

Diagnostic and Prognostic Value of Survivin in Pleural Effusion

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Abstract

Background: Survivin is an inhibitor of apoptosis that may be a novel diagnostic and prognostic marker of cancer. Our study is to investigate the diagnostic and prognostic value of survivin for pleural effusions. **Methods:** Sixty-five pleural effusion patients were enrolled prospectively. Pleural effusion samples were examined for survivin level by ELISA. Pleural effusions were divided into three groups: Group I, malignant pleural effusion (MPE) (n = 36); Group II, tuberculous pleurisy (TPE) (n = 18); and Group III, transudative pleural effusion (n = 11). The accuracy of diagnosis and the correlation between survivin level and survival in malignant pleural effusions (MPE) were analyzed. **Results:** Survivin level was 320.50 ± 228.24 pg/ml in MPE, 328.35 ± 146.79 pg/ml in TPE and 318.87 ± 208.39 pg/ml in transudative pleural effusion respectively. ROC curves for MPE versus TPE were analyzed, area under the ROC curve was 0.419, and for the cutoff value of 254.85 pg/ml sensitivity was 44.4% and specificity 55.6%. Survivin had no discriminative power in differentiating exudative effusions of MPE from non-MPE ($p = 0.648$). There was no correlation between survivin level and age, sex. However, statistically significant difference was found between primary lung carcinoma (238.66 ± 48.19 pg/ml) and extra-pulmonary metastatic carcinomas (435.09 ± 320.62 pg/ml) according to survivin level ($p = 0.033$). Survivin levels can distinguish patients who had poor prognosis (median survival 96 days) and those who had good prognosis (median survival 206 days) in MPE. **Conclusion:** survivin levels detected with ELISA had no discriminative power in differentiating exudative effusions included MPE and TPE. However, over-expression of survivin correlates with poor prognosis in cancer patients. Our results suggest that survivin may be a potential prognostic marker in MPE.

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Keywords

Survivin, Pleural Effusion, Diagnosis, Prognosis

1. Introduction

Pleural effusions have a variety of etiologies, including malignancies, pneumonia, tuberculosis, pulmonary embolism, cardiac failure and cirrhosis. Differentiating between malignant pleural effusion (MPE) and non-MPE often has important therapeutic implications [1] [2]. Malignant pleural effusion (MPE) is a common and important cause of cancer-related mortality and morbidity [3] [4]. Prompt diagnosis using minimally invasive test is important because the median survival after diagnosis of MPE is only 4 - 9 months. The sensitivity of cytologic examination of pleural effusion is variable with limited sensitivity [5] [6].

The initial diagnostic approach includes thoracentesis and cytological, histological and biochemical examinations. However, the sensitivity of these non-invasive techniques is unsatisfactory [7] [8] [9]. The sensitivity of conventional cytology for the detection of malignant cells in pleural effusion is insufficient, too [6]. Differentiating between malignant pleural effusion (MPE) and non-MPE often has important therapeutic implications.

Survivin is an inhibitor of programmed cell death; it mediates suppression of apoptosis by inhibition of the caspases 3 and 7, the terminal effectors in apoptotic protease cascades [10]. It is selectively up-regulated in many human tumors, where its over-expression correlates with poor outcome [11] [12] [13]. Nevertheless, survivin expression in pleural effusions of cancer patients is rarely reported. This study evaluated the diagnostic value of survivin levels in pleural effusions and explored the possible relationship between surviving levels and survival.

2. Materials & Methods

2.1. Study Design and Sample Selection

Between March 2017 and September 2017, a total of 65 patients admitted to our clinic were included in the study. All patients were follow-up for a period of 9 months, with telephone follow-up at least once for every month. Patients' demographics and tumor characteristics are summarized in **Table 1**. This study has been approved by the ethical committee and was in accordance with the ethical standards of the Committee for Human Experimentation, with the Helsinki Declaration of 1975 (revised in Tokyo 2004) and the Committee on Publication Ethics guidelines. All patients consecutively diagnosed with MPE, tuberculous pleurisy (TPE) and transudative pleural effusion were included. All patients were diagnosed according to criteria cited below which was considered as a reasonable standard for diagnosis. Medical history was taken from all patients included in the study. Physical examination was made, and poster-anterior

Table 1. Distribution of patients according to primary etiology.

	N (%)	Survivin (pg/ml)
Malignant pleural effusion	36	320.50 ± 228.24
Primary lung carcinoma	21	238.66 ± 48.19
Adeno	14	
Squamous	6	
Small cell carcinoma	1	
Metastatic other than lung	15	435.09 ± 320.62
Pancreas	1	
Esophagus	2	
Colon	4	
Gastrointestinal system	6	
Cervix	1	
Bladder	1	
Tuberculosis (TPE)	18	328.35 ± 146.79
Transudative pleural effusion	11	318.87 ± 208.39

No statistically significant difference was found between the three groups according to survivin level ($p = 0.989$).

chest X-ray ordered. Patients with pleural effusion routinely underwent diagnostic thoracentesis to obtain pleural fluid specimens for cell count; the measurement of total protein, lactate dehydrogenase (LDH) and cytological examination were checked. In addition, acid-fast bacilli were analyzed in effusions in the microbiology laboratory.

The diagnosis of TPE was done according to the following criteria; 1) Pathological demonstration of a necrotizing granulomatous inflammation in the pleural tissue sample taken with closed biopsy or Video Assisted Thoracoscopic Surgery (VATS); or 2) microbiologic isolation of mycobacterium tuberculosis in the pleural fluid; or 3) the routine examination of pleural fluid indicates lymphocyte-based exudates effusions with significant increase in adenosine deaminase (>40 U/L); plus exclusion of other possible diagnosis by clinical and radiological examination. The diagnosis of MPE was done according to the following criteria; malignant cells in the cytology of the pleural fluid and/or on histopathologic examination of the pleural tissue obtained by VATS or pleural blind biopsy, retrospective diagnosis in the follow-up observation. The diagnosis of transudative pleural effusion is based on medical history such as CHF, kidney failure and cirrhosis and so on, normally with polyserositis. Examination and detection of blood albumin, BNP and renal function help in the diagnosis of transudative pleural effusion. Left ventricular systolic dysfunction on echocardiography and response to diuretic therapy support the diagnosis of CHF, and hypoproteinemia regularly induce to transudative pleural effusion.

From each patient 10 ml of pleural fluid were transferred to 15 ml Eppendorf tubes. Following centrifugation at 4000 rpm for 10 min at 4°C supernatants were dispensed into 1 ml Eppendorf tubes and were refrigerated at -80°C until to work-up for the survivin measurement.

In this study, Human Total Survivin Enzyme Immunometric Assay Kit (Shanghai Lengton Bioscience Co. LTD., China) was used. According the package insert of the kit the dynamic range of the assay was between 5 and 1000 pg/ml. The sample will be doubling diluted if the content of surviving is above 1000 pg/ml. All pleural effusion samples and reagents were kept on bench until they reached to the room temperature. All the processes were carried out at room temperature according to instructions of the manufacturer.

2.2. Statistical Analysis

Patient demographics and disease characteristics were summarized using descriptive statistics. Sensitivity and specificity were calculated. All continuous data were expressed as mean and standard deviation, and categorical variables as frequency and percentage. Statistical mean difference between the groups was analyzed with Student t-test and in case of more than two groups with one-way ANOVA test. Kaplan-Meier was used in survival analysis and survival difference between groups was studied with the log-rank test. To analyze factors that effected survival, Cox regression test was used in which survivin level, age, sex, smoking, primary lung cancer and other than lung cancer were included as independent variables. Statistical significance was set at p value < 0.05. SPSS version 21 package program has been used for statistical analysis.

3. Results

Our study was carried out with 65 patients [M/F: 44/21, age (27 - 97 years)], referred to the affiliated Hospital of Hangzhou Normal University, and diagnosed with MPE, TPE and transudative pleural effusion between March 2017 and September 2017. Distribution of the patients according to the diagnoses is shown in **Table 1** and demographic characteristics in **Table 2**.

3.1. Survivin Levels in Subjects

Mean value of survivin in MPE, TPE and transudative pleural effusion were 320.50 ± 228.24 pg/ml, 328.35 ± 146.79 pg/ml, and 318.87 ± 208.39 pg/ml respectively. No statistically significant difference was found between the three groups ($p = 0.989$) (**Figure 1**). When the patients were divided into two groups as malignant and non-malignant pleural effusion, there is no statistically significant difference was found between the two groups too (320.50 ± 228.24 pg/ml versus 324.76 ± 169.15 pg/ml, $p = 0.648$) (**Figure 2**).

In the group of MPE, mean levels of survivin according to tumor origins are reported on **Table 1**. Patients with MPE were divided into two groups as primary lung cancer and extra-pulmonary metastatic carcinomas. Mean levels of

Table 2. Demographical features of patients.

	MPE (36)	TPE (18)	Transudative (11)	p
Age	70.72 ± 14.12	60.89 ± 21.81	70.63 ± 15.96	0.119
Age (min-max)	46 - 96	27 - 90	52 - 97	
Sex (F/M)	13/23	4/14	4/7	0.560
Smoking (y/n)	14/22	7/11	1/10	0.102

One-way ANOVA, χ^2 test, $p < 0.05$ is significant.

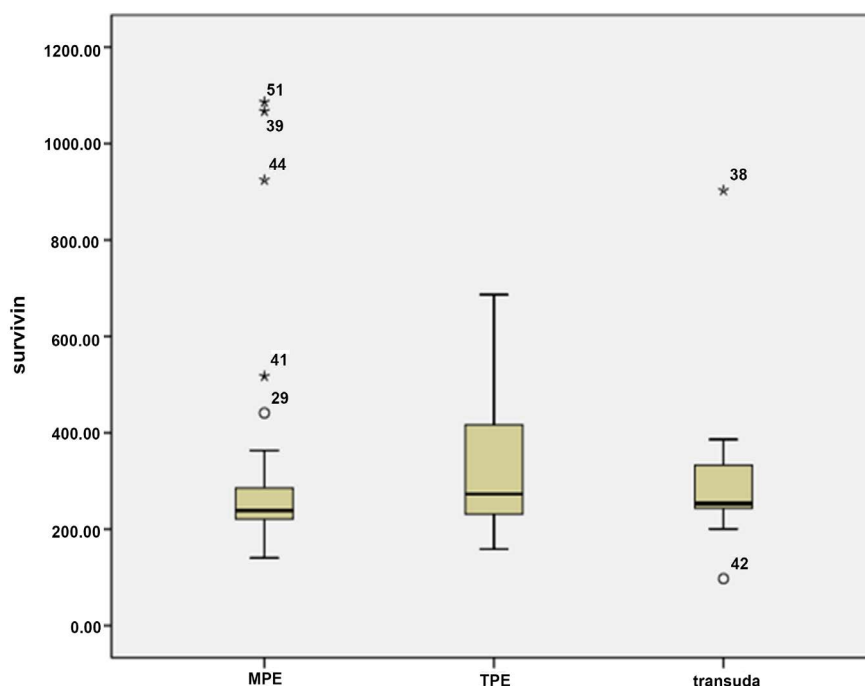


Figure 1. Mean value of survivin in MPE, TPE and transudative pleural effusion ($p = 0.989$).

survivin were found as 238.66 ± 48.19 pg/ml in primary lung carcinomas ($n = 21$) and 435.09 ± 320.62 pg/ml in extra-pulmonary metastatic carcinomas ($n = 15$). Statistically significant difference was found between the two groups according to survivin level ($p = 0.033$). However no significant correlation was found in terms of age and sex (**Figure 3**).

3.2. Discriminative Power of Survivin in MPE

ROC curve was created to find sensitivity and specificity of survivin level in MPE vs. non-MPE group. Area under the ROC curve was 0.422. Considering cutoff value as 254.85 pg/ml, sensitivity was found as 44.4% and specificity as 48.3%. According to the results, survivin had no discriminative power in differentiating MPE and non-MPE (**Figure 4**).

The ROC curve has also been drawn for MPE and TPE, which were two significant etiological reasons for exudative effusions. Area under the ROC curve

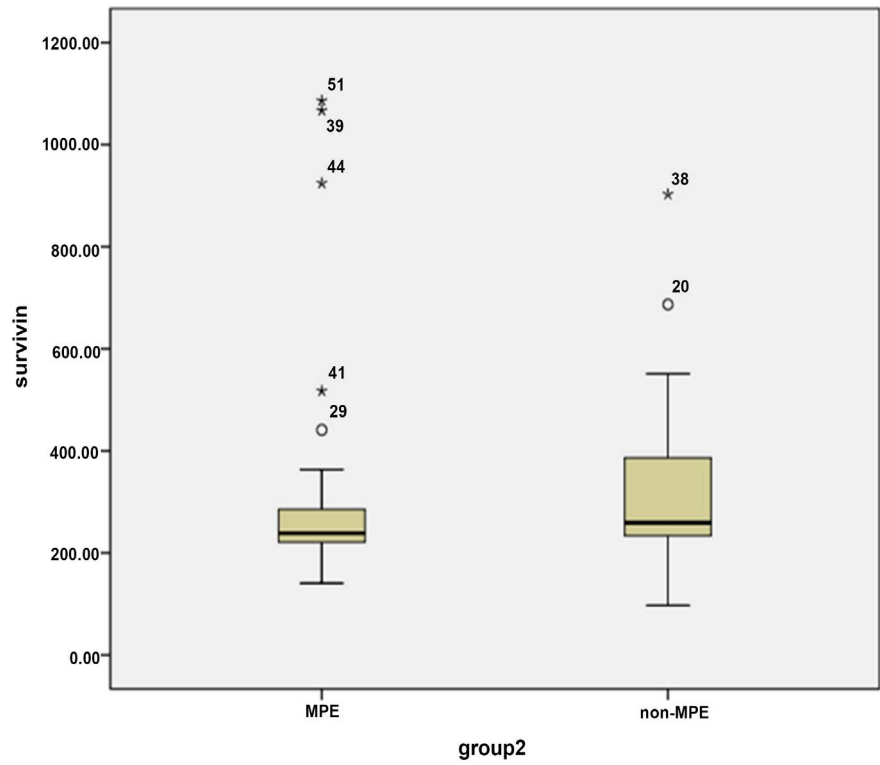


Figure 2. Comparisons of survivin levels between malignant and non-malignant effusions (320.50 ± 228.24 pg/ml versus 324.76 ± 169.15 pg/ml, $p = 0.648$).

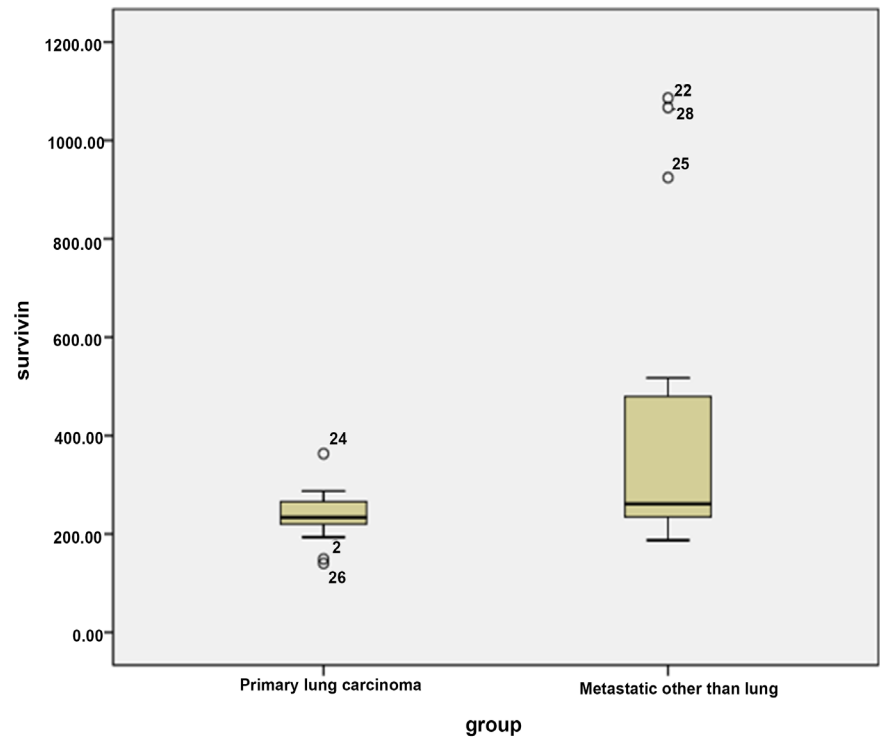


Figure 3. Comparisons of survivin levels between primary lungcarcinomas and extra-pulmonary metastatic carcinomas effusions (238.66 ± 48.19 pg/ml versus 435.09 ± 320.62 pg/ml, $p = 0.033$).

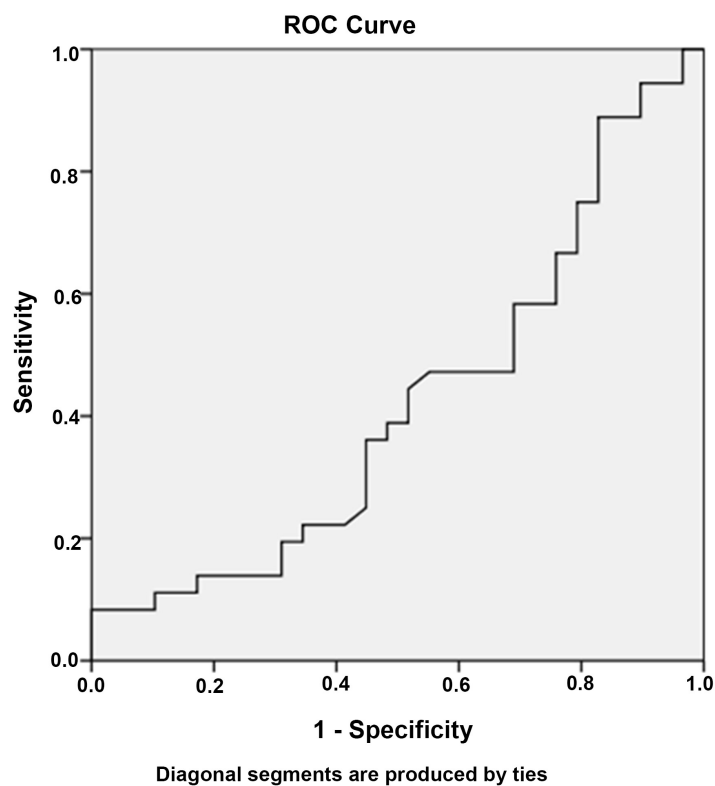


Figure 4. ROC analysis for survivin expression in MPE vs non-MPE group. The plot was constructed by computing the sensitivity vs. (1-specificity) for the different possible cutoff points of the survivin ELISA assay.

was 0.419, for the cutoff value of 254.85 pg/ml sensitivity was 44.4% and specificity 55.6%. According to the results, survivin had no discriminative power in differentiating exudative effusions of MPE and TPE all the same (**Figure 5**).

Similarly, when the ROC curve was drawn with MPE and transudative pleural effusion, area under the ROC curve was 0.428. Considering cutoff value as 254.85 pg/ml pg/ml, sensitivity was found as 44.4% and specificity as 54.5%. Same as above, survivin had no discriminative power in differentiating MPE and transudative pleural effusion.

However the dates indicated that metastatic other than lung cancer have more higher survivin expression than primary lung carcinoma in the pleural effusion. Statistically significant difference was found between metastatic other than lung (435.09 ± 320.62 pg/ml) and primary lung carcinoma (238.66 ± 48.19 pg/ml) groups according to survivin level. Area under the ROC curve was 0.686, considering cutoff value as 266.59 pg/ml, sensitivity was found as 60.0% and specificity as 71.6%, and suggesting a moderate overall accuracy (**Figure 6**).

3.3. Prognostic Value of Survivin in MPE

Kaplane-Meier survival analysis was performed in the MPE group.

Survivin levels (the median value of surviving with 238.85 pg/ml as cutoff value > 238.85 pg/ml indicate a high survivin expression and <238.85 pg/ml a

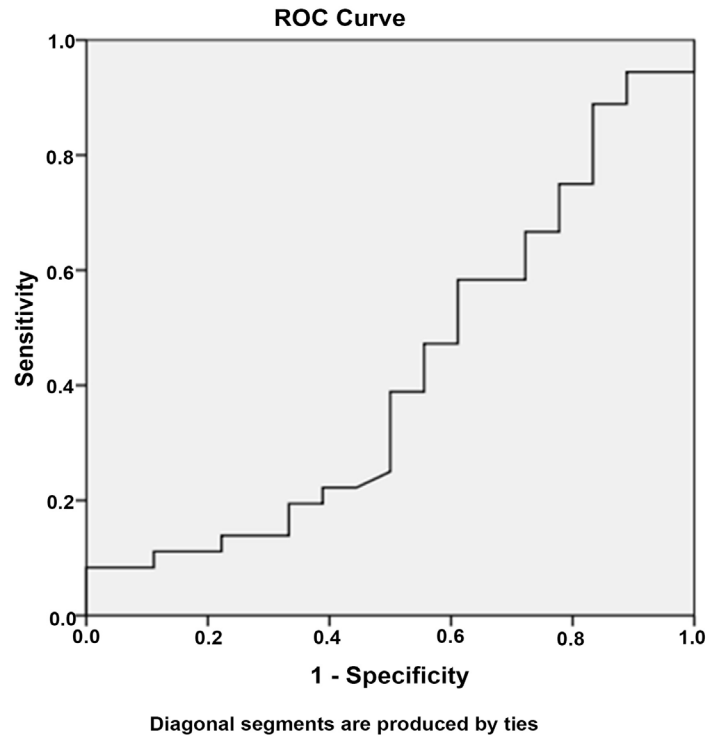


Figure 5. ROC analysis for survivin expression in MPE vs TPE group. The plot was constructed by computing the sensitivity vs. (1-specificity) for the different possible cutoff points of the survivin ELISA assay.

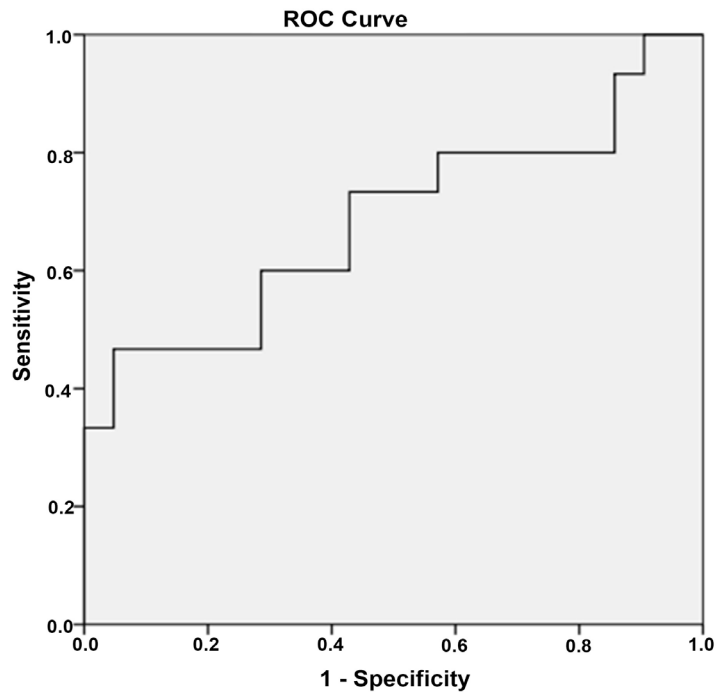


Figure 6. ROC analysis for survivin expression in Metastatic other than lung (435.09 ± 320.62 pg/ml) vs. Primary lung carcinoma (238.66 ± 48.19 pg/ml, $p = 0.004$). Considering cutoff value as 266.59 pg/ml, sensitivity was found as 60.0% and specificity as 71.6%.

low expression) can distinguish patients who had poor prognosis (median survival 96 days) and those who had good prognosis (median survival 206 days, $p = 0.041$). Elevated levels of surviving were related to reduced overall survival in Kaplan-Meier analysis (**Figure 7**, **Table 3**).

Cox regression analysis was carried out for significant factors influencing survival. Survivin level, age, sex, smoking, the group of primary lung cancer or other than lung cancer were included as independent factors. Survivin level, age, the group of primary lung cancer or not was retained as significant in backward elimination likelihood ratio test (**Table 4**).

4. Discussion

Survivin is a 16.5 kDa protein that inhibits apoptosis, promotes proliferation, and has a crucial role in the development of cancer. Survivin is expressed in a vast majority of human cancers [14] and is one of the key factors conferring and maintaining resistance to apoptosis [15], and its over-expression correlates with poor outcome [16] [17] [18]. But conflicting results have been published in association between survivin levels in serum and the prognosis of cancer [19] [20] [21] [22]. Furthermore, studies about survivin expression in pleural effusions are limited [23] [24]. Wu and colleagues [25] have analyzed pleural effusion specimens for survivin expression using ELISA. They reported remarkable sensitivity

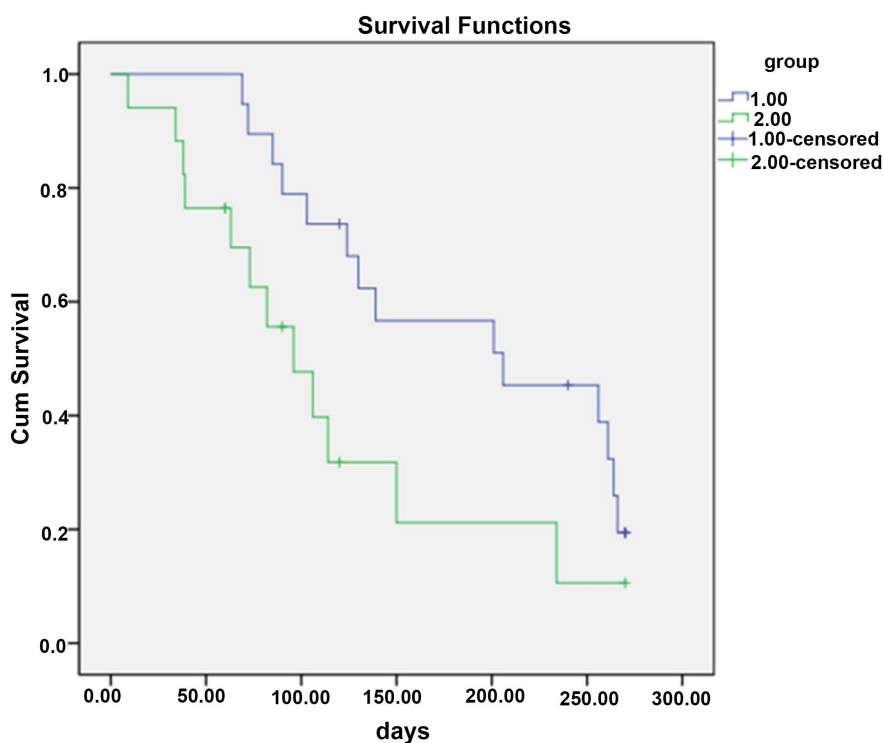


Figure 7. Kaplan-Meier survival curve showing the association between survivin expression and overall survival ($p = 0.041$). Curve 1: low expression of survivin with Survivin levels < 238.85 pg/ml; Curve 2: high expression of survivin with Survivin levels > 238.85 pg/ml.

Table 3. Test of equality of survival distributions for the different levels of group.

Overall Comparisons			
	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	4.172	1	.041

Table 4. Cox regression analysis data.

	B	SE	Wald	df	Sig.	Exp(B)
Age	0.047	0.023	3.949	1	0.047	1.048
Sex	-0.061	0.581	0.011	1	0.916	0.940
Smoking	0.115	0.824	0.019	1	0.889	1.121
Survivin	0.005	0.001	13.165	1	0.000	1.005
Primarylungornot	-2.945	1.158	6.472	1	0.011	0.053

and specificity with a cutoff value of 6.2 pg/ml. Interestingly, more than half of the TPE patients expressed survivin in the same study. It is known that TPE is frequently diagnosed as the cause of exudative pleural effusion in Asia and in our country. Our study included 18 TPE diagnosed, compared with MPE, according to our results, survivin had no discriminative power in differentiating exudative effusions of MPE from TPE. ROC curves for MPE versus TPE were analyzed and for the cutoff value of 254.85 pg/ml sensitivity was 44.4% and specificity 55.6%. Our results revealed that the sensitivity of survivin is low, which limits the clinical utility of survivin as a screening biomarker for MPE. Because in our country tuberculosis is endemic, discrimination between exudative effusions of MPE and TPE was very important and survivin had no discriminative power in such cases.

Interestingly, we were found that compared with primary lung carcinoma (238.66 ± 48.19 pg/ml), Survivin levels were elevated in the group of metastatic other than lung (435.09 ± 320.62 pg/ml, $p = 0.033$). No association has been detected between surviving levels of pleural effusion and age, sex, smoking states to our findings. According to previous studies increased survivin levels of mRNA expression in pleural effusion were associated with poor survival [23] [26]. Similarly, in our study, elevated levels of survivin was correlated with a reduced overall survival. Survivin levels can distinguish patients as a poor and good prognostic group.

In our study, survivin level was analyzed with ELISA technique. In the literature, survivin levels were studied with various methods such as mRNA with PCR, immune-blotting and ELISA. Analysis using various methods makes it difficult to compare the results.

5. Conclusion

In conclusion, the findings of this study suggest that survivin levels can be ele-

vated both in inflammation and malignancies. It can be suggested that positive values of survivin might be misleading in the regions with a high prevalence of TPE like in our country and cannot be used as a safe diagnostic tool in differentiation between TPE and MPE. However, beside its questionable diagnostic role aside, survivin has a potential role as a promising prognostic marker for MPE.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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