CA 125 level in serum. Statistically reliable data were not obtained regarding other tumour markers. The researchers cannot formulate an explanation for such results yet (*Ma et al.*, 2013). The calculations of the current study show that CA 125 level in MPE is statistically significantly different from parapneumonic pleurisy and cardiac hydrothorax level in effusion and serum, but there are no statistically significant differences between CA 125

level in effusion ($p = 0.850$), or serum ($p = 0.694$) in groups of cardiac hydrothorax and parapneumonic pleurisy.

3.2.5. BNP

Marinho and colleagues in 2011 compared cardiac hydrothorax (34 patients) with hepatic hydrothorax (10 patients), malignant effusion (21 patients) and tuberculous pleurisy (12 patients). It has been established that the thresholds in cardiac failure diagnosis was 132 pg/ml serum for BNP level (sensitivity 97.1 %, specificity 97.4 %) and 127 pg/ml for pleural effusion (sensitivity 97.1 %, specificity 87.8 %); it is therefore concluded that BNP levels both in serum and pleural effusion is a useful marker in diagnostics of cardiac failure (*Marinho et al.*, 2011). In the currently performed study, the sensitivity in effusion and serum was significantly lower (in both 50 %), but specificity was similar – 97.3 in pleural effusion and 95.9 % in serum, also values were significantly higher – 1097 pg/ml for effusion and 1631 pg/ml for serum.

In the meta-analysis of 10 studies which included 1120 patients in total, total sensitivity and specificity of NT-proBNP in identification of cardiac hydrothorax was 94 %, positive predictive value 15.2 and the negative predictive value – 0.06. According to the data of the authors, more than 85 % of patients with cardiac failure whose pleural effusion according to Light's criteria was exudate had high concentration of NT-proBNP. BNP diagnostic value, according to the authors' conclusions, was lower than NT-proBNP diagnostic value (*Porcel et al.*, 2007).

Kolditz and colleagues have surveyed 93 patients, 73 % of them with cardiac hydrothorax. When determining NT-proBNP level in pleural effusion and serum, it was established that in case of cardiac hydrothorax, it is statistically significantly increased, and the level of this marker in serum and pleural effusion closely correlated – Spearman correlation coefficient was 0.963, $p < 0.001$

(Kolditz *et al.*, 2006). In the current study, having analysed the ROC curves, it is established that the area under the curve for effusion and serum is 0.921, which indicates to high sensitivity and specificity of diagnostic method in both substrates; therefore, perhaps, BNP test in serum is sufficient in order to diagnose or exclude a transudate of cardiac etiology in pleural space, thus protecting patients from unnecessary invasive intervention and optimising examination costs.

3.2.6. PAI-1

There are not many studies that analyse the importance of PAI-1 in pleural effusion. In 1995, PAI-1 and D dimers in plasma and pleural effusion were determined in 10 patients with empyema, 9 – with tuberculous pleurisy, 31 – with MPE and 3 pleural effusion of uncertain etiology. It was established that both D dimer and PAI-1 level in pleural effusion is higher than in plasma. In patients with tuberculosis and empyema, PAI-1 level was higher than in patients with cardiac transudate or MPE (Philip-Joët *et al.*, 1995), which is fully in line with the currently obtained data, which have demonstrated that PAI-1 level in parapneumonic exudates is a statistically significantly higher than in malignant exudates and cardiac transudates.

In the study among other markers also PAI-1 was determined in pleural effusion of 19 patients with tuberculous pleurisy, 29 – with MPE, 30 – with parapneumonic pleurisy. Depending on the location of effusion in pleural space, patients were divided into two groups – loculated (42 patients) and a free pleural effusion (36 patients). PAI-1 level was significantly higher in the group with loculated pleural effusion – 114.9 vs 94.1 pg/ml; $p = 0.019$. Obviously, increased PAI-1 points to reduced fibrinolysis in loculated effusions (Chung *et al.*, 2005). In another study, 64 patients with parapneumonic pleurisy were divided into two groups – uncomplicated (26 patients) and complicated – loculated (38 patients)

parapneumonic pleurisy. The PAI-1 level in the uncomplicated parapneumonic pleurisy was 43 pg/ml, but in the complicated – 104 pg/ml ($p < 0.01$), which also points to reduced fibrinolytic activity in pleural space during fibrin septa formation (Chung *et al.*, 2013). In our study, patients were not divided according to effusion loculation in pleural space, but the median PAI-1 level in patients with parapneumonic pleurisy who were characteristic of fibrin formation and effusion loculation was statistically significantly higher – 291 ng/ml, $p < 0.001$.

In general, parapneumonic pleurisy is characterised by a fibrin formation and the liquid has a tendency to loculate in order to localised the inflammation process. The studies carried out so far have shown that effusions of inflammatory nature have indeed an increased level of PAI-1, which promoted the inhibition of fibrinolysis and formation of fibrin (Idell *et al.*, 1991, Lin *et al.*, 2005). This means that, perhaps, increased formation of PAI-1 is influenced by the inflammatory process. Also, the current study has shown that PAI-1 level in parapneumonic exudates is significantly higher than in malignant exudates.

Malignant pleural exudates have no tendency to loculate, which indicates that there is no or little fibrin production, which may be associated with a moderate PAI-1 activity (Lin *et al.*, 2005, Lu *et al.*, 2008). The findings of the current study suggest on significantly lower PAI-1 level in malignant pleural exudates when compared with parapneumonic effusions – 188 ng/ml and 291 ng/ml, $p < 0.001$. This leads to conclusion that the moderately high level of PAI-1 in MPE does not promote fibrin formation and effusion isolation as with parapneumonic pleurisy, but it promotes the spread of pathological process. The question about what inhibits PAI-1 formation in MPE and how it can be stopped remains unanswered. Since the palliative care of MPE is based on isolation of the process with iatrogenic fibrin precipitation, perhaps, the answer to this question could improve the treatment tactics of MPE.

According to the obtained data, PAI-1 level in serum is also statistically significantly higher in parapneumonic pleurisy than in MPE and cardiac transudates (204 ng/ml, p respectively 0.003 and < 0.001), which shows systemic activity of this fibrin inhibitor.

3.3. Factors influencing the study results

Results of the study and interpretation thereof has been influenced by several factor, such as the retrospective analysis of medical records, and thus reliance of appropriate recording of medical records and examination results by attending physicians was necessary. In the prospective study, only patients hospitalised in the Pulmonology Department were analysed, so patients with pleural effusion of another etiology (hepatic hydrothorax, hydrothorax due to hyperhydration or nephrotic syndrome, effusions due to pathology of abdominal cavity, PATE effusions, etc.) were not included in the study.

Interpretation of laboratory indicators could be influenced, perhaps, by the time spent to deliver the samples to the laboratory and the time for analysis, which did not depend on the performers of the study. The researchers were also limited in measurements of several parameters, e.g. adenosine deaminase, KL-6 and some others. Interpretation of some results could be influenced by lack of those measurements.

The interpretation of the results is further influenced by the relatively small group of patients that was studied prospectively, as well as the fact that there was no possibility to make thoracoscopy for patients and confirm MPE diagnosis also histologically.

4. Conclusions

1. The most frequent etiology of pleural effusion in all departments of internal diseases of REUH “Gaiļezers” was cardiac failure, but in the pulmonology department – malignant pleural effusion.
2. Hospital mortality of patients with pleural effusion is three times higher than the mortality of all hospitalised patients in total.
3. The examination methods of pleural effusion in the departments of internal diseases in REUH “Gaiļezers” were insufficient during the analysed period when compared with the methods recommended in the guidelines and medical literature.
4. Determination of CA 125 in pleural effusion can serve as a valuable additional diagnostic marker to differentiate malignant pleural effusion from pleural effusions of other etiology.
5. PAI-1 level in malignant pleural effusion was significantly lower than in parapneumonic pleurisy, so further studies of fibrinolytic system activities in MPE should be performed.
6. Circulating blood concentrations of BNP was a useful marker in the differentiation of cardiac hydrothorax, BNP in pleural effusion, however, did not provide additional diagnostic information.

5. Practical recommendations

To improve the diagnostics of pleural effusion, differential diagnosis and to facilitate timely and effective treatment, the following steps are needed:

1. All patients, where the clinical picture does not show any pathologies, which is complicated with transudate in pleural space (decompensated cardiac failure, decompensated hepatic failure, chronic renal disease) and who have pleural effusion, should have thoracentesis with ultrasound control.
2. Minimum amount of laboratory examinations:
 - a. Determination of LDH and protein in pleural effusion and serum;
 - b. Cytological examination of pleural effusion.
3. BNP or NT-pro-BNP in serum must be determined to confirm cardiac transudate.
4. In case of suspected cytological negative MPE, when the thoracoscopy is unavailable, determination of CA 125 in pleural effusion is recommended.

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ACKNOWLEDGEMENTS

I express the deepest gratitude to my scientific supervisor *Dr. med.* professor Aivars Lejnieks for his advice, patience, perseverance and inducements.

My heartiest thanks to my friend, colleague and advisor *Dr. med.* Associated Professor Inga Stukēna for her invaluable support in process of the research.

Thanks to Dr. Agnese Valdmāne-Zviedrītei for her assistance in summarization of the data.

Thanks to my great colleagues in P. Stradiņš KUS for their support and encouragement in moments when I was short of determination.

Thanks to Roche Academy of financial support.

Finally, thanks to my family, that is my principal motivation to achieve new goals.