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Chapter
Fibulin-3 as a Biomarker of Pleuric Involvement by Exposure to Fibers

Venerando Antonio Rapisarda and Caterina Ledda

Abstract

This chapter deals extensively with the role of Fibulin-3 (Fb-3) as early marker of malignant development, triggered by direct and long exposure to asbestos or asbestiform fibers. Asbestos has widely been used in many civic and industrial environments. Despite numerous countries, e.g., the European Union and the United States, have forbidden its production as well as utilization, still nowadays millions of tons of asbestos are manufactured worldwide. When inhaled, it causes the onset of malignant mesothelioma (MM) and several other types of cancer, including lung cancer. Health surveillance of subjects formerly exposed to asbestos is based on an early detection of major asbestos-related pathologies. However, the protocols adopted so far do not meet the sensitivity and specificity requirements needed to ensure an early diagnosis. Among the various eligible MM biomarkers, scientists have recently proposed Fb-3, which is a glycoprotein belonging to extracellular matrix proteins, coded through EFEMP-1 gene 2p 16 chromosome). Fb-3 is expressed by mesenchymal cells and plays a role in angiogenic processes as well-regulating cell-to-cell and cell-to-extracellular matrix communication. However, it is weakly expressed also in healthy tissues. Previous studies conducted on MM historically asbestos-exposed patients have shown, on several biological matrices such as serum and plasma, high Fb-3 concentrations. In the same way, high levels of circulating Fb-3 were observed in subjects exposed to a natural asbestiform fiber called fluoro-edenite (FE). Direct association between an increased Fb-3 expression and exposure to FE fibers has also been found in in-vitro and ex-vivo studies.

Keywords: fibulin-3, mesothelioma, asbestos, asbestos like fibers, biomarker

1. Introduction

Malignant mesothelioma (MM) is a malignant tumor originating from the mesothelial layer of the pleura, peritoneum, pericardium, and vaginal tunic and traditionally related to the exposure to asbestos fibers [1]. Asbestos includes different types of minerals: serpentine (chrysotile), and fibrous amphiboles cummingtonite-grunerite (amosite asbestos), actinolite, anthophyllite, riebeckite (crocidolite asbestos), anthracite, and tremolite. Such fibers represent an environmental health problem as chronic exposure to these minerals has been associated with respiratory diseases, including cancer. Additionally, exposure to several other types of mineral particles found in the natural environment and termed “naturally occurring asbestos” (NOA) such as fibers
of the minerals erionite, winchite, magnesio-riebeckite, Libby asbestos, richterite, antigorite, and fluoro-edenite (FE) have also been associated with MM [1, 2].

At present, MM is still considered a lethal cancer characterized by a considerable period of latency (≥30–60 years) and late diagnosis that determines bad prognosis and quality of life and unresponsiveness to presently available treatments [3]. To date, there are no diagnostic tools with high sensitivity and specificity that can be used to perform an early diagnosis of MM in asymptomatic people. Many biomarkers have been proposed for the screening and diagnosis of MM in exposed subjects [3–13]. Pathogenic mechanisms of lung illness were linked to the activation of different biomarkers including fibulin-3 (Fb-3) [3].

2. Fibulin-3 (Fb-3) gene expression

Fb-3 also known as Epidermal Growth Factor (EGF) Containing Fibulin Extracellular Matrix Protein-1 (EFEMP1) is an extracellular glycoprotein generally expressed in most tissues already in their embryonic phase. It is one of the seven proteins that belong to fibulinc family. Fibulins are characterized by EGF-like domain–couple calcium-binding-cb layout (epidermal growth factor) and a C-terminal fibulin type module. Fb-3 is codified by the EFEMP1 gene (also known as S1–5) present in chromosome 2p16. EFEMP1 contains 11 exons and codifies for a protein of 493 aminoacids with a 55 kDa molecular mass [14].

![Expression of Fb-3 in relation to the structures of the cellular matrix (by textbook of aging skin springer).](image-url)
The protein sequence contains a signaling peptide, five cbEGF domain couples preceded by a modified cbEGF domain and a fibulin-type C-terminal module. The modified cbEGF domain features an insert of 88 aminoacids. Under physiological conditions, Fb-3 is found in monomeric form. The recombinant Fb-3 shows a small shaft-like structure with a globule at one of its ends, which probably consists of the cbEGF modified domain [15].

Fb-3, like many other molecules that form the base membrane, has preserved itself best among the several species, keeping 92–94% of aminoacids identical in human, rats, and mice. During growth process, Fb-3 is expressed at mesenchyme level, especially in bone and cartilage structures.

In studies on Fb-3, the EFEMP1 gene was originally cloned by senescent fibroblasts taken from a subject with Werner Syndrome, a disease characterized by early aging, where an EFEMP1 mRNA overexpression can be observed. However, no mutation or fault in the EFEMP1 gene has been associated with Werner Syndrome or any other aging factors [16].

In adults, Fb-3 is largely distributed in various tissues, including the eyes. Particularly, a high expression of this glycoprotein can be observed in epithelial and endothelial cells, in their base membrane (see Figure 1) [1].

The latter play an essential role not only in structural or filtering functions, such as kidney glomerules, but also because they come into play in determining cell polarity and regulating cellular metabolic, proliferation, differentiation, and migration processes.

### 3. Fb-3 action mechanism

Fb-3 interacts with other base membrane proteins, such as extracellular matrix 1 protein (ECM1), the tissue inhibitor of metalloproteinase-3 (TIMP-3), endostatine (20 kDa C-terminal fragment of collagen XVIII), B hepatitis virus antigen X, tropoelastine (elastine monomeric subunit), etc. These interactions are likely to contribute to maintaining the base membrane integrity and anchoring other ECM structures, e.g., elastic fibers [17].

Fb-3 stimulates TIMP-1 and TIMP-3 expressions, but it inhibits expression and activities of (MMP)-2, MMP-3 and MMP-9 matrix metalloproteinases. It is associated with thinner elastic fibers, whereas it is not found in bigger elastic structures such as the aortic elastic lamina. Experimental studies have highlighted that EFEMP1 knockout rats show an early aging process and develop multiple tissue hernias, among which inguinal hernias, pelvic prolapse, and xiphoid process protrusions. In these guinea pigs, small-size elastic fibers of the connective tissue, including those of small blood vessel adventitia and vaginal tunics, are reduced both in size and resistance. A disgregation or a reduction of the elastic fibers in such tissues is probably responsible for phenotypes suffering from early aging and multiple hernias observed in knockout rats for EFEMP1.

Besides its role in maintaining ECM, Fb-3 also seems to have signaling functions. Indeed, Fb-3, by interacting with DA41, a protein, which binds the onco-suppressor DAN gene, can trigger DNA synthesis. The expression of EFEMP1 is thought to have a role in cell proliferation and tissue growth processes [18].

Inactivation of EFEMP1, through promoter methylation, is associated with lung and breast cancers. EFEMP1 undergoes a down-regulation in 60% of breast cancer cases and the promoter methylation of the stimulator just seems to be the main reason for this reduced expression. Analysis of
primary primitive, clinically well-characterized breast cancers has revealed a significant correlation between a reduced EFEMP1 expression and a reduction of the time span free from illness and of survival, generally. In the light of this evidence, one can assume that EFEMP1 might be used as molecular marker in lung and breast cancers.

An alteration of Fb-3, as an element of the base membrane, would seem to play an important role in tumor metastatic phenomena. Fb-3 would also seem to have a triggering action in cellular proliferation and migration processes. However, both the pathophysiological role of Fb-3 in base membranes and how the alteration and/or function failure of this protein may/may not have a part in causing pathologies are still to be ascertained.

4. Lab procedures to determine Fb-3

4.1 Fb-evaluation from tissues

Immunohistochemistry (IHC) is a laboratory technique, which enables to highlight the creation of antigen–antibody complexes inside a tissue.

Such diagnosis technique exploits the ability of some antibodies to recognize cellular proteins (like Fb-3), called antigens, which in tumoral cells may have expression characteristics (more or less apparent) other than those of ordinary cells. The sample, after formalin paraffin fixation and inclusion, is prepared for the immunohistochemical exam first by de-paraffining the sections, then remoisturizing them, and finally submitting them to antigenic unmasking. The sample is then incubated with the primary antibody and then with a biotine-streptavidina-kit detection system. To visualize the immunoreaction, Diaminobenzidine (DAB) is used as chromogen, which highlights the immunolabeling in brown. Densimetric and morphometric analyses of Fb-3 are obtained through optical microscope and image analysis software reading, in order to assess density in pixels (% of unit density) and the percentage of pixel immuno-labeled areas of the above quoted protein (see Figure 2) [2].

4.2 Assessment of Fb-3 from serum, plasma and other biological liquids

ELISA stands for Enzyme Linked Immuno Sorbent Assay. It is an immunological analysis technique used to assess any evidence of a particular antigen in a sample.

ELISA combines the specificity of the antigen–antibody reaction (immunological reaction) with the sensibility of a simple enzyme spectrophotometric dosage (see Figure 3).

Such technique is based on the assumption that, with adequate procedures, it is possible to conjugate the antibodies of a serum with some enzymes (peroxidase, alcalin phosphatase, beta-galactosidase) without altering their property to combine with the correspondent antigens. The enzyme used can catalyze a reaction on a suitable substratum with the formation of a colored terminal product, which allows highlighting the quantity of the antigen. In commercial formats, reactions are usually carried out inside polyvinyl or polystyrene wells (12 strip microplates with 8 wells each for a total of 96 wells) on which specific antibodies are attached for the antigen of interest or the antigen itself. The samples to analyze (plasma, serum, pleural liquid, broncho-aspirate, bronchoalveolar lavage, etc.) as well as reagents with interspersed
Figure 2. IHC determination of Fb-3 in lung tissues exposed to fluoro-edenite (FE). A–F: Sections of exposed lung tissue in which Fb-3 immunoeexpression was detected in intraparenchymal stroma around bronchioles, bronchiolar epithelium, interstitium between alveoli, alveolar epithelium, and macrophages. A1-F1: Image analysis by software in which an evident both high (red color) and low (green color) immunostaining was detected in exposed lung. A-F, original magnification 20x; scale bar: 100 μm.

Figure 3. Schematic drawing showing the antigen–antibody reaction (immunological reaction).
lavages needed to remove any excess are incubated inside these wells. Lastly, the substratum is added, which generates the colored product.

Positivity is assessed analyzing occurrence or not of the color, following the reaction catalyzed by the enzyme on the substratum. Immunoenzymatic technique can be used for researching both antigens and antibodies and lends itself to several variations for numerous applications likewise.

5. Fb-3 as biomarker in asbestos-related pathologies

One of the earliest studies involving the role of Fb-3 in cancer was carried out in 2009 in the United States on gliomas [19]. Following some preliminary investigations, the authors had hypothesized that gliomas’ local invasiveness could be caused by an interaction between mesenchymal proteins and some specific neural matrix proteins. In fact, unlike other central neuro system neoplasms (CNS), characterized by an expansive growth with shifting and compression of the surrounding parenchyma, gliomas show an infiltration type of growth. The extracellular matrix in the CNS normally contains high quantities of ialuronic acid and negatively charged proteoglycans, but low quantities of fibrillar proteins, which may support cellular adherence and mobility. In an attempt to identify the humoral signs, which could contribute to gliomas’ peculiar invasiveness (probably due to an altered relationship between cells and extracellular matrix), researchers have stimulated tumoral cells in-vitro, combining mesenchymal elements (fibronectine) with others, specific of the neural matrix (brevican) and, by using a micro-array, examined which genes came out overexpressed. Results showed a remarkable rise of Fb-3 expression in the glioma.

Successive studies about the role of Fb-3 showed mixed results: some observed an antagonist effect toward tumoral angiogenesis (reducing so its aggressiveness); others, as in the case of pancreatic adenocarcinomas and gliomas, observed Fb-3 expression rise associated with an increased vascular growth factor (VEGF) and tumoral growth. Table 1 reports some studies on Fb-3 and of different kind of cancers.

A research of 2011 on colorectal cancer detected an Fb-3 downregulation in the cancerous tissues compared with the adjacent healthy ones. Furthermore, the Fb-3 downregulation negatively correlated with the prognosis, tumor stage, lympho-node metastasis, and reduced time gaps free from the illness.

In intestinal tumors, Fb-3 downregulation seems then to show a worsened prognosis due to a reduced anti-angiogenic action.

In the light of what has been said, Fb-3 is thought to play, depending on the tumor type, a pro-angiogenic role (gliomas, cervix carcinoma) or an anti-angiogenic one (colon carcinoma). This apparent paradox may derive from a different behavior of this glycoprotein in relation to factors such as tissue histological characteristics and tumor micro-environment. The Fb-3 bond with TIMP-3 might interfere with that between VEGF to its type 2 receptor (VEGFR-2), causing the inhibition of tumoral angiogenesis. Besides, it has been observed that Fb-3 is able to competitively link the EGF receptor (EGFR) compared with EGF, so activating intra-cellular pathways (MAPK, Akt) in pancreatic adenocarcinoma. Finally, a few studies have concluded that the
EFEMP1 gene activation, due to the hypermethylation of its promoter, also occurs in several cancer types (lung, prostatic, colorectal, nasopharyngeal, and hepatocellular).

These data seem to point out that a reduced expression of this gene may be involved in carcinogenic and/or tumor growth processes. It seems however evident how the exact role of Fb-3 in tumoral growth still remains to be clarified and needs further research.

Table 1.
Studies exploring Fb-3 in relation to cancer type.
Recent studies on the pathophysiological role of Fb-3 in malignant mesothelioma (MM) are also taking into account this glycoprotein as a possible marker for early diagnosis and/or pathology follow-up.

This research falls within a larger assessment context of potential biomarkers in MM early diagnosis.

MM is a fatal tumor, with a long latency and aspecific symptoms, which often end up in a late diagnosis. MM is causally correlated with exposure to asbestos or asbestiform fibers. MM cases worldwide are definitely increasing. In Italy, an incidence peak is expected within 2025 [31, 32].

Actually 25% of MM is caused by professional exposure, 25% through indirect exposure of family members, and 50% from exposure to fibers in the surrounding environment [33].

MM patients survive averagely 6–18 months from diagnosis. However, it has been noticed that, if an early diagnosis is made, survival may even go beyond 5 years. Unfortunately, today there are still no effective prevention systems and screening procedures for this pathology [31–34].

Periodical X-ray exams have always been hard to do, due to: long latency of the disease (14–45 years); limited resolution of present techniques, especially for lesions at an early stage; exposure to ionizing radiations (justification principle). It is then clear how finding humoral biomarkers with high sensitivity and specificity might significantly enhance the prognosis of this disease.

Scientific debate on the eligible molecules has been going on for long, with no definite results. Indeed, none of the biomarkers studied seems to meet the requirements needed [35, 36].

The first study to propose using Fb-3 as a possible MM biomarker was conducted by Pass et al. [3]. The intent of the study was to analyze Fb-3 reliability compared with mesothelin, a protein already thoroughly studied as a biomarker, which had however shown no adequate sensitivity (47%) in recognizing MM cases. Plasma and effusion samples from patients with pleural MM, plasma samples from persons who had been exposed to asbestos but did not have MM, and plasma and effusion samples from patients with pleural effusions not due to MM were analyzed.

In this study performed on MM patients, sampling was carried out in the United States, at the Wayne State University, from 1998 to 2005, and at New York University Langone Medical Center, from 2005 to 2011, the “Detroit Cohort” and the “New York Cohort,” respectively.

The study also assessed patients with other neoplasms, in order to improve Fb-3 specificity. Altogether, 20 ovarian cancer, 20 glioblastoma, and 31 prostatic carcinoma patients were evaluated. Furthermore, 43 healthy subjects were used as control group (selection criteria included absence of previous exposure to asbestos and other neoplastic pathologies).

In conclusion, plasma Fb-3 levels can distinguish healthy persons with exposure to asbestos from patients with MM. In conjunction with effusion Fb-3 levels, plasma Fb-3 levels can further differentiate MM effusions from other malignant and benign effusions [3].

On the whole, there were 11 studies dealing with concentrations of Fb-3 in human beings, and they were performed on: MM tumoral tissue; pleural exudate; serum and plasma (see Table 2).

The surveys on MM patients’ tumoral tissues were conducted by Pass et al. [3] and Caltabiano et al. [6].
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In Pass et al. [3], the immunohistochemical analysis enabled to give a score to the nuclear as well as to the citoplasmatic positivity, taking into account both the number of positive cells and the positivity intensity. The authors detected Fb-3 nuclear and citoplasmatic expression in 100% of MM samples (26/26); the scores for intensity were similar both for the epithelial subtype, and the sarcomatoid and the sarcomatoid/epithelial mixed variant ones.

Comparison among the epithelial-histological, the epithelial-biphasic-histological and the sarcomatoid subtypes showed similar scores as far as the coloring intensity was concerned (mean score 7.7 ± 0.6 and 6.9 ± 0.8, respectively P = 0.87); and so did it with purely sarcomatoid- histological subtypes (6.6 ± 1.1; P = 0.62). The total coloring score (nuclear and citoplasmatic) turned out constantly higher in MM samples than in those detected in other pleural neoplastic forms (7.4 ± 0.5 vs. 2.4 ± 0.8; P < 0.001).

In Caltabiano et al. [6], Fb-3 immunohistochemical expression was assessed on tumoral tissues of six MM patients, previously exposed to fluoroedenite (FE); a natural, asbestiform fiber discovered in lava rock stone used as construction material in Biancavilla’s municipality, on the slopes of Mount Etna.

Outcomes showed immunoeexpression similar in the epithelial histological subtypes (three cases) and in the epithelial biphasic histological and sarcomatoid subtype (three cases) (see Figure 4).

The analysis of Fb-3 concentration in the pleural exudate was carried out in four studies: Pass et al. [3]; Creaney et al., [12]; Agha et al. [37]; Battolla et al. [8].

<table>
<thead>
<tr>
<th>Authors</th>
<th>Explored matrix</th>
<th>Patient’s pathology/exposure (n.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agha et al. [37]</td>
<td>Pleural exude and plasma</td>
<td>MM (25), pleural exude by no-MM neoplastic pathologies (11); benign pleura lesions (9).</td>
</tr>
<tr>
<td>Battolla et al. [8]</td>
<td>Pleural exude</td>
<td>MM (33); pleural exude by no-MM neoplastic pathologies (23); benign pleura lesions (64).</td>
</tr>
<tr>
<td>Creaney et al. [12]</td>
<td>Pleural exude and plasma</td>
<td>MM (82); pleural exude by no-MM neoplastic pathologies (36); benign pleura lesions (35).</td>
</tr>
<tr>
<td>Corradi et al. [13]</td>
<td>Plasma</td>
<td>MM (50); lung cancer (77); benign lung lesions (16); healthy control (66).</td>
</tr>
<tr>
<td>Demir et al. [36]</td>
<td>Serum</td>
<td>MM (42); healthy control not exposure to asbestos (48); healthy control exposure to asbestos (48);</td>
</tr>
<tr>
<td>Hassan et al. [5]</td>
<td>Serum</td>
<td>MM (45); lung cancer (63); benign lung lesions (63); benign pleura lesions (48); healthy control (60).</td>
</tr>
<tr>
<td>Jiang et al. [7]</td>
<td>Plasma</td>
<td>MM (15); benign lung lesions (29); benign pleura lesions (74); exposed to asbestos with no lesions (218); healthy control (94).</td>
</tr>
<tr>
<td>Kaya et al. [11]</td>
<td>Serum</td>
<td>MM (43); healthy control (40).</td>
</tr>
<tr>
<td>Kirschner et al. [10]</td>
<td>Plasma</td>
<td>MM (114); no MM cancers (37); benign pleura lesions (45); cardiac pathologies (34).</td>
</tr>
<tr>
<td>Napolitano et al. [9]</td>
<td>Plasma</td>
<td>MM (22); pleural exude by no-MM neoplastic pathologies (25); benign pleura lesions (33); healthy control (20).</td>
</tr>
<tr>
<td>Pass et al. [3]</td>
<td>Pleural exude and plasma</td>
<td>MM (92); exposed to asbestos with no lesions (136); benign pleura lesions (93); healthy control (43).</td>
</tr>
</tbody>
</table>

Table 2.
Studies exploring Fb-3 in pleural fluids and peripheral blood.
Pass et al. [3] observed significantly higher concentrations of Fb-3 in MM subjects’ pleural exudate than those detected in benign exudates or derived from other neoplasms. Therefore, the Fb-3 concentration allowed to tell MM subjects from all the others (area below the curve-AUC = 0.93), both in benign (AUC = 0.93) and in malignant pathologies (AUC = 0.94). Moreover, Fb-3 levels did not significantly differ between those patients (n = 22) who had received presurgery chemotherapy and those (n = 52) who had not (671.4 ± 72.5 vs 703.6 ± 42.6 ng/ml).

Fb-3 significantly correlated with the progress of the disease and made it possible to distinguish those patients (n = 54) who underwent citoreductive surgery in stage I-II (n = 21), from those (n = 33) with III-IV stage disease (576 ± 67 ng/ml vs. 765 ± 55 ng/ml, P = 0.04).

An Fb-3 = 733.4 ng/ml cutoff, measured at the time of surgery in all subjects (n = 69), correlated in an inverse proportional way with patients’ survival.

Creaney et al. [12] detected Fb-3 values and mesotheline in the pleural exudate of 153 patients: 82 had MM, 36 had pleural exudate caused by other neoplastic pathologies; 35 had benign exudates. The MM patients’ samples were collected within a month from diagnosis, prior to any kind of treatment. Fb-3 levels ranged between 17 and 5748 ng/ml. No significant difference was detected in Fb-3 levels among the three groups under exam; in detail, 63% of benign exudate samples exceeded the 346 ng/ml cutoff. Statistical analysis showed no difference in Fb-3 levels according to the pleural liquid protein composition (exudate or drained) and/or with blood.

Exudates coming from MM patients with biphasic or sarcomatoid histology showed significantly higher levels of Fb-3 (1331, range 538–2486 ng/ml) than the epithelial ones (426, range 171–1709 ng/ml; P = 0.018), also in those patients who had
had a cytological-based diagnosis (298, range 155–881 ng/ml; P = 0.002). No significant difference was observed in Fb-3 levels according to the stage of the disease. Altogether, the Fb-3 study results in the pleural exudate showed a 59% sensitivity and a 52% specificity, considering 346 ng/ml as threshold. The 0.588 AUC enabled to tell MM patients from all the others.

As regards mesothelin levels, they were remarkably higher in MM patients than in those with benign exudates (P < 0.001) and in others with exudates caused by other neoplasms (P < 0.001).

Creeaney and colleagues concluded that mesothelin gave out a 58% sensitivity and a 96% specificity, as well as a better diagnostic accuracy, compared with Fb-3 in pleural exudates of MM patients.

A study carried out by Agha et al. [37] analyzed 45 patients with pleural exudate, of whom: 25 MM cases, 11 secondary pleural metastases (3 cases of not-small-cell lung cancer, 2 breast cancers, 3 colon cancers, 1 case of kidney cancer, and 2 cases of lymphoma), and 9 patients with benign origin pleural exudates (5 tuberculosis, 1 pneumonia, and 3 pleurisy). MM patients showed significantly higher Fb-3 levels (331 ± 32.64 ng/ml) than those with pleural exudate derived from secondary metastases (153.01 ± 60.32 ng/ml). The difference between these parameters turned out to be statistically significant (P < 0.001).

The results highlighted that with a 150 ng/ml cut-off (AUC = 0.878; 72.3% sensitivity, 80% specificity), it was possible to tell MM patients from those with pleural metastatic pathology.

Besides, exploiting a 127.5 ng/ml cut-off (AUC = 0.909; sensitivity 88%, specificity 77.8%) it was possible to distinguish MM from the pleural benign exudate.

In a recent study, Battolla et al. compared Fb-3 and mesothelin levels in MM patients’ pleural liquid with that obtained from patients with pleural pathologies, both benign and malignant, other than MM. 120 subjects underwent thoracentesis between 2008 and 2011. Among these, 33 had MM, 64 had benign pleura lesions and 23 secondary pleural metastases. Fb-3 and mesothelin concentrations were assessed in ELISA. Results showed Fb-3 levels substantially similar in all subjects (P = 0.174), whereas mesothelin levels were significantly higher in MM subjects than others (P = 0.001).

The analysis of Fb-3 concentration in peripheral blood was conducted in seven surveys on plasma and three on serum.

In Pass et al’s study (2012), Fb-3 plasma values were assessed. The study sample included: 92 MM patients; 136 exposed to asbestos with no cancer; 93 patients with nonrelated MM pleural exudate; 43 healthy subjects as control group. The study was carried out in two separate cohorts: “Detroit cohort” and “New York cohort.”

Outcomes highlighted that Fb-3 average plasma levels enabled to significantly distinguish asbestos-exposed subjects from those with nonrelated MM exudate and from MM ones, in both cohorts. Fb-3 concentrations in MM “Detroit cohort” patients were similar to those of the “New York cohort” (105.0 ± 7.1 vs. 112.9 ± 7.6 ng/ml; P = 0.63). Fb-3 plasma levels did not significantly differ between the 44 MM patients, who had had presurgery chemotherapy and the 48 who had not (117.9 ± 8.1 vs. 101.1 ± 6.9 ng/ml; P = 0.12).

Fb-3 plasma level allowed to tell MM patients from those affected from other cancers or even those with pleural exudate (not MM-related), both benign and malignant. Finally, comparing the 28 patients at stage I-II of MM with the asbestos-exposed ones, with AUC = 0.99 and cutoff = 46.0 ng/ml, a 100% sensitivity [95% IC,
87.7–100] and a 94.1% specificity [95% IC, from 88.7 to 97.4] were reached. Fb-3 levels of MM patients went down after surgery in 100% of cases (18 out of 18).

Contrary to expectations, the authors found poor correlation between Fb-3 levels found in plasma samples and those detected in the pleural exudate of each MM patient (n = 17) (P = 0.98), as well as in the plasma and pleural exudate of 15 patients who had not MM-related exudate (P = 0.27).

Among the conclusions, the authors suggested using plasma samples instead of serum ones so as to assess Fb-3 blood levels, since the presence of two potential trombine cleavage sites could compromise the validity of the exam. Despite the encouraging results obtained by Pass's survey, further experiments gave out mixed outcomes.

A cohort of 153 patients (of whom 82 having MM) was investigated by Creaney et al., reporting a 22% sensitivity and a 95% specificity for plasma Fb-3 (cutoff = 52 ng/ml, AUC = 0.671). These values were definitely lower than those obtained, with the same patients for mesothelin (sensitivity 56%; specificity 95% — AUC = 0.816), which on the contrary seems to have a decisively better diagnostic accuracy on plasma samples. Although in this study mesothelin resulted superior to Fb-3 as to its diagnostic worth, the authors considered the latter superior from a prognostic point of view. Indeed, Fb-3 high levels correlated negatively with the patient’s prognosis. A possible explanation of this might depend on an Fb-3 higher expression by biphasic and sarcomatoid histotypes, which are generally characterized by a worse prognosis. Instead, mesothelin is mainly expressed by the epithelial histotype, with a better prognosis.

An Egyptian study [37] conducted on a small cohort of 45 subjects reported a 100% sensitivity and a 78% specificity in differentiating MM cases (n = 25) from nonmalignant pleural pathologies (n = 9), and an 88% sensitivity and 82% specificity in distinguishing MM from other forms of pleural cancer (n = 11). It is necessary, though, to point out that the authors, when evaluating Fb-3, used a nonspecified test and internally agreed cutoffs.

Corradi et al. assessed the concentration of Fb-3 and other protein biomarkers in the serum of four groups of patients: subjects previously exposed to asbestos and suffering from asbestosis; patients with MM; patients with not-small-cell lung carcinoma (NSCLC) and a control group, which showed no evidence of neoplastic pathologies. The results highlighted higher levels of Fb-3 in MM patients than the NSCLC group (P < 0.01) and the control (P < 0.05). However, Fb-3 values in MM patients did not significantly differ from those of subjects with asbestosis. The small number of patients in the study is the main weakness of these results.

A prospective survey carried out by Kaya et al. [11] examined 43 MM patients (primary involvement: 39 pleural, 4 peritoneal mesothelioma) and 40 controls. Results showed Fb-3 serum levels equal to 90.3 ± 42.1 and 17.8 ± 12.7 ng/ml, respectively (P < 0.001). A 36.6 ng/ml cutoff indicated a 93% sensitivity and a 90% specificity.

Napolitano et al. [9] analyzed levels of high mobility group box protein 1 (HMGB1) and Fb-3 in blood samples of 22 MM subjects, 20 others with documented exposure to asbestos, 13 with benign pleural exudate, 25 with malignant exudate (other than MM) and 20 controls. The authors concluded that the combination of HMGB1 and Fb-3 provided higher sensitivity and specificity in differentiating MM patients from others with benign or malignant pleural pathologies.

MM etiology usually involves professional and/or environmental exposure to asbestos. In an attempt to spot any possible differences among the MM types derived after environmental exposure, compared to the more frequently documented professional one, Demir et al. recruited a cohort of MM patients (n = 42) derived after
environmental exposure to asbestos and compared them with two control groups: the former composed of healthy individuals (n = 48) who had no previous, documented exposure to asbestos, with a normal chest X-ray exam; the latter, (n = 48) composed of subjects with documented environmental exposure to asbestos for at least 15 years, who showed no X-ray documented pleural plaques. The authors detected significantly higher values of Fb-3 in MM patients’ serum than in those who were just exposed to asbestos and the nonexposed control group.

Several investigations conducted by the working group (Caltabiano, Ledda and Loreto) directed by Rapisarda et al. [1, 6, 38, 39] analyzed the role of Fb-3 as biomarker in workers exposed to FE. Fb-3 plasma concentrations were measured in the blood of the FE-exposed workers and in a control group (non-exposed). In 52% of exposed subjects pleural plaques were detected. Fb-3 plasma concentrations resulted 12.96 e 5.29 ng/ml, respectively, in the exposed subjects compared to the control (P < 0.001).

The results highlighted a high predictive value of Fb-3 plasma levels in relation to the presence of pleural plaques.

Another survey revealed an Fb-3 increased expression in human mesothelial cells after exposure to FE. Moreover, the Fb-3 levels in the peripheral blood of 40 workers exposed to asbestos were analyzed and compared with those of professionally FE-exposed ones.

Also in this case, results showed Fb-3 higher levels in the FE-exposed group with pleural plaques than in those asbestos-exposed workers who did not show any pleural and/or parenchymal lesions.

At the same time, FE fibers were used to stimulate mesothelial cell cultures. Results showed an Fb-3 hyper-expression after exposure to FE even at low concentrations (see Figure 5).

6. Comparative analysis of Fb-3 with other biomarkers

As underlined afore, many surveys focused on researching new MM biomarkers. The reasons of such interest from the scientific community are not purely academic; in fact, even though in several Western countries exposure to asbestos seems to be confined to few professional contexts, in developing countries such as India and China asbestos is still extracted and exploited. For such reason MM continues to be a recurrent disease nowadays, also by reason of a few high-susceptibility population subgroups. About that, it has been demonstrated that hereditary mutations borne by the gene BRCA-associated protein 1 (BAP1) predispose for a higher incidence of some cancers, among which MM.
Several biomarkers have been proposed for diagnosing MM, among them metabolites, proteins and microRNAs (miRNAs). An ideal biomarker ought to spot selectively MM patients from those with other pathologies and/or asbestos-exposed subjects from non-exposed ones. With a view to an early diagnosis and implementation of surveillance programs, the ideal biomarker detection sample would be the blood, for its low invasiveness and better compliance; the pleural liquid would be less ideal, as it requires a more invasive collection technique.

Presently, mesothelin is MM’s only and most extensively studied biomarker, recognized by the American Food and Drug Administration (FDA) and by some EU countries. It is a protein precursor with a molecular weight of 71 kDa from whose cleavage, the *megakaryocyte potentiating factor* (MPF), which is then secreted into the blood and the *glycosylated phosphatidylinositol-linked glycoprotein*, a membrane protein, are originated. Physiologically, mesothelin is expressed at a low grade in mesothelial cells and almost in no way in other tissues; its overexpression is instead observed in several forms of cancer, such as MM, ovarian, lung cancer and pancreatic adenocarcinoma.

Some surveys highlighted the capability to identify MM patients through dosage of SMRPs (*soluble mesothelin-related peptides*) in the serum, getting a 60–90% sensitivity and an 80–85% specificity, as well managing to distinguish between MM subjects, asbestos-exposed ones, nonexposed subjects, and others with benign pleural pathologies. Other experiments showed the possibility to differentiate, through SMRPs serum values, MM patients from those with pleural secondary metastases. Studies on humans assessed SMRPs’ dosage in the pleural exudate (PE-SMRPs), reporting higher sensitivity values than those serum-obtained in spotting MM patients. A metanalysis compared data coming from 12 studies, basing itself on a total of 717 subjects suffering from mesothelioma and 2851 controls, among whom were healthy subjects as well as others with pleural pathologies. The results the authors reached showed an overall sensitivity of 64% and a specificity of 89% for the SMRPs measured in the serum.

It seems clear how mesothelin is the main term of comparison to ascertain Fb-3 effectiveness and its possible introduction in MM clinical routine.

A survey conducted by Creaney et al. [12] compared Fb-3 and mesothelin values in the plasma and pleural liquid of 202 subjects. The population examined included MM patients (n = 82), patients with benign asbestos-related diseases (n = 49), subjects with malignant exudate (n = 36), and others with benign exudate [35]. The authors underlined an enhanced diagnostic accuracy of mesothelin compared to Fb-3, both in plasma (AUC = 0.822 vs. 0.671) and in the pleural liquid (AUC = 0.815 vs. 0.588). However, the Fb-3 concentration in the pleural liquid turned out to be a predictive factor for the patient’s survival. MM subjects with Fb-3 lower levels in the pleural liquid than the average had significantly longer survival times than those with levels above the average (14.1 vs. 7.9 months). Mesothelin values and other parameters like neutrophil/lymphocyte ratio did not appear significantly correlated with the patients’ prognosis.

In a survey by Battolla et al., Fb-3 and SMRPs’ levels were contextually evaluated in pleural exudate of patients suffering from MM (n = 33), benign pleural lesions (n = 64) and secondary pleural metasteses (n = 23). Samples were analyzed by ELISA, and revealed Fb-3 values similar among MM subjects and the rest of the cohort (geometric mean = 68.1 vs. 66.2 ng/ml; P = 0.872) and significantly increased values of SMRPs in MM patients compared with the rest of the group (geometric mean = 14.6 vs. 3.2 nM; P < 0.001).
A survey conducted by Napolitano et al. compared HMGB1 values with mesothelin, Fb-3, and osteopontin (OPN) in the blood. The survey population included: a cohort of subjects who had been treated for pleural exudate derived from benign pathologies (n = 13), from MM (n = 22), and other malignant diseases (n = 25); a group of historically asbestos-exposed workers (n = 20); a group of healthy subjects with no documented exposure to asbestos (n = 20). The results of the study revealed that Fb-3 was the molecule with the highest sensitivity in telling MM subjects from those with other pleural pathologies, followed by HMGB1 hyper-acetylated form, by mesothelin and OPN. The authors concluded that the best diagnostic performance could be obtained combining HMGB1 and Fb-3 values.

Generally speaking, most studies in the literature report a better sensitivity of mesothelin compared with Fb-3, both in plasma and pleural liquid. Instead, comparative studies between Fb-3 and other potential MM biomarkers such as OPN and miRNAs are still missing.

7. Conclusions

MM is a fatal form of cancer derived from pleural mesothelial cells. Its etiology usually involves professional and/or environmental exposure to asbestos. Unluckily, early symptoms of this pathology are commonly nonspecific, and this generally entails a diagnosis of the disease at an advanced stage. There are several studies in literature dealing with potential biomarkers for MM early diagnosis and its differentiation from secondary pleural metastases, benign exudative forms and pleural plaques typical of subjects previously exposed to asbestos. If one considers what said so far, it appears clear that the use of reliable biomarkers (sensitive and specific) might be decisive for MM patients’ diagnosis, lengthening their life expectations.

The results of the various studies suggest that Fb3 may have a role in developing neoplastic as well as non-neoplastic diseases of the respiratory tract in subjects exposed to asbestos and/or asbestiform fibers. Moreover, some surveys are looking into the hypothesis that Fb-3 might be accounted for the malignant mutation of mesothelial cells after exposure to asbestos fibers.

In fact, chronic inflammation may induce cancer through the production of several cytokines and growth factors, which, as a consequence, may cause the apoptosis and cell proliferation process to alter. About this, it has been observed that p27, an onco-suppressor gene, often deactivated in tumors, gets downregulated in mesothelial cells after exposure to asbestiform fibers. In the same way, Fb-3 has been seen as significantly decreasing in several cancers, this suggesting its potential role as onco-suppressor gene and as antagonist to angiogenesis. However, conflicting scientific data point out a different role for Fb-3, like a “Dr Jekyll and Mr. Hyde” pattern, and suggest that Fb-3 may rather act as promoter of tumor invasion and survival, as in malignant gliomas, fostering angiogenesis. A reasonable interpretation of such pattern may be due to the aberrant methylation of Fb-3 promoter, since the Fb-3 expression is regulated by the hypermethylation of the promoter and/or by the interference of Fb-3 with the activation of kinase B protein (AKT).

In conclusion, circulating Fb-3 seems to be able to tell healthy asbestos-exposed subjects from MM patients. Fb-3 in the pleural liquid is thought to further differentiate MM subjects from those with benign and/or malignant effusions. To validate present results and test the effectiveness of Fb-3 combination with other possible biomarkers, it will be necessary to recruit larger numbers of patients.
The combined use of more biomarkers seems likely to guarantee more reliable results in terms of sensitivity and specificity so as to allow to tell, already at an early stage, MM from other pathologies of various nature. In the same way, using several biomarkers together with clinical-diagnostic exams, might contribute to carrying out the screening of populations exposed to asbestos/asbestiform fibers like in the above-mentioned case of subjects living in Biancavilla (CT), exposed to FE fibers released in the surrounding area.
References


[14] Lecka-Czernik B, Lumpkin CK Jr, Goldstein S. An overexpressed gene transcript in senescent and quiescent human fibroblasts encoding a novel


[28] Tong JD, Jiao NL, Wang YX, Zhang YW, Han F. Downregulation of fibulin-3 gene by promoter methylation in colorectal cancer


[34] Astoul P, Roca E, Galateau-Salle F, Scherpereel A. Malignant pleural mesothelioma: From the bench to the bedside. Respiration. 2012;83:481-493


