Early detection of malignant pleural mesothelioma

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Malignant pleural mesothelioma (MPM) is a rare tumour. Exposure to asbestos is a well-established aetiological factor for MPM. Patients typically present with shortness of breath due to pleural effusion or chest pain in a more advanced stage. The diagnosis is usually suggested by imaging studies (unilateral pleural thickening; pleural effusion). An occupational history must be obtained. Cytological examination of the effusion can be diagnostic, but often shows equivocal results. Therefore, histology, including immunohistochemistry, is the gold standard. Thoracoscopy, a video-assisted surgical procedure or open pleural biopsy in a fused pleural space may be necessary to provide sufficient material for accurate histological diagnosis. There are three main histological types (epithelial, sarcomatous and mixed) with ~60% being epithelial.

Data suggest the possible contribution of serum mesothelin-related proteins and osteopontin along with others as useful markers to support the diagnosis of mesothelioma; however, the precise role of these markers is yet to be defined.

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Introduction

Malignant pleural mesothelioma (MPM) is an aggressive neoplasm of the serosal lining of the pleural cavity arising from the mesothelial cells (i.e. from undifferentiated cells representing the adult remnants of the surface coelomic mesoderm) [1]. MPM is a rare tumor, but epidemiologic studies show a sharply rising incidence [2]. Although rare, its incidence is increasing, principally as a result of the long latency period of the disease [3]. Early diagnosis is a potential key factor to achieve significant progress in MPM management [4]. However, this is hampered by nonspecific presenting symptoms and diagnostic difficulties, including differentiation from reactive mesothelium, benign pleural lesions, and adenocarcinoma [5]. Early detection is limited by the long latency period, an inability of imaging to detect the disease at an early stage even when it is used as a screening strategy, and the lack of sensitive and specific blood-based markers [6].

Global burden of malignant pleural mesothelioma

The global mesothelioma burden is unclear [7]. Driscoll *et al.* [8] estimated that as many as 43 000 people worldwide die from the disease each year. Park *et al.* [9] suggested that one mesothelioma patient may be overlooked for every four to five recorded . However, to date, there is no established global baseline that can be used to evaluate trends in disease occurrence [10].

In industrialized countries

Mesothelioma incidence rates have been increasing throughout the industrialized world [11] (Figs 1 and 2). This reflects industrial exposure to asbestos, which was common up to the 1980s, combined with a latent period between exposure to asbestos and development of mesothelioma averaging 30–40 years [11]. The incidence is predicted to peak around 2020 [12].

In developing countries

Because of scant regulations of asbestos in the developing world, there is an emerging concern about the potential for a significant increase in asbestos-related disease [13]. Developing countries are still in the early stages of diagnosing mesothelioma and may be prone to misdiagnosis and reporting errors [14].

In Egypt

MPM is an increasing disaster in Egypt, which is underestimated and neglected [15]. Mesothelioma in Egypt is mainly attributed to an environmental origin — that is, exposure to asbestos, with a high incidence in women and young adults. Epidemiological data of 635 malignant mesothelioma patients over 4 years were collected from the National Cancer Institute, Cairo University and Abbassia Chest Hospital. This number is more than four times the number of patients that were diagnosed in the previous 11 years at National Cancer Institute [16].

Data obtained from the information network of the General Organization for Industrialization in Egypt showed that 14 asbestos factories were present in Egypt in the year 2004 [17]. These factories affect an area of ~5–7 km in radius, which explains the high

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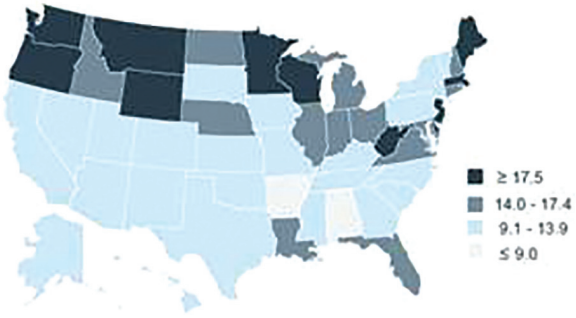
Fig. 1



1999–2005 malignant mesothelioma death rate per one million population in the USA.

incidence of mesothelioma in the neighborhood of these factories. Workers employed since 1948 by the Egyptian asbestos company Sigwart at the mills in greater Cairo (El-Maasara and Shubra El-Kheima) had an increased risk for mesothelioma (Fig. 3). In Egypt, the ministerial council decided to ban asbestos imports in 2004 and the Sigwart plants were closed in November 2004. Therefore, the predicted incidence of Mesothelioma in Egypt will reach its peak around 2040 [18].

Diagnosis of malignant pleural mesothelioma

The diagnosis of MPM is based on clinical presentation, history of asbestos exposure, radiological finding as well as pathological diagnosis [19].

History

Occupational asbestos exposure accounts for the majority of mesothelioma patients [20]. Thus, the diagnosis of mesothelioma in a worker should be viewed as a sentinel event, pointing to the need to obtain a comprehensive occupational history to elucidate possible occupational and nonoccupational asbestos exposures in the past [21].

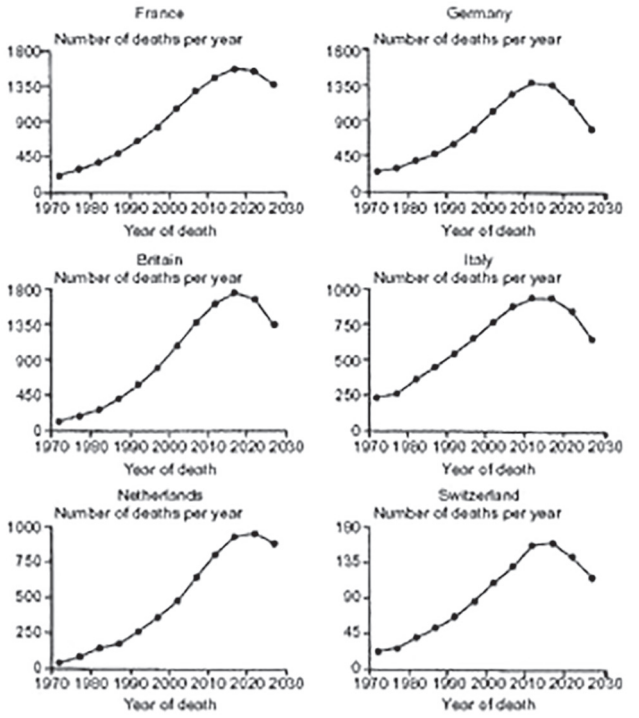
Clinical picture

Early in the course of the disease, dyspnea is the commonest symptom and is due to the presence of effusion. Chest pain may occur as the tumor locally invades the intercostal nerves. Eventually, the lung becomes encased by the tumor leading to worsening of dyspnea and chest tightness [22].

Radiology

The radiological diagnosis of pleural mesothelioma requires a high degree of clinical suspicion [23].

Fig. 2



Mesothelioma in Europe.

Chest radiography

The radiological manifestations tend to be those of pleural effusion and/or pleural thickening [24] (Fig. 4).

In late stages, ipsilateral shift of the mediastinum and retraction of the involved hemithorax are characteristic unless the tumor volume becomes very large [25].

Computed tomography scan

Pleural thickening or small focal pleural masses may be seen on computed tomography (CT). CT of the chest is useful in differentiating pleural fluid from pleural thickening [22] (Fig. 5).

The CT scan provides much greater sensitivity than the usual chest radiograph for identifying fluid and visualizing pleural-based masses [26].

Spiral computed tomography

The introduction of spiral CT into the field has yielded advantages in the diagnosis of MPM and its staging. Spiral CT combines the advantage of single breath-hold acquisition and three-dimensional reconstruction capabilities between bronchus, artery, vein, pleura, and lobular septum [27].

It is also better in visualization of the prediaphragmatic region in a single breath-hold, minimizing motion artifacts and thus better visualization of the diaphragmatic extension of MPM [28].

Magnetic resonance imaging

MRI showed tumor spread, tumor invasion of and through the diaphragm, and invasion of bony structures better than CT. Invasion of the chest wall and mediastinal soft tissue and tumor growth into the lung emphysema were equally well seen on both imaging methods [29] (Fig. 6).

Magnetic resonance imaging versus computed tomography scan

Recent studies comparing CT and MRI have shown that MRI is not significantly better than CT in defining local extent of the tumor. Therefore, CT remains the standard imaging study [30].

Position emission tomography

PET is a nuclear medicine imaging technique. PET scanning detects γ rays emitted by a tracer that is delivered into the body by a biologically active molecule, typically ^{18}F-FDG, a form of glucose. The effectiveness of the scan develops in part on how much ^{18}F-FDG is absorbed by the cancer cells, and hence can be seen on imaging [31] (Fig. 7).

Position emission tomography-computed tomography

PET-CT seems to be superior to other imaging modalities in detecting more extensive disease involvement and in identifying unsuspected occult distant metastases [32].

Gallium scan

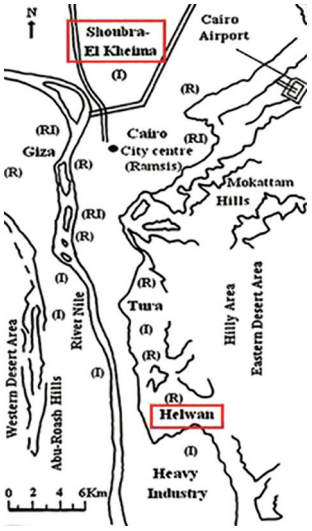
Mesothelioma is reported to take up gallium 76. Gallium 76 scans in seven patients obtained before resection were compared with pathology, where no definite correlation was found between gallium 76 uptakes and the histological type [33] (Fig. 7).

In another study, uptake of gallium citrate by mesothelioma was positive in 43 of the 49 patients (88%) with pleural mesothelioma [25] (Fig. 8).

Thallium 201

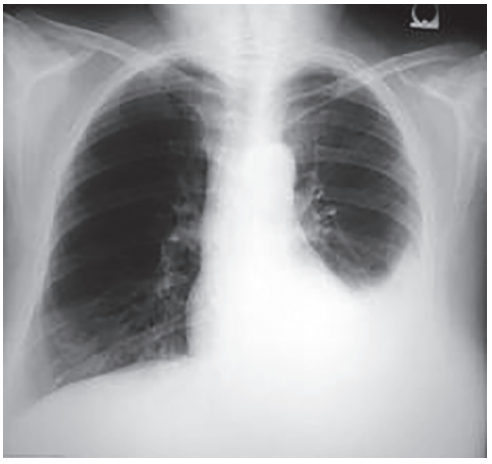
Planar thallium 201 scintigraphy in a single mesothelioma patient revealed diffuse pleural accumulation. It can demonstrate the exact tumor location [34].

Fig. 3



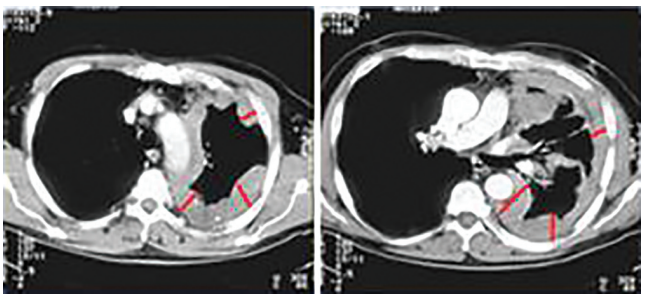
Map of Greater Cairo. fx1 Industrial areas highly polluted with asbestos.

Fig. 4



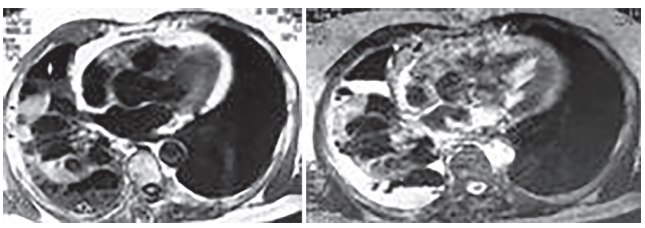
Chest radiograph showing left-sided pleural mesothelioma.

Fig. 5



Appearance on chest computed tomography of malignant pleural mesothelioma. Red bars measure the thickness of pleural disease.

Fig. 6



MRI showing right-sided malignant pleural mesothelioma.

Additional imaging studies

Bone scans are indicated to evaluate specific symptoms (i.e. localized bone pain or laboratory abnormalities such as an elevated alkaline phosphatase) [25].

Thoracocentesis

Thoracocentesis is often the initial diagnostic intervention. Cytological diagnosis of MPM from pleural fluid is, however, unreliable, as reactive mesothelial cells and cells from other malignant tumors such as sarcomas and adenocarcinomas are often very difficult to distinguish from malignant mesothelioma cells [35].

Pleural biopsy

Tissue sampling must be carried out to diagnose MPM correctly [23].

Closed pleural biopsy

Percutaneous pleural biopsy yields a diagnosis of malignancy in up to one-third of patients [30].

The relatively low yield of blind pleural biopsies is owing to several factors that may be relevant to the stage of the disease when pleural biopsy is performed, to the absence of visualization of the area being sampled, and to operator inexperience [36].

Computed tomography-guided cutting needle biopsy

CT-guided cutting needle biopsy of the pleural tissue associated with a pleural effusion is a relatively new technique compared with Abram's biopsy [37]. Results of observational series suggest that this technique might improve the diagnostic sensitivity to about 80% for pleural malignancy [38].

Thoracoscopy

Thoracoscopy is an excellent tool to diagnose pleural disease definitively and yields almost a 100% success rate [39]. Video-assisted thoracoscopy has allowed improved visualization of the pleura and offers the ability to perform biopsies from multiple sites [40] (Fig. 9).

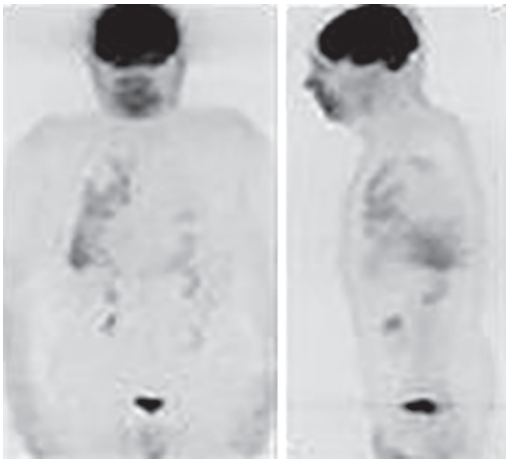
Open (surgical) pleural biopsy

Surgical biopsy would be an ultimate possibility to explore the pleural space, providing both excellent visualization of the target areas and the largest possible biopsy size. In addition, it may be combined with a potentially curative therapeutic approach by partial pleurectomy and/or partial chest wall resection. Open biopsy has a sensitivity of 97% and specificity of 56% for identifying epithelial MPM [39].

Pathological diagnosis

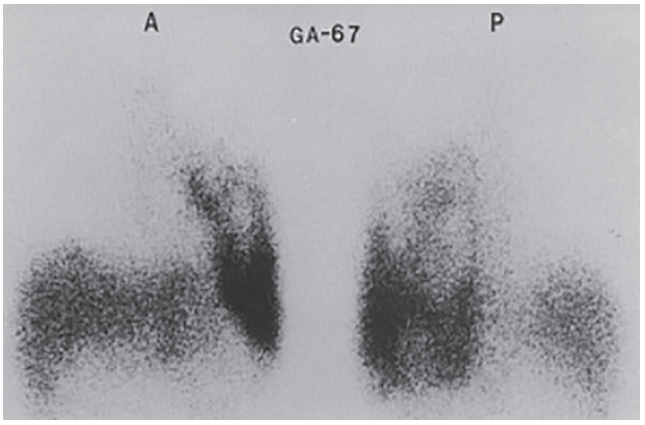
Diagnosis is usually achieved by the application of a combination of techniques, including histopathology, histochemistry, immunohistochemistry, ultrastructural examination, and mesothelial markers [41].

Fig. 7



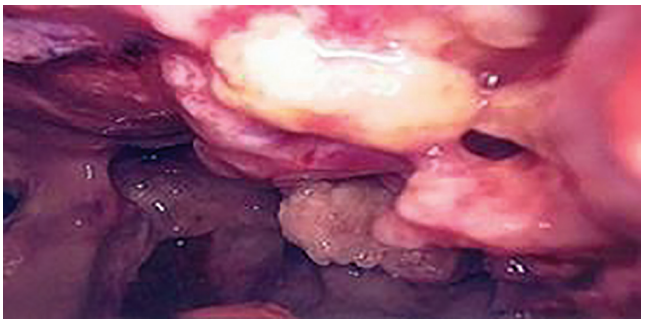
PET scan for a patient with malignant pleural mesothelioma.

Fig. 8



Anterior and posterior scan images show diffuse Ga-67 uptake in the left middle and lower lung fields.

Fig. 9



Video-assisted thoracoscopy showing malignant pleural mesothelioma.

Histopathology

Mesothelioma is classified into the following three histological subtypes: epithelial, sarcomatoid, and biphasic (Fig. 10).

The epithelial subtype is the most common and carries the most favorable prognosis [42].

Histochemical techniques

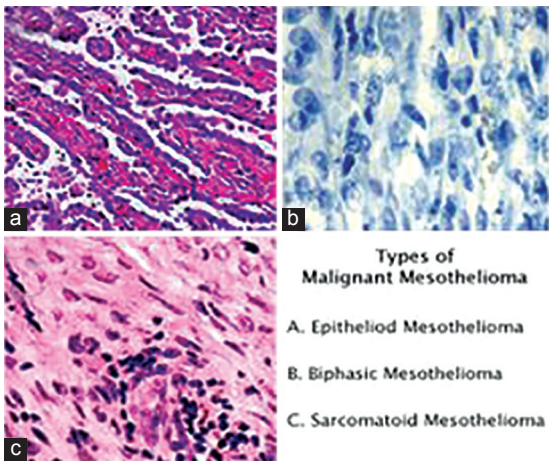
The most frequently used biochemical stain is the Periodic acid Schiff stain with diastase. The presence of strong Periodic acid Schiff positivity in tumor cells is indicative of epithelial mucin, which strongly argues the diagnosis of malignant mesothelioma. Alcian blue stains hyaluronic acid positively in mesothelioma [41].

Immunohistochemistry

Immunohistochemistry is currently the most widely applied technique in establishing the diagnosis of mesothelioma. Epithelial mesothelioma typically stains positive for calretinin, cytokeratins, and vimentin, and negative for CEA and thyroid transcription factor-1 [43] (Fig. 11).

A pathology panel was convened at the International Mesothelioma Interest Group biennial meeting (October 2006) to develop the practical guidelines for the pathologic diagnosis of malignant mesothelioma. The International Mesothelioma Interest Group recommends that markers have either sensitivity or specificity greater than 80% of the lesions in questions. Interpretation of positivity generally should take into account the localization of the stain (e.g. nuclear vs. cytoplasmic) and the percentage of cells staining (>10% is suggested for cytoplasmic membranes markers). These guidelines are meant to be a practical reference for the pathologist [44].

Fig. 10



Types of malignant pleural mesothelioma. (a) Epithelioid mesothelioma. (b) Biphasic mesothelioma. (c) Sarcomatoid mesothelioma.

Ultrastructural examination

The epithelial variant is composed of polygonal cells with numerous long slender branching surface microvilli, desmosomes, abundant tonofilaments, and intracellular lumen formation. In sarcomatoid variant, elongated nuclei and abundant rough endoplasmic reticulum are found. The biphasic nature of mesothelioma is characterized by stromal cells separated by matrix containing collagen fibers, which appear spindle or ovoid [45] (Fig. 12).

Microarray

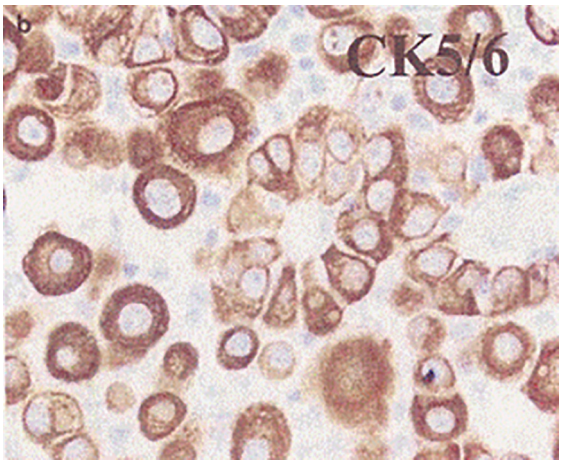
A microarray or gene chip measures the expression level of a gene by determining the amount of messenger RNA (mRNA) that is present. A microarray allows the simultaneous analysis of the expression levels of hundreds, thousands, or even tens of thousands of genes in a single experiment.

mRNA is copied into labeled cDNA with reverse transcriptase so that the relative abundance of individual mRNAs is reflected in the cDNA product. This method has proven to provide excellent specificity and reproducibility using this technique, based on the expression levels of a small number of genes, and can be useful in the early and accurate diagnosis of MPM [46].

Biomarkers

Improved detection methods for the diagnosis of asymptomatic MPM are essential for an early and reliable detection and treatment of this type of neoplastic diseases. Thus, focus has been on finding tumor markers in the blood that can be used for noninvasive detection of malignant mesothelioma [47].

Fig. 11



Stains used to diagnose mesothelioma. CK5, cytokeratin 5/6.

Mesothelin

Mesothelin is a 40-κD cell surface glycosylated phosphatidylinositol-anchored glycoprotein, which functions in cell-to-cell adhesion [48]. Mesothelin is expressed by normal mesothelial cells; however, it is highly overexpressed in cancers such as MPM, pancreatic, or ovarian carcinoma [49].

Soluble mesothelin-related peptide (SMRP) is another protein product of the human mesothelin gene [50]. SMRP can be detected in blood, and has been found highly increased in blood of patients with mesothelioma [51]. The first report of SMRP as a marker of mesothelioma suggested excellent values for sensitivity (84%) and specificity (virtually 100%) [51]. Evidence has been published indicating that an enzyme-linked immunosorbant assay using two antibodies to SMRP, OV569 and 4HS, holds promise for detection of MPM [52].

Megakaryocyte potentiating factor (MPF)

MPF is a soluble protein produced by proteolytic cleavage of the mesothelin precursor protein and is secreted by the mesothelioma cell lines [52]. Holleroet *et al.* found that MPF can be used as a serum biomarker of malignant mesothelioma. At 95% specificity, MPF had a sensitivity of 68% [53].

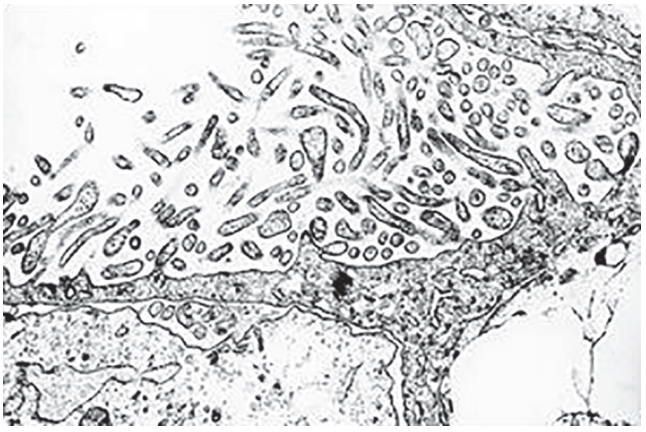
Osteopontin

Osteopontin, also known as early T-lymphocyte activation 1 (Eta-1), is a secreted multifunctional glycoprotein. Its putative functions include roles in bone metabolism, urine regulation, wound healing, cell survival, and tumor progression [54].

Serum osteopontin levels are higher in patients with mesothelioma than in those with asbestos-related, nonmalignant pleural diseases [54]. Osteopontin levels are elevated in patients with both the epithelioid and sarcomatoid subtypes as well as in patients with several other malignancies and nonmalignant conditions [55]. Osteopontin has been seen as a promising biomarker because of its expression on gene expression arrays to predict survival and recurrence patterns in patients with pleural mesothelioma [56], and it seems especially interesting as a potential early diagnostic marker because it has been shown to differentiate asbestos-exposed patients from stage I mesothelioma patients [57].

Tables 1 and 2 summarize the data on the previous biomarkers.

Fig. 12



Transmission electron micrograph of an epithelial mesothelioma showing long microvilli.

Angiopoietin-1

Angiopoietin-1 (Ang-1) is a counteracting ligand for the endothelial-specific receptor tyrosine kinase Tie-2 and is an important regulator of blood vessel growth, maturation, and function. Ang-1 promotes angiogenesis, induces vascular maturation, and decreases vascular permeability [59].

Tabata *et al.* [60] demonstrated that Ang-1 stimulated the cell growth and migration of MPM cells in in-vitro studies. They also demonstrated that patients with MPM had significantly higher serum levels of Ang-1 in comparison with a population with a history of asbestos exposure that did not develop MPM. The authors suggest that Ang-1 could be a novel useful serum prognostic factor.

Table 1 Serum biomarkers for mesothelioma [58]

Question	SMRP	MPF	Osteopontin
What is it?	A splice variant of mesothelin	A splice variant of mesothelin; stimulates megakaryocyte colony formation *in vitro*	A tumor-associated glycoprotein; regulates cell–matrix interaction and cellular signaling
Elevated in epithelial MM?	Yes	Yes	Yes
Elevated in sarcomatoid MM?	No	No	Yes
Elevated in other cancer?	Ovarian, pancreatic, and lung carcinomas, non-Hodgkin lymphoma	Cervical, ovarian, and pancreatic cancer	Breast, colorectal, gastric, lung, melanoma, ovarian, prostate cancer
Elevated in other benign conditions?	No	No	Coronary artery disease, interstitial pneumonia, and other benign lung disease

SMRP, soluble mesothelin-related peptide.

Table 2 Potential uses for mesothelioma serum biomarkers [58]

Variables	SMRP	MPF	Osteopontin
Screening in asbestos-exposed population (retrospective study)	Yes	Not done	Yes
Screening in asbestos-exposed population (prospective study)	High false-positive ratio	Not done	Not done
Monitoring treatment effect	Yes	Yes	Yes
Predicting prognosis	Yes	Yes	Yes
Differentiating MM from benign pleural disease	Yes	Yes	Yes
Differentiating MM from other cancers	Yes	Not done	No
Determining duration of asbestos exposure	Not done	Not done	Yes
Sensitivity for detecting MM (at a specificity of 95%)	73%	34%	47%

SMRP, soluble mesothelin-related peptide.

Fibulin-3

Fibulin-3 is a highly conserved member of the extracellular glycoprotein fibulin family encoded by the gene, epidermal growth factor-containing fibulin-like extracellular matrix protein (EFEMP1), on chromosome $2p^{16}$ [61]. Plasma fibulin-3 levels can distinguish healthy persons with exposure to asbestos from patients with mesothelioma. In conjunction with effusion fibulin-3 levels, plasma fibulin-3 levels can further differentiate mesothelioma effusions from other malignant and benign effusions [3].

Fibronectin

Fibronection is a high-molecular-weight glycoprotein, which binds to receptors known as integrins. It exists in two forms, an insoluble form in the extracellular matrix and a soluble form found in the plasma [62]. Its role as a tumor marker in malignant mesothelioma has been investigated by Emri *et al.* [63].

The level of fibronectin in the pleural fluid and the plasma was elevated in patients with malignant mesothelioma but was not statistically significant in comparison with tuberculous and other nonmesotheliomatous effusions. Thus, measurement of serum fibronectin may not add much to the existing diagnostic aids.

CYFRA21-1

CYFRA21-1 is a soluble cytokeratin 19 fragment and can be used as a tumor marker in many cancers [62].

Paganuzzi *et al.* [64] observed a significantly higher level of CYFRA21-1 in the serum of patients with malignant mesothelioma in comparison with patients with any benign pulmonary disease. It can serve as a marker for follow-up of patients with established malignant mesothelioma, as it is found to have a significant prognostic value for survival [65]. The sensitivity of serum CYFRA21 levels for the diagnosis of MPM has been stated to be 40% [65].

Platelet derivative growth factor (PDGF)

PDGF is a protein secreted from the alpha granules of the platelets and mesenchymal cells. It regulates cell division and growth and also has a role in migration and angiogenesis. It exists as a dimer with the isoforms: AA, AB, and BB. PDGF AB was found to be significantly higher in patients with mesothelioma compared with their healthy controls [66].

The levels showed a correlation with the histological subtypes, higher levels seen in the epitheloid subtype. Serum PDGF level can be used as a marker to assess the prognosis in patients diagnosed with malignant mesothelioma [62].

Tissue polypeptide antigen

Tissue polypeptide antigen is a growth and proliferative cell marker. It is a polypeptide composed of cytokeratin 8, 18, and 19. It is found to be elevated in many cancers including squamous cell carcinoma of the head and neck and thyroid cancer. Its role in malignant mesothelioma has been investigated in few studies [62]. It was found to be a useful marker in predicting disease progression and survival [67].

Serum hyaluronan

Hyaluronan is a glycosaminoglycan distributed widely in the body. It is found in the synovial fluid connective tissue and vitreous humor of the eye. Serum and pleural fluid hyaluronan levels have been found to be increased in patients with malignant mesothelioma. It can serve as a potential tumor marker or as a marker of progression of the disease [68].

The literature reports the sensitivity and specificity of serum hyaluronan to be 65% and 85%, respectively, as an indicator of disease progression [69].

Conclusion

The diagnosis of mesothelioma can be challenging. If the diagnosis of mesothelioma is suspected, a careful occupational history must be obtained. Improved detection methods for the diagnosis of asymptomatic MPM are essential for an early and reliable detection and for treatment of the disease. MPM should be considered in any patient with either pleural fluid or pleural thickening, especially if chest pain is present. Thoracoscopy and open surgical biopsy remain the best diagnostic procedures in suspected MPM. Identification of tumor markers and development of assay to measure them are important goals in oncology.

The proposed markers have insufficient accuracy to replace cytohistology and cannot be presently proposed as screening tools. The usefulness of biological markers should be further evaluated in selected highly exposed population including the involuntary surveillance protocol. Therefore, new mesothelioma-specific biomarkers are needed to detect MPM at an earlier stage.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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