Predicting Malignant Mesothelioma by Analyzing Serum N-ERC/Mesothelin, C-ERC/Mesothelin, Hyaluronan, Osteopontin, and Syndecan-1 Levels

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Abstract

Objective: Tumor biomarkers are promising study areas for the early or differential diagnosis of malignant pleural mesothelioma (MPM). This study aimed to determine the effectiveness of analyzing serum N-ERC/mesothelin, C-ERC/mesothelin, hyaluronan, osteopontin, and syndecan-1 levels for distinguishing patients with MPM from those with metastatic malignant pleural diseases (MMPDs), benign pleural diseases (BPDs), and benign asbestos pleurisy (BAP).

Methods: Tumor biomarker levels of serum samples of 230 cases were analyzed by enzyme-linked immunosorbent assays.

Results: All investigated biomarkers did not reveal sufficient diagnostic information to distinguish MPM from MMPD. N-ERC/mesothelin showed moderate ability to distinguish MPM from BPDs and particularly BAP (sensitivities of 67% and 73%, respectively, and specificities of 84% and 86%, respectively). C-ERC/mesothelin had a lower efficacy than N-ERC/mesothelin, whereas osteopontin had a high specificity for distinguishing MPM from other pleural diseases (80%) but with a poor sensitivity (32%). Hyaluronan and syndecan-1 had only limited effects as individual biomarkers. However, logistic regression analysis indicated that all the studied biomarkers could contribute, and a logistic model improved their performance, with the receiver operating characteristic curve plot showing an area under the curve of 0.75. Thus, the investigated biomarkers were unable to provide sufficient sensitivity and specificity levels; however, they all may contribute as a basis for an expanded logistic multiparameter model.

Conclusion: Patients with high N-ERC/mesothelin and C-ERC/mesothelin levels have a high risk for MPM; appropriate invasive procedures should be performed. The patients who have high tumor biomarker levels and undefinite histopathological investigation results at the first-line procedure, should be managed using further invasive procedures.

Keywords: Hyaluronan, megakaryocyte potentiating factor, mesothelin, mesothelioma, osteopontin, syndecan-1

INTRODUCTION

Malignant pleural mesothelioma (MPM) is a highly aggressive and incurable mesenchymal cancer. The global incidence of MPM continues to increase (1). The clinical symptoms of MPM are not suggestive during the early stages of the disease, and most patients are diagnosed at later stages (1-3). Therefore, in most cases, chemotherapy is the only option for anti-tumor treatment. The median survival time with chemotherapy series is approximately 13 months (2-4). However, in selected patients with the epithelial subtype and early stage disease who undergo a multimodality treatment schedule, increased survival rates and median survival times were reported (5-7). Therefore, early diagnosis is a potential key factor for managing MPM. There is currently increased interest in the search for biomarkers that would be of value for diagnosing and clinical monitoring of MPM (8-12). Promising biomarkers introduced in recent studies are hyaluronan (13-15), osteopontin (13-20), megakaryocyte potentiating factor (MPF) (15, 18), and mesothelin (13-15, 19-20).

A precursor protein encoded by human mesothelin and having a weight of 71 kDa has two physiological fragments. C-ERC/mesothelin is the 40-kDa C-terminal fragment of mesothelin and is also known

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as the soluble mesothelin-related peptide or as mesothelin. N-ERC/mesothelin is the 31-kDa N-terminal fragment of mesothelin and is also known as MPF (19). To date, studies have focused more on the C-ERC/mesothelin fragment. However, it was also demonstrated that N-ERC/mesothelin was physiologically secreted into blood; N-ERC/mesothelin was also proposed to be a promising candidate tumor biomarker for MPM.

Hyaluronan has been suggested to be a useful predictive diagnostic and prognostic biomarker in pleural effusion of patients with MPM, both as a single biomarker and in combination with mesothelin (13,14). Once hyaluronan reaches the blood stream, it is rapidly turned over, and its role as a serum biomarker along with osteopontin remains debated (13-17).

Syndecan-1 (CD138), a transmembrane heparan sulfate proteoglycan, is a major proteoglycan in epithelial cells (21, 22). Syndecan-1 was noted to be a prognostic indicator in MPM (21, 23). However, data describing the role of syndecan-1 in the diagnosis of MPM are sparse, and syndecan-1 indicates a carcinoma, which is a negative biomarker for mesothelioma compared with adenocarcinoma (14, 22, 24).

In this study, we analyzed the efficiencies of analyzing serum MPF, soluble mesothelin, hyaluronan, osteopontin, and syndecan-1 levels, both individually and in combination, for distinguishing patients with MPM from those with other pleural diseases such as metastatic malignant pleural diseases (MMPDs), benign pleural diseases (BPDs), and benign asbestos pleurisy (BAP) in a research and clinical center located to an area where mesothelioma and other asbestos-related lung diseases are endemic.

METHODS

This study was conducted at the Department of Chest Diseases in a medical faculty in Middle Anatolian Region from January 2004 to December 2010. The study was approved by the Eskişehir Osmangazi University Ethical Committee. The trial registration ID is NCT02029105.

In 2004, a database for pleural diseases was constructed to be prospectively filled in the department. The findings, outcome features, and characteristics of follow-up for all patients with pleural diseases were recorded in this database. From the date of the first database establishment, a "tissue, blood, serum, and fluid specimen bank" was constructed; blood, serum, and pleural fluid samples of patients, which were taken at the beginning of the diagnosis process, were stored in this bank at −80°C. The blood, serum, and pleural fluid samples of the patients were stored during the diagnosis process.

Patients and Follow-up

Consecutive patients were admitted to participate in the study if they met the following criteria: (i) evidence of exudative pleural effusion for which a specific diagnosis could be determined by cytological, histopathological, microbiological, or clinical; radiological; and other examinations and (ii) a willingness to participate in the study. A final histopathological diagnosis was obtained for all patients with MPM. A final cytological or histopathological diagnosis was also obtained for patients with MMPD. Patients without a final diagnosis (malignant or benign) were excluded. Patients diagnosed with BAP were those whose pleural spaces were monitored by medical thoracoscopy, following clinical, laboratory, and radiological diagnostic procedures, and whose tissue samples were determined to have "fibrinous pleuritis" after histopathological examination. All the patients were followed up for at least 48 months to track the diagnosis. All the patients were thoroughly informed about the study, and their written consent was obtained.

Histopathological Evaluation of Samples

Biopsy samples were evaluated by the pathology department of our medical faculty. The cases were primarily categorized as benign and malignant; the malignant samples were further categorized according to their cellular properties. Positive and negative mesothelial immunomarkers were used to differentiate tumors of mesothelial origin from those of epithelial origin. In the granulomatous lesions, periodic acid–Schiff and Ziehl–Neelsen histochemical staining were performed to investigate the presence of fungi and acid-resistant bacilli (*Mycobacterium tuberculosis*), respectively.

Examination of Tumor Biomarkers in Serum Samples

MPF, soluble mesothelin, hyaluronan, osteopontin, and syndecan-1 levels in the serum samples were estimated by enzyme-linked immunosorbent assays (ELISAs) at the Division of Pathology, Department of LABMED, Karolinska Institutet, Stockholm, Sweden.

The serum samples were diluted 1:10 because of preoptimization tests. All analyses were performed in duplicates by researchers who were unaware of the patients' diagnosis. The human N-ERC/mesothelin (catalog no. 99666/7-16 assay) and osteopontin (catalog no. 27158) ELISA kits were supplied by Immuno-Biological Laboratories Co. Ltd. Japan; the C-ERC/mesothelin ELISA kit was supplied by (MESOMARK™) FDI Fujirebio Diagnostics, Inc.; the hyaluronan ELISA kit (Ref. 029-001) was supplied by Corgenix company; and the syndecan-1 ELISA kit (catalog no. 950.640.096) was supplied by the Diaclone company. Analyses were conducted using the sensitivity TM XS Microplate Sample Processor (Bio-Tek Instruments Inc., Vermont, USA), according to the manufacturer's instructions. C-ERC/mesothelin levels were determined at nanomolar levels because of the design of the relevant test kit; all the other biomarkers were determined in ng/ml.

Statistical Analysis

Study data were analyzed using Statistical Package for the Social Sciences for Windows (Version 13.0) and the JMP 11 software (SPSS Inc.; Chicago, IL, USA). The study data were presented as means±standard deviation (SD), medians, interquartile range counts, and percentage values. The χ^2 test was used for comparing frequencies, and t-test or one-way ANOVA test was used for comparing the averages. To analyze whether there was a difference in terms of blood parameter values, the conformity of distributions to normal distributions was first analyzed. For the analysis, Kolmogorov–Smirnov conformity to normal distribution test was performed, and conformity to normal distribution graphs were drawn.

Because none of the analyzed biomarker levels demonstrated a normal distribution, a logarithmic transformation was performed to approximate to normal distribution. A normal distribution conformity test was then again performed for the data. Mann–Whitney U test was used for comparing the two groups for variables that did not demonstrate normal distribution, and Kruskal–Wallis test was used for comparing more than two groups. Wilcoxon signed ranks test

with Bonferroni correction was used to identify the group that resulted in the difference using Kruskal–Wallis test.

Receiver operating characteristic (ROC) curve analysis was conducted to establish the predictive values for MPF, mesothelin, hyaluronan, osteopontin, and syndecan-1 for distinguishing malignant mesothelioma from benign groups or other malignancies. The area under the curve (AUC) and SD were estimated. The predictive power of the biomarkers was compared using the method by DeLong et al. (25) and by logistic regression analysis. The sensitivity, specificity, and 95% confidence intervals were calculated for each of the established predictive values.

First, the natural logarithms of the biomarker values were established for comparing the predictive power of the biomarker combinations, and then, biomarker standardization was performed on the basis of the benign group. The weight values (β) of each biomarker were established in MPM determination by a logistic regression analysis. Each biomarker was multiplied by the logistic regression coefficient and added to the combined biomarker value. AUC, cut-off, and predictive values of the new variable were calculated. P values of <0.05 were considered to be statistically significant.

RESULTS

The 230 cases included in the study were divided into three groups according to the study aims (Table 1).

The serum levels of the biomarkers are demonstrated in Figure 1 according to the patients' groups. Table 2 shows the distribution of the mean and median values of N-ERC/mesothelin, C-ERC/mesothelin, hyaluronan, osteopontin, and syndecan-1 tumor biomarker levels, analyzed in the serum samples of patients, and the interquartile range levels according to the study groups.

Table 1. Characteristics of patients based on groups

Characteristics	Number
Number of patients	**230**
Age (year) X±SD (range)	60.3±13.0 (19–85)
Male (%)	142 (61.7%)
Female (%)	88 (38.3%)
Malignant pleural mesothelioma	**91 (39.6%)**
Epithelial	65(71.4%)
Mixed	14(15.4%)
Sarcomatous	9(10.9%)
Undefined	3(3.2%)
Metastatic malignant pleural diseases	**74 (32.2%)**
Lung cancer	39(52.7%)
Breast cancer	11(14.9%)
Metastasis from various sites	24(32.4%)
Benign asbestos pleurisy	**22(9.5%)**
Benign pleural diseases	**43 (18.7%)**
Tuberculous pleurisy	24(55.8%)
Other benign causes	19(44.2%)

SD: Standart deviation

The serum N-ERC/mesothelin and C-ERC/mesothelin levels were significantly higher in patients with MPM than in those with MMPD, BPD, and BAP.

The mean serum hyaluronan level was found to be significantly higher in patients with MPM than in those with BAP. No difference in serum hyaluronan levels was observed among patients with MPM, MMPD, and BPD. The mean serum osteopontin level was significantly higher in patients with MMPD than in those with MPM. There was no difference in serum osteopontin levels among patients with MPM, BPD, and BAP. Serum syndecan-1 levels were significantly higher in patients with MMPD and BPD than in those with MPM; no significant differences were observed between patients with MPM and BAP.

The ROC curves obtained to evaluate the efficiency of analyzing individual biomarker levels for distinguishing patients with MPM from those with MMPD, BPD, and BAP are shown in Figure 2. The cut-off, AUC, and related sensitivity and specificity values of the biomarkers are listed in Table 3.

For distinguishing patients with MPM from those with other pleural diseases, the highest AUC value among all biomarkers was obtained for N-ERC/mesothelin (0.72). However, serum N-ERC/mesothelin levels had moderate sensitivity (71%) and specificity (71%) at a specified cut-off value. Although the AUC value for osteopontin was low, it was remarkable that at a specified cut-off value, it had a lower sensitivity (32%) but a relatively higher specificity (80%) (Table 2).

When the different patient groups were compared with MPM, none of the individual biomarkers had sufficient sensitivity and specificity (Table 3). To evaluate possible combinatory effects, the logarithmic values were taken for logistic regression analysis. The possible diagnostic values of the logistic models were tested according to

$$p=e^{(b_0+\Sigma x_i*b_i)}/(1+ e^{(b_0+\Sigma x_i*b_i)},$$

where b_0 is the intercept, b_i is the individual parameter estimate and x_i is the obtained logarithmic (ln) concentration of the respective biomarker.

The results (Table 4) indicate that all studied parameters contribute diagnostic information, thus distinguishing MPM from all non-MPM. The most significant estimates were obtained with N-ERC/mesothelin, osteopontin, and syndecan-1. As observed from these estimates, elevated serum hyaluronan, N-ERC/mesothelin, and N-ERC/mesothelin levels support the diagnosis of MPM, whereas serum osteopontin and syndecan-1 levels favor alternative diagnoses. P values, obtained from a logistic model that included all five parameters, slightly improved the diagnostic capacity (sensitivity, 61%; specificity, 80%), with the ROC curve having an AUC value of 0.75. When the model was reduced by removing the two less significantly contributing biomarkers, AUC was still 0.73.

For distinguishing patients with MPM from those with BPD, the highest AUC values among the biomarkers were obtained for N-ERC/mesothelin (0.76), followed by a close value for mesothelin (0.74). However, the sensitivity and specificity values for N-ERC/mesothelin were higher than those for mesothelin. Furthermore, the AUC value for the combination N-ERC/mesothelin and syndecan-1 was 0.56, which is lower than that of N-ERC/mesothelin alone.

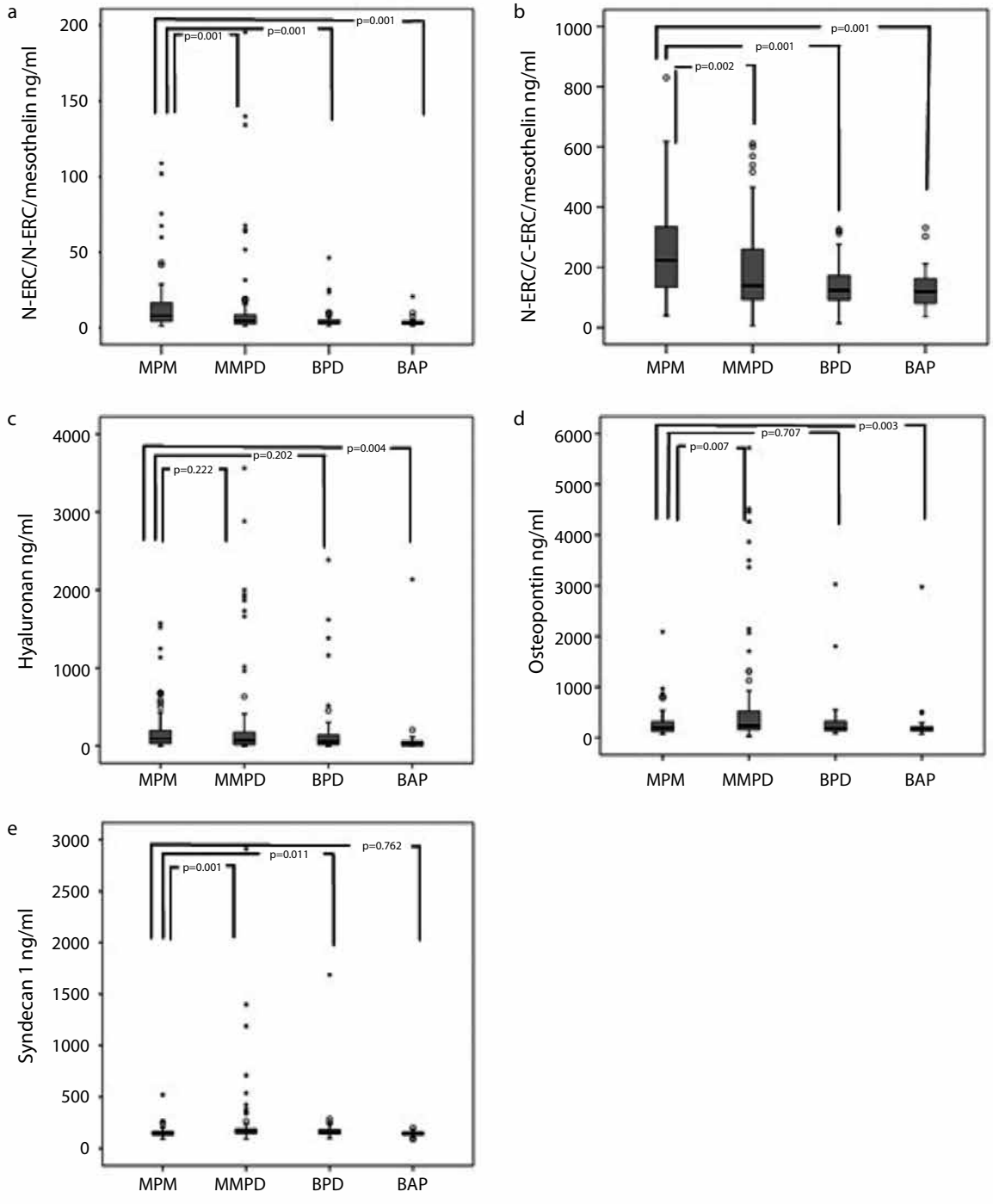

Figure 1. a-e. (a) The serum levels of the markers according to the patients' groups (a) N-ERC Mesothelin (b) C-ERC Mesothelin (c) Hyaluronan (d) Osteopontin (e) Syndecan-1

For distinguishing patients with MPM from those with BAP, the highest AUC value among all the biomarkers was obtained for N-ERC/mesothelin (0.81). Thus, N-ERC/mesothelin has a moderate sensitivity (73%) and a relatively higher specificity (86%). For the same purpose, the AUC value of the combination N-ERC/mesothelin and hyaluronan was lower than that of N-ERC/mesothelin alone.

In our study, N-ERC/mesothelin has higher AUC, sensitivity, and specificity values compared with C-ERC/mesothelin, which is one of the two fragments of same molecule. With regard to the subtypes of mesothelioma, N-ERC/mesothelin (p=0.004) and C-ERC/mesothelin (p=0.0058) levels were found to be higher in the epithelial subtype of mesothelioma than in the other subtypes.

DISCUSSION

In our study, the mean serum N-ERC/mesothelin and C-ERC/mesothelin levels were significantly higher in patients with MPM than in those with MMPD, BPD, and BAP (Figure 1). However, logistic regres-

Table 2. Distribution of serum tumor biomarker levels according to the study groups

Tumor biomarker	Malignant pleural mesothelioma	Metastatic malignant pleural diseases	Benign asbestos pleurisy	Benign pleural diseases
N-ERC/mesothelin* X±SD (ng/mL) (min–max)	14.3±19.0 (1.4–108.9)	15.2±32.9 (1.2–195.7)	4.4±4.1 (1.4–20.7)	5.9±7.9 (1.2–46.3)
Median (interquartile range)	7.9 (4.5–16.8)	4.6 (2.7–10.0)	3.1 (2.2–10.3)	3.6 (2.2–10.3)
C-ERC/mesothelin* X±SD (nM) (min–max)	254.6±153.5 (40.1–229.7)	193.7±148.6 (6.4–611.2)	132.9±75.5 (36.6–331.6)	140.8±74.6 (14.6–325.2)
Median (interquartile range)	223.8 (132.8–339.7)	139.9 (92.8–261.6)	119.8 (104.9–274.8)	124.0 (66.4–274.8)
Hyaluronan* X±SD (ng/mL) (min–max)	203.4±311.5 (0.1–1572.3)	335.6±707.5 (0.1–3563.0)	135.4±450.0 (0.1–2137.6)	231.1±491.4 (0.1–2388.1)
Median (interquartile range)	94.0 (29.7–200.5)	69.2 (13.6–178.4)	29.2 (15.7–181.4)	51.4 (15.7–181.4)
Osteopontin* X±SD (ng/mL) (min–max)	274.8±265.7 (74.3–2090.2)	803.1±1295.7 (32.9–5718.0)	324.3±602.1 (70.6–2973.7)	332.2±498.4 (89.9–3026.3)
Median (interquartile range)	199.3 (131.0–319.7)	238.0 (163.0–556.5)	178.2 (141.0–342.6)	189.4 (141.0–342.6)
Syndecan-1* X±SD (ng/mL) (min–max)	154.0±50.2 (91.7–520.2)	251.7±374.5 (91.7–2910.8)	145.6±27.5 (88.2–202.2)	201.5±234.5 (100.1–1686.2)
Median (interquartile range)	149.3 (133.1–165.5)	164.0 (147.1–188.8)	150.5 (112.7–175.2)	159.5 (11.7–175.2)

Logarithmic transformation was provided to approximate to normal distribution during estimations.

*p values for each biomarker; N-ERC/mesothelin: MPM–MMPD: p=0.001; MPM–BPD: p=0.001; MPM–BAP: p= 0.001. C-ERC/mesothelin: MPM–MMPD: p=0.002; MPM–BPD: p=0.001; MPM–BAP: p=0.001. Hyaluronan: MPM–MMPD: p=0.222; MPM–BPD: p=0.202; MPM–BAP: p=0.004. Osteopontin: MPM–MMPD: p=0.007; MPM–BPD: p=0.707; MPM–BAP: p=0.402. Syndecan-1: MPM–MMPD: p=0.001; MPM–BPD: p=0.011; MPM–BAP: p=0.762

BAP: Benign asbestos pleuritis; BPD: benign pleural diseases; C-ERC: C-ERC/mesothelin; MMPD: metastatic malignant pleural diseases; MPM: malignant pleural mesothelioma; N-ERC: N-ERC/mesothelin; SD: standart deviation

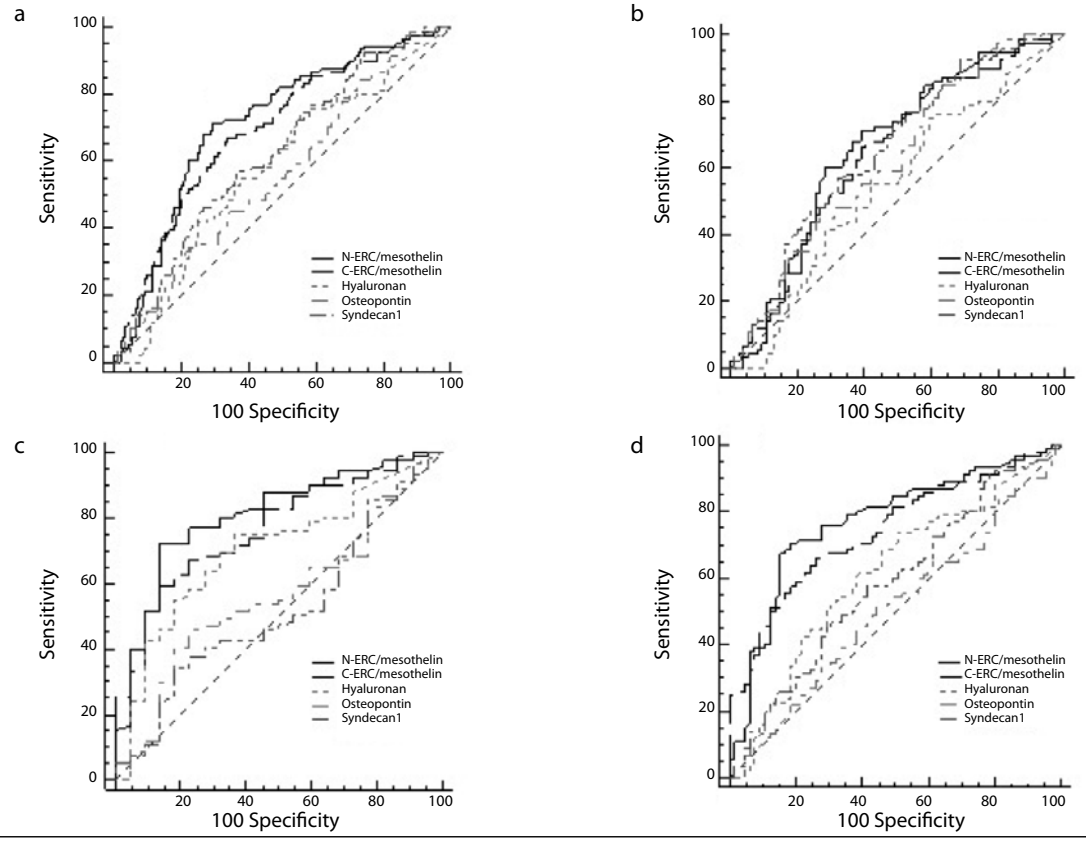

Figure 2. a-d. The ROC curves obtained to evaluate the efficiency of individual markers in distinguishing MPM patients from other patients. (a) MPM versus other pleural diseases. (b) MPM versus MMPD. (c) MPM versus BAP. (d) MPM versus BPD.

BPD: Benign pleural diseases; MMPD: metastatic malignant pleural disease; MPM: malignant pleural mesothelioma; ROC: receiver operating characteristic

Table 3. Cut-off, AUC, related sensitivity, specificity, PPV, and NPV values of biomarkers for distinguishing patients with MPM from those with other pleural diseases

Biomarker		N-ERC/mesothelin (ng/mL)	C-ERC/ mesothelin (nM)	Hyaluronan (ng/mL)	Osteopontin (ng/mL)	Syndecan-1 (ng/mL)
MPM versus other pleural diseases	Cutoff	>5.4	>162.9	>37.9	≤139.1	≤151.5
	AUC 95% CI	0.72 0.65–0.77	0.69 0.63–0.71	0.58 0.51–0.64	0.56 0.50–0.63	0.62 0.55–0.68
	Sensitivity 95% CI	71.4 61.0–80.4	67.0 56.4–76.5	74.7 64.5–83.3	31.9 22.5–42.5	57.1 46.3–67.5
	Specificity 95% CI	70.5 62.2–77.9	66.2 57.7–74.0	44.6 36.2–53.3	79.9 72.2–86.2	63.3 54.7–71.3
MPM versus MMPD	Cutoff	>5.4	>164.7	>37.9	≤349.5	≤151.5
	AUC 95% CI	0.66 0.58–0.73	0.64 0.57–0.72	0.55 0.47–0.62	0.62 0.54–0.70	0.65 0.57–0.72
	Sensitivity 95% CI	71.4 61.0–80.4	65.9 55.3–75.5	74.7 64.5–83.3	83.5 74.3–90.5	57.1 46.3–67.5
	Specificity 95% CI	60.8 48.8–72.0	60.8 48.8–72.0	40.5 29.3–52.6	37.8 26.8–49.9	67.6 55.7–78.0
MPM versus BAP	Cutoff	> 5.4 ng/mL	>180.9	>35.0	>216.1	>159.4 ng/mL
	AUC 95 % CI	0.81 0.72–0.88	0.76 0.68–0.84	0.70 0.60–0.78	0.56 0.46–0.65	0.52 0.42–0.62
	Sensitivity 95% CI	72.5 62.2–81.4	59.3 48.5–69.5	74.7 64.5–83.3	46.2 35.6–56.9	34.1 24.5–44.7
	Specificity 95% CI	86.4 65.1–97.1	86.4 65.1–97.1	63.6 40.7–82.8	77.3 54.6–92.2	81.8 59.7–94.8
MPM versus BPD	Cutoff	> 5.6 ng/mL	>167.5	>22.9	<139.1	<151.5
	AUC 95% CI	0.76 0.67–0.83	0.74 0.65–0.81	0.57 0.48–0.65	0.52 0.43–0.61	0.64 0.55–0.72
	Sensitivity 95% CI	67.4 56.8–76.8	64.1 53.5–73.9	75.0 64.9–83.4	31.5 22.2–42.0	57.6 46.9–67.9
	Specificity 95% CI	83.7 69.3–93.2	74.4 58.8–86.5	41.9 27.0–57.9	76.7 61.4–88.2	65.1 49.1–79.0

AUC: Area under the curve; BAP: benign asbestos pleuritis; BPD: benign pleural diseases; C-ERC: C-ERC/mesothelin; CI: confidence interval; MMPD: metastatic malignant pleural diseases; MPM: malignant pleural mesothelioma; N-ERC: N-ERC/mesothelin; NPV: negative predictive value; PPV: positive predictive value

Table 4. Logistic regression analysis of logarithmic transformed biomarker concentrations in serum. All studied parameters may contribute to the diagnosis of MPM, with N-ERC/mesothelin, osteopontin, and syndecan-1 being most important

	Estimate	Wald χ^2	p
Intercept	7.196		
Hyaluronan	0.095	2.230	0.135
N-ERC/mesothelin	0.613	3.562	0.059
C-ERC/mesothelin	0.675	2.657	0.103
Osteopontin	–0.705	9.866	0.0017
Syndecan-1	–1.709	8.675	0.0032

C-ERC: C-ERC/mesothelin; MPM: malignant pleural mesothelioma; N-ERC: N-ERC/mesothelin

sion analysis also indicated that hyaluronan, osteopontin, and syndecan-1 may contribute to the diagnosis.

Several neoplasms cause increase serum hyaluronan levels. High serum hyaluronan levels were mostly detected in the advanced stage of the disease in patients with MPM (26). Grigoriu et al. (27) found that when the serum hyaluronan cut-off level was set at 26.5 µg /l, the sensitivity was 83% and the specificity was 42%. At higher concentrations, the positive predictive value for mesothelioma increased, and when the cut-off value was set at 100 µg/l, the specificity increased to 85% for distinguishing patients with MPM from those with MMPD and to 95% for distinguishing patients with MPM from those with BPD. However, at this cut-off value, the sensitivity values greatly decreased. In some other studies, the sensitivity of hyaluronan has been reported to be 40%–70% (26, 28). In our study, the cut-off value of serum hyaluronan suggested by Grigoriou had moderate sensitivity but a poor specificity to be used as a single parameter in clinical conditions.

Serum osteopontin levels have been reported to be elevated in several types of cancer and MPM (16, 29-31). Pass et al. (16) first described that the serum osteopontin level was a useful biomarker

for diagnosing MPM. Patients with MPM had significantly increased serum osteopontin levels compared with controls from asbestos-exposed and non–asbestos-exposed populations. Thus, osteopontin is a more general biomarker for malignancy, and patients with MPM could be distinguished from those with benign asbestos pleuritis with a sensitivity of 78% and specificity of 86%. In contrast, subsequent studies have failed to support this view. In a recent study, no difference in serum osteopontin levels was observed when subjects with MPM were compared with those with BPDs, which were caused by asbestos exposure (17). Serum osteopontin levels can be elevated in subjects with benign asbestos-related disorders, and the level can also be affected by other nonmalignant conditions (30-32). In our study, the average serum osteopontin level was higher in patients with MMPD than in those with MPM (Figure 1). Specificity was relatively high (80%), but sensitivity was too low for distinguishing patients with MPM from other patients.

In the first publication that defined mesothelin as a useful biomarker for diagnosing MPM, elevated serum mesothelin levels, exceeding the determined cut-off value, were found in 84% of patients with MPM, with a specificity of 100% (33). Later, Scherpereel et al. (34) obtained a sensitivity of 80% and specificity of 83% when distinguishing patients with MPM from those with BPDs on the basis of serum mesothelin levels. In addition, the sensitivity was 56% and specificity was 73% when distinguishing patients with MPM from those with MMPD. Similar results were reported by other studies that tested serum or pleural effusion mesothelin levels (35-38). Studies analyzing the efficiency of serum mesothelin levels for diagnosing patients with MPM reveal various specificity and sensitivity values; sensitivity values varied between 40% and 80% (9, 27, 34, 37), whereas specificity values varied between 83% and 99% (34, 35). The reasons for this variance included small sample sizes (particularly for samples of non-MPM cases exposed to asbestos), use of different cut-off levels, selection bias inherent in hospital-based populations, retrospective studies of prospectively collected samples, assay variations, and possible changes in the molecular concentrations of samples stored at −80°C for a long period of time (10, 33, 36, 38, 39).

These early studies were performed with a reagent labeling an epitope on the cell membrane-associated C-terminal mesothelin fragment (C-ERC/mesothelin). Reagents binding to the shed N-terminal fragment (N-ERC/mesothelin) are also currently commercially available, and studies of this mesothelin fragment are emerging (40).

The serum N-ERC/mesothelin level was elevated in most patients with MPM of the epithelial subtype, with a sensitivity of 71% and a specificity of 93% (19). In another study that compared serum C-ERC/mesothelin and N-ERC/mesothelin levels with those of other patient groups, the ROC curve analysis did not reveal a significant difference between these fragments for distinguishing patients with MPM from other patients (18). Onda et al. (41) found that their N-ERC/mesothelin assay had a sensitivity of 91% and a specificity of 100% for diagnosing MPM. However, the results of these studies were based on samples from patients with advanced-stage MPM and healthy control subjects. In a study by Hollevoet et al. (18), which included 507 cases and used the same assay for diagnosing MPM, serum N-ERC/mesothelin with a cut-off level of 19.1 ng/ml has been reported in a sensitivity and specificity of 53% and 99%, respectively. Similar to C-ERC/mesothelin, the sensitivity and specificity values obtained with N-ERC/mesothelin are affected by the study design and the assay used (15, 40, 42).

Similar to C-ERC/mesothelin in our cohort, N-ERC/mesothelin is a moderately effective tumor biomarker for distinguishing patients with MPM from those with other pleural diseases. Thus, the levels of the two fragments only co-vary to some extent, and there are obviously differences in how they reach the blood stream. While N-ERC/mesothelin is enzymatically cleaved off from the cell surface to enter the blood stream, the C-ERC/mesothelin fragment is released by other mechanisms such as cell deterioration. Therefore, it is interesting to observe that one of them does not hide the possible effect of the other in the logistic regression analysis, i.e., they rather appear to complement each other.

An important common finding of previous studies is that elevated serum mesothelin levels in patients with MPM were observed in epithelioid or mixed MPM subtypes and that there was no such increase among patients with MPM of the sarcomatous subtype (33-35, 39). In our study, the serum levels of both mesothelin fragments were significantly higher in patients with MPM of the epithelioid subtype than in those of other subtypes. This is an important limitation and a possible contributing factor to the low sensitivity.

In MPM cells, the expression of syndecan-1 correlates with the inhibition of growth and migration of tumor cells (43). It has been reported that syndecan-1 might be related to the differentiation stage of mesothelioma, that syndecan-1 was strongly expressed in MPM with the epithelioid and biphasic subtype, and that the expression of syndecan-1 was lower in the sarcomatous subtype (21, 24). The cell surface expression of syndecan-1 was correlated with the long-term survival, and an increase in syndecan-1 might be an indicator for a better prognosis (21). However, it should be noted that syndecan-1 is abundant in epithelial tissues and is overexpressed by several carcinomas, even more so than by epithelioid MPM. In our study, average syndecan-1 levels were higher in patients with MMPD and BPD than in those with MPM. In the differential diagnosis of MPM, the specificity and sensitivity values obtained for syndecan-1 indicated that it would not be useful as a single serum biomarker. The logistic regression analysis also showed that syndecan-1 can be a a biomarker to exclude the diagnosis of MPM. However, further studies with regard to post-translational modifications and subtype specificity of syndecan-1 may better assess its role in MPM.

The tumor biomarkers tested to date with the aim of distinguishing MPM from other pleural diseases could not reach achieve the desired specificity and sensitivity, and accordingly, some studies analyzed the efficiency of combining tumor biomarkers. In the study by Grigoriu et al. (27), which analyzed mesothelin and hyaluronan in serum and pleural effusion, the combination of these two biomarkers did not increase the efficiency for distinguishing patients with MPM from those with other pleural diseases. In another study, combining the serum mesothelin and plasma osteopontin biomarker levels using a logistic regression model did not significantly increase AUC-ROC (31). In a recent study, serum mesothelin level remains the most specific biomarker for diagnosing mesothelioma, and biomarker combinations using a logistic regression model have not increased the efficiency of diagnosis of MPM (15). In the current study, we showed that

all five analyzed biomarkers contributed with diagnostic information. Although the joint result from such a model remains insufficient, the analyzed compounds may well form the basis for an expanded future battery, which also includes other candidate biomarkers.
The presently analyzed biomarkers may have a place in clinical situations as follows. In patients with a clinical suspicion of MPM, a high mesothelin or hyaluronan level can themselves motivate further exploratory diagnostic measures. However, the sensitivities are only moderate, and lower levels do not exclude the diagnosis, making clinical, radiological, and other laboratory findings for later examination more important for the final diagnosis. Although not examined in this study, these measures may be examined over time to monitor the amount of tumor tissue and the possible effect of treatments.

During clinical practice, one of the most frequent problems for diagnosing MPM is patients with BAP. BAP cannot be histopathologically diagnosed; however, the diagnosis can be implemented by the harmony of epidemiological, clinical, radiological, and sometimes thoracoscopic findings. A critical question for patients who were considered to have BAP after certain evaluation, is that how can we decide at the crossroad? Which patient will undergo further surgical diagnostic procedures such as VATS or thoracotomy because of a higher concern for MPM, which patient will be selected for "wait and see"? (44-46). At this point, we suppose that serum mesothelin can be useful. If the serum level is higher than the decided cut-off value, video-assisted or open thoracic surgical biopsy can be performed without any loss of time. This is also valid for other BPDs.

Another useful area for tumor biomarkers can be the early diagnosis of MPM. A high-risk cohort of asbestos-exposed individuals can be screened with a longitudinal follow-up with repeated biomarker analyses. Increasing levels would then indicate an early disease, perhaps with better chances for a curative therapy. However, with our estimates of sensitivity and specificity for the presently available biomarkers, the utility of individual biomarkers would be limited. Thus, the positive predictive values of elevated biomarker levels, either as isolated analyses or in the logistic model, remain low (47).

CONCLUSION

Therefore, the present biomarkers are not sufficient for screening healthy high-risk groups. However, this possibility is important for an earlier diagnosis of MPM, and further studies should focus on finding new additional biomarkers, perhaps combined in a diagnostic multiparameter battery.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Eskişehir Osmangazi University (Trial Registration ID: NCT02029105).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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