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# Asbestos-Related Diseases and Blood Biomarkers

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## Abstract

Asbestos-related diseases, including asbestosis, benign pleural diseases, lung cancer, other types of cancer, and especially malignant mesothelioma (MM), still represent an enormous problem all over the world and are among the most investigated occupational diseases. Considering that MM is a highly aggressive and severe malignant cancer of pleura, peritoneum and other serosal surfaces, new blood biomarkers for earlier diagnosis, following response to treatment and disease progression, have been intensively investigated. Several studies suggested that soluble mesothelin-related peptides, fibulin-3, survivin, osteopontin, vimentin, calretinin, and many others could be helpful in diagnosis, detecting the progression of MM and evaluating tumour response to treatment; however, these biomarkers have not been validated in clinical practice. Therefore, search for novel better stand-alone or composite biomarkers is under way. The aim of this chapter is to present the importance of blood biomarkers in evaluating the risk of developing asbestos-related diseases, early diagnosis, following the response to treatment and progression of these diseases, with special emphasis on MM.

**Keywords:** asbestos-related diseases, malignant mesothelioma, blood biomarkers

## 1. Introduction

Although the asbestos production and usage have been banned in many countries, the asbestos-related diseases still represent an enormous public health problem all over the world [1–3]. Occupational and environmental exposure to asbestos fibres has been associated with the development of asbestosis, pleural diseases such as pleural plaques, diffuse pleural thickening, pleural effusion, malignant mesothelioma (MM) of the pleura, peritoneum and other serosal surfaces, lung cancer, and some other types of malignant diseases, including cancer of the larynx, cancer of the ovary, and possibly also cancers of the buccal mucosa, the pharynx, the gastrointestinal tract, and the kidney [1–10]. The asbestos-related diseases are considered to be among the most investigated occupational diseases [1–3, 7, 8, 10]. In particular, MM, a highly aggressive cancer, causes serious concerns because of its dismal prognosis, poor therapeutic strategies, and fatality [11–13]. Therefore, search for novel better stand-alone or composite biomarkers is under way. This is especially important for high-risk populations with a known history of asbestos exposure.

The aim of this chapter is to present the importance of blood biomarkers in evaluating the risk of developing asbestos-related diseases, early diagnosis,

following the response to treatment and progression of these diseases, with special emphasis on MM.

## **2. Blood biomarkers in asbestos-related diseases**

It has been proposed that blood biomarkers, such as mesothelin, fibulin-3, osteopontin, vimentin, and many others, could enable noninvasive and early detection of asbestos-related diseases and could be particularly helpful in diagnosing MM, detecting the progression of this cancer and evaluating tumour response to treatment.

### **2.1 Mesothelin**

One of the most investigated biomarkers in MM is mesothelin, a circulating form of a glycoprotein attached to the cell surface, that is considered to have a role in cell adhesion, proliferation, invasion, and possibly in cell-to-cell signalling. Mesothelin is highly expressed in MM as well as in several other cancers [14–18]. It exists in different forms that can be detected in serum in the form of soluble mesothelin-related peptides (SMRP) by enzyme-linked immunosorbent assay (ELISA) using monoclonal antibody techniques [19]. Many studies have investigated mesothelin as a possible tumour biomarker for diagnosing MM, evaluating response to treatment, as well as for detecting the progression of this malignoma [16, 20–26].

Robinson et al. proposed SMRP as a marker for diagnosis and monitoring progression of the disease [20]. Later, the same group also suggested that SMRP may also be useful for monitoring MM progression and may prove useful for screening asbestos-exposed individuals for early MM [16].

Different mesothelin-related antibodies were tested in studies to detect different forms of mesothelin. Maeda et al. found that the soluble N-terminal fragment N-ERC/mesothelin is a very stable and plentiful in the blood [27]. Shiomi et al. identified N-ERC/mesothelin as a potential biomarker for MM and used newly developed ELISA system to gain data on N-ERC/mesothelin levels in different clinical settings. In their study, serum N-ERC/mesothelin levels showed that the median values from MM patients were extremely high as compared to levels obtained from other subjects (e.g., healthy volunteers and asbestos-related non-malignant diseases) [28].

Several other studies also reported higher levels of SMRP in subjects with MM and proposed that SMRP could be a useful tumour biomarker for diagnosing MM and monitoring the disease progression [21–24].

Franko et al. found that pre-treatment SMRP levels were significantly higher than in stable disease, partial response, and complete response, as were SMRP levels in progressive disease compared to stable disease, partial response, and complete response. The findings of this study also suggested that SMRP may be a useful tumour marker for detecting the progression of MM and evaluating tumour response to treatment [25].

A study of Hollevoet et al. investigated the diagnostic accuracy and use of serum mesothelin in early diagnosis by performing an individual patient data meta-analysis. The results of the study showed that in patients suspected of having MM, a positive blood test for mesothelin at a high-specificity threshold presented a strong incentive to urge further diagnostic steps. On the other hand, they reported that the poor sensitivity of mesothelin clearly limits its added value to early diagnosis [26].

The overall diagnostic accuracy of SMRPs in serum and the pleural fluid was also investigated in meta-analysis of Cui et al. [29]. The authors concluded that

SMRPs in serum and pleural fluid are helpful biomarkers for diagnosing MM, and that they have a similar diagnostic accuracy. However, they stressed that negative results of SMRP determinations are not sufficient to exclude MM, while the positive test results indicate that further invasive diagnostic steps might be necessary for the diagnosis of MM [29].

The meta-analysis by Gillezeau et al. studied the mean differences of mesothelin, osteopontin, and fibulin-3 in blood and pleural samples. A total of 32 studies with mesothelin levels were included. Statistically, significant mean differences have been found between MM patients and all the other comparison groups for mesothelin blood and pleural levels. It has been concluded that based on the findings, mesothelin levels seem to be significantly lower in all control groups compared with those with MM, suggesting a possible role of mesothelin as a screening biomarker for MM [30].

## 2.2 Fibulin

Human fibulin-3, also known as epidermal growth factor containing fibulin-like extracellular matrix protein 1 (EFEMP1), has also been investigated as a potential biomarker for asbestos-related diseases, especially for MM [31–33]. It is a member of a family of extracellular matrix glycoproteins [34] that have been proposed to be important in the regulation of cell proliferation and migration and to act as tumour suppressors or activators in different cancers [34–38]. Fibulin-3 is predominately localised in the extracellular matrix of elastic tissue, and it has restricted expression in the body [37].

Several studies showed that levels of fibulin-3 expression decreased in several types of cancer and were correlated with poor survival of patients with breast cancer [39], hepatocellular carcinoma [40], and lung cancer [41, 42]. On the contrary, an increase in fibulin-3 was found in cervical carcinomas [43], pancreatic cancer [44], and malignant gliomas [45].

Fibulin-3 was first investigated as a potential tumour biomarker of MM in the study of Pass et al. who found that plasma fibulin-3 levels can distinguish asbestos-exposed healthy persons from patients with MM [31]. Their results showed that plasma fibulin-3 levels in conjunction with fibulin-3 levels in pleural effusions can differentiate MM effusion from other malignant and benign effusions [31].

Several further studies investigated the possible role of fibulin-3 in the diagnosis of MM, but the results were not consistent. Kaya et al. proposed that real use of serum fibulin-3 was not for prognosis but for diagnosis of MM [46].

Ren et al. performed a systematic review and a meta-analysis of eight studies to evaluate the diagnostic value of fibulin-3 in plasma, serum, and pleural effusion. They found that the overall sensitivity and specificity for blood fibulin-3 were 0.87 [95% confidence interval (CI) 0.58-0.97] and 0.89 (95% CI 0.77-0.95), respectively. Based on these results, they concluded that fibulin-3 is a useful diagnostic biomarker for MM [47]. Similarly, Pei et al. reported that fibulin-3 confers a relatively high diagnostic efficacy and could be acceptable as an auxiliary biomarker to aid in MM identification [32].

Jiang et al. investigated the utility of fibulin-3 not only for MM but also for other asbestos-related diseases, therefore including patients with pleural plaques, asbestosis, and MM. The results showed that median plasma fibulin-3 level of subjects in the MM group was higher than that in other groups. The results also showed that subjects in the asbestosis group had a higher median fibulin-3 level compared to those in the control group. Their study proposed that fibulin-3 could be a potential biomarker for early screening of MM, but not for other asbestos-related diseases [33].



The meta-analysis of Gillezeau et al., which includes nine studies with fibulin-3 levels, also presented a statistically significant difference in both blood and pleural levels of fibulin-3 in MM patients compared with those of all other groups [30].

On the other hand, some other studies suggested that plasma fibulin-3 levels have low diagnostic accuracy [48–50]. The study of Creaney et al. identified soluble mesothelin as a superior diagnostic biomarker for MM compared to fibulin-3, whereas fibulin-3 provided superior prognostic information compared to mesothelin [48]. Kirschner et al. reported that plasma fibulin-3 level was significantly elevated in MM patients from the Sydney cohort, but not the Vienna cohort; however, the diagnostic accuracy was low. The data confirmed the potential prognostic value of pleural effusion fibulin-3 [49]. The same applies to the study of Ledda et al. who reported that fibulin-3 did not show a superior diagnostic performance [51].

The study of Kovac et al. aimed to evaluate the potential applicability of fibulin-3 plasma levels as a biomarker of response to treatment and its prognostic value for progressive disease within 18 months. The results of the study showed significantly higher fibulin-3 levels in progressive disease in comparison with the levels before treatment, in complete response to treatment, and in stable disease, which indicated that fibulin-3 could be helpful in identifying the progression of MM. On the contrary, no significant difference was observed between the fibulin-3 levels before treatment in comparison with the levels in complete response to treatment, partial response to treatment, and stable disease. The findings of this study suggest that fibulin-3 could be helpful in detecting the progression of MM [52].

### **2.3 Survivin**

Survivin is a member of the inhibitor of the apoptosis protein (IAP) family and is known to have a role in the regulation of cell division and apoptosis (programmed cellular death). Survivin was first described as an inhibitor of caspase -9. However, several studies found that the role of survivin in pathogenesis of malignant diseases involves not only apoptosis but also the regulation of the mitotic spindle checkpoint, as well as chemoresistance and promotion of angiogenesis. This protein is commonly not expressed in normal differentiated tissues; however, it was found to be expressed in some cancers. Survivin is related to increased tumour aggressiveness, both in pleural fluid and in tissue [53].

Few studies investigated the role of survivin in asbestos-related diseases, or more precisely in MM. In their study, Hmeljak et al. performed on tissue samples aimed to elucidate whether survivin expression is associated with tumour cell proliferation and apoptosis and to investigate the prognostic and predictive value of survivin expression in MM. The results indicated that survivin expression might contribute to prediction of treatment response. However, the survivin expression in pleural MM did not show to have prognostic significance [54].

The only study so far that included blood (serum) samples is the study of Goricar et al. who investigated the influence of serum survivin levels on the outcome of cisplatin-based chemotherapy in patients with MM. The findings suggested that serum survivin levels could serve as a biomarker predicting response to treatment in MM before and during chemotherapy [55].

### **2.4 Osteopontin**

Osteopontin is an extracellular cell adhesion protein that is involved in several biological processes, including cell-matrix interaction, cell-signalling and migration, immunological regulation, as well as in tumour development [56–60].

Elevated levels of serum osteopontin have been found in several cancers, such as colon cancer [61], breast cancer [62], lung cancer [63], as well as in MM [64]. Accordingly, serum osteopontin has been suggested to be a possible biomarker of early detection of MM [64–66].

Pass et al. investigated the presence of osteopontin in pleural MM and determined serum osteopontin levels in three populations: in asbestos-exposed subjects without cancer, subjects without cancer who were not exposed to asbestos, and in asbestos-exposed subjects with MM. Based on the results, the authors concluded that serum osteopontin levels could be used to distinguish asbestos-exposed individuals who do not have cancer from asbestos-exposed individuals with pleural MM [65].

The diagnostic performance of osteopontin was investigated in several other studies of asbestos-related diseases, but the results were not consistent [67–71].

Paleari et al. investigated the role of plasma osteopontin in diagnosis of pleural MM; however, their results suggested that plasma osteopontin levels cannot discriminate between chronic inflammatory and malignant lung disease [67].

The potential role of serum and plasma osteopontin in pleural MM diagnosis was reported by Cristaudo et al. [68]. Their results suggested that plasma osteopontin and serum osteopontin are not influenced by confounders such as age, smoking, and asbestos exposure. Moreover, plasma and serum osteopontin were proposed to be useful biomarkers in the diagnosis of epithelial MM in addition to radiological examination [68].

Comparison of plasma versus serum levels of osteopontin in patients with MM was performed by Creaney et al., who found that plasma osteopontin has a superior diagnostic accuracy to serum [69].

Osteopontin as the diagnostic biomarker was investigated in the cross-sectional study. The analysis showed that serum osteopontin levels in MM were higher than in benign asbestos-related diseases and healthy exposed subjects [70].

A systematic review and meta-analysis by Hu et al. aimed to evaluate the diagnostic accuracy of circulating osteopontin in pleural MM. Based on the analysis of six studies, the overall diagnostic sensitivity was 0.65 (95% CI 0.60–0.70) and specificity 0.81 (95% CI 0.78–0.85). The authors concluded that osteopontin is an effective marker for diagnosis of pleural MM [71].

Regarding peritoneal MM, osteopontin was studied as a potential circulating biomarker of diffuse peritoneal MM by Bruno et al. who reported that at multivariate analysis, osteopontin was related with survival. However, the authors concluded that osteopontin warrants further investigation as a prognostic marker for diffuse peritoneal MM [72].

Considering pleural plaques, Mastrangelo et al. investigated in their study whether plasma osteopontin was an indicator of asbestos exposure or effect. Their results suggested that osteopontin cannot be a reliable biomarker of asbestos exposure or effect (presence of pleural plaques) [73].

## 2.5 Calretinin

MM diagnosis is usually made at the advanced stages of the disease, which contributes to poor prognosis and short survival of MM patients [74, 75]. To confirm MM diagnosis, an immunohistochemical analysis investigating a panel of markers on tissue samples is required [75]. Among the positive immunohistochemical MM markers that can discriminate between malignant and mesothelial cells with the highest sensitivity and specificity are calretinin, cytokeratin5/6, and WT1 [76]. As biomarkers that would enable an earlier noninvasive diagnosis of MM are widely

studied, recent studies evaluated if soluble calretinin could also be used as a biomarker in MM [75, 77–80].

Calretinin is a 30-kDa calcium-binding protein that belongs to the EF-hand family [81]. It acts as a calcium-buffering protein and calcium sensor. It plays an important role in the neurons, but it is also expressed in the mesothelial cells [81]. Calretinin was already shown to promote the invasiveness, proliferation, and migration of mesothelial cells [82]. It may also be involved in activating the focal adhesion kinase (FAK) signalling pathway and epithelial-to-mesenchymal transition [82].

Studies showed that calretinin was increased in plasma and serum of MM patients compared to patients with other asbestos-related diseases and healthy controls [75, 79, 80]. Interestingly, patients with asbestosis also had slightly higher serum calretinin compared to patients with pleural plaques [75]. The ELISA assay developed by Raiko et al. is highly sensitive when used to detect calretinin in plasma or serum and is robust enough to detect calretinin in retrospective samples regardless of storage time [75, 79]. However, as calretinin is mostly expressed in epithelioid and biphasic MM, but only in around 30% of sarcomatoid MM [81], its usefulness as a soluble biomarker is limited in this histological subtype [75].

Studies also suggest that using a combination of calretinin and mesothelin can increase the sensitivity for detecting MM [75, 77]. In asbestos-exposed subjects that developed MM, calretinin was increased already in prediagnostic plasma samples (even more than a year prior to the clinical diagnosis) compared to asbestos-exposed subjects that did not develop MM, especially in samples closer to the diagnosis [77]. Even though sensitivity was limited to an individual biomarker, using a combination of both calretinin and mesothelin had better predictive ability and could also be important as a screening biomarker in asbestos-exposed subjects [77].

## **2.6 Other biomarkers**

Apart from the most frequently studied biomarkers described above, some studies investigated other serum or plasma factors in asbestos-related diseases [83–85]. Among protein biomarkers, megakaryocyte potentiating factor and high mobility group box 1 (HMGB1) were increased in MM patients compared to healthy individuals or patients with benign asbestos-related diseases [84, 85].

Additionally, novel studies suggest microRNA (miRNA) expression could also serve as a diagnostic or prognostic biomarker in MM [84–86]. Kirschner et al. compared cell-free miRNA profiles in plasma from MM patients with healthy controls and proposed the potential role of miRNA-29c\* and miRNA-92a as a candidate tumour biomarkers, and indicated that miRNA-625-3p is a promising novel diagnostic marker for MM [86]. Micolucci et al. in their systematic review and a quantitative meta-analysis compared the data from asbestos-exposed and MM subjects and suggested that the most promising candidates for a multimarker signature were circulating miRNA-126-3p, miRNA-103a-3p, and miRNA-625-3p in combination with mesothelin [87]. Mozzoni et al. aimed to identify a pattern of miRNA (mi-RNA-16, miRNA-17, mi-RNA-126, and miRNA-486) as a possible diagnostic biomarker for patients with pleural MM and asbestosis and as prognostic biomarkers for patients with pleural MM. The results showed that all miRNA levels were decreased in patients with pleural MM or asbestosis, which has been suggested to support the role of circulating miRNAs as potential biomarkers for asbestos-related diseases. Additionally, miRNA-16 was directly related to prognosis of patients with pleural MM, indicating its possible use as prognostic factor in patients with pleural MM [88]. Santarelli et al. performed a study to identify miRNAs associated with asbestos-induced malignances. In this study, four serum miRNAs (mi-RNA-126, miRNA-205, miRNA-222, and miRNA-520g)



were implicated in asbestos-related malignant diseases and could be utilised for screening in asbestos-exposed populations [89].

As individual biomarkers that have been proposed in asbestos-related diseases have some limitations, it was suggested that a combination of different factors might be a better diagnostic or prognostic biomarker in asbestos-related diseases [83–85].

### **3. The role of composite blood biomarkers in asbestos-related diseases**

Several studies investigated the potential role of composite blood biomarkers in asbestos-related diseases, and many of them included mesothelin together with various other biomarkers [72, 90].

Felten et al. assessed the influence of age and asbestos exposure on the blood levels of the proposed tumour markers, mesothelin, and osteopontin and determined the change of these markers over time. The results showed that age had a strong influence on biomarker levels. On the other hand, there was no association between asbestos exposure duration or benign asbestos-related diseases and biomarker levels. The researchers concluded that fixed cut-off values for deciding between intensive clinical work-up and continued surveillance appeared inadequate for evaluating markers [91].

In addition to evaluating the potential applicability of fibulin-3 plasma levels as a biomarker of response to treatment and progression of disease, the study of Kovac et al. also assessed the potential applicability of fibulin-3 in comparison with or in addition to SMRP. The results indicated that in addition to SMRP, fibulin-3 could also be useful in detecting MM progression [52].

Bonotti et al. evaluated the usefulness of SMRP, plasma osteopontin, and vimentin as markers of the clinical response to treatment in patients suffering from epithelioid MM. In their study, SMRP, osteopontin, and vimentin showed statistically significant differences between the disease categories: stable disease, partial response, and disease progression. Based on the results, it has been concluded that the time course of SMRP and vimentin was strongly associated with disease status, and so was the time course of osteopontin, although to a lesser extent. The researchers suggested that these markers appear to be particularly effective in cases of partial response and disease progression, even though their possible use in stable disease should be better elucidated [90].

In a recent study that evaluated soluble mesothelin, calretinin, and megakaryocyte potentiating factor, the use of composite of these biomarkers improved the performance for diagnosis of pleural MM compared to population controls [78]. The combination of calretinin and megakaryocyte potentiating factor had the highest sensitivity in men, while the combination of calretinin and mesothelin had the highest sensitivity in women [78].

In an Italian cohort, the diagnostic performance of fibulin-3 against SMRP was compared in patients with pleural effusion from MM. The results of the study showed that the levels of fibulin-3 were similar in pleural effusions from pleural MM and pleural effusion from other pathologies in contrast to SMRP levels, which were significantly higher in pleural effusion from pleural MM. A further analysis confirmed that SMRP showed a good performance, whereas fibulin was not able to discriminate pleural MM from other pathologies. The conclusion was that fibulin detection in pleural effusion, contrary to SMRP detection, is not useful as a biomarker for the diagnosis of pleural effusion from pleural MM [92].

Bruno et al. assessed the diagnostic and prognostic values of mesothelin, osteopontin, CEA, CA19-9, CA125, and CA15-3 in diffuse peritoneal MM and



other peritoneal malignancies. The conclusion was that when assessing peritoneal surface malignancies of unknown origin, elevated mesothelin with low CA19-9 may increase the suspicion index for diffuse peritoneal MM. As for the role of osteopontin, further research is needed [72].

#### **4. Conclusions**

Considering that asbestos-related diseases, and in particular MM, still represent a huge health problem and economic burden, the investigation of potential biomarkers for evaluating the risk for developing asbestos-related diseases, earlier diagnosis of asbestos diseases, evaluating response to treatment and progression of these diseases, is of great importance. Biomarkers for assessing risk of developing asbestos diseases are of considerable significance especially in high asbestos-exposed populations. As presented in the chapter, the results of the studies are not consistent, therefore further research is needed to clarify inconsistency and find reliable biomarkers that could be used in clinical practice and would enable better outcome of asbestos-related diseases and increase survival in MM.

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## References

- [1] Stayner L, Welch LS, Lemen R. The worldwide pandemic of asbestos-related diseases. *Annual Review of Public Health*. 2013;**34**:205-216
- [2] Takahashi K, Landrigan PJ, Collegium R. The global health dimensions of asbestos and asbestos-related diseases. *Annals of Global Health*. 2016;**82**(1):209-213
- [3] Visona SD, Villani S, Manzoni F, Chen Y, Ardissino G, Russo F, et al. Impact of asbestos on public health: A retrospective study on a series of subjects with occupational and non-occupational exposure to asbestos during the activity of Fibronit plant (Broni, Italy). *Journal of Public Health Research*. 2018;**7**(3):1519
- [4] Hillerdal G, Henderson DW. Asbestos, asbestosis, pleural plaques and lung cancer. *Scandinavian Journal of Work, Environment & Health*. 1997;**23**(2):93-103
- [5] Cugell DW, Kamp DW. Asbestos and the pleura: A review. *Chest*. 2004;**125**(3):1103-1117
- [6] Antao VC, Larson TC, Horton DK. Libby vermiculite exposure and risk of developing asbestos-related lung and pleural diseases. *Current Opinion in Pulmonary Medicine*. 2012;**18**(2):161-167
- [7] Vainio H. Epidemics of asbestos-related diseases--something old, something new. *Scandinavian Journal of Work, Environment & Health*. 2015;**41**(1):1-4
- [8] Marsili D, Terracini B, Santana VS, Ramos-Bonilla JP, Pasetto R, Mazzeo A, et al. Prevention of asbestos-related disease in countries currently using asbestos. *International Journal of Environmental Research and Public Health*. 2016;**13**(5):1-19
- [9] Solbes E, Harper RW. Biological responses to asbestos inhalation and pathogenesis of asbestos-related benign and malignant disease. *Journal of Investigative Medicine*. 2018;**66**(4):721-727
- [10] Dolzan V, Dodic-Fikfak M, Franko A. Gene-environment interactions: The case of asbestosis. In: Korhan O, editor. *Occupational Health*. Rijeka: InTech; 2017. pp. 159-178
- [11] Robinson BWS, Lake RA. Medical progress - advances in malignant mesothelioma. *The New England Journal of Medicine*. 2005;**353**(15):1591-1603
- [12] Miller JM, Thompson JK, MacPherson MB, Beuschel SL, Westbom CM, Sayan M, et al. Curcumin: A double hit on malignant mesothelioma. *Cancer Prevention Research (Philadelphia, Pa.)*. 2014;**7**(3):330-340
- [13] Rossini M, Rizzo P, Bononi I, Clementz A, Ferrari R, Martini F, et al. New perspectives on diagnosis and therapy of malignant pleural mesothelioma. *Frontiers in Oncology*. 2018;**8**:91
- [14] Chang K, Pastan I. Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers. *Proceedings of the National Academy of Sciences of the United States of America*. 1996;**93**(1):136-140
- [15] Scholler N, Fu N, Yang Y, Ye Z, Goodman GE, Hellstrom KE, et al. Soluble member(s) of the mesothelin/megakaryocyte potentiating factor family are detectable in sera from patients with ovarian carcinoma. *Proceedings of the National Academy of Sciences of the United States of America*. 1999;**96**(20):11531-11536

- [16] Robinson BW, Creaney J, Lake R, Nowak A, Musk AW, de Klerk N, et al. Soluble mesothelin-related protein--a blood test for mesothelioma. *Lung Cancer*. 2005;**49**(Suppl 1):S109-S111
- [17] Tian L, Zeng R, Wang X, Shen C, Lai Y, Wang M, et al. Prognostic significance of soluble mesothelin in malignant pleural mesothelioma: A meta-analysis. *Oncotarget*. 2017;**8**(28):46425-46435
- [18] He X, Wang L, Riedel H, Wang K, Yang Y, Dinu CZ, et al. Mesothelin promotes epithelial-to-mesenchymal transition and tumorigenicity of human lung cancer and mesothelioma cells. *Molecular Cancer*. 2017;**16**(1):63
- [19] Peake MD, Entwisle J, Gray S. Malignant pleural mesothelioma: clinical presentation, radiological evaluation and diagnosis. In: O'Byrne K, Rusch V, editors. *Malignant Pleural Mesothelioma*. Oxford, New York: Oxford University Press; 2006. pp. 35-60
- [20] Robinson BW, Creaney J, Lake R, Nowak A, Musk AW, de Klerk N, et al. Mesothelin-family proteins and diagnosis of mesothelioma. *Lancet*. 2003;**362**(9396):1612-1616
- [21] Beyer HL, Geschwindt RD, Glover CL, Tran L, Hellstrom I, Hellstrom KE, et al. MESOMARK: A potential test for malignant pleural mesothelioma. *Clinical Chemistry*. 2007;**53**(4):666-672
- [22] Scherpereel A, Grigoriu B, Conti M, Gey T, Gregoire M, Copin MC, et al. Soluble mesothelin-related peptides in the diagnosis of malignant pleural mesothelioma. *American Journal of Respiratory and Critical Care Medicine*. 2006;**173**(10):1155-1160
- [23] Grigoriu BD, Scherpereel A, Devos P, Chahine B, Letourneux M, Lebailly P, et al. Utility of osteopontin and serum mesothelin in malignant pleural mesothelioma diagnosis and prognosis assessment. *Clinical Cancer Research*. 2007;**13**(10):2928-2935
- [24] Cristaudo A, Foddìs R, Vivaldi A, Guglielmi G, Dipalma N, Filiberti R, et al. Clinical significance of serum mesothelin in patients with mesothelioma and lung cancer. *Clinical Cancer Research*. 2007;**13**(17):5076-5081
- [25] Franko A, Dolzan V, Kovac V, Arneric N, Dodic-Fikfak M. Soluble mesothelin-related peptides levels in patients with malignant mesothelioma. *Disease Markers*. 2012;**32**(2):123-131
- [26] Hollevoet K, Reitsma JB, Creaney J, Grigoriu BD, Robinson BW, Scherpereel A, et al. Serum mesothelin for diagnosing malignant pleural mesothelioma: An individual patient data meta-analysis. *Journal of Clinical Oncology*. 2012;**30**(13):1541-1549
- [27] Maeda M, Hino O. Molecular tumor markers for asbestos-related mesothelioma: Serum diagnostic markers. *Pathology International*. 2006;**56**(11):649-654
- [28] Shiomi K, Hagiwara Y, Sonoue K, Segawa T, Miyashita K, Maeda M, et al. Sensitive and specific new enzyme-linked immunosorbent assay for N-ERC/mesothelin increases its potential as a useful serum tumor marker for mesothelioma. *Clinical Cancer Research*. 2008;**14**(5):1431-1437
- [29] Cui A, Jin XG, Zhai K, Tong ZH, Shi HZ. Diagnostic values of soluble mesothelin-related peptides for malignant pleural mesothelioma: Updated meta-analysis. *BMJ Open*. 2014;**4**(2):e004145
- [30] Gillezeau C, van Gerwen M, Ramos J, Liu B, Flores R, Taioli E. Biomarkers for malignant pleural mesothelioma: A meta-analysis. *Carcinogenesis*. 2019. DOI: 10.1093/carcin/bgz103. [Epub ahead of print]

- [31] Pass HI, Levin SM, Harbut MR, Melamed J, Chiriboga L, Donington J, et al. Fibulin-3 as a blood and effusion biomarker for pleural mesothelioma. *The New England Journal of Medicine*. 2012;**367**(15):1417-1427
- [32] Pei D, Li Y, Liu X, Yan S, Guo X, Xu X, et al. Diagnostic and prognostic utilities of humoral fibulin-3 in malignant pleural mesothelioma: Evidence from a meta-analysis. *Oncotarget*. 2017;**8**(8):13030-13038
- [33] Jiang Z, Ying S, Shen W, He X, Chen J, Xia H, et al. Plasma fibulin-3 as a potential biomarker for patients with asbestos-related diseases in the Han population. *Disease Markers*. 2017;**2017**:1725354
- [34] Timpl R, Sasaki T, Kostka G, Chu ML. Fibulins: A versatile family of extracellular matrix proteins. *Nature Reviews. Molecular Cell Biology*. 2003;**4**(6):479-489
- [35] Argraves WS, Greene LM, Cooley MA, Gallagher WM. Fibulins: Physiological and disease perspectives. *EMBO Reports*. 2003;**4**(12):1127-1131
- [36] Kim IG, Kim SY, Choi SI, Lee JH, Kim KC, Cho EW. Fibulin-3-mediated inhibition of epithelial-to-mesenchymal transition and self-renewal of ALDH+ lung cancer stem cells through IGF1R signaling. *Oncogene*. 2014;**33**(30):3908-3917
- [37] Kobayashi N, Kostka G, Garbe JH, Keene DR, Bachinger HP, Hanisch FG, et al. A comparative analysis of the fibulin protein family. Biochemical characterization, binding interactions, and tissue localization. *The Journal of Biological Chemistry*. 2007;**282**(16):11805-11816
- [38] Zhang Y, Marmorstein LY. Focus on molecules: Fibulin-3 (EFEMP1). *Experimental Eye Research*. 2010;**90**(3):374-375
- [39] Sadr-Nabavi A, Ramser J, Volkmann J, Naehrig J, Wiesmann F, Betz B, et al. Decreased expression of angiogenesis antagonist EFEMP1 in sporadic breast cancer is caused by aberrant promoter methylation and points to an impact of EFEMP1 as molecular biomarker. *International Journal of Cancer*. 2009;**124**(7):1727-1735
- [40] Nomoto S, Kanda M, Okamura Y, Nishikawa Y, Qiyong L, Fujii T, et al. Epidermal growth factor-containing fibulin-like extracellular matrix protein 1, EFEMP1, a novel tumor-suppressor gene detected in hepatocellular carcinoma using double combination array analysis. *Annals of Surgical Oncology*. 2010;**17**(3):923-932
- [41] Yue W, Dacic S, Sun Q, Landreneau R, Guo M, Zhou W, et al. Frequent inactivation of RAMP2, EFEMP1 and Dutt1 in lung cancer by promoter hypermethylation. *Clinical Cancer Research*. 2007;**13**(15 Pt 1):4336-4344
- [42] Xu S, Yang Y, Sun YB, Wang HY, Sun CB, Zhang X. Role of fibulin-3 in lung cancer: in vivo and in vitro analyses. *Oncology Reports*. 2014;**31**(1):79-86
- [43] En-lin S, Sheng-guo C, Hua-qiao W. The expression of EFEMP1 in cervical carcinoma and its relationship with prognosis. *Gynecologic Oncology*. 2010;**117**(3):417-422
- [44] Seeliger H, Camaj P, Ischenko I, Kleespies A, De Toni EN, Thieme SE, et al. EFEMP1 expression promotes in vivo tumor growth in human pancreatic adenocarcinoma. *Molecular Cancer Research: MCR*. 2009;**7**(2):189-198
- [45] Hu B, Thirtamara-Rajamani KK, Sim H, Viapiano MS. Fibulin-3 is uniquely upregulated in malignant gliomas and promotes tumor cell



- motility and invasion. *Molecular Cancer Research: MCR*. 2009;7(11):1756-1770
- [46] Kaya H, Demir M, Taylan M, Sezgi C, Tanrikulu AC, Yilmaz S, et al. Fibulin-3 as a diagnostic biomarker in patients with malignant mesothelioma. *Asian Pacific Journal of Cancer Prevention : APJCP*. 2015;16(4):1403-1407
- [47] Ren R, Yin P, Zhang Y, Zhou J, Zhou Y, Xu R, et al. Diagnostic value of fibulin-3 for malignant pleural mesothelioma: A systematic review and meta-analysis. *Oncotarget*. 2016;7(51):84851-84859
- [48] Creaney J, Dick IM, Meniawy TM, Leong SL, Leon JS, Demelker Y, et al. Comparison of fibulin-3 and mesothelin as markers in malignant mesothelioma. *Thorax*. 2014;69(10):895-902
- [49] Kirschner MB, Pulford E, Hoda MA, Rozsas A, Griggs K, Cheng YY, et al. Fibulin-3 levels in malignant pleural mesothelioma are associated with prognosis but not diagnosis. *British Journal of Cancer*. 2015;113(6):963-969
- [50] Sun HH, Vaynblat A, Pass HI. Diagnosis and prognosis-review of biomarkers for mesothelioma. *Annals of Translational Medicine*. 2017;5(11):244
- [51] Ledda C, Caltabiano R, Vella F, Matera S, Marconi A, Loreto C, et al. Fibulin-3 as biomarker of malignant mesothelioma. *Biomarkers in Medicine*. 2019;13(10):875-886
- [52] Kovac V, Dodic-Fikfak M, Arneric N, Dolzan V, Franko A. Fibulin-3 as a biomarker of response to treatment in malignant mesothelioma. *Radiology and Oncology*. 2015;49(3):279-285
- [53] Mita AC, Mita MM, Nawrocki ST, Giles FJ. Survivin: Key regulator of mitosis and apoptosis and novel target for cancer therapeutics. *Clinical Cancer Research*. 2008;14(16):5000-5005
- [54] Hmeljak J, Erculj N, Dolzan V, Pizem J, Kern I, Kovac V, et al. Is survivin expression prognostic or predictive in malignant pleural mesothelioma? *Virchows Archiv*. 2013;462(3):315-321
- [55] Goricar K, Kovac V, Franko A, Dodic-Fikfak M, Dolzan V. Serum survivin levels and outcome of chemotherapy in patients with malignant mesothelioma. *Disease Markers*. 2015;2015:316739
- [56] Wai PY, Kuo PC. The role of Osteopontin in tumor metastasis. *Journal of Surgical Research*. 2004;121(2):228-241
- [57] Frey AB, Wali A, Pass H, Lonardo F. Osteopontin is linked to p65 and MMP-9 expression in pulmonary adenocarcinoma but not in malignant pleural mesothelioma. *Histopathology*. 2007;50(6):720-726
- [58] Ohashi R, Tajima K, Takahashi F, Cui R, Gu T, Shimizu K, et al. Osteopontin modulates malignant pleural mesothelioma cell functions in vitro. *Anticancer Research*. 2009;29(6):2205-2214
- [59] Tajima K, Ohashi R, Sekido Y, Hida T, Nara T, Hashimoto M, et al. Osteopontin-mediated enhanced hyaluronan binding induces multidrug resistance in mesothelioma cells. *Oncogene*. 2010;29(13):1941-1951
- [60] Chen RX, Xia YH, Xue TC, Ye SL. Osteopontin promotes hepatocellular carcinoma invasion by up-regulating MMP-2 and uPA expression. *Molecular Biology Reports*. 2011;38(6):3671-3677
- [61] Coppola D, Szabo M, Boulware D, Muraca P, Alsarraj M, Chambers AF, et al. Correlation of osteopontin protein expression and pathological stage across a wide variety of tumor histologies. *Clinical Cancer Research*. 2004;10(1 Pt 1):184-190

- [62] El-Tanani MK, Yuen HF, Shi Z, Platt-Higgins A, Buckley NE, Mullan PB, et al. Osteopontin can act as an effector for a germline mutation of BRCA1 in malignant transformation of breast cancer-related cells. *Cancer Science*. 2010;**101**(6):1354-1360
- [63] Rouanne M, Adam J, Goubar A, Robin A, Ohana C, Louvet E, et al. Osteopontin and thrombospondin-1 play opposite roles in promoting tumor aggressiveness of primary resected non-small cell lung cancer. *BMC Cancer*. 2016;**16**:483
- [64] Chen Z, Gaudino G, Pass HI, Carbone M, Yang H. Diagnostic and prognostic biomarkers for malignant mesothelioma: An update. *Translational Lung Cancer Research*. 2017;**6**(3):259-269
- [65] Pass HI, Lott D, Lonardo F, Harbut M, Liu Z, Tang N, et al. Asbestos exposure, pleural mesothelioma, and serum osteopontin levels. *The New England Journal of Medicine*. 2005;**353**(15):1564-1573
- [66] Ivanov SV, Ivanova AV, Goparaju CM, Chen Y, Beck A, Pass HI. Tumorigenic properties of alternative osteopontin isoforms in mesothelioma. *Biochemical and Biophysical Research Communications*. 2009;**382**(3):514-518
- [67] Paleari L, Rotolo N, Imperatori A, Puzone R, Sessa F, Franzi F, et al. Osteopontin is not a specific marker in malignant pleural mesothelioma. *The International Journal of Biological Markers*. 2009;**24**(2):112-117
- [68] Cristaudo A, Foddìs R, Bonotti A, Simonini S, Vivaldi A, Guglielmi G, et al. Comparison between plasma and serum osteopontin levels: Usefulness in diagnosis of epithelial malignant pleural mesothelioma. *The International Journal of Biological Markers*. 2010;**25**(3):164-170
- [69] Creaney J, Yeoman D, Musk AW, de Klerk N, Skates SJ, Robinson BW. Plasma versus serum levels of osteopontin and mesothelin in patients with malignant mesothelioma--which is best? *Lung Cancer*. 2011;**74**(1):55-60
- [70] Bayram M, Dongel I, Akbas A, Benli I, Akkoyunlu ME, Bakan ND. Serum biomarkers in patients with mesothelioma and pleural plaques and healthy subjects exposed to naturally occurring asbestos. *Lung*. 2014;**192**(1):197-203
- [71] Hu ZD, Liu XF, Liu XC, Ding CM, Hu CJ. Diagnostic accuracy of osteopontin for malignant pleural mesothelioma: A systematic review and meta-analysis. *Clinica Chimica Acta*. 2014;**433**:44-48
- [72] Bruno F, Baratti D, Martinetti A, Morelli D, Sottotetti E, Bonini C, et al. Mesothelin and osteopontin as circulating markers of diffuse malignant peritoneal mesothelioma: A preliminary study. *European Journal of Surgical Oncology*. 2018;**44**(6):792-798
- [73] Mastrangelo G, Marangi G, Ballarin MN, Michilin S, Fabricio AS, Valentini F, et al. Osteopontin, asbestos exposure and pleural plaques: A cross-sectional study. *BMC Public Health*. 2011;**11**:220
- [74] Kovac V, Zwitter M, Zagar T. Improved survival after introduction of chemotherapy for malignant pleural mesothelioma in Slovenia: Population-based survey of 444 patients. *Radiology and Oncology*. 2012;**46**(2):136-144
- [75] Johnen G, Gawrych K, Raiko I, Casjens S, Pesch B, Weber DG, et al. Calretinin as a blood-based biomarker for mesothelioma. *BMC Cancer*. 2017;**17**(1):386
- [76] Husain AN, Colby TV, Ordonez NG, Allen TC, Attanoos RL, Beasley MB, et al. Guidelines for pathologic diagnosis

of malignant mesothelioma 2017 update of the consensus statement from the international mesothelioma interest group. *Archives of Pathology & Laboratory Medicine*. 2018;**142**(1):89-108

[77] Johnen G, Burek K, Raiko I, Wichert K, Pesch B, Weber DG, et al. Prediagnostic detection of mesothelioma by circulating calretinin and mesothelin - a case-control comparison nested into a prospective cohort of asbestos-exposed workers. *Scientific Reports*. 2018;**8**(1):14321

[78] Jiménez-Ramírez C, Casjens S, Juárez-Pérez CA, Raiko I, Del Razo LM, Taeger D, et al. Mesothelin, Calretinin, and Megakaryocyte Potentiating Factor as Biomarkers of Malignant Pleural Mesothelioma. 2019. DOI: 10.1007/s00408-019-00244-1. [Epub ahead of print]

[79] Raiko I, Sander I, Weber DG, Raulf-Heimsoth M, Gillissen A, Kollmeier J, et al. Development of an enzyme-linked immunosorbent assay for the detection of human calretinin in plasma and serum of mesothelioma patients. *BMC Cancer*. 2010;**10**:242

[80] Aguilar-Madrid G, Pesch B, Calderon-Aranda ES, Burek K, Jimenez-Ramirez C, Juarez-Perez CA, et al. Biomarkers for predicting malignant pleural mesothelioma in a Mexican population. *International Journal of Medical Sciences*. 2018;**15**(9):883-891

[81] Blum W, Schwaller B. Calretinin is essential for mesothelioma cell growth/survival in vitro: A potential new target for malignant mesothelioma therapy? *International Journal of Cancer*. 2013;**133**(9):2077-2088

[82] Worthmuller J, Blum W, Pecze L, Salicio V, Schwaller B. Calretinin promotes invasiveness and EMT in malignant mesothelioma cells

involving the activation of the FAK signaling pathway. *Oncotarget*. 2018;**9**(91):36256-36272

[83] Creaney J, Robinson BWS. Malignant mesothelioma biomarkers: From discovery to use in clinical practice for diagnosis, monitoring, screening, and treatment. *Chest*. 2017;**152**(1):143-149

[84] Cristaudo A, Bonotti A, Guglielmi G, Fallahi P, Foddìs R. Serum mesothelin and other biomarkers: What have we learned in the last decade? *Journal of Thoracic Disease*. 2018;**10**(Suppl 2):S353-S359

[85] Foddìs R, Bonotti A, Landi S, Fallahi P, Guglielmi G, Cristaudo A. Biomarkers in the prevention and follow-up of workers exposed to asbestos. *Journal of Thoracic Disease*. 2018;**10**(Suppl 2):S360-S368

[86] Kirschner MB, Cheng YY, Badrian B, Kao SC, Creaney J, Edelman JJ, et al. Increased circulating miR-625-3p: A potential biomarker for patients with malignant pleural mesothelioma. *Journal of Thoracic Oncology*. 2012;**7**(7):1184-1191

[87] Micolucci L, Akhtar MM, Olivieri F, Rippo MR, Procopio AD. Diagnostic value of microRNAs in asbestos exposure and malignant mesothelioma: Systematic review and qualitative meta-analysis. *Oncotarget*. 2016;**7**(36):58606-58637

[88] Mozzoni P, Ampollini L, Goldoni M, Alinovi R, Tiseo M, Gnetti L, et al. MicroRNA expression in malignant pleural mesothelioma and asbestosis: A pilot study. *Disease Markers*. 2017;**2017**:9645940

[89] Santarelli L, Gaetani S, Monaco F, Bracci M, Valentino M, Amati M, et al. Four-miRNA signature to identify asbestos-related lung malignancies. *Cancer Epidemiology, Biomarkers & Prevention*. 2019;**28**(1):119-126

[90] Bonotti A, Simonini S, Pantani E, Giusti L, Donadio E, Mazzoni MR, et al. Serum mesothelin, osteopontin and vimentin: Useful markers for clinical monitoring of malignant pleural mesothelioma. *The International Journal of Biological Markers*. 2017;**32**(1):e126-e131

[91] Felten MK, Khatab K, Knoll L, Schettgen T, Muller-Berndorff H, Kraus T. Changes of mesothelin and osteopontin levels over time in formerly asbestos-exposed power industry workers. *International Archives of Occupational and Environmental Health*. 2014;**87**(2):195-204

[92] Battolla E, Canessa PA, Ferro P, Franceschini MC, Fontana V, Dessanti P, et al. Comparison of the diagnostic performance of Fibulin-3 and Mesothelin in patients with pleural effusions from malignant mesothelioma. *Anticancer Research*. 2017;**37**(3):1387-1391