ORIGINAL ARTICLE

Cancer ratio and other new parameters for differentiation between malignant and nonmalignant pleural effusions

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KEY WORDS

adenosine deaminase, cancer ratio, Fas ligand, lactate dehydrogenase, pleural effusion

ABSTRACT

INTRODUCTION In contrast to tuberculous pleurisy (TP), no accurate and commonly accepted biochemical marker of malignant pleural effusion (MPE) has been established.

OBJECTIVES We aimed to evaluate the ability of a previously reported cancer ratio (CR) to discriminate between MPEs and non–MPEs; to test whether age may have additional value in differentiating MPEs from non–MPEs; and if so, to combine lactate dehydrogenase (LDH) and age with other TP biomarkers in search of an index useful in the identification of MPEs.

PATIENTS AND METHODS A retrospective analysis of data from 140 patients with malignant (n = 74), tuberculous (n = 37), and parapneumonic (n = 29) pleural effusions was performed. The diagnostic performance of a test to discriminate between MPEs and non–MPEs was evaluated using the receiver operating characteristic curve analysis.

RESULTS Three ratios showed the largest area under the curve (AUC): serum LDH to pleural fluid soluble Fas ligand, age to pleural fluid adenosine deaminase (ADA), and serum LDH to pleural fluid interleukin 18; moreover, the ratios were characterized by high sensitivity (95%, 93.2%, and 92.9%, respectively) and fair specificity (64.8%, 71.2%, and 58.5%, respectively) for differentiating MPEs from non–MPEs. The AUC for CR was lower and showed a sensitivity of 94.6% and a specificity of 68.2%.

CONCLUSIONS Our study showed a lower specificity of the CR for discriminating between MPEs and non–MPEs than previously reported. We demonstrated that the combinations of serum LDH with other pleural fluid biomarkers of TP have a similar diagnostic performance. We also found that age might be an important factor differentiating between MPEs and non–MPEs and proposed a new age to pleural fluid ADA ratio which has a discriminative potential similar to that of the CR.

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INTRODUCTION Pleural effusion is a common clinical entity affecting approximately 1.5 million patients per year in the United States.[1] There is a large number of diseases which may be associated with pleural effusion formation. This includes both local conditions affecting the pleura (eg, tuberculous pleurisy [TP], pleural mesothelioma), as well as extrapulmonary diseases with secondary pleural involvement (eg, chronic heart failure, liver cirrhosis). To date, differentiation between 2 types of pleural effusion—exudate and transudate—is the most common initial diagnostic approach to patients with pleural effusion.[2] Further differentiation between the specific diseases associated with formation of pleural exudate usually requires a more in-depth analysis of pleural fluid, including total and differential cell count, pH and glucose levels, adenosine deaminase (ADA) activity, as well as cytological and microbiological examinations.[3] If inconclusive, the tests are followed by more invasive diagnostic procedures.

In some patients the clinical, radiological, and laboratory results might be confusing and not particularly helpful in making a reliable diagnosis. This refers not only to patients with rare diseases but also to those with common causes of pleural

involvement, such as malignant pleural effusion (MPE). Although MPE can be diagnosed by simple pleural fluid cytology, this method has significant limitations, including a highly variable sensitivity, ranging from as low as 11.6% to as high as 71%.[4-6] In contrast to other common causes of pleural effusion such as TP, no accurate biomarkers of MPE have been established. Several tumor markers were extensively evaluated, including carcinoembryonic antigen, cytokeratin-19 fragments, and cancer antigen 125,[7] but none of them were found sensitive and specific enough to be implemented in routine clinical practice. Interestingly, some authors reported a relationship between low ADA levels and MPE, but there are insufficient data on the true diagnostic performance of the above relationship.[8] Due to the above limitations of noninvasive or minimally invasive diagnosis of MPE, a substantial proportion of patients require more invasive diagnostic tests. This carries the risk of complications and, in some cases, may lead to further delay in cancer diagnosis.

Recently, Verma et al[9,10] reported a new biochemical parameter which showed a high diagnostic accuracy for pleural malignancies. This is a quotient of serum lactate dehydrogenase (LDH) and pleural fluid ADA, termed by the authors as the "cancer ratio" (CR). At the cut-off level of more than 20, the CR showed high sensitivity and specificity for identifying patients with MPE. The high diagnostic performance of this parameter is based on the observations that MPE is usually associated with high serum LDH levels, while TP—with high pleural fluid ADA levels. It should be noted, however, that other studies, including that by Lee et al,[11] revealed that older patients may have tuberculous pleural effusion (TPE) with low ADA levels. Therefore, interpretation of low pleural fluid ADA levels in older patients suspected of TP should be cautious. The relationship between age and pleural fluid ADA level was also reported by Abrao et al,[12] who found a significant moderate negative correlation between these 2 variables in 309 patients with pleural effusion. Importantly, TP was the underlying disease in 56.3% of the 174 patients included in the study. Hence, the authors concluded that the use of lower ADA cut-off value in older patients can reduce the number of false-negative results of ADA in TPE.

Considering the above reports, the objectives of our study were: to assess the ability of the CR to discriminate MPE from other causes of exudate; to test whether age should be included in ratios discriminating between MPEs and non–MPEs; and, if so, to combine LDH and age with other biomarkers of TP in search of an index useful in the identification of MPE.

PATIENTS AND METHODS

Study design This study was part of an ongoing research project focused on the diagnostic utility of different pleural fluid biomarkers. A unified diagnostic approach to patients with pleural effusion has been implemented in our institution, with all relevant clinical and laboratory data stored in an electronic database. This approach was presented in some earlier publications.[13,14] Briefly, all patients underwent: 1) clinical examination, which included signs and symptoms and medical history taking; 2) standard blood tests; 3) electrocardiogram; 4) chest radiograph; and 5) diagnostic thoracentesis with pleural fluid analysis. Effusions were classified as transudates or exudates using Light's criteria.[2] Pleural fluid samples (average pleural fluid volume, 100 ml) for measurements of biomarker levels were collected during the diagnostic thoracentesis. The samples were centrifuged at 2000 RPM for 10 minutes and the supernatant was frozen at –70°C. The activity or the levels of predefined biomarkers were later measured in thawed pleural fluid samples.

Additional diagnostic procedures, including blood tests, echocardiography, thoracic and abdominal computed tomography scan, abdominal ultrasound, positron emission tomography bronchoscopy, mammography and/or breast ultrasound, and pleural biopsy or thoracoscopy, were at the discretion of the attending physician.

The current study included a retrospective analysis of clinical and laboratory data of patients with MPEs, TPEs, and parapneumonic pleural effusions (PPEs) treated between 2011 and 2016. Patients with pleural transudates and those with exudative effusions of untypical origin were excluded from the analysis.

The following parameters were evaluated: patient age, final diagnosis, basic pleural fluid biochemical parameters (pH, glucose, total protein, LHD), pleural fluid total and differential cell count and pleural fluid levels of 9 well-established or potential TP biomarkers: ADA, interferon γ (IFN-γ), soluble interleukin-2 receptor (sIL-2R), IFN-γ–induced protein 10 (IP-10), interleukin-12 subunit p40 (IL-12p40), interleukin 18 (IL-18), tumor necrosis factor (TNF) and soluble Fas ligand (sFasL).[14-16] ADA activity was determined with colorimetric method by Giusti,[17] while the concentrations of IFN-γ, sIL-2R, IP-10, IL-12p40, IL-18, TNF, and sFasL were measured with respective enzyme-linked immunosorbent assay kits (R&D System, Minneapolis, Minnesota, United States) according to the manufacturer's instructions.

The study protocol was approved by the institutional review board of the Medical University of Warsaw and all patients signed an informed consent to participate in the study.

Statistical analysis Statistical analysis was performed with Statistica 13.1 (StatSoft, Tulsa, Oklahoma, United States) and MedCalc 18.0 (MedCalc Software, Ostend, Belgium) software packages.[18] Data were presented as median and interquartile range (IQR). Differences between continuous variables were tested using the Kruskal–Wallis or the Mann–Whitney test. A multivariate logistic regression analysis with pleural malignancy as the outcome was performed. The diagnostic performance of a test to discriminate between

TABLE 1 Age and selected biochemical parameters in patients with malignant, tuberculous, and parapneumonic pleural effusions

Parameter	MPE (n = 74)	TPE (n = 37)	PPE (n = 29)	*P* value		
				MPE vs TPE	MPE vs PPE	TPE vs PPE
Age	69.0 (60.0–77.0)	52.0 (35.0–75.0)	59.0 (51.0–69.0)	<0.01	0.02	1.0
Pleural fluid protein, g/dl	4.3 (4.0–4.7)	5.0 (4.6–5.7)	4.1 (3.6–4.8)	<0.001	1.0	<0.001
Pleural fluid/serum protein	0.6 (0.6–0.7)	0.70 (0.67–0.77)	0.68 (0.60–0.72)	<0.001	0.49	0.03
Pleural fluid LDH, U/l	784.0 (402.0–490.0)	865.0 (601.0–1656.0)	3755.0 (1097.0–14 110)	0.58	<0.001	0.03
Serum LDH, U/l	530.9 (390.9–749.0)	424.0 (327.9–536.3)	476.2 (436.5–588.6)	0.04	1.0	0.23
Pleural fluid LDH/serum LDH	1.3 (0.8–2.5)	2.43 (1.5–3.8)	7.68 (1.92–30.3)	<0.01	<0.001	0.34

Data are presented as median (interquartile range).

Abbreviations: LDH, lactate dehydrogenase; MPE, malignant pleural effusion; PPE, parapneumonic pleural effusion; TPE, tuberculous pleural effusion

TABLE 2 Multivariate logistic regression analysis with pleural malignancy as the outcome variable

Parameter	Coefficient	Standard error	*P* value
Step 1: stepwise analysis (age, pleural fluid cells, and chemistry)			
Age	0.29	0.07	<0.001
Serum LDH	0.16	0.07	0.03
Variables not included in the model: pleural fluid protein, serum protein, pleural fluid/serum protein, pleural fluid LDH, pleural fluid/serum LDH, pleural fluid cells			
Step 2: enter analysis (age, serum LDH, subsequent biomarker)			
Pleural fluid ADA	−0.46	0.08	<0.001
Pleural fluid IFN-γ	−0.44	0.07	<0.001
Pleural fluid sIL-2R	−0.04	0.08	0.61
Pleural fluid IP-10	−0.48	0.08	<0.001
Pleural fluid IL-12p40	−0.11	0.08	0.19
Pleural fluid IL-18	−0.30	0.09	<0.01
Pleural fluid TNF	−0.32	0.09	<0.001
Pleural fluid sFasL	−0.21	0.10	0.04
Pleural fluid IL-23	−0.14	0.10	0.17

Abbreviations: ADA, adenosine deaminase; IFN-γ, interferon γ; IL-12p40, interleukin-12 subunit p40; IL-18, interleukin 18; IL-23, interleukin 23; IP-10, interferon-γ–induced protein 10; sFasL, soluble Fas ligand; sIL-2R, soluble interleukin-2 receptor; TNF, tumor necrosis factor; others, see TABLE 1

MPEs and non–MPEs was evaluated using the receiver operating characteristic (ROC) curve analysis, which included the calculation of the area under the curve (AUC) and 95% confidence intervals (CIs). The DeLong's test was used to compare the ROC curves representing the diagnostic performance of different tests.[19] All *P* values were 2-tailed, and a *P* value of less than 0.05 was considered significant.

RESULTS The study included 140 patients with MPEs, TPEs, and PPEs (76 men [54.3%] and 64 women [45.7%]; median age, 64.5 [IQR, 54–75] years). There were 74 patients with MPEs (52.9%), 37 patients with TPEs (26.4%), and 29 patients with PPEs (20.7%). The malignancies associated with pleural effusion were as follows: lung cancer (38 patients, 51.4%), breast cancer (12 patients, 16.2%), ovarian cancer (6 patients, 8.1%), gastric cancer (3 patients, 4.1%), lymphoma (5 patients, 6.8%), pancreatic cancer (4 patients, 5.4%), mesothelioma (2 patients, 2.7%), laryngeal cancer (1 patient, 1.4%), colorectal cancer (2 patients, 2.7%), cervical cancer (1 patient, 1.4%). Comparative characteristics of patients with MPE, TPE, and PPE are presented in TABLE 1.

In the multivariate logistic regression analysis, pleural fluid ADA, IFN-γ, IP-10, IL-18, TNF, and sFasL levels were found to be negative predictors of MPE. In contrast, age and serum LDH levels were positive predictors of MPE (TABLE 2). Pleural fluid IP-10, ADA, and IFN-γ levels were characterized by the highest coefficient values: −0.48, −0.46, and −0.44, respectively (*P* <0.001). Pleural LDH levels and lymphocyte or neutrophil counts did not predict the malignant origin of pleural fluid.

The sensitivity, specificity, and AUC for different diagnostic parameters and their ratios are demonstrated in TABLE 3. The 3 indices with the largest AUC were serum LDH/pleural fluid sFasL, age/pleural fluid ADA, and serum LDH/pleural fluid IL-18. These ratios showed a high sensitivity and fair specificity for discriminating MPE from TPE and PPE (TABLE 3). The ROC curves for the 3 most accurate ratios are presented in FIGURES 1–3. In our study, the AUC for the CR was 0.826 and was smaller than the respective values for serum LDH/pleural fluid sFasL, age/pleural fluid ADA, and serum LDH/pleural fluid IL-18 ratios. The age/pleural fluid IL-18 ratio and pleural fluid IL-18 alone were the most specific parameters differentiating between MPEs and other types of pleural effusions. The data are presented in TABLE 4. The development of more complex ratios that included the above parameters resulted in similar or slightly higher diagnostic

TABLE 3 Comparison of the area under the curve for different parameters differentiating between malignant and nonmalignant pleural effusion

Parameter	AUC	95% CI	Cutoff value	Sensitivity, %	Specificity, %	+LR (95% CI)	–LR (95% CI)
Serum LDH/pleural fluid sFasL	0.849	0.747–0.922	>11.6	95.0	64.8	2.7 (1.9–3.9)	0.08 (0.01–0.5)
Age/pleural fluid ADA	0.847	0.776–0.902	>2.62	93.2	71.2	3.24 (2.2–4.8)	0.10 (0.04–0.2)
Serum LDH/pleural fluid IL-18	0.830	0.751–0.892	>2.18	92.9	58.5	2.24 (1.7–3.0)	0.12 (0.05–0.3)
Serum LDH/pleural fluid ADA	0.826	0.753–0.885	>16.4	94.6	68.2	2.97 (2.1–4.2)	0.08 (0.03–0.2)
Age/pleural fluid IFN-γ	0.826	0.752–0.884	>1.36	97.3	57.6	2.29 (1.7–3.0)	0.05 (0.01–0.2)
Age/pleural fluid TNF	0.826	0.752–0.884	>1.4	97.3	57.6	2.5 (1.8–3.5)	0.11 (0.04–0.3)
Pleural fluid ADA	0.821	0.747–0.881	<29.6	97.3	68.2	3.06 (2.1–4.4)	0.04 (0.01–0.2)
Serum LDH/pleural fluid TNF	0.821	0.733–0.889	>12.1	100.0	67.7	3.1 (2.2–4.4)	0.00
Age/pleural fluid sFasL	0.821	0.714–0.900	>1.6	90.0	66.7	2.7 (1.8–4.0)	0.15 (0.04–0.6)
Serum LDH/pleural fluid IFN-γ	0.811	0.736–0.872	>2.45	100.0	54.5	2.20 (1.7–2.9)	0.00
Pleural fluid IFN-γ	0.804	0.729–0.866	<75	98.6	56.1	2.25 (1.7–3.0)	0.02 (0.003–0.2)
Pleural fluid sFasL	0.802	0.693–0.885	<23.5	75.0	79.6	3.68 (2.1–6.6)	0.31 (0.1–0.7)
Pleural fluid TNF	0.798	0.708–0.870	<31.2	92.9	67.7	2.88 (2.0–4.2)	0.11 (0.03–0.3)
Age/pleural fluid IL-18	0.791	0.708–0.859	>2.41	49.1	95.4	10.6 (3.4–33.2)	0.53 (0.4–0.7)
Pleural fluid IL-18	0.776	0.692–0.847	<23.5	49.1	93.8	7.98 (3.0–21.4)	0.54 (0.4–0.7)
Serum LDH/pleural fluid IP-10	0.758	0.679–0.827	>0.04	91.9	62.1	2.43 (1.8–3.3)	0.13 (0.06–0.3)
Age/pleural fluid IP-10	0.753	0.673–0.822	>0.0027	100.0	49.9	1.89 (1.5–2.4)	0.00
Pleural fluid IP-10	0.739	0.658–0.810	<9922	90.5	57.6	2.13 (1.6–2.9)	0.16 (0.08–1.3)

Units for the variables presented in the table: age, years; pleural fluid ADA, U/l; pleural fluid IFN-γ, pg/ml; pleural fluid IL-18, pg/ml; pleural fluid IP-10, pg/ml; pleural fluid sFasL, pg/ml; pleural fluid TNF, pg/ml; serum LDH, U/l.

Abbreviations: AUC, area under the curve; LR, likelihood ratio; others, see TABLES 1 and 2

TABLE 4 Comparison of the area under the curve for complex ratios differentiating between malignant and nonmalignant pleural effusion

Complex ratio	AUC	95% CI	Cut-off value	Sensitivity	Specificity	+LR (95% CI)	–LR (95% CI)
Pleural fluid LDH × pleural fluid ADA/serum LDH	0.837	0.765–0.894	<70.8	94.6	68.2	2.97 (2.1–4.2)	0.08 (0.03–0.2)
Pleural fluid LDH/(serum LDH × age)	0.761	0.681–0.828	<0.55	87.8	60.6	2.2 (1.6–3.0)	0.2 (0.1–0.4)
(Serum LDH × age)/pleural fluid ADA	0.850	0.780–0.905	>917.0	98.6	66.7	3.0 (2.1–4.2)	0.02 (0.003–0.1)
(Serum LDH × age)/pleural fluid sFasL	0.866	0.766–0.934	>925.9	95.0	74.1	3.7 (2.3–5.8)	0.07 (0.01–0.5)
(Serum LDH × age)/pleural fluid IL-18	0.836	0.758–0.897	>167.5	87.7	64.6	2.5 (1.8–3.5)	0.2 (0.09–0.4)
(Serum LDH × age)/(pleural fluid LDH × pleural fluid ADA)	0.845	0.775–0.901	>1.11	95.9	65.1	2.9 (2.0–4.1)	0.06 (0.02–0.2)
(Serum LDH × age)/(pleural fluid LDH × pleural fluid sFasL)	0.809	0.702–0-891	>3.03	100	54.5	2.2 (1.6–2.9)	0.00
(Serum LDH × age)/(pleural fluid LDH × pleural fluid IL-18)	0.830	0.751–0.892	>2.87	75.4	78.5	3.5 (2.2–5.7)	0.31 (0.2–0.5)

Units and abbreviations: see TABLES 1, 2, and 3

accuracy measured as the AUC (TABLE 4). The highest diagnostic performance was found for the serum LDH × age)/pleural fluid sFasL ratio.

Differences between selected AUCs for the age/biomarker ratio and AUCs for biomarker alone (presented in TABLE 3) are shown in TABLE 5. In the multivariate logistic regression, age was a positive predictor of MPE. A paired comparative analysis of the diagnostic accuracy of age and various TP biomarkers vs biomarkers alone revealed significant differences in 3 pairs. The highest difference was observed between pleural fluid ADA and age/pleural fluid ADA ROC curves. In the other 2 pairs, the difference was smaller but still significant (TABLE 5).

DISCUSSION Our study, undertaken to test the diagnostic performance of the recently reported CR and to evaluate the potential usefulness of other typical markers of TPE in diagnosing MPE, showed some interesting relationships between various biomarkers and pleural malignancies. Besides pleural fluid ADA, we also found pleural fluid IFN-γ, IP-10, IL-18, TNF, and sFasL to be negative predictors of MPE. In contrast, positive relationships between serum LDH, patient's age, and malignant origin of pleural effusion were

TABLE 5 Differences between the area under the curve of pleural fluid adenosine deaminase, interferon γ, and interferon-γ–induced protein 10 and age-related quotients

Parameter	Difference between AUCs	SE	95% CI	*P* value
ADA	+0.0257	0.0108	0.0045–0.0469	0.02
Age/pleural fluid ADA				
Pleural fluid IFN-γ	+0.0215	0.0180	−0.0137 to 0.0567	0.23
Age/pleural fluid IFN-γ				
Pleural fluid IP-10	+0.0140	0.0059	0.0025–0.0256	0.02
Age/pleural fluid IP-10				
Pleural fluid IL-18	+0.0150	0.0070	0.0011–0.0288	0.03
Age/pleural fluid IL-18				
Pleural fluid TNF	+0.0276	0.0146	−0.0010 to 0.0563	0.06
Age/pleural fluid TNF				
Pleural fluid sFasL	+0.0190	0.0177	−0.0157 to 0.0536	0.28
Age/pleural fluid sFasL				

The "+" sign denotes a positive difference between the AUC for age/biomarker ratio and AUC for biomarker alone.

Abbreviations and units: see TABLES 2 and 3

demonstrated. To our knowledge this is the first study which tested the diagnostic performance of the CR in an external cohort. We confirmed that the CR can be used to identify MPEs with high sensitivity and an AUC of 0.826. On the other hand, the specificity of the CR in our study was significantly lower than that reported by Verma et al.[9] Our study also demonstrated that a diagnostic performance similar to that of the CR can be achieved using other simple ratios or even single biomarkers. These include age/pleural fluid ADA ratio, age/pleural fluid IFN-g, age/pleural fluid TNF, or pleural fluid ADA alone. Thus, we believe that our study shows that not only serum LDH but also patient's age might be an important factor combined with ADA or other markers of TPE to distinguish between MPEs and non–MPEs. In this context, we add new and complementary data to the interesting results previously reported by Verma et al.[9,10] Moreover, we found that some modifications in the CR with the substitution of ADA with sFasL or IL-18 instead of ADA may result in even higher diagnostic performance than the original CR. Interestingly, in our earlier publication we reported that pleural fluid sFasL but not IL-18 was highly sensitive and specific for TPE.[13]

Although several different biomarkers were tested in our study, it should be underlined that relatively high diagnostic performance was demonstrated for parameters (ratios) which combined simple and widely available data. The results of our multivariate logistic regression analysis confirmed the earlier observation that the serum LDH level was an independent factor associated with the risk of pleural malignancies.[9] LDH is an enzyme that catalyzes the lactate to pyruvate conversion. It is particularly important in cancer cell energy production, which relies on glycolysis in a process known as the Warburg effect.[20] Although clinical observations or previous studies did not find that the serum LDH level alone is sensitive and specific enough to detect malignancies, its increased serum activity is common in patients with malignant diseases, especially with leukemias.[21] Brindley et al[22] showed that the changes in serum LDH levels in 91 patients with various solid malignant tumors reflected changes in tumor size. Combining serum LDH with pleural fluid ADA increased the diagnostic performance of LDH alone and turned out to be useful, at least potentially, for differentiating patients with MPEs from those with other pleural pathologies.[9,10]

Our multivariate logistic regression analysis showed that patient's age is even more closely related to MPE (coefficient, 0.29; $P < 0.001$) than the serum LDH level. This seems to be easy to explain, as the incidence rates of most malignancies increase with age.[23] In Poland, most malignant tumors reported between 2011 and 2013 were diagnosed in patients aged 60 years or older (70% in men and 60% in women). The malignancy incidence rate increased with age and was highest in patients between 80 and 90 years old.[24] The majority of lung cancer cases were diagnosed in patients above 50 years of age (96% of men and 95% of women), with a peak incidence observed in the ninth decade for men and seventh decade for women, respectively.[25] These statistics are concordant with data from other European countries, for example, in the United Kingdom 50% of lung cancer cases were diagnosed in patients at the age of 70 years or older.[26]

As regards tuberculosis (TB), its incidence rate in developed countries also increases with age. However, the epidemiology of extrapulmonary TB, including TP is more complex and depends on the specific TB location and other variables. In general, TP is believed to develop in younger patients. A large epidemiological study by Zhang et al[27] demonstrated that pleural TB was a predominating form of extrapulmonary TB in Denmark and Greenland, while in Somalia and Asia it was far less common than lymphatic TB and bone/joint TB. Altogether, pleural TB affected mostly young people aged between 15 and 24 years.[27] Also, numerous previous publications on pleural effusion reported the relationship between younger age and TP. These included significant difference between the age of patients with TPE vs those with non–TPE[13] as well as the association between the younger age and the risk of TPE. Therefore, younger age was included in different predictive models for TPE.[28,29]

Given all of the above, we believe that the use of age in ratios tested in our study was supported not only by our own results but also by a large body of earlier published data. It should be noted, however, that the diagnostic performance of ratios that includes patient's age might significantly change when applied in populations in which

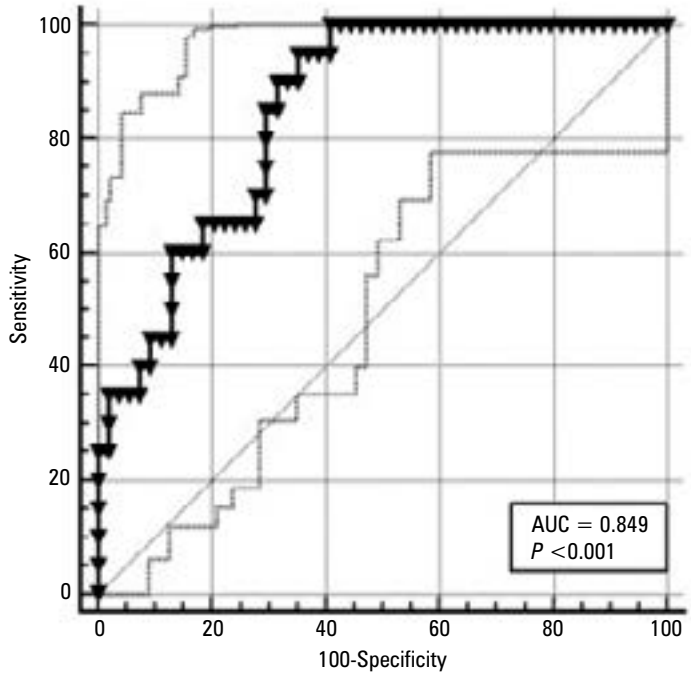

FIGURE 1 Receiver operating characteristic (ROC) curve for serum lactate dehydrogenase / pleural fluid soluble Fas ligand. Thick line with black triangles represents the ROC curve. Thin dotted lines represent 95% CI. AUC indicates the area under the ROC curve.

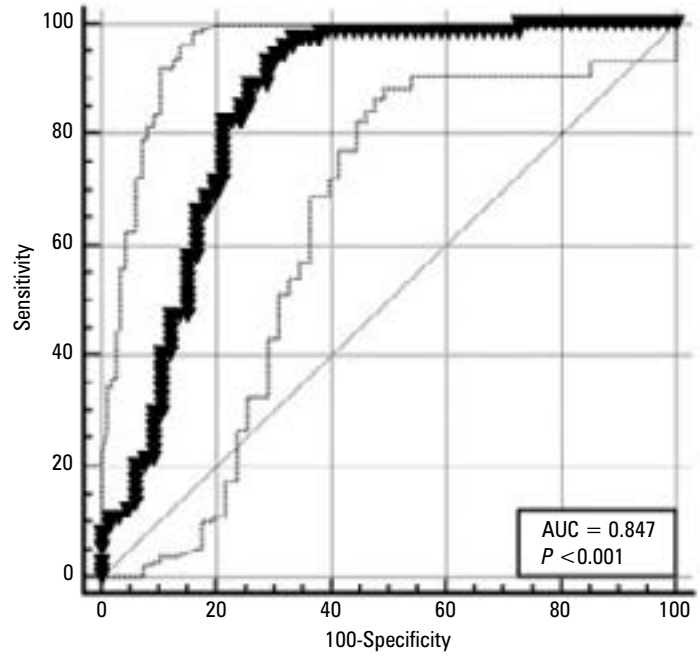

FIGURE 2 Receiver operating characteristic (ROC) curve for age/ pleural fluid adenosine deaminase. Thick line with black triangles represents the ROC curve. Thin dotted lines represent 95% CI. AUC indicates the area under the ROC curve.

pleural TB affects older patients. Nonetheless, our study showed that a new, previously unreported age/ADA ratio may be a useful parameter differentiating between MPE and TPE.

Although the highest diagnostic performance was found for serum LDH/pleural fluid sFasL level, we realize that this ratio will probably not gain a wide acceptance as a marker of MPE. This also refers to serum LDH level/pleural fluid IL--18 level. This is because neither sFasL nor IL--18 are routinely tested in pleural effusion. On the other hand, it should be stressed that the difference between the AUC for the 2 above ratios was only slightly different than the respective AUC for the age/pleural fluid ADA ratio and CR. Therefore, for practical reasons, the 2 latter ratios seem to be more promising diagnostic tools than the others.

The difference between the specificity of CR found in the studies by Verma et al[9,10] and in the current study seems to be an interesting issue. The difference is quite significant, as Verma et al[9,10] reported the specificity of 0.94 and 0.85 (in 2 studies), while the specificity calculated in our study was only 0.68. The difference may probably be explained by different inclusion criteria and different characteristics of the study group. In our study, the proportions of patients with MPE, TPE, and PPE were 53%, 26%, and 21%, respectively, while there was a significant predominance of patients with MPE and TPE (87% of all) in the first study by Verma et al.[9] They included only 8.5% of patients with PPE and also 5.5% of

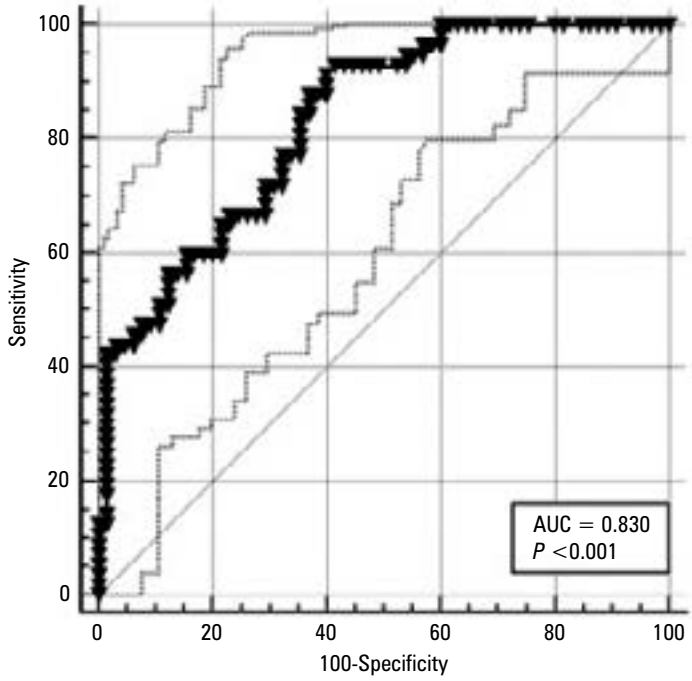

FIGURE 3 Receiver operating characteristic (ROC) curve for serum lactate dehydrogenase/pleural fluid interleukin 18. Thick line with black triangles represents the ROC curve. Thin dotted lines represent 95% CI. AUC indicates the area under the ROC curve.

patients with undiagnosed pleural effusion. In contrast to our study, the second study by Verma et al[10] included only patients with MPE and TPE. Moreover, in both cited studies, there was a significant predominance of patients with lung cancer among patients with MPE (95% and 97.6%, respectively).[9,10] In our study, patients with lung cancer constituted only 51.4% of patients with MPE. As serum LDH levels may be associated with the tumor type and spread, the inclusion of patients with more advanced and more aggressive tumors can probably result in higher specificity of the CR.

When discussing the role of various biomarkers in the diagnosis of MPE, it is important to perceive this problem in a wider context of the differential diagnosis of pleural effusion. Although a high-quality guideline on the investigation of the unilateral pleural effusion has been published, the diagnosis of pleural effusion can still be challenging.[30] The general approach is to prefer minimally invasive diagnostic strategy, with thoracentesis and pleural fluid analysis as the major and critically important diagnostic step. However, the sensitivity of pleural fluid analysis, including cytology, is limited. Although according to a recent survey pleural fluid cytology is requested in as many as 93% of patients with suspected MPE,[31] it has a mean sensitivity of only approximately 60%. Thus, a substantial proportion of patients with MPE may require more invasive diagnostic procedures, including percutaneous pleural biopsy or thoracoscopy. The use of various pleural fluid and/or serum biomarkers may be an attractive alternative to more invasive diagnostic procedures or may facilitate patient selection for pleural biopsy and thoracoscopy.

Our study has several limitations. First, it was a single-center retrospective study. Second, it included only patients with MPE, TPE, and PPE but no patients with other causes of exudative pleural effusion, such as pulmonary embolism or drug-induced pleural effusion. Patients with other underlying diseases were not included because their number was relatively low and they formed a highly heterogeneous group. Nevertheless, a real-life scenario should probably include patients with other, difficult to diagnose causes of pleural exudate. Third, our analysis was limited to patients with MPE as a whole group, with no subanalysis of patients with different tumor types and stages. We could not perform such an analysis due to a small number of patients in different subgroups defined by tumor type and the stage of the disease. Nevertheless, we believe this analysis might reveal new and interesting data showing the tumor types and stages of the disease associated with MPE, which are particularly difficult to differentiate from other underlying diseases. This refers not only to serum LDH levels but also to pleural fluid ADA. High pleural fluid ADA levels were reported in patients with lymphatic malignancies.[32] We are aware of the fact that the new tools (ratios) proposed in our study should undergo external validation in larger, possibly multicenter, prospective studies.

Conclusions Our study confirmed a possible role for the CR as a tool differentiating between MPEs and non–MPEs. However, the specificity of this test was lower than in previous studies. We also found that similar diagnostic performance might be achieved when combining the serum LDH level with other pleural fluid markers of TP. We demonstrated that a new age/pleural fluid ADA ratio has a discriminative potential similar to that of the CR when differentiating between MPEs and non–MPEs. The CR and age/pleural fluid ADA ratio are easy to use in everyday practice because they include only basic information and the results of

widely available and routinely performed tests. Future prospective studies should be undertaken to evaluate the role of the age/pleural fluid ADA in routine clinical practice.

CONTRIBUTION STATEMENT PK and MM conceived the concept of the study and performed the literature search. PK, MM, RWL, and RK contributed to the design of the study. PK, RK, and AS were involved in patient recruitment and data collection. AS was responsible for pleural fluid processing and measurements of pleural fluid biomarkers. PK and MM performed data analysis. PK, MM, and RK contributed to data interpretation. PK and MM wrote the first draft of the manuscript. All authors critically reviewed the manuscript, edited, and approved its final version.

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