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Factors influencing malignant evolution and long-term survival in solitary fibrous tumours of the pleura

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Summary. Solitary pleuro-pulmonary fibrous tumours are relatively uncommon neoplasms that are difficult to manage therapeutically and which, cytogenetically, have been poorly studied.

The aim of the present work was to analyse the characteristics of a series of consecutive operated solitary pleural fibrous tumours in an attempt to discover a malignant pattern of evolution.

This was a retrospective observational study of 19 cases. Samples were studied for clinical, histological, immunohistochemical and cytogenetic characteristics (aCGH, FISH). Descriptive statistics were used: the Kapplan-Meyer log-rank test and the Cox-regression model for survival analysis. Analysis of malignant evolution was achieved using 2x2 tables; significant factors were included in a binary logistic regression model.

Parietal pleural implantation of the primary tumour, high mib1 expression, and low p53 expression were seen to be statistically significant factors for survival. We recommend a close follow-up for patients with a malignant primary tumour and low p53 expression and a regular long-term follow-up for benign primary tumours with a high mib1 index, high positive p53, and deletions. These findings need confirmation in more extensive series.

Key words: Solitary pleural fibrous tumour, Malignant pleural tumour, MIB-1 index prognostic value, Genetic analysis, aCGH and FISH analysis

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Introduction

Solitary pleural fibrous tumours (SPFT) are infrequent mesenchymal neoplasms and account for 5 and 10% of all pleural tumours (Cardillo et al., 2009; Park et al., 2011). First described by Klemperer and Rabin in 1931 (England et al., 1989), around 1000 cases have been reported in the literature. Their histogenesis is unknown and bears no relationship to exposure to asbestos or smoking. They are often detected in routine diagnoses for check-ups or other causes, in which about 50% of patients are asymptomatic. Complete surgical resection is the treatment of choice, followed or not by adjuvant radiotherapy.

Macroscopically, an SPFT is a non-encapsulated well-circumscribed mass, pedunculated or sessile. SPFTs have multiple microscopic growth patterns, which can be classified as solid-spindle or diffuse-sclerosing (Moran et al.,1992; Zhang et al., 2004). Although the histogenesis of such tumours is unknown, protein expression is characteristically positive for vimentin, CD34 and bcl2, and is negative for cytokeratins (CKs). Some authors have linked the expression of p53 and ki67 (mib1) to a high degree of aggressiveness (Park et al., 2011).

Few cytogenetic analyses of SPFT have been reported (Fletcher, 1997; Torabi et al., 2008; Swelam et al., 2009; Torres-Olivera et al., 2009) and they have proved to be poorly consistent, with controversial results, because they are based on studies of short series or isolated cases (Schirosi et al., 2008). In the cases that have been reported (Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer, 2012), no consistent cytogenetic abnormalities were detected.

Thus, it seems that knowledge of the cytogenetics and genomic changes occurring in SPFTs could be important to determine the pathogenesis of this type of neoplasm and its treatment using new therapeutic targets.

Most of these tumours show a biologically benign behaviour, although up to 30% of them eventually show local relapses and as many as 12% display malignant behaviour (Abu Arab, 2012). Despite the different classifications proposed (England et al., 1989; de Perrot et al., 2002), no definitive clinical, histological, immunohistological or genomic criteria have been reported to attempt to classify patients who will develop a malignant pattern.

The aim of the present work was to analyze the clinical, histological, immunohistochemical and cytogenetic characteristics of a series of consecutive pleural fibrous tumours operated at a tertiary hospital with the aim of discovering a malignant pattern of evolution so that close monitoring can be offered to patients at risk.

Material and methods

This was a retrospective observational study based on a series of SPFTs operated between July 1995 and March 2011 at the University Hospital of Salamanca, Spain. All clinical variables and outcomes used in this analysis were recorded prospectively on a customized database.

The series comprised 19 operated patients and these afforded a total of 23 samples to study: 19 primary tumours and 4 relapses. All cases were fixed in 10% formalin-buffer and embedded in paraffin. In order to obtain robust specimens for tissue-array composition, a careful selection of the most representative areas was performed in triplicate by a senior consulting pathologist. Histological and immunohistochemical analyses were performed in all specimens. In 21 samples from 19 tumours DNA was collected for cytogenetic studies.

The following variables belonging to different areas were recorded for study:

1.-Clinical variables: age, sex, presenting symptoms, number and description of surgical procedures, death due to tumour relapse.

2.-Histological variables: size, position of implantation on the pleura, sessile or pedunculated, a benign or malignant diagnosis, and the de Perrot classification of the tumours (de Perrot et al., 2002).

3.-Immunohistochemical variables: presence or lack of expression of CKs, vimentin, CD34, CD99, ALK, Calretinin, S100, actin, bcl 6, bcl 2 and the degree of expression of mib1, p53, PDGFR α and PDGFR ß. p53 expression was considered positive when at least 5% of the cells reacted with the specific antibody with an intensity of 2+. The intensity of expression of mib1 (ki67) was classified as 0 (negative) when less than of 5% of the cells reacted with the specific antibody; as 1 (low) when the reaction was seen in 5-20% of the cells, and as 2 (high) when it was seen in more than 20% of the cell population. The PDGFR index (for α and ß receptors) was calculated after measuring the intensity of expression (0 low, 1 medium, 2 high) and the percentage of cells that reacted with the specific antibody. The other antibodies were considered positive when 5% or more of the tumour cells reacted with the specific antibody.

4.-Genetic variables: genomic changes observed as gains and/or losses of DNA, identifying copy-number alterations (CNAs) and FISH values.

5.-Patient status: the final status of all patients was determined in December 2011 on the basis of either the patient's most recent clinical visit or hospitalization or by means of a telephone interview.

DNA isolation

Genomic DNA was extracted from FFPET samples in which at least 70% of the cells visible in the section were tumour cells. Tissue sections (3 μm) were deparaffinized. DNA was quantified using a Nanodrop spectrophotometer (ND-1000, NanoDrop Technologies, Wilmington, DE, USA) and its quality was assessed by the 260:280 ratio and by agarose gel visualization.

Comparative genomic hybridisation study

Comparative Genomic Hybridisation (CGH) was performed using oligonucleotide microarrays (Roche NimbleGen, Inc., Reykjavik, Iceland) in order to identify CNAs. The whole genome oligonucleotide microarray was provided by NimbleGen. 1 μg gDNA from each FFPE sample and reference sample was labelled using the NimbleGen. Dual-Color DNA Labeling Kit and hybridized to a NimbleGen Human CGH 12x135K. Sample preparations and hybridizations were carried out according to the manufacturer's protocols (NimbleGen Arrays User's Guide-CGH Analysis, Roche NimbleGen, Inc.).

Data analysis

Raw data on fluorescence intensity were obtained from scanned images of the oligonucleotide arrays, using NIMBLESCAN 2.3 extraction software (NimbleGen Systems). The data were normalized and analyzed using the segMNT algorithm in NimbleScan v2.6 Software. Additionally, we used the CGHweb interface, which generates a heatmap panel of the segmented profiles according to the different methods used, as well as a consensus profile (Lai et al., 2008).

Fluorescence in situ hybridization (FISH)

Interphase FISH was performed on all the samples using commercially available probes with the LSI 22q11.2 (DiGeorge Sind/ VCFS Dual Color), LSI c-myc (8q24.12-q241.3) Spectrum Orange and CEP3, CE7, CEP 17 and LSI 9p2 and the LSI p53 (17p13). We used

cosmid 9-4 and 4-1 to test for the PDGFR ß gene in the 5q31-33 region as well as probes for the PDGFR α/FIP1L1 fusion (these probes were kindly provided by Professor P. Marynen, Centre for Human Genetics, Leuven, Belgium). Interphase FISH used the method described previously (Robledo et al., 2009). FISH signals in morphologically intact, non-overlapping nuclei were counted. The images were captured on an Olympus BX60 epifluorescence microscope coupled with a CCD camera and then evaluated with Cytovision software (Applied Imaging). Approximately 400 non-overlapping tumour cells were evaluated.

Statistical analysis

Descriptive analyses of the main patient characteristics, their clinical symptoms, and the macroscopic features of the tumours and relapses are reported. Categorical data are presented using the number of cases and (%); continuous normally distributed data are expressed as means ± SD, while continuous non-normally distributed data are presented as medians (range).

Univariate analysis of survival probability was calculated with the Kaplan-Meier method, using the log-rank test to analyze differences between groups. Then, a multivariate Cox regression model was implemented, including significant variables in the previous univariate analyses to identify any adverse prognostic factors.

The relationship between variables and a malignant pattern of evolution was analyzed using comparative tests to calculate the odds ratio and its 95% confidence interval. If a positive relationship was found, a binary logistic regression model was developed using the statistically significant variables. $P<0.05$ was considered significant.

All calculations were performed with SPSS-15 for Windows.

Results

Clinical data

19 patients were operated. Most of them were female (13 out of 19) and the mean age of the series was 57.4±14.6 years (range: 34-78 years). In 6 cases (31.2%) the primary tumour was discovered due to thoracic pain but in 5 (26.3%) it was asymptomatic and was discovered by chance in a radiological study. In 4 patients (21.1%) dyspnoea and in 2 cases (10.5%) cough were the reasons for consulting. Two patients had paraneoplastic syndromes: one had recurrent hypoglycaemia and other hypertrophic pulmonary osteoarthropathy.

The mean size of the primary tumours was 12.57cm±5.5 (range: 3-22cm). Most tumours were pedunculated and had grown from the visceral pleura. 84.2% of the primary tumours were benign and 52.6% were classified as stage 0 of the de Perrot classification (Table1). Only in one case, case 11, did the tumour appear as multiple benign lesions growing from the visceral pleura of the left inferior pulmonary lobe. It consisted of a large central mass 7 cm in diameter and two smaller satellite nodules. In this initial surgery, the procedures varied from simple resection of the tumour (7 cases) to extrapleural right pneumonectomy with replacement of the pericardium and diaphragm. In all cases, surgery was considered macroscopically and microscopically complete because no positive margins were observed in the final pathological reports. The mortality of the series at 30 days was zero.

Six cases had a tumour relapse. In 2 cases, an extensive local and distant relapse plus a poor general situation precluded surgery and both died due to progression of the illness at two and three years after the first surgical intervention respectively. A further 2 cases, in which a first local relapse was operated, currently have extensive local non-surgically treatable relapses and are receiving chemotherapy. Finally, one malignant

Table 1. Summary of macroscopic and microscopic characteristics of the primary tumors.

		n=19
Localization	Visceral pleura	12
	Parietal pleura	2
	Mediastinic pleura	4
	Multiple	1
Implantation type	Pedunculated	13
	Sessile	6
Histology	Benign	16
	Malignant	3
de Perrot's stages	0	10
	1	6
	2	2
	3	1
	4	0

Table 2. Inmunohistochemical characteristics of the tumors.

		Nº	%
P53	Positive	15	78.9
	Negative	4	21.1
Mib1	<5%	12	63.1
	5-20%	3	15.8
	>20%	4	21.1
Bcl 6	Positive	8	42.9
	Negative	11	57.1
		Mean	Range
PDGFR$^{\alpha}$		0.24±0.4	0-1.6
PDGFR^{ß}		0.35±0.5	0-1.6

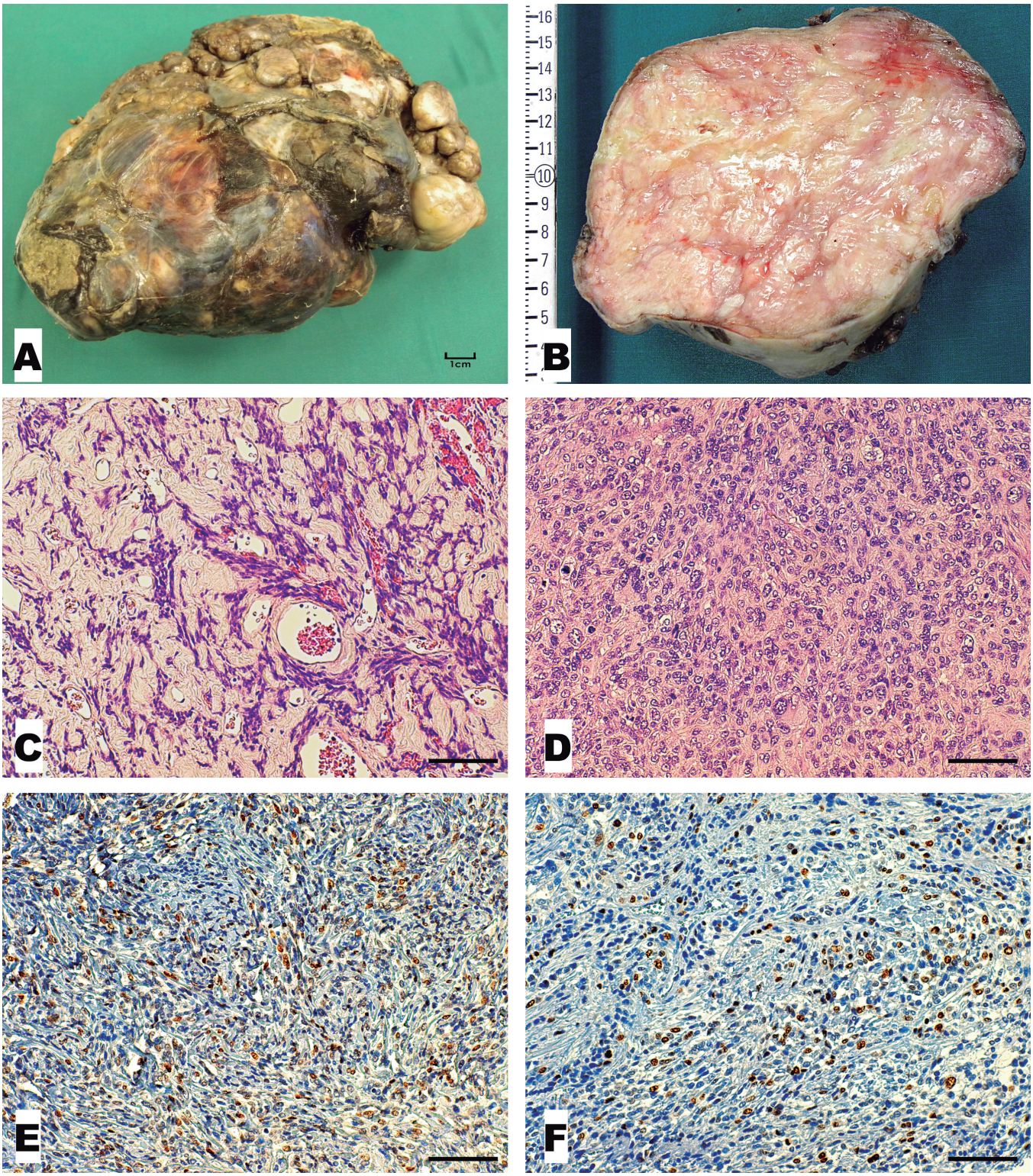

Fig. 1 A. External surface of a solitary pleural fibrous tumour (SPFT), a non-encapsulated well-circumscribed and consistently firm mass. **B.** Cut surface of a SPFT with a lobulated appearance. **C.** Histology of a benign. **D.** Histology of a malignant SPFT, displaying increased cellularity and atypical features (Hematoxilin-eosin). **E.** Malignant SPFT immunostained for mib1 (immunohistochemistry for mib1). **F.** High p53 index in a malignant SPFT (Immunohistochemistry for p53). Scale bar 50 μm.

and one benign case of relapse were operated and the patients are currently free of illness at one and four years after the second surgery, respectively.

The mean size of the relapsed tumours was 7.9±3.7 cm (range: 5-14). Most were sessile (3 out of 4) and multiple (4 out of 6), 2 cases having local and extrapleural tumors: one in the chest wall and the other in the abdominal wall. All but one were classified as stage 3 or 4 of de Perrot's classification. The surgical procedures in this group (4 cases) were more complex than in the first operation, requiring different degrees of lung resection and adjacent tissue removal to guarantee complete surgical excision of the lesions. The mortality of the second procedures was zero.

Morphological and immunohistochemical data (Table 2).

Macroscopically, most of the tumours were well-delimited, had a consistent firmness and were lobulated in section (Fig. 1a,b). 16 of the primary tumours and only 1 of the relapses were classified as histologically benign (England et al., 1989). The others, 3 primary tumours and 3 relapses, were classified as malignant (England et al., 1989) (Fig. 1d). 81% of the tumours were predominantly cellular and only in 2 cases was the stroma predominant (Zhang et al., 2004). The most frequent clinical picture was the "paternless pattern", observed in 14 specimens, followed by the haemangiopericytoma-like pattern (Fig. 1c).

All tumours displayed a homogeneous immunoexpression of CD34, Bcl2, vimentin and no expression of cytokeratin, CD99, calretinin or ALK. p53 showed a positive expression in 78.9% of the primary tumours (15 specimens) and in all relapses (Fig. 1f). Mib1 expression in the primary tumours was 0 (negative) in 12 cases, 1 (low) in 3 cases, and 2 (high) in 4 cases (Fig. 1e); while it was variable in the relapses.

PDGFR analysis in the group of primary tumours revealed that 7 patients had a negative Haura Index (HI) (Haura et al., 2005) for PDFGRα and 6 cases were HI-negative for PDFGRß. By contrast, 1 case of PDFGRα and 2 cases of PDFGRß had a high index of 1.6. The distribution of the values for PDFGRα had a mean of 0.24±0.4 (range 0-1.6) while for PDFGRß the mean was 0.35±0.5 (range: 0-1.6).

FISH and aCGH data

To identify sites of CNAs, aCGH was performed. In 37% of cases (7/19) we observed genomic changes. Losses were more frequent than gains (27 vs. 16). The most frequent losses were seen for 1p34.2 and 8p23.3 in 3/7 cases and 7q21 in 2/7 cases (Table 3).

According to the FISH analyses, 3 of 15 cases presented trisomy for LSI C-Myc in 40%, 30% and 30% of the cells. In most cases no aberrations were revealed by FISH on chromosomes 3, 7, 17, 9p21 and 22q11. Two cases showed trisomy 3 in 30% of cells and only one case showed + 7 (Fig. 2). We did not identify any patients with rearrangement on 4q12 or 5q31-q33. In two cases, the deletion of p53 was observed in 38% and 44% of the nuclei.

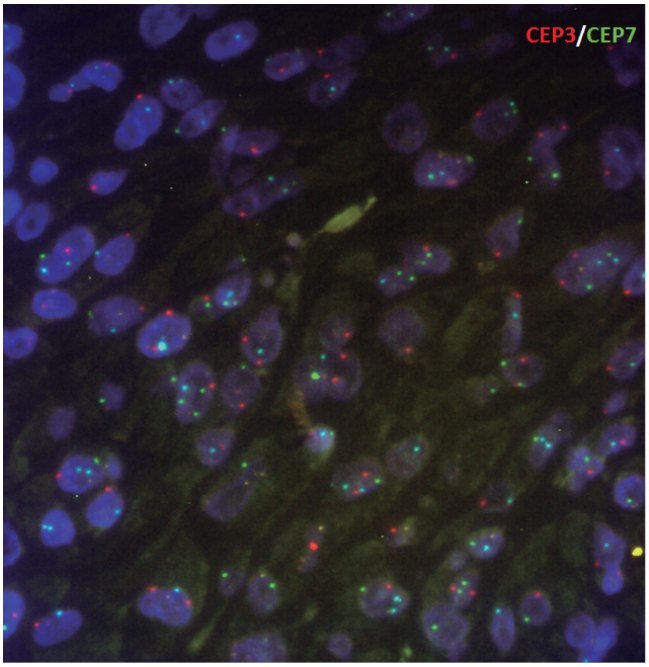

Fig. 2. Paraffin section interphase FISH. FISH performed on a paraffin-embedded tissue section of an SPFT with probes for the centromeric region of chromosome 3 CEP 3 (orange signals) and 7 (green signals). 10x1000

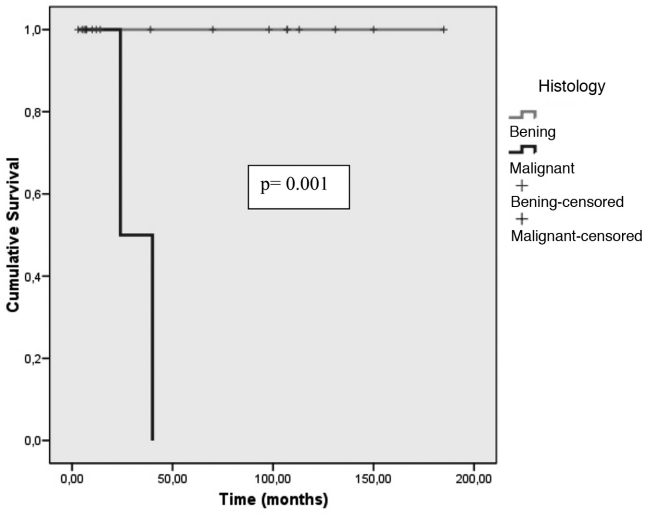

Fig. 3. Survival curves showing the influence of the diagnosis of malignancy of the primary tumour on tumour-related survival.

Survival data

At the time of the present analysis, only two patients had died of tumour progression; another one died of an unrelated cause; two are currently receiving chemotherapy treatment, and the rest are alive and free of illness. The global follow-up period of the series is 17 years and 3 months, and the mean follow-up is 59.4±58.4 range (3-185) months.

The median survival time of the series has not been reached yet, and the mean survival time is 155.7±18.6 months (95IC%: 119.1-192.3). The median disease-free interval of the cases that underwent a relapse is 17±14 months, in contrast to a disease-free interval of 120±54 months of the rest of the series. Histological classification as benign or malignant (p=0.001) (Figure

Table 3. Cases with genomic changes detected by aCGH.

Case number	Losses	Gains
1		
2		
3		
4	1p34.2	
5		
6		
7	1p13.3 7q21.11 7q33 8p23.3 16q22.3 19p13.2	2q32.1 9q21.33 10p14 15q25.1 15q26.1 18q22.3 22q12.3-q13.1
8	1p34.3-p34.2 2p25.1 2p24.1 2p11.2 2q11.1-q11.2 2q37.2 3p25.1 3p14.2 4q35.1 7q21.12-q21.13 13q14.2 14q31.1	2q37.3 11q12.1 13q12.11-q12.12 20q13.33
9		
10		
11		
12	4q28.3	
13	7p15.1 8p23.3-p23.1 15q25.2 17p12	3q25.3 4p11-q11 13q14.3 17p13.1 17q11-1-q11.2
14		
15		
16		
17	1p34.2	
18		
19	8p23.3 12q24.32	

Table 4. Binary logistic model for recurrence.

Variable	Wald	Significance	ß	ß95%CI
Parietal pleura implant	6.17	0.013	0.46	0.004-0.52
MIB1 expression	4.42	0.035	9.02	1.16-70.02

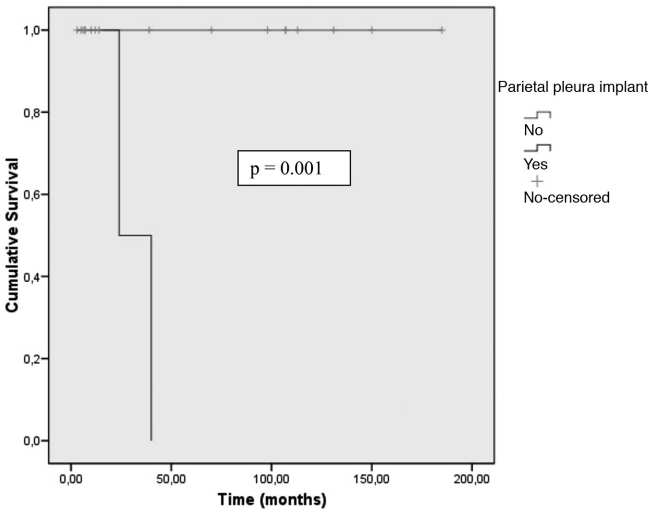

Fig. 4. Survival curves showing the influence of parietal implantation of the primary tumour on survival.

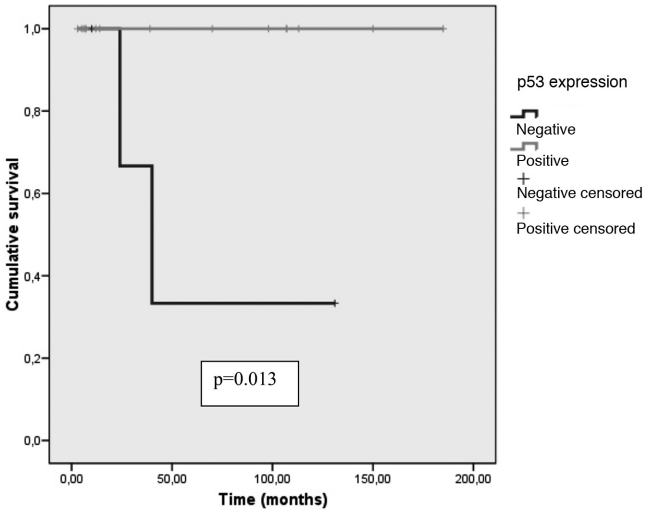

Fig. 5. Influence of p53 expression on cumulative survival. Survival is reduced in non-p53-expressing tumours.

3), parietal pleural implantation of the primary tumour (p=0.001) (Fig. 4), minimum/negative p53 expression (Fig. 5) and mib1 high expression (p=0.003) (Fig. 6) were significant factors for survival in the univariate analysis; these were not supported in the multivariate analysis owing to the small sample size and the short number of tumour-related deaths that had occurred.

No relationship was found between survival and the rest of the variables recorded: clinical, histological, immunohistochemical or other cytogenetic alterations.

Relationship to malignant evolution

All variables were tested again to attempt to find a common pattern for predicting a malignant evolution of the primary lesion, defined as the occurrence of relapse. In an individual analysis, the following variables showed a statistically significant relationship with recurrence: parietal pleural implantation of the primary tumour (χ^2=4.84; bilateral p=0.02; OR: 0.235 95%CI (0.1-0.554)) and high expression of mib1 (X^2=14.7; bilateral p=0.001; OR: 6 95%CI (1.03-35.9). In the binary logistic regression, both variables maintained their statistical significance (Table 4).

Even though we only found a statistical relationship of the probability of recurrence with the parietal position of the primary tumour and a high mib1 expression, when the cases were ordered according to the histology of the primary tumour and the final occurrence of relapse an almost homogeneous pattern appeared (Table 5). According to this table, it seems that patients with a benign primary tumour showing a medium-high mib1 expression, a high positive p53 expression and the presence of cytogenetic losses have a higher risk of recurrence as a group (χ^2=10.3; bilateral p=0.005; OR: 0.14 95%CI (0.040-0.515)).

Discussion

SPFT is an infrequent lesion and at our Hospital we have seen only 23 cases in the last 16 years. Consistent with reports in the literature, most of them were benign. However, the behaviour of these tumours is still difficult to predict, and hence, long-term follow-up is

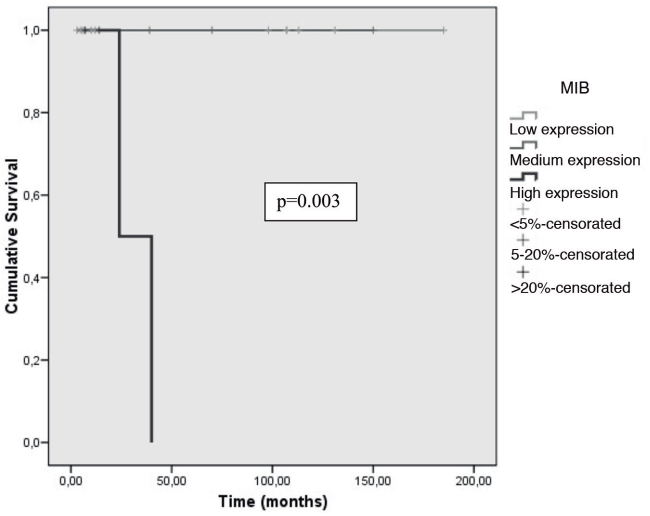

Fig. 6. Survival curves showing the influence of mib1 expression. Survival is decreased in high mib1-expressing cases.

Table 5. Tidied cases according to the primary tumour histology and the occurrence of relapse.

Case number	Histology of primary tumor	de Perrot's stage	Location	Histology of relapses	Gene Losses >0'6 MB	Mib 1 index	P53 index	Deaths
1	Benign	1	Visceral pl.	-	-	5%	20%	-
4	Benign	0	Visceral pl.	-	+	5%	5%	-
5	Benign	0	Visceral pl.	-	-	5%	50%	-
6	Benign	1	Visceral pl.	-	-	5%	60%	-
7	Benign	0	Visceral pl.	-	+	5%	50%	-
8	Benign	0	Visceral pl.	-	+	5%	60%	-
12	Benign	1	Visceral pl.	-	-	5%	50%	-
13	Benign	1	Visceral pl.	-	+	5%	5%	-
14	Benign	0	Mediastinum	-	-	5%	20%	-
16	Benign	0	Mediastinum	-	-	5%	30%	-
17	Benign	0	Visceral pl.	-	+	5%	20%	-
18	Benign	1	Visceral pl.	-	-	5%	25%	-
3	Benign	0	Visceral pl.	Benign	-	10%	30%	-
9	Benign	0	Mediastinum	Malignant	+	10%	50%	-
11	Benign	1	Multiple	Malignant	-	10%	50%	-
19	Benign	0	Parietal pl.	Malignant	+	20%	30%	-
2	Malignant	2	Parietal pl.	Malignant	-	20%	5%	+
10	Malignant	2	Parietal pl.	Malignant	-	40%	5%	+
15	Malignant	3	Visceral pl.	-	-	10%	20%	-

recommended (de Perrot et al., 2002; Schirosi et al., 2008).

In consonance with the literature reviewed (Swelam et al., 2009), we found that primary parietal pleural implantation of the tumour is a very negative factor for survival. In our results, this was one of the most significant prognostic factors since it revealed the statistically significant influence on survival and on the probability of recurrence. According to the data shown in table 5, the power of this variable is related to the two deceased malignant cases whose original tumours were in this position and to case number 19, in which the benign primary lesion recurred later on, in contrast to the rest of the series. Upon analyzing our series, we detected two distant metastases without local relapse, which is an uncommon finding. This has been addressed previously by Cardillo et al. (2012), who reported that the majority of relapses occur locally.

We consider that both the definition of the criteria of malignancy proposed by England et al. (1989) and the classification system of de Perrot et al. (de Perrot et al., 2002) are useful for pathologists and clinicians. In their review, Cardillo et al. (2012) found that sessile malignant tumours had a poor prognosis although long term survival was also possible. In our series most of the cases were benign, with a low de Perrot stage (Table 1). Although it was surprising that all the malignant first-resected tumours showed a low de Perrot stage, the medium survival time of the patients with those malignant tumours was only 24 months. The malignant potential may have masked any other possible prognostic factors.

The reviews performed by Schirosi et al. (2008) and Yokoi et al. (1998) suggested that a high mitotic index and an overexpression of p53 are markers of a poor prognosis, linking the latter with recurrence and invasion. In our cases, mib1 expression lay between 5 and 20% in the patients with primary benign tumours who relapsed (14.29%), and was higher than 20% in the cases in which the tumours were malignant and had the worst prognosis (23.81%) (Table 5).

In our series, p53 had a variable expression but it was clearly overexpressed in the cases with a primary benign histology who relapsed. In contrast, p53 was almost negative in the two already deceased malignant cases. These two cases showed a chromosomal deletion of p53 in 38 and 44 % of their nuclei. The presence of genetic losses in gliomas and lymphomas has been related to shorter survival (Camacho et al., 2001; Burton et al., 2002). This aggressive malignant behaviour explains the significance of this factor as regards survival (Fig. 3).

We were surprised by case number 3, which was a benign tumour with a benign relapse. We speculate that both lesions were present at the moment of surgery but because of its tiny size one of them went unnoticed.

In a complete analysis of 26 solitary fibrous tumours, Walters et al. (2011) found that 50% of their cases expressed bcl6 with no rearrangement or amplification in FISH analyses. In their series, positive bcl6 expression was significantly more frequent in malignant tumours as compared with benign or uncertain SPFTs. In our series, we found no significant relationship between bcl6 expression and a malignant histology.

The genetic background of SPFTs is poorly known. The data published about CGH and aCGH report chromosomal anomalies in 42-53% of cases (Krismann et al., 2000; Bertucci et al., 2013; Mohajeri et al., 2013). In our study we observed that the SPFTs showed genomic changes in 7/19 (38%) cases at the time of diagnosis. In 3/7 (43%) cases we identified two common deletion clusters in the 8p23.3 and 7q21.3 subbands, which are "hot deletion regions". Deletions of 8p23.3 and 7q21 have been reported previously in tumours such as colon, breast and urothelial carcinomas. This observation makes these areas strong candidates for tumour suppressor regions.

A loss on 8p23.3 has been reported in previous studies in colon, breast and urothelial carcinomas (Wood et al., 2007; Cooke et al., 2008; Williams et al., 2010), which makes it a strong candidate as a tumour suppressor region. The genes affected by the minimum common region of deletion, and therefore candidates, are CLN8, C8orf61, has-mir-596 and ARHGEF10. Functional data about ARHGEF10 (Rho guanine nucleotide exchange factor (GEF)) support a role for it in processes of carcinogenesis that are initiated by extracellular stimuli that work through G protein-coupled receptors. It activates RhoB, which is down-regulated in many tumour types (Mazieres et al., 2004) and is necessary for apoptosis in response to DNA damage in transformed cells (Cooke et al., 2008).

It has been proposed that the larger the tumour, the more frequent the CNAs, suggesting that genomic changes may promote tumour growth. Thus, CGH mainly reveals genomic changes in tumours larger than 10 cm (Abu Arab, 2012). In our study we found no tumour size-related DNA copy-number changes.

To our knowledge, only 9 of pleural SPFTs have been studied by conventional cytogenetics (England et al., 1989). These studies have shown that t (4;15) (q13;q26) and gains on chromosomes 8 and 21 were the most frequent anomalies. In our cases, chromosomal losses were more frequent than gains but, in agreement with previous findings, we found gains in 8. We failed to find any direct association between losses or multiple cytogenetic changes (> 0'6 MB) and survival or relapse, but the presence of cytogenetic losses in benign primary tumours seems to be a factor warranting regular follow-up.

According to CGH analyses the samples in our series showed no genomic changes detected by FISH of 4q12 and 5q, where the *PDFGRα* and *PDFGRβ* are located respectively, and these are target molecules for Imatinib (Rossi et al., 2006). The literature reports one case of an SPFT treated with this drug. Imatinib is a tyrosine kinase inhibitor that acts on mutations of exons

9 and 11 of the *c-kit* gene and also in no-D842V mutations of *PDGFRα* in patients with gastrointestinal stromal tumours (Rossi et al., 2006). It exerts a dual activity, blocking TGFß (transforming growth factor) and PDGFR receptors in TPFS *in vitro* and *in vivo* (Prunotto et al., 2009). More exhaustive knowledge of the cytogenetic alterations of these tumours would allow the use of drugs of proven effectiveness or the development of new target therapies.

The main limitation of our study is the sample size, which may contribute to a loss of statistical significance in multivariate tests. We have estimated that 259 patients in each study arm would be needed to adequately assess the influence of a high expression of mib1 on long-term survival with a power of 90% (α error=0.05, RR=3). Such sample sizes are hard to access given the low incidence of SFTPs, and hence a broader sample, which could be achieved through multicentre cooperation, would be needed to confirm or reject our univariate results.

Complete surgical removal is the treatment of choice, but many patients relapse, long-term follow up being essential. Indeed, there is a ten-year relapse in our series after the first surgery. Nonetheless, no individual patient displays the same behaviour and hence it is necessary to establish criteria to allow patients with a low risk of relapse to be distinguished from those who need constant monitoring. The classifications made until now, such as that of de Perrot et al. (2002) and that of Abu Arab et al. (2012), are based on macroscopic and microscopic criteria, and recommend long-term follow up, with different intervals, for all patients.

From our analysis, on considering primary benign tumours that have been completely excised we established two different groups featuring an opposite behaviour of the disease based on morphological criteria (location of implantation), on immunohistochemical data (high mib1 expression and high p53 expression) and on cytogenetic criteria (losses) (Table 5). Thus, patients with a single visceral pleural implantation, a low de Perrot stage, low mib1 immunoexpression, low p53 and no losses of 0.6 MB or more will need few check-ups after surgery. However, patients who show multiple or parietal pleural implantations, mib 1>10 %, high p53 expression and cytogenetic losses would need a more exhaustive follow up because of higher risk of recurrence and the poorer prognosis. All malignant cases need to be followed up closely, especially if p53 is negative, due to the aggressiveness of the tumour.

Conclusions

To conclude, according to the results of our studies primary tumour histology, parietal pleural implantation of the primary tumour, high mib1 expression and low p53 expression are statistically significant factors for survival. Additionally, we recommend a close follow-up of patients that present with a malignant primary tumour with a low p53 expression and we recommend a regular long-term follow-up for cases with completely excised benign primary tumours with a high mib1 index, high positive p53 expression and the deletion of 17p13. All these findings need to be confirmed in more extensive series.

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