Abstract. Background: In the literature, there exist conflicting data on the value of fibulin-3 (FBLN3) for the diagnosis of pleural effusion (PE) in malignant pleural mesothelioma (MPM). Therefore we compared the diagnostic performance of FBLN3 against that of soluble mesothelin-related peptide (SMRP) in a cohort of Italian patients. Materials and Methods: FBLN3 and SMRP were detected in PE from 33 patients with MPM, 64 with pleural benign lesions and 23 with non-MPM pleural metastases using a commercial enzyme-linked-immunosorbent (ELISA)-assay kit according to manufacturers’ instructions. Results: Levels of FBLN3 were similar in PE from MPM and PE from other pathologies (geometric mean=68.1 vs. 66.2 ng/ml; p=0.872) in contrast to SMRP levels, which were significantly higher in PE from MPM (geometric mean=14.6 vs. 3.2 nM; p<0.001). Receiver operating characteristic analysis confirmed that SMRP showed a good performance (area under the curve=0.79, p<0.001), whereas FBLN3 was not able to discriminate MPM from other pathologies (area under the curve=0.44, p=0.838). Conclusion: FBLN3 detection in PE, in contrast to SMRP detection, is not useful as a biomarker for the diagnosis of PE from MPM.

Malignant pleural mesothelioma (MPM) is an asbestos-related tumor, arising in the pleural cavity, with a poor prognosis (1, 2) and a worldwide incidence expected to increase in the next 10 years (3, 4).

Pleural effusion (PE) is often the primary manifestation of MPM; it is obtained by thoracentesis and may be important for initial diagnosis (5). However, PE is a common event, being present in a large variety of neoplastic and benign diseases although sometimes its diagnosis can be difficult (6).

A reliable diagnostic soluble biomarker for MPM is an area of intense research because an ideal biomarker has not yet been identified (7, 8). Among such biomarkers, fibulin-3 (FBLN3) (9, 10) and soluble mesothelin-related peptide (SMRP) (11-17) have received considerable attention. FBLN3 is a glycoprotein encoded by the epidermal growth factor-containing fibulin-like extracellular matrix protein 1 gene that plays a role in cell proliferation and migration (9, 10). FBLN3 has low expression in normal tissues, whereas it is overexpressed in different types of cancers, including MPM, and is secreted in body fluids (9, 10). SMRP is a 40-kDa cell-surface glycoprotein with putative function in cell-to-cell adhesion, cell-to-cell recognition and signaling (18, 19). SMRP is expressed by normal mesothelial cells and overexpressed by various cancer types, including MPM (20, 21). In addition, SMRP can be released and detected in serum and PE (11-17).

However, while SMRP detection in effusion is clearly documented and currently considered the best marker usable for routine diagnostic purposes, the value of FBLN3 must be confirmed because conflicting data exist in the literature (22-25).

In order to clarify the value of FBLN3 in the diagnosis of PE from MPM, we compared its performance with that of SMRP. To our knowledge, this is the first study analyzing the performance of FBLN3 in PE specimens collected from a cohort of Italian patients.
Materials and Methods

Patients and samples. The study protocol was approved by the Ethics Committee of the Liguria Region (PR. 207REG2014) and all patients were enrolled in the study after their informed consent was given. All patients underwent thoracentesis at the Division of Pneumology (Sarzana, La Spezia, Italy) between March 2008 and July 2011. The definitive diagnosis for all patients was made on the basis of clinical signs, imaging data, cytological examination of PE and by examination of hematoxin-and-eosin-stained biopsy sections combined with immunohistochemistry (26).

The study was performed on 120 PE samples collected prior to any treatment. The study enrolled 33 patients with MPM, 64 patients with pleural benign lesions and 23 patients with non-MPM pleural metastases (Table I).

FBLN3 and SMRP detection assay. Aliquots from PE were centrifuged at 1,500 × g for 10 min at 4°C and the supernatant was stored at −20°C until the analysis was performed. FBLN3 levels were detected by Fibrulin-3 ELISA kit (USCN Life Science Inc., Houston, TX, USA) and SMRP levels by MesoMark ELISA Assay Kit (Cis-Bio International Gif-sur-Yvette, France) according to the manufacturers’ instructions. The lowest sensitivity threshold of the assay was 0.183 ng/ml and 0.3 nM for FBLN3 and SMRP, respectively. All PE samples were tested in duplicate.

Statistical analyses. The FBLN3 and SMRP levels in PE are reported as the geometric mean (GM). The diagnostic performance of SMRP was estimated through receiver operating characteristic (ROC) analysis and the area under the ROC curve (AUC) was used as a measure of accuracy of patients’ classification (27). The Mann–Whitney test was applied to assess whether each AUC was statistically greater than 0.50 (level of non-discrimination or chance line). In addition, exact binomial standard error of AUC was used to compute corresponding 95% confidence limits (95% CL) (28). All tests were two-tailed and a p-value of less than 0.05 was considered statistically significant. All analyses were performed using Stata (Stata Statistical Software, release 11.2; Stata Corporation, College Station, TX, USA).

Results

Comparison of FBLN3 and SMRP levels in PE from MPM versus PE from other pathologies. We evaluated the concentration of FBLN3 and SMRP in PE. The group of patients with MPM was found to have FBLN3 concentrations similar to those observed for the group with benign disease and that with metastases (p=0.174). In contrast, significantly different SMRP concentrations were observed in PE from MPM compared to the group with benign disease and that with metastases (p<0.001) (Table II).

Moreover, the results found in the single subgroups were confirmed by comparing the MPM group with the benign and metastatic groups combined (Table II).

Comparative analysis of FBLN3 and SMRP diagnostic performances in MPM-PE. The diagnostic accuracy of FBLN3 in PE from MPM was compared with that of SMRP by ROC analysis (Figure 1). The ROC analysis confirmed a lower performance of FBLN3 as compared to that of SMRP.

Discussion

SMRP can be considered the best soluble marker currently available for the diagnosis of PE from MPM (11-17). However, SMRP has some limitations that do not make it an ideal marker. SMRP detection in PE is characterized by high specificity but by modest sensitivity (MPM vs. benign disease, optimal cut-off=9.3 nM, specificity=93.0%, sensitivity=75.0.0%) (13).

In addition, as pointed out in the US Food and Drug Administration approval of Mesomark in 2007 (29), SMRP should be used in epithelioid or biphasic histology MPM, thus excluding the sarcomatoid histology (about 20% of total MPM cases) (13) in which SMRP has low expression and poor secretion by malignant MPM cells (20, 21). Therefore, research is concentrated on the evaluation of new markers with higher diagnostic power and validity for all MPMs, which could replace or complement use of SMRP (7, 8).
Among the various molecules, FBLN3 generated great interest following an article published in 2012 by Pass et al. in which FBLN3 was described as a marker with great diagnostic accuracy for both sera and PE (24). The results were subsequently corroborated by Agha et al. (22) in 2014. However, these data were not confirmed in two articles published in other prestigious journals. Indeed, again in 2014, Creaney et al. reported a much lower performance of FBLN3 (23), and Kirchner et al. reiterated its modest diagnostic accuracy in 2015 (25).

In our work, we evaluated the diagnostic power of FBLN3 in PE, using ROC curve analysis, in comparison to that of SMRP on the same group of patients. The ROC curve provides a precise and valid measure of diagnostic accuracy as the AUC value is indicative of the diagnostic power of the test in the sense that the higher the AUC, the greater is the discriminatory power of the marker.

According to the traditional academic point system for interpreting AUC values, an AUC has a perfect discriminatory power if its value is 1.0, excellent if 0.9-1.0, good between 0.8 and 0.9, fair at 0.7-0.8, poorly accurate at 0.6-0.7, and failed between 0.5 and 0.6. No discrimination exists if the value is 0.5 (27).

For FBL3 we found AUC=0.44 (Figure 1, panel C). Thus, in accordance with the reported above scale, FBLN3 is not able to discriminate PE arising from MPM from that arising from other causes. In other words, the concentration of FBLN3 in PE has no diagnostic or clinical relevance and therefore is not recommended for routine use.

Our results are therefore consistent with those reported by Creaney et al. (23), in which the PE-FBLN3 test was to be considered poorly accurate (AUC=0.588), and by Kirchner et al. (25), who did not report the AUC value, but only a small difference between the average levels of FBLN3 in the PE from MPM and non-PM. In contrast, our results do not agree with those reported by Pass et al. (AUC= 0.93) (24) and by Agha et al. (AUC=0.909) (22) who suggested detection of FBLN3 in PE as an excellent test. We are not able to provide an explanation for the discrepancy on data for FBLN3 in PE in the literature, but we can make two observations. The first is that all the studies used a kit provided by the same manufacturer (MesoMark ELISA kit, see Materials and Methods) for the determination of FBLN3 levels hence the uniformity of the methodology should ensure uniformity of the results. The second is that the various studies were carried out on small groups of patients from five cities of three different continents [Detroit/New York, USA: 74 MPM vs. 93 non-PM (24); Shebin Elkom, Egypt: 25 MPM vs. 9 benign (22); Sidney, Australia: 82 MPM vs. 71 non-PM (23); Sidney, Australia: 30 MPM vs. 60 non-PM (25); present report, La Spezia, Italy: 33 MPM vs. 87 non-PM]. Therefore, due to the low number of patients, the various studies do not allow definitive statistical conclusions to be drawn. In addition, these studies were performed on patients of different geographical origin who may have individual characteristics (biological, genetic, clinical, etc.) that justify the differences found in each report. We believe that in order for results to be supported by a solid statistical basis, it is necessary to take into account the individual characteristics and, simultaneously, to define a sample size able to discern between random variability and variability due to the clinical conditions that are compared.

Finally, it has been unanimously reported by various authors that the level of FBLN3 in PE indeed has a prognostic value in survival of patients with MPM (23-25). Due to the limited number of our patients with MPM (n=33), we have not delved into this aspect, which will be evaluated in a further study.

In conclusion, our results show that FBLN3 determination in PE does not have sufficient diagnostic power for its use in clinical routine. However, for a definitive judgment on the value of the determination of FBLN3 in PE, further investigations are needed on other cohorts of patients to explain the differences reported in the various studies so far carried out.

**Conflicts of Interest**

All Authors declare that no potential conflicts of interest exist with any companies/organizations whose products or services may be discussed in this article.
Figure 1. Diagnostic performance estimated through the receiver operating characteristic analysis of mesothelin and fibulin-3 levels in pleural effusion. Fibulin-3 levels were detected by the Fibulin-3 ELISA kit (USCN Life Science Inc., Houston, TX, USA) and mesothelin by the MesoMark kit (Cis-Bio International Gif/Yvette, France). All samples were tested in duplicate. AUC: Area under the ROC curve; 95% CL: 95% confidence limits of AUC. P: median test p-value.
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