Original Article

Expression of estrogen receptor beta (ERβ) and its prognostic value in pleural mesothelioma

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Background: Overexpression of estrogen receptors in malignant pleural mesothelioma has shown an independent relation with a better prognosis of survival, and the use of selective estrogen receptor beta (ERβ) agonists increases the susceptibility to antitumor treatment.

Methods: This was a retrospective single center study that analyzed the response of malignant pleural mesothelioma with an expression of ERβ to first-line chemotherapy. The study included patients with pleural mesothelioma pathologically confirmed between 2013 and 2016 at the National Institute for Respiratory Disease (INER), who underwent an immunohistochemistry assay for ERβ (mouse monoclonal antibody PPG5/10). The primary endpoint was the response to chemotherapy based on RECIST 1.1 according to the ERβ expression; secondary outcomes were the overall survival (OS) and progression-free survival (PFS).

Results: We included 22 patients, regarding the expression of ERβ, 17 (77.2%) patients had high or moderate degree, while 5 (22.7%) had low degree or null expression. The response to treatment as by RECIST 1.1, 12 (54.5%) had partial response, 5 (22.7%) had stable disease, and 3 (13.6%) had progression. None of the patients had a complete response. Of those who had a partial response, 9 (75%) had a high or moderate degree of ERβ expression in tumor cells, and 3 (25%) had a low or null degree of expression.

Conclusions: High and moderate expression of ERβ group with advanced clinical stage malignant pleural mesothelioma was associated with a tendency of higher OS and better response to chemotherapy treatment resulting in longer PFS although statistical significance was not achieved.

Keywords: Mesothelioma; estrogen receptor beta (ERβ); prognosis

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Introduction

Malignant mesothelioma is an aggressive tumor which may originate in the mesothelial tissue of either pleural or abdominal cavities, the tunica vaginalis or pericardium. Diagnosis occurs more frequently during advanced stages, appearing as an acute admission to hospital due to breathlessness, chest pain and unilateral pleural effusion (1) with a negative prognosis for the majority of patients despite treatment (2-6). Statistics related to its morbidity and mortality rates are limited; in 2013, 50,400 new cases of malignant pleural mesothelioma (MPM) were estimated, and 33,700 (66.9%) deaths due to this disease (7,8).

At the United States, there is a medium rate of incidence with close to 3,300 new cases reported and 2,700 deaths every year (9). In Mexico, this tumor is ranked 34th among malignant neoplasms (10), during the period of 1979 to 2000 a study reported 793 deaths by MPM in the country, of which 62% were male patients and 38% female. These statistics concur with those collected at the Instituto Nacional de Enfermedades Respiratorias "Ismael Cosío Villegas" (INER), a third-level care center specialized in pulmonary pathology, including lung and pleural cancer, which are representative of the epidemiology within the country's central region. Between 1991 and 2007 a total of 247 new diagnoses were reported (74.7% male) (11), followed by a significant increase in incidence between 2006 and 2009 of 149 new cases (71.1% male) (12). In both studies, the age at presentation varied between 51 and 70 years old and exposure to asbestos of any kind was documented in up to 91% of cases (11,12).

To date, there is no malignancy that has a more causal relation with a defined carcinogen than MPM with asbestos (up to 90%), after an approximate 30-year latency period of exposure (13-16). According to the International Agency for Research on Cancer there are at least 13 cohort studies and 18 case-control studies probing the relation between asbestos exposure and mesothelioma (17), however, the latency time is long but the survival at diagnosis is short, regardless of the exposure time (18-20). Unlike the reduction in the use of asbestos in developed countries, Asia and Latin America has become an increasingly common trend resulting in a continuous growth in the incidence of mesothelioma in the next 10 to 20 years (21-24). Environmental exposure could be also due to other asbestiform fibers (such as Libby amphibole and Fluoro-edenite) into air by routine human activities or natural weathering processes, this exposure may vary across the area by task and according to the spatial distribution of contamination, soil, vegetation type, and environmental conditions (25,26). Besides the asbestos exposure, some patients do not report known exposure (27,28), so other types of factors have been studied such as different mineral fibers other than asbestos, radiation, chronic serosal inflammatory conditions, simian virus 40, BAP-1 cancer predisposition syndrome, contributing to the development of MPM (29-31). In different studies, the factors involved in carcinogenesis already mentioned above result in alteration of immunocompetent cells to result in a decline of tumoral immunity. Asbestos can induce chronic inflammation due to the production of reactive oxygen/nitrogen (32) more pathogenic in *in vivo* than in *in vitro* by macrophage activation (33) that results in increased NF-κB activity, a signaling pathway that plays a role orchestrating the inflammatory response as well as cell proliferation (34). This state of inflammation has tried to be demonstrated in different ways such as the verification of the presence of antinuclear autoantibodies (35) or elevation of biomarkers such as serum mesothelin in those exposed to asbestos fibers (36).

MPM is hard to stage due to the lack of consensus on the staging system, however, particularly among patients that do not undergo surgery, it is a common practice to use the American Joint Committee on Cancer (AJCC) TNM (tumor, nodes, metastasis staging system) (37-39).

A biopsy to have a definitive diagnosis is of high importance to begin treatment expeditiously (40-42). Recently, the role of estrogen receptor expression in different human tumors has remained controversial (43), however, in malignant mesothelial tissue there is evidence of its usefulness as a prognostic factor (44-46). Considering that this tumor is less frequent in women and that they have a more favorable prognosis (47), it may be hypothesized that development of this tumor could be related to the expression of estrogen receptors (48). A study that analyzed 78 samples of MPM tissue and 21 samples of normal pleural tissue and demonstrated that there was no expression of ERα in any of the samples, either malignant or normal. On the other hand, both tissue types presented expression of ERβ. This same study demonstrated that overexpression of this receptor is independently related with a better survival prognosis, most notably in the epithelioid histological subtype. Moreover, it was demonstrated that expression of this receptor inhibits the growth of tumor cells promoting the expression of proteins p21 and p27 and inhibiting the expression of cyclin B1 (49). It has even been possible to link the expression of ERβ to the cell's metabolic state, where a higher lactate concentration in the intracellular space results in a greater expression of ERβ (50).

Conversely, two studies have demonstrated that using

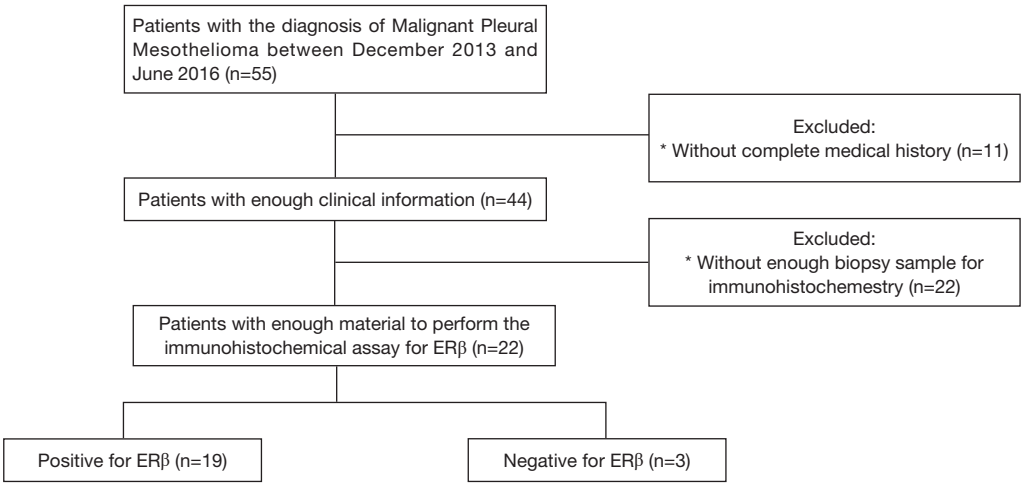

Figure 1 Flow diagram of the study population. *, bullet point.

a selective agonist of ERβ, in this case, a molecule known as KB9520 decreases the growth of MPM cells in both *in vitro* and in murine models (50,51). In addition, there is evidence that the selective agonism of this receptor could increase the sensitivity to the antitumor treatment. In one study an increased sensitivity to cisplatin was reported *in vitro* together with a protective effect to this cytotoxic agent in normal mesothelial cells (51-53). In a similar fashion, another study produced sensitivity to the use of the EGFR tyrosine-kinase inhibitor gefitinib, which seems to diminish its rate of internalization when the agonist KB9520 was added to an *in vitro* model (54). The aim of this study was to assess the response rate to first-line chemotherapy in malignant pleural mesothelioma with an expression of ERβ.

Methods

A retrospective study was performed at the Thoracic Oncology and Pathological Anatomy departments at INER, in Mexico City. The study design was approved by INER's Institutional Ethics Board in accordance with the Declaration of Helsinki, Fortaleza Brazil 2013 (approval document: C56-18). Patients older than 18 years old with a histopathological diagnosis of malignant pleural mesothelioma were included, diagnosed between December 2013 and June 2016, with at least one chest imaging study prior to the start of chemotherapy. Exclusion criteria were underage patients, those that underwent resection or radiotherapy with curative intent prior to chemotherapy, and those without a complete medical history or enough paraffin blocks to perform immunohistochemical studies as shown in *Figure 1*. The primary endpoint was the rate of response to first-line chemotherapy within the first 6 months, and the secondary endpoints were the percentage of resectability, progression-free survival (PFS), and overall survival (OS).

The following clinical and pathological variables were determined: gender, age, expositional, mesothelioma pattern, TNM clinical stage, first-line chemotherapy scheme, response as per RECIST 1.1, OS and PFS.

In addition, immunohistochemical analysis was performed on the biopsy samples to determine the expression of ERβ by our pathologist, who was blinded to the treatment received by the patient, as well as his survival rate. An immunohistochemical assay with a murine monoclonal antibody aimed at the region C-terminal of isoform 1 of the human ERβ was used (GTX47720, PPG5/10, GeneTex Inc., Indio, CA, USA). An automated, standardized, immunohistochemistry VENTANA Benchmark XT system (Ventana Medical Systems, Roche Inc., Tucson, AZ, USA) was also used. To determine the degree of positive staining by direct observation, a qualitative scale was used as follows: 1+ for weak staining, 2+ for intense staining, and 3+ for very intense staining; a percentage of cells that were stained with antibody was also calculated. High and moderate expression of ERβ, considered at the same group, was determined when more than 50% of cells were stained with an intensity greater than 1+. SPSS software version 24.0 (IBM software, Armonk, NY, USA) was used for analysis. The variables were expressed as the median values, as well as total values and percentages. OS and PFS were graphed using a Kaplan-

Table 1 General characteristics of the population according to his ERβ expression (n=22)

Characteristic	High or moderate ERβ expression (n, %)	Low or null ERβ expression (n, %)
Gender		
Male	9 (40.9)	4 (18.2)
Female	6 (27.3)	3 (13.6)
ECOG performance status		
0	0	1 (4.5)
1	5 (22.7)	2 (9.1)
2	10 (45.5)	2 (9.1)
3	0	2 (9.1)
Comorbidities		
Systemic arterial hypertension	6 (27.3)	1 (4.5)
Diabetes mellitus	2 (9.1)	2 (9.1)
Smoking		
Yes	9 (40.9)	3 (13.6)
No	4 (18.2)	2 (9.1)
Passive	2 (9.1)	2 (9.1)
Clinical stage		
IA	1 (4.5)	0
IB	0	0
II	1 (4.5)	0
III	5 (22.7)	3 (13.6)
IV	8 (36.4)	4 (18.2)
Histological strain		
Epithelioid	12 (54.6)	6 (27.3)
Sarcomatoid	0	0
Mixed	3 (13.6)	1 (4.5)

Meier plot. The criterion for statistical significance was $P<0.05$. The immunohistochemical and all the financial related issues were absorbed by the investigation group from the study.

Results

A total of 55 patients were identified with a diagnosis of MPM starting December 2013. Of these, 37 patients were male (67.3%) and 18 were female (32.7%). The average age in years was 64.1 (standard deviation 9.94) with a range of 41 to 84 years. Only 44 patients (80%) had enough information in either paper or electronic charts at INER in order to be considered for the statistical analysis.

Regarding the histopathological characteristics of the tumors, 42 out of the 44 cases had an epithelioid histology (95.5%) with the rest being of mixed histology (4.5%). There were no cases of only sarcomatoid histology. Out of the 44 cases, only 22 (50%) had enough material to perform the immunohistochemical assay to determine the expression of estrogen receptor beta (ERβ). According to the TNM classification the 22 cases had the following clinical stage distribution at the moment of the diagnosis: stage IV 54.6%, stage III 36.3%, stages II and IA each with 4.5%. Amongst the patients that were statistically analyzed as per their ERβ expression, exposure to asbestos was only documented in 9 out of 22 cases (40.9%). It was possible to perform surgical resection after treatment with chemotherapy in 4 patients (18%) and 3 patients received radiotherapy (14%). The median PFS in this population was 9.85 months, while the median OS was 14.35 months. *Table 1* lists the characteristics of the population that was included in the ERβ expression analysis.

Of the 22 tissue samples available for ERβ immunohistochemical analysis, 19 were positive for this receptor (86.4%) and in 15 of these 100% of cells were stained with different degrees of intensity (68.2%). *Table 2* shows the response to first-line chemotherapy as well as the possibility to provide definitive treatment to patients, with either chemotherapy or radiotherapy, after chemotherapy treatment. *Table 2* also lists the PFS and OS as they relate to ERβ expression. The response to treatment as by RECIST 1.1, 12 (54.6%) had a partial response, 5 (22.7%) had stable disease, and 3 (13.6%) had progression. None of the patients had a complete response. Of the patients who had a partial response, 9 (75%) patients had a high or moderate degree of ERβ expression in tumor cells, and 3 (25%) had a low or null degree of expression. According to the criterion used to define high and moderate ERβ expression group (positive staining in 50% of cells), it was observed that patients with partial response after first-line chemotherapy and high or moderate ERβ expression were 41% of the population (9 patients) while those with low or null ERβ expression were 14% (3 patients). No patient had a full response. The percentage of resectability after chemotherapy in patients with either high or moderate and

Table 2 Patient outcomes and survival according to ERβ expression

ERβ expression	Partial response	Resected patients	PFS (months)	OS (months)
High or moderate	9 (41%)	2 (9%)	12.2	19.3
Low or null	3 (14%)	2 (9%)	9.3	10.3

ERβ, estrogen receptor beta; PFS, progression-free survival; OS, overall survival.

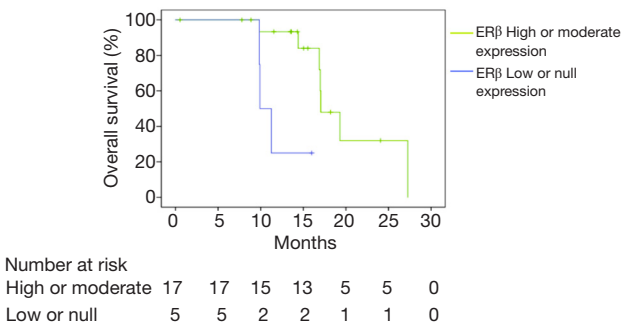

Figure 2 Overall survival, in months, comparing the patients with high or moderate ERβ expression and those with low or null ERβ expression. ERβ, estrogen receptor beta.

low or null ERβ expression was 9% in both groups. Neither the difference in partial response nor the percentage of resectability achieved statistical significance. The median PFS for patients with high or moderate ERβ expression was 12.2 months, compared to 9.3 months amongst those with low or null expression (P=0.67, 95% CI, 4.8–12.9). The median OS had a statistical tendency to be greater in patients with high or moderate ERβ expression by 9.2 months since the difference of the medians was 19.5 and 10.3 months for patients with high or moderate and low or null expression, respectively (P=0.054, 95% CI, 9.79–10.01). This relationship is shown in *Figure 2*.

Exploratory analyses to identify risk factors for OS were performed with the use of a multivariate logistic-regression model (age, gender, ECOG performance status, smoking index, histological strain, clinical stage, degree of ERβ expression and chemotherapy regimen), with no significant predictors for OS were found in this analysis.

Discussion

The general characteristics of the population agree with previous reports described in the literature. The average age in years was 64.1 (range, 41 to 84), being the stage IV the most prevalent clinical stage at the moment of the diagnosis (54.5%). Since most diagnoses are made in advanced stages, it becomes of broad interest the identification of non-invasive molecular markers for an early diagnosis. The most studied biomarker is mesothelin, characterized by a good specificity, but it has low sensitivity. Other protein markers had reported interesting results such as the HMGB1 (55) and microRNAs expression (56) as promising diagnostic biomarkers, notwithstanding the above, none of the markers available today are sufficiently reliable to be used in the surveillance of subjects exposed to asbestos or in the early detection of MPM. Exposure to asbestos was only documented in 41% of the studied population, which represents a lower percentage of what is described in the literature. This may be due to underestimation while questioning the patient or to unknown exposure to these compounds (mainly in construction materials), that is subsequently not reported. Finally, tobacco use was reported by slightly more than half of the analyzed patients. Although there is a greater expression of ERβ in men, this may be due to the prevalence of MPM in men without a clear relationship between the degree of ERβ expression and gender. It is noteworthy to see that those patients with worse ECOG had higher expression of ERβ. Regarding the histological strain, those with mixed subtype showed lower expression of ERβ possibly explained due to the aggressiveness and poor differentiation of this subtype.

There is an apparent relationship between the rate of response to treatment and a high or moderate ERβ expression, although it did not achieve statistical significance. It is important to mention that no direct comparison was made between the type of chemotherapy used as first-line of treatment and the obtained response. No relationship was found between resectability after chemotherapy treatment and ERβ expression, or a statistically significant relationship between ERβ expression and PFS. However, a tendency was found that links a better OS to patients with high or moderate ERβ expression. This is correlated with studies reported in the literature where the expression of this receptor leads to a better prognosis in OS.

It is important to mention that group of investigators

decided to use the PPG5/10 antibody due to validations previously made by Saunders *et al.*, 2002 (57), Shaaban *et al.*, 2008 (58) and Wu *et al.*, 2012 (59), however, a year later from the processing and collection of our samples, Andersson *et al.*, 2017 (60) and Nelson *et al.*, 2017 (61) published studies where the validity of different anti-ERβ antibodies was compared generating controversy about the specificity of the PPG5/10 antibody. This is of relevance since due to current financial issues of the research group, our interest of verifying ERβ expression through the use of other antibodies such as PPZ0506 or CWK-F12 is limited, being aware of the need for a future prospective study using the antibodies of interest already mentioned.

This study highlights that the response to treatment is apparently not related to ERβ expression. However, studies that showed an increased response to treatment with either cisplatin or a tyrosine-kinase inhibitor used an agonist specific to this receptor. Therefore, with our results, it may not be possible to discard the fact that, apart from being a potential prognostic marker, ERβ expression could have predictive value for treatment with a selective agonist (51,54). The main limitations of this work include the sample size which is modest compared to other literature reports that analyzed up to 78 patients (50,51,54). This imposes a limitation to the statistical power of our findings despite being partially in agreement with reports in the literature and in other studies.

Some other limitations were the inclusion of a single hospital, the loss of patients due to incomplete medical charts, and the lack of sufficient tissue samples, which resulted in 60% of patients diagnosed with mesothelioma during the study period not being included in the final analysis of ERβ expression. Finally, the retrospective nature of the study impacts the ability to generalize the results.

Amongst the strengths of this study, stands out the fact that this is the first time that ERβ expression in advanced mesothelioma is studied in Mexico. In addition, this study linked ERβ expression to patient prognosis and showed results that agree with international literature reports. The inclusion of more patients to this cohort and expansion into a prospective study will improve its statistical power, which would certainly demonstrate the survival benefit with a degree of significance greater than just a tendency. Moreover, it would be possible to include in a future study the use of a selective agonist of ERβ concomitant to chemotherapy in order to compare the response to treatment *in vivo* in a clinical setting, and not just *in vitro* as reported so far in the literature (51,54).

Conclusions

A high or moderate expression of ERβ as measured by immunohistochemistry was related to a higher response to chemotherapy treatment in patients with advanced malignant pleural mesothelioma, although this result was not statistically significant. It was not possible to link the expression of ERβ with a higher rate of resectability in patients with advanced malignant pleural mesothelioma.

A tendency was found for higher OS in patients with advanced malignant pleural mesothelioma and high or moderate ERβ expression.

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Footnote

Conflicts of Interest: Dr. Jeronimo Rafael Rodríguez-Cid has educational, investigational and advice relations with MSD, Bristol Myers, Roche, Takeda, Amgen, Abvie, Aztra Zeneca, Boehringer Ingelheim, Pfizer, Celgen, Novartis and Bayer; Dr. Orlando García-Acevedo works today for Aztra Zeneca as MSL; Dr. Jorge Arturo Alatorre-Alexander has educational, investigational and advice relations with MSD, Bristol Myers, Roche, Takeda, Aztra Zeneca, Boehringer Ingelheim and Pfizer. The other authors have no conflicts of interest to declare.

Ethical Statement: The study design was approved by INER's Institutional Ethics Board in accordance with the Declaration of Helsinki, Fortaleza Brazil 2013 (approval document: C56-18).

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