Biomarkers in routine diagnosis of pleural effusions

Uporaba bioloških označevalcev pri rutinskem diagnosticiranju plevralnih izlivov

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Key words: biomarkers; pleural effusion; Light's criteria; malignant pleural effusion; paramalignant effusion

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Abstract

Background: Pleural fluid biochemical analysis is the first step in pleural effusion (PE) diagnostics. Our purpose was to analyse the utility of the biomarkers used at our clinic in the routine diagnosis of PE.

Methods: We retrospectively reviewed the PE levels of proteins, lactate dehydrogenase (LDH), alpha amylase (AA), pH and glucose in 433 patients who were treated at the University Clinic Golnik in a one-year period and compared these values with the final identified aetiology of the effusions.

Results: The majority of the effusions were determined to be a consequence of malignancy (n = 154) or infection (n = 108). In 94 cases the aetiology of the effusions was heart failure and in 54 cases other diseases, while 23 effusions remained aetiologically undetermined. Considering Light's criteria, the vast majority of the effusions were correctly classified as exudates or transudates (97.1 %). Comparing paramalignant and malignant effusions, we detected significantly lower values of pleural fluid LDH ($p < 0.0005$) and proteins ($p < 0.0005$), and higher pH ($p < 0.0005$) values in the paramalignant effusions.

Conclusion: We have found that pleural LDH and proteins are the most helpful biochemical parameters in our routine diagnosis of pleural effusions and helped us to correctly narrow the aetiological spectrum. Furthermore, significantly higher pleural LDH and protein values and a pH below 7.32 additionally facilitated distinguishing between malignant and paramalignant effusions. Parameters such as glucose and AA are useful in selected cases and have a limited role in routine diagnostics.

Izvleček

Izhodišče: Biokemijska analiza plevralnega izliva (PI) je prvi korak pri diagnosticiranju PI. Namen študije je bil ugotoviti uporabnost bioloških označevalcev, ki se uporabljajo pri rutinskem diagnosticiranju.

Metode: V retrospektivno analizo smo vključili plevralne vrednosti proteinov, laktat dehidrogenaze (LDH), alfa amilaze, pH in glukoze 433 bolnikov, ki so bili obravnavani na Univerzitetni kliniki za pljučne bolezni in alergijo Golnik v obdobju enega leta, in jih primerjali glede na vzrok izliva.

Rezultati: Vzrok PI so bile v večini primerov maligne (n = 154) ali infekcijske bolezni (n = 108). Pri 94 bolnikih je bil vzrok srčno popuščanje, v 54 primerih druge bolezni, v 23 primerih pa vzrok PI ni bil določen. Z uporabo Lightovih meril je bila večina PI pravilno opredeljena kot transudat ali eksudat (97,1 %). V skupini paramalignih plevralnih izlivov smo zaznali pomembno nižje plevralne vrednosti LDH ($p < 0.0005$) in proteinov ($p < 0.0005$) ter višje vrednosti ph ($p < 0.0005$) v primerjavi z malignimi plevralnimi izlivi.

Zaključek: Z raziskavo smo potrdili, da z določitvijo plevralnih vrednosti LDH in proteinov pomembno zmanjšamo spekter diferencialne diagnostike PI, zato sta med najpomembnejšimi biokemičnimi parametri v rutinski diagnostiki. Statistično značilne visoke vrednosti plevralnega

LDH in proteinov ter vrednosti pH pod 7.32 nam lahko služijo kot dodaten pripomoček pri ločevanju malignih od paramalignih izlivov. Določanje glukoze in AA v PI je smiselno in uporabno le v nekaterih primerih.

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1. Introduction

Pleural effusions (PEs) are a common medical problem and have more than 50 recognised causes. The most common conditions resulting in PEs are congestive heart failure, pneumonia and malignancy (1,2).

Thoracentesis with further specific analyses is the cornerstone of diagnosis in most cases of pleural effusions, except for a clinically and radiologically convincing heart failure, which responds to proper treatment. The first recommended step in PE management is determining whether the effusion is a transudate or an exudate, according to the Light's criteria. If at least one of the following criteria is present (ratio of the pleural to serum protein values > 0.5, ratio of the pleural to serum lactate dehydrogenase (LDH) values > 0.6, or pleural LDH values > 0.66 upper normal serum level), the effusion is classified as an exudate. The reported diagnostic accuracy of Light's criteria is 68–95 %, with a sensitivity of 97.5 % and a specificity of 73.8 % (3-6). Approximately 25 % of transudates are erroneously identified as exudates, which occurs most frequently in patients with heart failure after receiving diuretics (5).

If effusion is an exudate, additional biochemical markers such as pH, glucose, alpha amylase (AA), cholesterol, and triglycerides can be helpful; in selected cases, also further cytological and microbiological investigations to detect carcinoma cells or bacterial strains are required (7-10).

The aim of our study was to evaluate the potential utility of each biochemical marker used at the tertiary clinic in the routine diagnosis of a PE.

2. Method

In this retrospective study, we analysed usefulness of biochemical tests in PEs in real diagnostic situations and investigated the further role of biochemical markers.

We included patients who were treated at the University Clinic Golnik, in whom a thoracentesis for diagnostic purposes was performed in the period from 1 Januay 2011 to 31 December 2011. Patient's pleural fluid and serum samples were immediately transported to biochemistry, cytology and microbiology laboratories and analysed within one hour. Biomarkers in pleural fluid, including LDH, proteins, pH, glucose and AA, were measured using standard routine methods. In the cytological laboratory, differential blood counts were performed, and malignant cells were detected. Bacterial cultures, acid-fast bacillus smears and cultures were performed according to culture recommendations.

Three pulmonologists carefully evaluated the diagnostic, treatment and follow-up results of the patients in a one--year period and defined aetiological diagnoses of the PEs.

Table 1: Aetiology of pleural effusions

Aetiology of pleural effusion	Number (%)
Malignancy	154 (35.5 %)
Pleural carcinosis	84
Mesothelioma	16
Lymphoproliferative diseases	10
Paramalignant effusion	44
Infection	108 (25.0 %)
Parapneumonic	77
Tuberculosis	16
Empyema	15
Heart failure	94 (21.7 %)
Other	54 (12.5 %)
Pulmonary embolism	7
Posttraumatic effusion	9
Post-operative effusion	11
Systemic connective tissue disease	11
Trapped lung	6
Chylothorax	4
Ascites	3
Chronic pancreatitis	2
Tumour in the right atrium	1
Idiopathic effusion	23 (5.3 %)

Pleural malignancy was confirmed by the detection of malignant cells in the PEs and biopsies obtained through a thoracoscopy or a blind pleural biopsy. In polymetastatic lung carcinomas with positive computed tomography (CT) or positron emission tomography (PET) scans of pleural carcinosis, it was decided to classify those patients in the carcinosis group despite the lack of cytological confirmation of carcinoma cells. Effusions secondary to lung cancer without any evidence of pleural invasion were considered paramalignant. In acute febrile illness with an ipsilateral parenchymal infiltrate and resolution of the PE with antibiotic treatment, the PE was classified as parapneumonic. Empyema was diagnosed according to either the colonisation of bacteria from the PE or a macroscopically purulent effusion. Tuberculosis pleuritis was diagnosed if *Mycobacterium tuberculosis* was cultured from the effusion or pleural biopsies. A diagnosis of PE secondary to pulmonary embolism was determined when a pulmonary embolism or infarction was observed on CT angiography and no other abnormality suggesting pneumonia or cancer was discovered. Heart failure (HF) was confirmed by clinical and radiological signs of HF (right heart decompensation with elevated central venous pressure, peripheral oedema, pulmonary venous congestion, enlarged heart, bilateral effusion, a diagnosis of previous heart disease and the absence of inflammatory pulmonary infiltrates or malignancy). Other rarer aetiologies were determined according to the patients' history, clinical picture, laboratory and imaging tests.

PEs were classified as transudates or exudates according to Light's criteria. Furthermore, the values of different bi-

Table 2: Levels of biochemical markers in malignant and paramalignant effusions

Aetiological group /Biochemical marker	LDH [µkat/l]	Proteins [g/l]	pH	Glucose [mmol/l]	Alpha amylase [µkat/l]
Malignant effusions	**7.5 (± 0.7)**	**43.0 (± 1.0)**	**7.30 (± 0.01)**	**5.83 (± 0.28)**	**1.39 (± 0.22)**
Plural carcinosis	7.8 (± 0.9)	42.8 (± 1.1)	7.31 (± 0.01)	6.26 (± 0.33)	1.65 (± 0.29)
Mesothelioma	6.4 (± 1.5)	43.1 (± 3.0)	7.21 (± 0.05)	3.44 (± 0.53)	0.58 (± 0.12)
Lymphoproliferative disorders	7.3 (± 2.3)	43.9 (± 2.1)	7.35 (± 0.05)	5.50 (± 0.68)	0.61 (± 0.11 =)
Paramalignant effusion	**3.3 (± 0.4)**	**36.0 (± 1.4)**	**7.39 (± 0.01)**	**7.44 (± 0.38)**	**0.68 (± 0.06)**

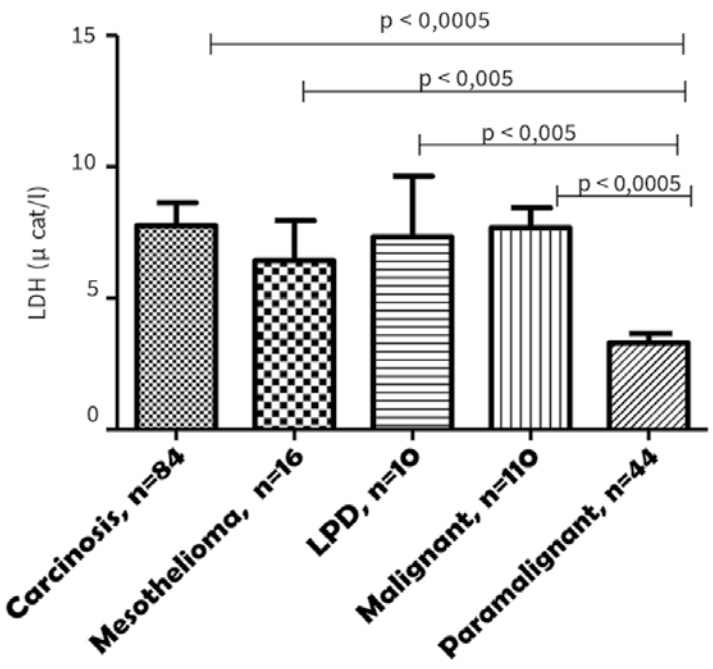

Figure 1a: LDH levels in pleural carciosis, mesothelioma, LPD-lymphoproliferative disorder and paramalignant effusion. In malignant group are all patients with pleural carcinosis, mesothelioma and LPD.

omarkers were compared between the different aetiological groups.

In statistical analysis, quantitative variables are presented as mean with standard error and qualitative variables as frequencies and percentages. To assess the difference in variables between the groups we used the Mann-Whitney unpaired t-test. Correlations were performed by Pearson's rank-order method. Statistical analyses were performed with Excel and GraphPad Prism 5. We considered p-value of < 0.05 as statistically significant.

3. Result

Out of the 475 patients in whom thoracentesis was performed, 42 patients were excluded from the study due to the lack of biochemical PE tests. The mean age of the 433 remaining patients was 69.7 (± 0.7) years. In total, 264 patients (61 %) were male, and 169 patients (39 %) were female.

The most common aetiologies of PEs were malignant disease in 154 cases (35.5 %), infection in 108 cases (25.3 %) and heart failure in 94 cases (21.7 %). Other causes were present in 54 cases and further division of main three aetiologies are presented in Table 1. In 23 patients (5.3 %), the cause of the PE was not determined despite extensive evaluation.

LDH and proteins were measured in all patients, glucose was measured in 417 patients (96.3 %), AA in 378 patients (87.3 %), pH in 313 patients (72.3 %) and haemoglobin in 61 effusions (14.1 %). Cytology and microbiology tests were performed in all cases.

In accordance with Light's criteria, out of 410 effusions with identified causes, 305 (74.4 %) were classified as exudates, and 105 (25.6 %) as transudates with an accuracy of 97.1 %, the positive predictive value (PPV) for exudates of 99.0 % and the PPV for transudates of 92.1 %. According to the final diagnosis, 2.9 % of cases were misclassified. In three patients with pleural carcinosis, effusions were wrongly classified as transudates, one of them had pH < 7.30, one had normal pH and in one pH was not measured. In nine patients with heart failure, the effusions were classified as exudates according to Light's criteria. All of these patients were receiving diuretic therapy prior to thoracentesis, were polymorbid and four of these patients had chronic renal failure.

Further, pleural LDH values expressed in µkat/l were significantly elevated (7.5 ± 0.7) in malignant compared with paramalignant effusions (3.3 ± 0.4) (p < 0.0005) (Table 2, Figure 1a). The difference in pleural protein values expressed in g/l was also significant, with levels of proteins 43.0 ± 1.0 in malignant and

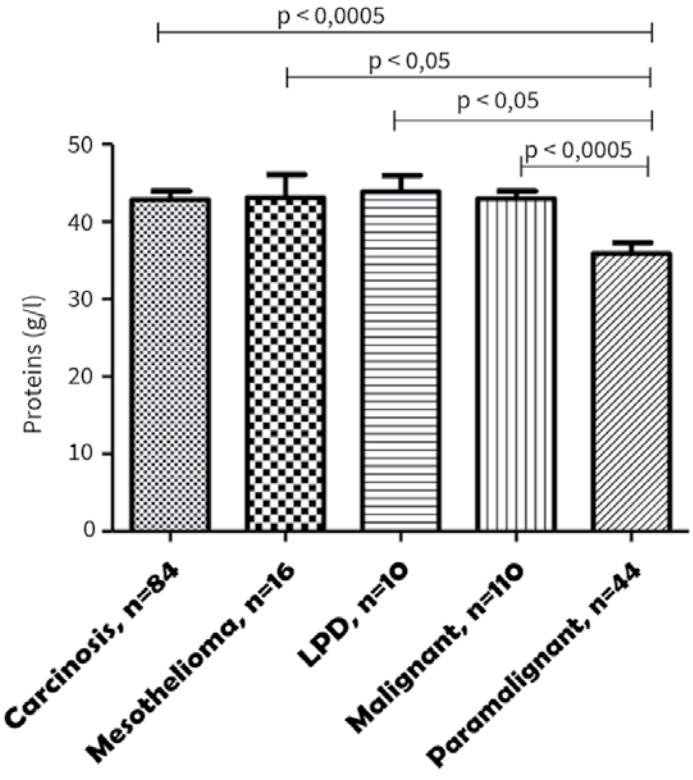

Figure 1b: Protein levels in pleural carciosis, mesothelioma, LDH-lymphoproliferative disorder and paramalignant effusion. Malignant group includes all patients with pleural carcinosis, mesothelioma and LPD.

36.0 ± 1.4 in paramalignant effusions. There were no significant differences in LDH or protein levels between different types of malignant pleural involvement.

We found significantly lower pH values in the PEs in all infectious aetiologies (6.88–7.29), in the combined malignant group (7.30 ± 0.01), pleural carcinosis (7.31 ± 0.01) and mesothelioma group (7.21 ± 0.05). Combined malignant effusions had significantly lower pH values compared to paramalignant PEs (7.39 ± 0.01)(Figure 1c).

Glucose levels, expressed in mmol/l, were significantly lower in mesothelioma (3.4 ± 0.54) and empyema (1.3 ± 0.52) compared to other groups. Moreover, glucose values were lower in all malignant effusion compared to paramalignant effusion, but the difference was significant only in mesothelioma subgroup. In all paramalignant effusions, the glucose levels were greater than 3.4 mmol/l.

The AA levels were expressed as μkat/l; considering all pleural malignancies combined, we did not find any significant differences between the malignant (1.39 ± 0.22) and paramalignant involvement of the pleura (0.68 ± 0.06).

4. Discussion

The results of this study have confirmed the usefulness of thoracentesis and Light's criteria, which effectively separate exudative from transudative effusions. In case of transudate, the pleura is unaffected and PE is in most cases a result of a HF, nephrosis or liver cirrhosis. In contrast, in case of exudate, PE is a result of pleural disease, and malignancy is suspected, so more extensive diagnostic procedure is required (2,3,5,11). Our results have shown high accuracy of Light's criteria, which was 97.1 %, being comparable with previously reported accuracy of 68–95 % (2,6,12). In three patients with pleural carcinosis, effusions were identified as transudates, which actually present only a small proportion of patients with pleural carcinosis, but reminds us that in highly suspicious cases further diagnostics should be performed despite biochemical characteristics of a transudate. Additionally, in nine (2.1 %) patients with a HF, effusions were misclassified as exudates. All of these patients had more comorbidities, including chronic renal failure, and were using diuretics, a situation that was already reported when PE in HF presented with characteristics of an exudate (5).

In our retrospective one-year period of pleural fluid analyses, we found that the most common cause of PE was ma-

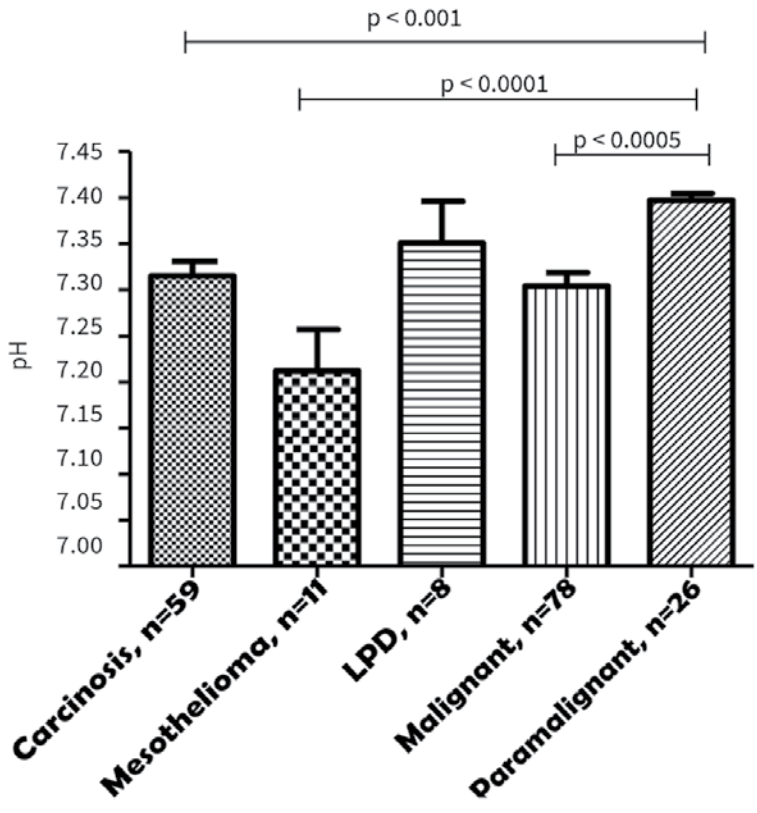

Figure 1c: pH levels in pleural carciosis, mesothelioma, LDH-lymphoproliferative disorder and paramalignant effusion. Malignant group includes all patients with pleural carcinosis, mesothelioma and LPD.

lignancy (32.9 %), followed by infection (24.3 %) and HF (23.3 %). Our results cannot be directly compared with the reported overall proportions of different aetiologies because this analysis excluded a large proportion of patients with obvious heart decompensation in whom thoracentesis was not performed. Moreover, the study was conducted in a tertiary centre, where we can expect a higher percentage of patients with more severe diseases (malignancies and infections). The resulting proportions of different aetiologies among subgroups of pleural exudates are comparable to other reported studies (2,3,13).

As mentioned previously, pleural biochemical test have crucial role in the differentiation between exudates and transudates, but criteria to separate malignant from benign exudates have not been established. There are reports suggesting that glycosaminoglycans (GAGs), VEGF, various tumour markers (CA-125, CEA, CYFRA 21–1 and NSE) and mesothelin could be helpful, but the accuracy of these markers has not yet reached the confidence level for clinical utility (14-19). Our study focused on biochemical markers, which are already routinely used in diagnostics of a PE and concentrated on PEs, which are result of malignant diseases. We found that the levels of proteins and LDH were significantly higher in pleural malignant involvement compared to paramalignant PEs. An additional biochemical marker that differed between mentioned groups was the level of pH, with significantly lower values in effusions with malignant pleural involvement (combined malignant PE, carcinosis and mesothelioma). In only one (3.5 %) patient with paramalignant PE pleural pH was lower than 7.32. Therefore, higher pleural LDH and protein levels and lower pH could offer additional help in a lung cancer staging in borderline cases or where other diagnostic tools are not feasible. Until now, only one study reported difference in basic laboratory values between paramalignant and malignant effusions, where they detected significantly higher levels of serum proteins in patients with malignant effusion compared to paramalignant (20).

The clinical importance of other biomarkers is limited to specific cases. We have not found any significant correlations between AA and glucose levels and malignant or paramalignant effusions.

5. Conclusion

In conclusion, we confirmed the accuracy of Light's criteria for distinguishing transudates from exudates. Furthermore, significantly higher pleural LDH and protein levels and lower pH could be useful in differentiation between paramalignant and malignant PEs.

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