MISS ELENA ARELLANO-ORDEN (Orcid ID : 0000-0003-0581-8947)

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Survivin is a negative prognostic factor in malignant pleural effusion

Elena Arellano-Orden (1), Beatriz Romero-Romero (1), Verónica Sánchez-López (1), José Martín-Juan (1), Francisco Rodríguez-Panadero (1, 2), Remedios Otero-Candelera (1,2)

Institutions

1. Unidad Médico-Quirúrgica de Enfermedades Respiratorias. Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocío/Universidad de Sevilla, Seville, Spain
2. CIBER de Enfermedades Respiratorias (CIBERES), Instituto de Salud Carlos III, Madrid, Spain

Correspondence:

Elena Arellano-Orden, Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocío, Avda. Manuel Siurot, s/n. 41013 Seville, Spain. Tel.: +34 955923063. E-mail: marellano-ibis@us.es

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Take home: High pleural fluid survivin levels are an adverse predictor of pleurodesis and survival in malignant pleural effusion

ABSTRACT

Survivin is a well-known member of the inhibitor of apoptosis family, and has been related with increased tumour aggressivity, both in tissue and in pleural fluid.

Objectives: In patients with malignant pleural effusion, we sought to investigate the changes in pleural fluid survivin concentrations induced by talc instillation into the pleural space. Those changes were also examined in relation to pleurodesis outcome and patient survival.

Methods: We investigated 84 patients with malignant pleural effusion who underwent talc pleurodesis. Of them, 32 had breast cancer, 25 lung cancer, and 27 mesothelioma. Serial samples of pleural fluid were obtained before thoracoscopy (baseline) and 24 h thereafter.

Results: Survivin levels were successfully quantified in all pleural fluid samples, and they were significantly higher in samples obtained after thoracoscopic talc poudrage compared with baseline ($p < 0.001$). Patients with higher pleural fluid survivin levels at baseline had a significantly poorer pleurodesis outcome ($p = 0.004$). A 30 pg/ml cutoff for baseline survivin in pleural fluid predicted failure of pleurodesis with a 54% sensitivity and 79% specificity ($p = 0.009$). Moreover,

median post-pleurodesis survival of patients with baseline survivin levels ≥30 pg/mL was 4 months (range: 0.1-38), compared with 13 months (range: 0.1-259) in patients below that cut-off ($p < 0.001$).

Conclusion: Elevated pleural fluid survivin concentrations are useful to predict failure of pleurodesis and are associated with shorter survival in patients with malignant pleural effusion.

KEYWORDS: Malignant pleural effusion, pleurodesis, survival, survivin.

INTRODUCTION

A malignant pleural effusion (MPE) is defined by an increase in the volume of pleural fluid accompanied by the presence of malignant cells. MPE may develop because of a primary pleural malignancy, e.g., mesothelioma, but more commonly occurs following metastatic cancer. The number of new patients diagnosed with MPE diagnosed in Europe each year is over 100,000 (1), with lung cancer being the most common underlying etiology (accounting for approximately one third of all cases). Other secondary malignancies that may cause MPE include breast cancer, lymphoma, ovarian cancer, colon cancer, and other solid tumors (2). Because pleural involvement generally reflects the presence of advanced-stage tumors, the treatment for MPE is aimed to prevent recurrences by obliterating the pleural cavity; this can be achieved by introducing a sclerosing agent into the pleural space (pleurodesis) (3-5). It is expected that approximately 60% of patients with MPE will require pleurodesis to prevent fluid re-accumulation (6), especially in presence of large and/or recurrent pleural effusions. Owing to its good safety profile, talc remains the most commonly used sclerosing agent for recurrent MPE (7-10). Specifically, calibrated talc has gained popularity for chemical pleurodesis (11, 12). The mechanisms of talc-induced artificial obliteration of the pleural space include the

induction of apoptosis (13, 14), and the inhibition of tumor-associated angiogenesis (15, 16).

Apoptosis is a key homeostatic mechanism to maintain cell populations in tissues, and deregulation of normal apoptosis is involved in malignant cell transformation (17, 18). Inhibitors of apoptosis-related proteins (IAP) and the Bcl-2 family of proteins are among the most important regulators of apoptosis (19, 20). Survivin − a well-known member of the IAP family encoded by the *BIRC5* gene − inhibits apoptosis and promotes cell proliferation (21). It is frequently overexpressed in human cancers (22), potentially serving as a tumor biomarker (23). Mechanistically, survivin forms dimers and binds to the activated form of caspase-3 to inhibit apoptosis (24).

In the present study conducted in a sample of patients with MPE, we sought to investigate the dynamic changes of pleural fluid survivin concentrations following talc instillation in the pleural space. By measuring survivin levels in pleural fluid samples both before and after talc-induced pleurodesis, we aimed to investigate whether talc could exert proapoptotic effects, in relation with pleurodesis outcome and patient survival.

MATERIALS AND METHODS

Patient population

The study sample consisted of 84 patients with a diagnosis of MPE who were scheduled for pleurodesis by thoracoscopic talc poudrage. The procedural protocol has been previously described in detail (25). In brief, all the participants underwent thoracoscopy performed by the same physicians in a respiratory endoscopy suite. Both local and intravenous anesthetics (mepivacaine and pethidine, respectively) were used. After complete removal of the pleural fluid, 5 g of asbestos-free, sterile

talc (Novatech, La Ciotat, France) was used to induce pleurodesis in each patient. The study protocol was approved by the local Institutional Review Board, and written informed consent was obtained from all participants. Reporting of the study conforms to STROBE statement along with references to STROBE statement and the broader EQUATOR guidelines (Simera et al. January 2010 issue of EJCI).

Clinical data collection

Demographic data, lung reexpansion and pleurodesis outcome were systematically collected in all patients. Patients were classified as *"naïve"* or *"non-naïve"* regarding absence or presence of chemo or radiotherapy prior to pleurodesis, respectively. The outcome of pleurodesis was defined in accordance with the criteria described in the European Respiratory Society/American Thoracic Society consensus statement (26). All patients were followed until death, or up to 22 years (in some exceptional cases with breast cancer and very good response to chemotherapy). Pleurodesis was considered as failed when the effusion recurred at any time. Lung re-expansion in the first two days after pleurodesis was checked (blindly to the pleurodesis outcome) on serial chest X ray (CXR), and rated as complete, partial or failed. The LENT prognostic score was calculated for all participants as previously described (27). Pleural tumor burden – including rating in visceral pleura- was assessed at thoracoscopy using a specific scale previously developed by our group (28). Survival from onset of pleural effusion (*"cumulated survival"*) and from time of pleurodesis (*"survival post-talc"*) was also recorded in each patient.

Pleural fluid sampling and survivin quantification

Serial samples of pleural fluid were obtained from all participants before thoracoscopy (baseline) and 24 h thereafter, and supernatants obtained from centrifugation were kept frozen until analysis. Concentrations of survivin in pleural fluid samples were determined with a commercially available enzyme-linked immunosorbent assay (Quantikine, R&D Systems, Minneapolis, MN, USA). According to manufacturer´s specifications, the minimum detectable dose of human survivin is 1.58 pg/ml.

Statistical analysis

Clinical variables are expressed as means and standard deviations, standard errors of the mean (SEM) or 95% confidence intervals (CI), medians (with range), or absolute and relative frequencies, as appropriate. Intergroup comparisons of continuous variables were performed by one-way analysis of variance (ANOVA) preceded by the Levene test for variance homogeneity. When the equal variance test failed, we used the Kruskal-Wallis test for continuous variables and the χ^2 test for categorical variables, and correlations between continuous variables were investigated using the Spearman's rank correlation coefficient (ρ). The Mann–Whitney *U*-test was used to compare selected pairs of groups. Survival curves were plotted with the Kaplan-Meier method and compared with the log-rank (Mantel-Cox) test. Cox regression analysis was used to investigate the possible impact of different tumour types -and also the possible influence of chemo and/or radiotherapy prior to pleurodesis- on pleural fluid survivin levels and on patient´s survival. Receiver operating characteristic (ROC) curves were used to identify the optimal cutoff values for pleural fluid survivin concentrations in relation to both pleurodesis outcome and

patient survival. All calculations were performed with the Statistical Package for the Social Sciences, version 20.0 (SPSS, IBM Corporation, Somers, NY, USA). Two-tailed p values < 0.05 were considered statistically significant.

RESULTS

Study patients

The general characteristics of the 84 study patients with MPE are summarized in Table 1. There were 32 patients with breast cancer, 25 with lung cancer, and 27 with mesothelioma. The latter group included 20 epithelial, four sarcomatous, and three mixed-type mesotheliomas. One patient with epithelial mesothelioma (who underwent talc-induced pleurodesis, extrapleural pneumonectomy, and multimodal therapy) had a 100-month survival.

All the patients with mesothelioma (100%), 17/25 with lung cancer (68%), only two of the 32 patients with breast cancer (6%) and 46/84 in the total series (55%) were naïve for previous treatment (chemo or radiotherapy). Post-pleurodesis median survival was 9.4 months in non-naïve patients (range: 0.1-259) vs. 8.8 in the naïve ones (range: 0.1-100), with no significant differences in the total series.

Survivin levels in pleural fluid

Survivin levels were significantly higher in pleural fluid samples obtained after thoracoscopic talc poudrage compared with baseline ($p<0.001$; Fig. 1A). Such differences were evident even when the analysis was conducted in different tumor types. Notably, patients with breast cancer had significantly higher concentrations of survivin in their pleural fluid than in those with lung cancer or mesothelioma ($p = 0.004$), both at baseline and 24 h after thoracoscopic talc poudrage. In contrast,

baseline survivin concentrations were lower in mesothelioma than in other tumor types (Fig. 1B). When different mesothelioma subtypes were compared, baseline survivin in the pleural fluid did not show significant differences, the only exception being a trend toward higher levels in the sarcomatous type than in the mixed and epithelial mesothelioma types (data not shown). Pleural fluid concentrations of survivin measured at 24 h after thoracoscopic talc poudrage were positively correlated with tumor burden in the pleural cavity ($\rho = 0.257$, $p=0.019$).

Pleural fluid concentrations of survivin in relation to patient survival

We used ROC analysis to identify the optimal cutoff level of pleural fluid survivin for the prediction of patient survival. The areas under curve (AUC) for baseline and 24 h post-pleurodesis pleural fluid survivin levels were 0.719 (95% CI = 0.6−0.838, $p = 0.001$) and 0.646 (95% CI = 0.519–0.771, $p = 0.03$), respectively (Fig. 2A). The AUC for survival from the time of pleurodesis was superior to AUC for cumulated survival from onset of pleural effusion (0.715 vs. 0.676, respectively) (Fig. 2B). For this reason, we chose post-pleurodesis survival for further comparisons in the present study.

Median post-pleurodesis survival of patients with baseline survivin levels ≥30 pg/mL was 4 (range: 0.1-38) months vs. 13 months (range: 0.1-259) in patients below that cutoff ($p < 0.001$) (Fig. 2C).

Patients with breast cancer had a median survival of 5 vs. 18 months, respectively, for baseline survivin above/below the 30 pg/ml cutoff ($p < 0.001$), whereas the median survival was 2 vs. 7 months for those with lung cancer, and 8 vs. 10 months for mesothelioma, respectively (Table 2). Also, patients with baseline survivin > 30 pg/ml had a significant higher tumor burden in the pleural cavity ($p<0.05$).

Pleural fluid concentrations of survivin in relation to pleurodesis outcome

The outcome of pleurodesis was classified based on the criteria described in the "Methods" section. Overall, pleurodesis was successful in 69% when complete lung reexpansion (as observed on CXR) was obtained, and failed in 71% of the cases with uncomplete lung reexpansion ($p = 0.008$). Regarding tumour type, an 87%, 50% and 69% successful pleurodesis was observed when complete lung reexpansion was achieved in patients with breast, lung cancer and mesothelioma, respectively. High visceral pleura tumour burden was associated with failed pleurodesis in 53% of the cases in the total series ($p < 0.04$), and in 42%, 89% and 50% of patients with breast, lung cancer and mesothelioma, respectively.

Patients with higher pleural fluid survivin levels at baseline had a significantly poorer pleurodesis outcome ($p = 0.004$; Fig. 3A). This observation was consistent when sub-analyses were performed according to different tumour types, albeit not significantly so (Fig. 3B).

Pleurodesis failed in 67% of patients with baseline survivin ≥ 30 pg/ml in the overall series ($p = 0.002$), and in 54%, 87% and 67% in breast, lung cancer and mesothelioma, respectively. The 30 pg/ml baseline survivin cutoff predicted failure of pleurodesis with a 54% sensitivity and 79% specificity (AUC = 0.668, 95% CI: 0.549-0.786, $p = 0.009$).

Notably, the outcome of pleurodesis showed a positive correlation with pleural fluid pH ($\rho = 0.419$, $p = 0.002$) and an inverse association with the LENT score ($\rho = -0.272$, $p=0.02$). A similar relationship was observed regarding patient survival from the date of pleurodesis. Specifically, a positive association was evident between survival and pleural fluid pH ($\rho = 0.446$, $p = 0.004$), whereas an inverse relationship with the LENT score was identified ($\rho = -0.367$, $p = 0.015$).

DISCUSSION

Although previous reports suggested that the intrapleural application of talc induces apoptosis (14, 15, 29), the exact mechanisms by which this phenomenon occurs have not yet been fully elucidated. In the present study, we systematically investigated pleural fluid concentrations of survivin − an inhibitor of apoptosis – in patients with MPE who underwent talc-induced pleurodesis. Published studies have investigated both survivin gene expression and protein concentrations in malignant and benign pleural effusion, suggesting a potential role of this molecule as a diagnostic biomarker of MPE (30-32). There is also growing evidence that investigation of survivin concentrations in blood may be helpful for diagnostic and prognostic purposes in cancer patients (33-35). Lan *et al.* (35) have previously demonstrated that survivin mRNA expression in the pleural fluid is inversely associated with survival in cancer patients. Our current results confirm and expand previous observations by showing that survivin levels in the pleural fluid may predict both pleurodesis outcome and patient survival in MPE. In this regard, median post-pleurodesis survival of patients with baseline survivin levels ≥30 pg/mL was 4 (range: 0.1-38) months vs. 13 months (range: 0.1-259) in patients below that cutoff ($p < 0.001$). Also, patients with baseline survivin > 30 pg/ml had a significant higher tumor burden in the pleural cavity ($p<0.05$), and the same cutoff predicted failure of pleurodesis with a 54% sensitivity and 79% specificity.

Pleurodesis was successful in 69% in the global series when complete lung reexpansion (as observed on CXR) was obtained. Regarding tumour type, an 87%, 50% and 69% successful pleurodesis was observed when complete lung reexpansion was achieved in patients with breast, lung cancer and mesothelioma, respectively. Despite complete reexpansion, lung cancer had worse pleurodesis

results than the other groups, and we speculate that tumor-related massive lung involvement and/or stiffness of the lung parenchyma might have a negative influence on pleurodesis outcome. On the other hand, breast cancer had the best results, possibly due to the influence of chemo and/or radiotherapy.

Our results indicate that survivin quantification in the pleural fluid might be helpful as a clinical tool for selecting the ideal candidates for pleurodesis, as well as for predicting their prognosis in the post-procedural phase.

Mechanisms involved in pleurodesis for malignant pleural effusions are poorly understood, and there are some clues suggesting that benign mesothelial tissue remaining in the pleural space of patients with malignant pleural effusion plays a pivotal role in achieving a successful pleurodesis. Nasreen *et al.* (14) have previously shown that talc was capable of inducing apoptosis in malignant pleural mesothelioma cells but not in benign mesothelial cells. In addition, Lee and coworkers (29) treated lung adenocarcinoma cells and benign cells obtained from patients with talc, doxycycline, and bleomycin. Apoptosis as determined by flow cytometry was observed in lung adenocarcinoma cells exposed to talc, doxycycline, and bleomycin, with talc producing the highest apoptotic rates.

In an ongoing *in vitro* study from our Group (yet unpublished), we observed that benign human mesothelial cells -when incubated with pleural fluid samples from patients with malignant pleural effusion after talc application- produced higher survivin levels when compared with malignant pleural mesothelioma and human lung adenocarcinoma cell lines co-incubated with the same post-talc pleural fluid samples. These observations suggest that benign mesothelial cells are protected against talc-induced apoptosis. Because pleurodesis outcomes are inversely associated with pleural tumor burden, the protection of *benign pleural mesothelium*

against talc-induced apoptosis may be viewed as beneficial for promoting pleurodesis. Taken together, these results suggest that – within the context of MPE – survivin could exert different effects on benign and neoplastic cells. Specifically, survivin production elicited by talc in benign mesothelial cells may promote benign cell proliferation and successful pleurodesis. In contrast, an increased production of survivin by malignant cells might protect them against apoptosis, ultimately promoting a greater pleural tumor burden, a higher likelihood of pleurodesis failure, and a shorter survival expectancy. Thus, the preservation of a benign mesothelium seems essential to promote a successful pleurodesis.

The outcome of talc-induced pleurodesis showed a negative correlation with both pleural fluid survivin levels and the LENT score, which in turn predicted patient survival. In contrast, both pleurodesis outcome and patient prognosis had a positive association with pleural fluid pH, an observation which is in line with the published literature (36). Intriguingly, our study showed a previously unreported negative correlation between baseline levels of survivin and pleural fluid pH ($\rho = -0.314$, $p = 0.015$). These data indicate that both elevated pleural fluid survivin levels and a low pleural pH can reflect the presence of more biologically aggressive tumors. Accordingly, a low extracellular pH has been related to local hypoxia and an increased tumor aggressiveness through the suppression of apoptosis in malignant cells (37).

In conclusion, the results of our current study demonstrate that elevated pleural fluid survivin concentrations are useful to predict failure of pleurodesis and are also associated with shorter survival in patients with malignant pleural effusion.

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LEGENDS:

Table 1. Patients with malignant pleural effusion submitted to talc pleurodesis.

Table 2. Survival of patients regarding baseline survivin levels in pleural fluid

Figure 1. Levels of survivin in pleural fluid before and 24 hours after talc pleurodesis (Panel A). Levels of survivin (pg/ml) in pleural fluid before and 24 hours after talc pleurodesis in patients with breast cancer, lung cancer and mesothelioma (Panel B). p value was calculated with U-Mann Whitney test.

Figure 2. Panel A: ROC analysis for survivin levels before and 24 hours post-talc pleurodesis in patients with malignant pleural effusion (see text for explanations). Panel B: ROC analysis regarding survival post-talc pleurodesis and cumulated survival (from onset of pleural effusion) with baseline survivin cutoff = 30 pg/ml. Area under the curve (AUC) for post-pleurodesis survival = 0.715 (95% CI: 0.599-0.830), and for cumulated survival = 0.676 (95% CI: 0.553-0.799). Panel C. Post-pleurodesis survival of patients with malignant pleural effusion, depending on levels of baseline survivin in pleural fluid.

Figure 3. Survivin basal levels in pleural fluid regarding outcome of pleurodesis in the global series (Panel A). Survivin basal levels of baseline in pleural fluid regarding outcome of pleurodesis in different types of tumor (Panel B). p value was calculated with U-Mann Whitney test.

Table 1. Patients with malignant pleural effusion submitted to talc pleurodesis.

		Breast cancer (n = 32)	**Lung cancer (n = 25)**	**Mesothelioma (n = 27)**	**Entire cohort (n = 84)**	**P value***
Males, n (%)		1 (3.1%)	21 (80.8%)	25 (96.2%)	47 (56%)	<0.05
Age, years (Mean ± SD)		58.28 ± 10.5	65.12 ± 12.7	62.15 ± 9.7	61.6±11.2	NS
Survival after pleurodesis, (months) (Mean, 95% CI)		29.3 (13–51)**	10.8 (6–15)	14.1 (9–23)	18.9 (13–28)	
median (range)		13 (0.6–259)	4.8 (0.1–40)	9 (0.3–100)	8.8 (0.1–259)	
Cumulated survival*** (months)(Mean, 95% CI)		38 (21-59)**	14.4 (9-19)	18.3 (12-28)	24.6 (18-34)	
Median (range)		19 (2.4-262)	8.6 (2-42)	13.1 (1.7-106)	13.5 (1.7-262)	
Outcome of pleurodesis	Success	21/29 (72%)	10/20 (50%)	15/25 (60%)	46/74 (62%)	NS
	Partial	6/29 (21%)	6/20 (30%)	5/25 (20%)	11/74 (23%)	
	Failure	2/29 (7%)	4/20 (20%)	5/25 (20%)	11/74 (15%)	
Total tumor burden (mean) (95% CI)		5.4 (4.8-6)	5.6 (5-6)	6.1 (5.6-6.6)	5.7 (5.3-6)	NS
Visceral pleura tumor burden mean (95% CI)		1.6 (1.3-1.8)	1.9 (1.7-2.1)	1.8 (1.6-2)	1.7 (1.6-1.9)	NS
LENT score (Mean ± SEM)		2.3 ± 0.1	3.6 ± 0.2	1.3 ± 0.1	2.4 ± 0.1	<0.001
Pleural fluid pH (mean) (95% CI)		7.31 (7.26–7.36)	7.30 (7.25–7.35)	7.27 (7.22–7.32)	7.29 (7.26–7.32)	NS

*P values were calculated with χ^2 test or Student's *t*-test for unpaired data, as appropriate.
** Survival of patients with breast cancer was significantly longer than in the other two groups.
*** Cumulated survival was computed from onset of pleural effusion until death.
Abbreviations: NS, not significant; SD, standard deviation; CI, confidence interval; SEM, standard error of the mean.

Table 2. Survival of patients regarding baseline survivin levels in pleural fluid

	Survivin < 30 pg/ml (N = 54)			**Survivin ≥ 30 pg/ml (N = 30)**		
SURVIVAL POST-TALC (months, Mean (95% CI) Median (range)	25 (15-38)** 13 (0.1-259)			8 (5-11) 4 (0.1-38)		
CUMULATED SURVIVAL* Mean (95% CI) Median (range)	31 (21-44) 15 (2-262)			13 (9-18) 9 (2-41)		
	Breast (19)	**Lung (17)**	**MPM (18)**	**Breast (13)**	**Lung (8)**	**MPM (9)**
SURVIVAL POST-TALC (months, Mean (95% CI) Median (range)	44 (19-75)*** 18 (2-259)	13 (7-20) 7 (0.1-40)	16 (8-29) 10 (1-100)	8 (3-14)*** 5 (0.6-38)	5 (1-11) 2 (0.1-18)	11 (4-19) 8 (0.3-31)
CUMULATED SURVIVAL* Mean (95% CI) Median (range)	54 (28-84)*** 29 (5-262)	13 (5-24) 8 (2-41)	19 (12-33) 14 (5-106)	14 (9-20)*** 13 (2-40)	9 (4-16) 5 (2-29)	18 (12-28) 12 (2-41)

* Cumulated survival was computed from onset of pleural effusion until death.

** Survival of patients with baseline survivin < 30 pg/ml in pleural fluid was significantly longer than in patients above this cut-off ($p < 0.001$, Log Rank Mantel-Cox).

*** Survival of patients with breast cancer and baseline survivin < 30 pg/ml in pleural fluid was significantly longer than in patients above this cut-off ($p < 0.001$, Log Rank Mantel-Cox).