Diagnostic and prognostic significance of survivin levels in malignant pleural effusion

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Received 29 April 2012; accepted 9 April 2013
Available online 18 June 2013

KEYWORDS
Survivin;
Pleural effusion;
Prognosis;
Malignant

Summary
We aimed to evaluate the diagnostic and prognostic value of measuring survivin levels, which is an inhibitor of apoptosis in pleural effusions. Methods: Group I, malignant (MPE) (n = 51); Group II, tuberculosis (TPE) (n = 18); Group III transudative (TE) (n = 9) effusions were enrolled prospectively. We used ELISA to analyze 78 effusions. The value for the differential diagnosis and the correlation between survivin and survival in MPE were analyzed. Results: Survivin level was 41.75 ± 76.20 in MPE, 15.83 ± 10.92 in TPE and 8.33 ± 8.67 in TE. When the patients divided two groups as malignant and non-malignant pleural effusion (non-MPE), survivin level was significantly higher in MPE (41.75 ± 76.20) than in non-MPE (13.33 ± 2.05) (p = 0.012). The cutoff value for survivin levels detected by ROC curve analysis was 7.5 pg/ml, with sensitivity and specificity values of 72%, 44%, respectively. Survivin had no discriminative power in differentiating exudative effusions of MPE from TPE (p = 0.405). There was no correlation between survivin level and age, sex, location, fluid pH, glucose, protein, albumine and ADA level while there was significant moderate correlation with fluid LDH (r = 0.49; p < 0.001). Survivin levels can distinguish patients who had poor prognosis (median survival 75 days, n = 24) and those who had good prognosis (median survival 219 days, n = 27, p = 0.03) in MPE.

In conclusion, survivin expression levels detected with ELISA had no discriminative power in differentiating exudative effusions included MPE and TPE. Elevated survivin levels are associated with poor survival in MPE. Our results suggest that survivin may be a potential prognostic marker in MPE.

Published by Elsevier Ltd.

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0954-6111/$ - see front matter Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.rmed.2013.04.011
Introduction

Malignant pleural effusion (MPE) is a common and important cause of cancer-related mortality and morbidity. Prompt diagnosis using minimally invasive test is important because the median survival after diagnosis is only 4–9 months. The sensitivity of cytologic examination of pleural effusion is variable with limited sensitivity and not predictive of prognosis. Consequently, many patients need to undergo invasive diagnostic tests such as thoracoscopic pleural biopsy. Besides, none of the prognostic marker has been validated until now for MPE.

Survivin is an inhibitor of apoptosis that may be a novel diagnostic and prognostic marker of cancer. It is selectively upregulated in many human tumors, where its overexpression correlates with poor outcome. Tissue expression of survivin has a critical role for diagnosis, prognosis and the prediction of response to therapy. But, there are limited data on the expression and prognostic role of survivin in malignant pleural effusion. So, its value in the analysis of biological fluids such as pleural effusion is not known.

We aimed to determine the discriminative power of survivin in proven cases of MPE and non-MPEs diagnosed by conventional cytopathologic and histopathologic methods, and testing the prognostic value of survivin levels in MPE.

Materials and methods

Subjects

Between October 2009 and July 2010, a total of 78 patients [51 with malignant, 18 with tuberculosis and 9 with congestive heart failure (CHF)] with pleural effusion admitted to our clinic were included in the study. All patients consecutively diagnosed with MPE, tuberculous pleurisy (TPE) and CHF were included. All patients were diagnosed according to criteria cited below which was considered as a reasonable standard for diagnosis. Distribution of patients according to the primary etiology has been shown in Table 1.

Medical history was taken from all patients included in the study. Physical examination was made, and posteroanterior chest X-ray ordered. Thoracentesis was carried out in all patients. Total protein, albumin, LDH, glucose and pH were measured in the pleural fluid and blood sample, and ADA levels also were studied in pleural fluid. In addition, fluid cell formula and 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) (17 patients); or (2) microbiologic isolation of Mycobacterium tuberculosis in the pleural fluid (2 patients); 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 (27 patients) and/or on histopathologic examination of the pleural tissue obtained by VATS (6 patients) or pleural blind biopsy (18 patients).

The diagnosis of CHF is based on medical history, physical examination and detection of cardiomegaly in chest X-ray. Left ventricular systolic dysfunction on echocardiography and response to diuretic therapy was also used in the confirmation of the diagnosis.

Methods

Study has been approved by the ethical committee and informed consents were obtained from all participants. From each patient 5 ml aliquots of pleural fluid were

<table>
<thead>
<tr>
<th></th>
<th>N (%)</th>
<th>Survivin levels (pg/ml)</th>
<th>Survival, median ± SE (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant pleural effusion</td>
<td>51 (65)</td>
<td>41.75 ± 76.20</td>
<td></td>
</tr>
<tr>
<td>Primary lung carcinoma</td>
<td></td>
<td>52 ± 95a</td>
<td>75 ± 4 (67–82)</td>
</tr>
<tr>
<td>Adeno</td>
<td>22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small cell carcinoma</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metastatic other than lung</td>
<td>14</td>
<td>25 ± 20</td>
<td>45 ± 67 (0–177)</td>
</tr>
<tr>
<td>Breast</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal system</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostate</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenoid cystic carcinoma</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervix</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown primary</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>8</td>
<td>18 ± 22</td>
<td>181 ± 29 (122–239)</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>5</td>
<td>86 ± 124</td>
<td>215 ± 119 (0–449)</td>
</tr>
<tr>
<td>Tuberculosis (TPE)</td>
<td>18 (23)</td>
<td>15.83 ± 10.92</td>
<td></td>
</tr>
<tr>
<td>Congestive Heart Failure (CHF)</td>
<td>9 (12)</td>
<td>8.33 ± 8.67</td>
<td></td>
</tr>
</tbody>
</table>

*No statistically significant difference was found between the tumor groups according to survivin level (p = 0.22).
received in 2 ml Eppendorf tubes and were refrigerated at −80 °C until to work-up. In this study, Human Total Survivin Enzyme Immuno-Metric Assay Kit (TiterZyme EIA, Assay Design, Inc., Ann Arbor, MI, USA; Catalog # 900-111) was used. According the package insert of the kit the dynamic range of the assay was between 31.25 and 1000 pg/ml and the lower limit of detection was 3.6 pg/ml. All pleural effusion samples and regents 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.

Statistical Analysis

Patient demographics and disease characteristics were summarized using descriptive statistics. Sensitivity, specificity, positive predictive value, and negative predictive value 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. In case of a significant difference with one-way ANOVA test, Tukey HSD was used for post-hoc analysis to define the groups from which the difference arose. Chi-square test was used in categorical variables. The groups were properly combined, and the analysis was repeated if expected frequency was less then 5 in one of the cells. In bivariate correlations studied with survivin, Spearmen’s correlation test was used as the distribution of survivin was not normal. 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, fluid LDH level and tumor types were included as independent variables. In the first model all independent variables were included and in the second model backward elimination with likelihood ratio was used to construct the model. Statistical significance was set at p < 0.05. SPSS for Windows, version 16 package program has been used for statistical analysis.

Results

Our study was carried out with 78 patients [M/F: 48 (61%)/ 30 (39%), age (18–80 years)], referred to Yedikule Chest Disease and Thoracic Surgery Training and Research Hospital, and diagnosed with MPE, TPE and CHF between October 2009 and July 2010. Of the MPE cases, 27 (53%) were diagnosed with fluid cytology, 18 (34%) with blind pleural biopsy and 6 (13%) with VATS. Acid-fast bacillus was found in fluid smear and Mycobacterium tuberculosis isolated in culture of two patients, chronic granulomatous inflammation in pleural biopsy of 17 patients with TPE, and beside that definitive diagnosis was made ruling out the other possibilities. Distribution of the patients according to the diagnoses is shown in Table 1 and demographic characteristics in Table 2.

Survivin levels in study populations

Mean value of survivin in MPE, TPE and CHF were 41.75 ± 76.20 pg/ml, 15.83 ± 10.92 pg/ml, and 8.33 ± 8.67 pg/ml respectively. Mean value of survivin tended to be higher in MPE, but no statistically significant difference was found between the three groups (p = 0.182). When the patients were divided into two groups as malignant and non-malignant pleural effusion, mean level of survivin was found to be significantly higher in malignant effusions compared to non-malignant group (41.75 ± 76.20 versus 13.33±10.69, p = 0.012) (Fig. 1).

In the group of MPE, mean levels of survivin according to tumor origins are reported on Table 1. No statistically significant difference was found between the groups according to survivin level (p = 0.22). Patients histologically diagnosed with an adenocarcinoma were divided into two

Table 2 Demographical features of patients.

<table>
<thead>
<tr>
<th></th>
<th>(MPE) (n = 51)</th>
<th>(TPE) (n = 18)</th>
<th>CHF (n = 9)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean)</td>
<td>60.20 ± 13.91</td>
<td>34.22 ± 14.39</td>
<td>75.22 ± 8.25</td>
<td>0.000**</td>
</tr>
<tr>
<td>Age (min-max)</td>
<td>20–80</td>
<td>18–63</td>
<td>59–85</td>
<td></td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>22/29</td>
<td>7/11</td>
<td>1/8</td>
<td>0.135</td>
</tr>
<tr>
<td>Smoking (p/y)</td>
<td>30 (73.5%)</td>
<td>8 (44.4%)</td>
<td>5 (55.6%)</td>
<td>0.945</td>
</tr>
<tr>
<td>Alcohol</td>
<td>5 (9.4%)</td>
<td>2 (11.1%)</td>
<td>0</td>
<td>0.383</td>
</tr>
<tr>
<td>Asbest expose</td>
<td>19 (35.8%)</td>
<td>0</td>
<td>0</td>
<td>0.000**</td>
</tr>
<tr>
<td>ECOG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>19</td>
<td></td>
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<tr>
<td>3</td>
<td>13</td>
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<tr>
<td>4</td>
<td>4</td>
<td></td>
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</tr>
</tbody>
</table>

One-way ANOVA, χ² test, p < 0.05 is significant **.
groups as having primary lung carcinomas and metastatic lung cancer. Mean levels of survivin were found as 54.9/C6 98.9 in primary lung adenocarcinoma (n = 21) and 31.44/C6 21.76 in extrapulmonary metastatic adenocarcinoma (n = 9). No statistically significant difference was found between the groups according to survivin level (p = 0.31).

When all patients were evaluated, a significant moderate correlation was found between survivin level and fluid LDH (r = 0.49; p < 0.001) levels. In the MPE group, moderate correlation was found between survivin level and fluid LDH (r = 0.47; p = 0.001) levels. No significant correlation was found in terms of age, gender, side of fluid, amount, appearance, pH, glucose, protein, albumin and ADA of fluid.

**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 0.63 ± 0.06 (SE) (p = 0.066) (%95 CI 0.505—0.747). Considering cutoff value as 7.5 pg/ml, sensitivity was found as 72% and specificity as 44%. ROC curve is shown in Fig. 2. The ROC curve has also been drawn for MPE and TPE which were two significant etiological reason for exudative effusions. Area under the ROC curve was 0.566 ± 0.067 (p = 0.405) (%95 CI 0.434—0.698). According to our results, survivin had no discriminative power in differentiating exudative effusions of MPE and TPE.

When the ROC curve was drawn with MPE and CHF area under the ROC curve was 0.75 ± 0.08(SE) (p = 0.019) (%95 CI 0.596—0.897). Considering cutoff value as 7.5 pg/ml, sensitivity was found as 72% and specificity as 78%.

**Prognostic value of survivin among MPE patients**

Kaplan—Meier survival analysis was performed in the MPE group. Survival was better in combined lymphoma and mesothelioma groups compared to malignant fluids due to combined primary lung cancer and metastatic lung cancer groups (p = 0.015, Log-rank (Mantel—Cox)). The worst median survival was found in malignant fluids related with extrapulmonary primary tumors. Considering the patients diagnosed with an adenocarcinoma subtype in MPE group, no statistically significant difference was found between primary lung and other than lung groups in survival analysis. Survivin levels (cutoff determined by the median value of survivin ie >20 pg/ml vs <20 pg/ml) can distinguish patients who had poor prognosis (median survival 75 days, n = 24) and those who had good prognosis (median survival 219 days, n = 27, p = 0.03). Elevated levels of survivin were related to reduced overall survival in Kaplan—Meier analysis (Fig. 3).

Cox regression analysis was carried out for significant factors influencing survival. Survivin level, fluid LDH level and tumor type were included as independent factors. Only survivin level was retained as significant in backward elimination likelihood ratio test. (Table 3)

**Discussion**

Survivin is a 16.5 kDa protein that inhibits apoptosis, promote proliferation, and has a crucial role in the development of cancer. Survivin is selectively upregulated in many human tumors, where its overexpression correlates with poor outcome6—9 and chemotherapy/radiotherapy resistance.7,8 In a recent meta-analyses, it was suggested that the survivin −31G>C promoter polymorphism might be
associated with an increased risk of cancer especially in the Asian populations. Conflicting results have been published in the association between survivin levels in serum and the risk/prognosis of cancer. However, survivin detection in urine appears to be a highly predictive molecular marker of bladder cancer.

Only a few studies have been conducted in pleural effusion for evaluating a diagnostic and prognostic role of survivin. Dong et al. showed that the sensitivity of the diagnosis for lung cancer and negative predicted value increased when cytological examination of pleural effusion and detection of survivin mRNA in pleural effusion was combined. Lan et al. have reported that survivin mRNA levels were significantly higher in MPEs. Overexpression of survivin mRNA correlated with poor prognosis in cancer patients.

Wu et al. had been the first authors to have analyzed pleural effusion specimens for survivin expression using ELISA. They reported remarkable sensitivity and specificity with a cutoff value of 0.0062 ng/ml. The limitation of their study was that non-malignant group has limited number of TPE patients (n = 9). Interestingly, more than half of the TPE patients (5/9) expressed survivin in the same study. Specificity can reach 100% when TPE patients were excluded from 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 by biopsy or culturing of the mycobacterium. Survivin levels were higher in MPE group (41.75 pg/ml) when compared with TPE (15.83 pg/ml). However, according to our results, survivin had no discriminative power in differentiating exudative effusions of MPE from TPE (%95 CI 0.434–0.698).

ROC curves for MPE versus non-MPE were analyzed and for the cutoff value of 7.5 pg/ml sensitivity was 72% and specificity 44%. When TPE cases were excluded, for the same cutoff value of 7.5 pg/ml sensitivity was 72% and specificity was improved to 78% and was statistically significant. Our results showed increased specificity when TPE patients were excluded, although our group included limited number of CHF transudative effusions. Since this study was made in a country where tuberculosis is endemic, discrimination between exudative effusions of MPE and TPE was very important and survivin had no discriminative power in such cases.

No association has been detected between survivin levels of pleural effusion and age, sex, performance status, stage of tumor and subgroup of disease similar to our findings. The correlation between survivin levels in pleura and fluid LDH was moderate in our study ($r = 0.49; p < 0.001$). There are studies that report the prognostic importance of LDH levels. However in our study, Cox regression analysis did not retain LDH levels as a significant factor in survival and only survivin was found significant. It may be that survivin may overshadow the prognostic effect of LDH because of their correlation.

Mesothelioma and lymphoma have better long-term survival compared with metastatic effusion related with lung and other solid tumors ($p = 0.015$). In addition patients with mesothelioma had lower levels of survivin though comparison between tumor types did not reach statistical significance. No statistically significant difference of survivin was found between adenocarcinoma patients according to primary site in lung or others ($p = 0.31$).
However, in Cox regression analysis, tumor type was not retained as significant factor in survival. According to previous studies increased survivin levels or mRNA expression in pleural effusion were associated with poor survival. In our study, elevated levels of survivin was correlated with a reduced overall survival. Survivin levels can clearly distinguish patients as a poor and good prognostic group.

In our study, survivin level was analyzed with ELISA technique. In studies of a limited number in the literature, survivin levels were studied with various methods such as mRNA with PCR, IHC, immunoblotting and ELISA. Analysis using various methods limits the studies and makes it difficult to compare results.

In conclusion, survivin levels can be elevated 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 our country and cannot be used as a safe diagnostic tool in differentiation between TPE and MPE. Therefore, TPE group limits the use of survivin in differential diagnosis of MPE can lead to false-positive results. However, its questionable diagnostic role aside survivin has a potential role as a remarkable prognostic marker for MPE.

Conflict of interest statement

None declared.

References