PROGNOSTIC FACTORS IN MALIGNANT PLEURAL MESOTHELIOMA

FATTORI PROGNOSTICI NEL MESOTELIOMA PLEURICO MALIGNO

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Parole chiave: mesotelioma pleurico maligno, fattori prognostici, genetica

Abstract

Malignant pleural mesothelioma is an extremely aggressive neoplasm with a poor prognosis. Conventional medical, physical and surgical treatments and their combinations are basically ineffective. Just a few subjects experience some benefit. In the present review we evaluated the impact on prognosis of some conventional factor (i.e. age, gender, histology, staging) as well as relatively new clinical factors such as quality of life, positron emission tomography assessment, occult residual disease. Furthermore, many biological and genetic markers have been recently recognized at the bases of the onset and growth of mesothelioma. These novel factors may play an important role in defining the prognosis of the disease and eventually they might have an hypothetical usefulness in addressing therapy.
Prognostic factors in malignant pleural mesothelioma

Abstract

Il mesotelioma pleurico maligno è una neoplasia estremamente aggressiva, con una prognosi severa. I trattamenti convenzionali medici, fisici e chirurgici e le loro combinazioni sono praticamente inefficaci e solo pochi soggetti riferiscono qualche beneficio. Nella presente rassegna abbiamo valutato l’impatto sulla prognosi di alcuni fattori classici (es. età, sesso, istologia, staging) come pure quello dei nuovi fattori clinici, quali qualità di vita, tomografia ad emissione di positroni, malattia occulta residua. Inoltre, molti fattori biologici e genetici sono stati recentemente riconosciuti come responsabili dell’origine e dello sviluppo della malattia. Questi nuovi fattori possono esercitare un ruolo importante nella definizione della prognosi e potrebbero avere una ipotetica utilità nella formulazione di una terapia mirata.

Introduction

Malignant pleural mesothelioma are rare and severe tumors originating from the mesothelial cells of the pleura (1). The association between asbestos and mesothelioma is a classic epidemiologic model of exposure leading to the disease within a period of 10-30 years (2, 3). Although the complete ban of asbestos was adopted since the end of the 80s, this long latency explains the persistent high incidence of the disease (4, 5). The majority of the new patients belongs to the large number of individuals exposed during the 1930s to 1960s in asbestos mines and asbestos-related industries, before the causal relationship between asbestos and malignant pleural mesothelioma was recognized (6, 7). An estimated 2,000 to 3,000 cases occur annually in the United States. In Western Europe alone, incidence ranges between 15 to 36 new cases every 100,000 inhabitants with a peak expected for the year 2015 (4) and a quarter of a million deaths are projected over the next 30 years (5).

Malignant pleural mesothelioma is classically characterized by local aggressiveness and poor patient prognosis. The insidious presentation of the disease as well as its diffuse nature make mesothelioma difficult to treat even at early stage. Survival averaging approximately 8-10 months from diagnosis (8, 9). The limited knowledge of the biology of this tumor and its poor response to conventional therapy has resulted in a variety of therapeutic approaches. Despite these efforts the research of an effective therapy remains a frustrating challenge for both oncologists and surgeons. Mesothelioma arises from mesothelium, which is an extensive monolayer of flattened mesothelial cells that line the serous cavities such as pleura, peritoneum, pericardium and testicular tunica vaginalis (9, 10). The natural history of the disease follows sequential phases (11). The tumor usually originates from the parietal pleura and remains localized for a variable period. Usually the growth of the neoplasm follows a continuous pattern. Notwithstanding, at the final stages a distant spread may also take place.

The differential diagnosis is mainly with pleural effusions and thickening (12). Age, gender, associated symptoms, asbestos exposure, positive cytology in pleural effusion and appearance on chest computed tomography (CT) may orientate the diagnosis. However, the presence of benign pleural plaques, which are often correlated to asbestos exposure, may simulate at CT a mesothelioma (13). Furthermore, pleural fluid cytology findings are often negative despite repeated thoracentesis. Therefore, definitive diagnosis can be made only by incision biopsy (14).

Preoperative diagnosis still represents a great challenge (13). A localized disease is infrequently symptomatic at presentation, mainly reporting cough or indistinct chest pain. Chest radiographs usually reveal unilateral pleural abnormalities with a pleural effusion. Chest CT is the main imaging procedure for the evaluation of pleural effusion and thickening. It provides a good evaluation of the size, density and rapport to neighboring intrathoracic organs such as the great vessels, lungs, heart and pericardium.

The merit of positron emission tomographic (PET) scan is currently being assessed (15). The degree of uptake on PET scan should correlate with the most aggressive disease associated with strongly PET positive and viceversa.

This review is aimed at illustrating the present role of the traditional prognostic factors and the hypothetical usefulness of the novel biologic (16) and genetic markers (17). The early identification of poor prognostic indicators may be useful in reducing the heterogeneity of the clinical response and addressing a more targeted therapy.
Clinical prognostic factors

It is general experience that conventional medical, physical and surgical treatments and their combinations are basically ineffective and just a few subjects experience some benefit. So far, the individuation of the best responders to therapy was essentially based on clinical factors. The difficulties in making early diagnosis and accurate staging in a so heterogeneous disease have considerably hindered the formulation of definite prognostic classes. Classic clinical prognostic factors have been recently integrated with other new variables including quality of life status and modern diagnostic techniques. New acquisitions about all these factors were reviewed and analyzed in the following sections.

Asbestos exposure

The inhalation of asbestos fibers would promote the carcinogenic process leading to the onset of mesothelioma (3). According to this rationale, it has been hypothesized that duration and intensity of asbestos exposure may have a prognostic impact (18). Nonepithelial mesothelioma specimens usually present a more elevated asbestos fiber count (19). This finding could be associated to a more elevated and prolonged exposure to asbestos, which happens more in occupational than indoor-outdoor environment (20). This higher asbestos burden might justify the relatively greater proportion of men among patients with nonepithelial histology as well as the rarity of these histotypes in women, which are involved only to background or household exposures (21, 22, 23, 24, 25). As consequence the load and duration of the exposure may represent an indirect prognosticator.

Gender and age

Gender and age have per se a scant relevance as prognostic factors, but they might assume a role since indirectly related to other more effective prognosticators. As reported in the previous paragraph, the asbestos exposure influences the correlation of sex and age with some histotypes. According to this account female subjects are exposed to a minor load of asbestos and tend to develop relatively less aggressive forms. On the contrary, the relatively smaller amount of women among patients with nonepithelial histology may be correlated to the higher asbestos load in men due to direct occupational exposure (20).

The impact of the age on the prognosis is differently considered. Similarly to other tumors the early onset of a mesothelioma may imply a more aggressive disease. However, the development of the tumor in the younger subset of the population would be associated to background or household exposure. In these conditions the most frequent histology is epithelial histology and therefore the prognosis less severe. Several authors have recently included the advanced age as a good predictor of lower survival indicating various cutoff thresholds such as 50 (21), 60 (26), 70 (27) and 75 (28) years.

Performance status and quality of life

Performance status or other measures of quality of life are an indirect evidence of the status of the disease and may have a certain correspondence with the prognosis (27). As a matter of fact two major prognostic score systems European Organization for Research and Treatment of Cancer (EORTC) (29, 30) and Cancer and Leukemia Group B (CALGB) (25) include the poor performance status in the number of the parameters to complete the score. Performance status can be classically measured by Karnofsky index (31) and this parameter was documented as one of the most sensible indicator of prognosis. Tanrikulu et al proved effective with the Karnofsky index a threshold of 60 at both uni and multivariate analysis in 363 patients diagnosed from 1989 to 2010 (26). The other scale of performance status is represented by the Eastern Cooperative Oncology Group (ECOG) and the cutoff value was considered greater than 0 (8).

Due to the high mortality of the disease the improvement of quality of life is often considered the most important endpoint of therapy. Nevertheless, general health scales such as the SF-36 Health Survey (32) can also become prognostic instruments. Few papers dealt with the role of quality of life index as prognostic factor. The significant amelioration after extrapleural pneumonectomy was documented as associated with a longer survival. In addition, the presence of a postoperative low physical component summary SF-36 was significantly correlated to a lower survival rate after extrapleural pneumonectomy (33).

Histology

Mesothelioma is classically divided into three histologic subtypes: epithelial, mixed or biphasic and sarcomatoid (34). These last types are correlated to a more severe prognosis. As mentioned above the onset of certain histotypes may be correlated to the amount and the period of asbestos exposure. In a wide review Hillerdal et al (35) reported that 50% were epithelial, 34% mixed and 16% sarcomatoid types. It is common knowledge that the epithelial subtype is
the most common and its presence may strongly influence the therapeutic strategy towards more aggressive procedures. Thus, it would be desirable to achieve an histological diagnosis before to start any form of treatment. According to the morphologic features it is frequently difficult to distinguish between an epithelial mesothelioma and an adenocarcinoma, or between a sarcomatoid mesothelioma and a sarcoma. No single immunohistochemical stain is diagnostic; a panel of both positive and negative stains is required. Epithelial mesotheliomas typically stain positive for calretinin, cytokeratins, and vimentin, and negative for carcinoembryonic antigen and thyroid transcription factor (10).

**Staging**

Several pathologic staging system have been proposed (36, 37, 38, 39), but none of them provided optimal survival stratification among patients treated with surgery. In 1976 Butchart (36) formulated the first pathologic staging system on the basis of the findings during extrapleural pneumonectomy. This system had a limited value due to the restrict number of patients suitable to this operation and the high postoperative mortality. The Brigham and Women’s Hospital system represented an evolution of the previous system. It was formulated according to the results of extrapleural pneumonectomy in trimodality therapy (38). Important prognostic factors were the involvement of surgical margins and extrapleural lymph nodes although the latter were difficult to determine (39, 40).

In order to uniform the system with other neoplastic forms a TNM classification by the International Mesothelioma Interest Group was proposed since 1995 (39) (Table 1).


<table>
<thead>
<tr>
<th>pT</th>
<th>Description</th>
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<tbody>
<tr>
<td>T1a</td>
<td>Tumour limited to the <strong>ipsilateral parietal pleura</strong> including mediastinal and diaphragmatic pleura. No involvement of the visceral pleura.</td>
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<tr>
<td>T1b</td>
<td>Tumour involving the <strong>ipsilateral parietal pleura</strong>, including mediastinal and diaphragmatic pleura. Scattered <strong>foci of tumour</strong> involving the <strong>visceral pleura</strong>.</td>
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<tr>
<td>T2</td>
<td>Tumour involving each of the ipsilateral pleural surfaces (parietal, mediastinal, diaphragmatic, and visceral pleura) with at least one of the following features:</td>
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<td></td>
<td>· involvement of <strong>diaphragmatic muscle</strong></td>
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<td></td>
<td>· <strong>confluent visceral pleural</strong> tumour (including the fissures) or extension of tumour from visceral pleura into underlying <strong>pulmonary parenchyma</strong></td>
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<tr>
<td>T3</td>
<td>Describes locally advanced but potentially resectable tumour.</td>
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<td></td>
<td>Tumour involving all of the ipsilateral pleural surfaces (parietal, mediastinal, diaphragmatic, and visceral pleura) with at least one of the following features:</td>
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<td></td>
<td>· involvement of the <strong>endothoracic fascia</strong></td>
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<td></td>
<td>· extension into the <strong>mediastinal fat</strong></td>
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<td></td>
<td>· <strong>solitary</strong>, completely resectable focus of tumour extending into the <strong>soft tissues of the chest wall</strong></td>
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<td></td>
<td>· <strong>non-transmural</strong> involvement of the <strong>pericardium</strong></td>
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<tr>
<td>T4</td>
<td>Describes locally advanced technically unresectable tumour.</td>
</tr>
<tr>
<td></td>
<td>Tumour involving all of the ipsilateral pleural surfaces surfaces (parietal, mediastinal, diaphragmatic, and visceral pleura) with at least one of the following features:</td>
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<tr>
<td></td>
<td>· <strong>diffuse extension or multifocal masses</strong> of tumour in the <strong>chest wall</strong>, with or without associated <strong>rib</strong> destruction</td>
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<td>· direct transdiaphragmatic extension of tumour to the <strong>peritoneum</strong></td>
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<td>· direct extension of tumour to the <strong>contralateral pleura</strong></td>
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<td>· direct extension of tumour to one of more <strong>mediastinal organs</strong></td>
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<td>· direct extension of tumour into the <strong>spine</strong></td>
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<td></td>
<td>· tumour extending through to the <strong>internal surface of the pericardium</strong> with or without a pericardial effusion, or tumour involving the <strong>myocardium</strong></td>
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Prognostic factors in malignant pleural mesothelioma

**pN**

- **N0** No regional node metastases
- **N1** Metastases in the ipsilateral bronchopulmonary or hilar lymph nodes
- **N2** Metastases in the subcarinal or ipsilateral mediastinal lymph nodes, including the ipsilateral mammary nodes
- **N3** Metastases in the contralateral mediastinal, contralateral internal mammary, ipsilateral or contralateral supraclavicular lymph nodes

**pM**

- **M0** No distant metastasis
- **M1** Distant metastasis present

<table>
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<tr>
<th>Stage summary</th>
<th>Stage</th>
<th>Description</th>
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<tr>
<td>Stage Ia</td>
<td>T1a</td>
<td>N0</td>
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<tr>
<td>Stage Ib</td>
<td>T1b</td>
<td>N0</td>
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<tr>
<td>Stage II</td>
<td>T2</td>
<td>N0</td>
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<tr>
<td>Stage III</td>
<td>Any T3</td>
<td>Any N1</td>
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<td>Any N2</td>
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<tr>
<td>Stage IV</td>
<td>Any T4</td>
<td>Any N3</td>
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<td>Any M1</td>
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It incorporated data on the natural growth of the disease. The T parameter described the progressive invasion of the thoracic structures defining with T3 advanced but potentially resectable disease and with T4 unresectable one. All patients with nodal involvement either hilar or mediastinal presented the same prognosis and therefore they were all included into a same stage. Unfortunately, also this staging system failed to predict survival in a large series of patients undergoing radical multimodality therapy evidencing groups with same TN status and different prognosis (41).

This system has been recently revised restricting the analysis only to mesotheliomas with epithelial histology (42). Involvement of lung parenchyma and diaphragm were upstaged from T2 to T3, and involvement of mediastinal fat from T3 to T4. Conversely, invasion of endothoracic fascia, chest wall at a single focus and into pericardium were downstaged from T3 to T2. Risk of residual disease was also considered on the basis of pathologic examination staging as T2 the presence of the tumor within 1mm of any margin, as T3-T4 the involvement of specific surgical margins and as T4 the seeding of the tumor within prior chest tube sites. The new TN combinations resulted in improved stage survival stratification: median values were 51 months at stage I, 26 at II, 15 at III and 8 at IV, respectively (42).

At present accurate staging requires complete assessment of histotype, tumor extent, resection margin and nodal metastases and still remains a very demanding challenge in mesothelioma. A revision for nodal station-dependent status has been recently proposed (43). Despite refinement in diagnostic devices a consistent discrepancy in defining specific characteristics between non-invasive and intraoperative/pathological staging still persists. Furthermore the relatively low number of patient per year hinders a prospective analysis which is mandatory for validation.

**PET scan staging**

The use of PET as prognostic instrument is current topic of discussion in oncology. In theory, the entity of metabolism is directly proportional to the cellular reduplication activity, thus it is conceivable that the intensity of tracking-signal related to $^{18}$fluorodeoxyglucose uptake should be a good predictor of the aggressiveness of the disease. The role of PET as staging and prognostic device in malignant pleural mesothelioma has been widely investigated since 1998 (44).

In 1999 Bénard et al (45) demonstrated an evident difference in standard uptake value (SUV) in the pleural primary lesions between short and long-survivors. Many studies in the literature confirmed the importance of SUV in predicting the prognosis. Ceresoli et al (46) reported a correlation between decrement of SUV after mid-term chemotherapy and the less aggressive evolution of the disease. Lee et al (47) have recently showed the relationship between SUV of the primary lesion and the propensity to metastatize. However, there is a not substantial agreement about the most significant threshold value. Flores et al (48) in a large series selected 10 as the best SUV, in the same year Meigan et al (49) indicated 4 as the most predictive value.
Surgery
The prognostic impact of the extent of the resection still represents a critic topic of discussion. Although in theory extrapleural pneumonectomy can be considered a more radical procedure, it did not affect survival more significantly than pleurectomy/decortication. A recent comparative analysis in 663 consecutive patients demonstrated no statistical difference in survival by procedure at any stage (50). On the contrary, multivariate analysis showed a marginal survival benefit for pleurectomy/decortication (51).

The substantial equivalence between the two operations may be explained in different ways. Pleurectomy/decortication is a less radical operation and this belief is confirmed by the observation of a higher local recurrence rate (65%) (52). This negative impact on prognosis is partially counterbalanced by the slightly lower operative mortality of the procedure. Furthermore, in patients with bulky tumor or confluent pleural tumor, an extrapleural pneumonectomy is necessary to achieve complete resection. Therefore, extrapleural pneumonectomy may be more frequently performed in the most extended tumors, which has worse prognosis.

Occult residual disease
Both extrapleural pneumonectomy and radical pleurectomy/decortication are performed with the aim of extirpate all gross tumor (53). Nevertheless none of the two procedures is able to eradicate all residual microscopic tumors. The prognostic impact of occult disease after extrapleural pneumonectomy for malignant mesothelioma was investigated in resection margins and extrapleural nodal status. Sugarbaker et al demonstrated in a wide series that pre-resectional evaluation of extrapleural nodes may individuate those patients suitable for radical therapy (54). Same authors showed that the presence of microscopic tumor foci in the resection margins affects long-term survival, highlighting the need for further investigation of locoregional control. Patients with margin-negative, extrapleural node-negative resection demonstrated a longer survival [40]. Cervical mediastinoscopy was useful in all patients considered for extrapleural pneumonectomy (55, 56, 57).

The presence of occult disease in resection margins and lymph nodes after extrapleural pneumonectomy has been investigated in a recent multiinstitutional study by immunohistochemical staining with anticalretinin and antimesothelin monoclonal antibodies (58). Occult disease was significantly correlated with rapid disease recurrence and with a worse prognosis.

Multimodality therapy
Multimodality approach combines chemotherapy and/or radiation with surgery. It is usually deemed necessary since neither extrapleural pneumonectomy nor pleurectomy/decortication eradicates all residual microscopic tumors. Initially multimodality therapy outlined maximal cytoreductive surgery followed by radiotherapy and chemotherapy (38). Although multimodality therapy produced an evident effect in prolonging survival, the risk of local recurrence was very high (59) with scant benefits on general prognosis.

Present trials introduced a neoadjuvant approach, consisting of three to four cycles of a platinum agent with either gemcitabine or pemetrexed, followed by surgery (60). Radiotherapy is usually performed after resection to allow high-doses irradiation without involvement of the underlying lung. A multicenter neoadjuvant Swiss study (61) documented a positive response in 74% of the patients who underwent extrapleural pneumonectomy in a multimodality strategy; however median survival time did not exceed 23 months. In a recent American trial (60), 33% of patients responded to the chemotherapy and in 70% was possible an extrapleural pneumonectomy, but even in this study median overall survival time reached only 16.8 months.

Optimizing the multimodality treatment should always consider the level of chemotherapeutic response. At present chemotherapeutic drugs are chosen on empiric bases. Mujoomdar et al (62) have recently investigated the prevalence of in-vitro drug resistance on a large sample of resected mesothelioma specimens. The authors found a significant proportion of extreme/intermediate resistance to cisplatin, gemcitabine and vinorelbine, which are credited as the most active drugs against mesothelioma. It is conceivable that these patients may show a marginal drug-response also in vivo, thus reducing feasibility of surgery and negatively influencing the prognosis. Similarly, Zucali et al (63) have described the prediction of responsiveness to pemetrexed could be correlated to expression of thymidylate synthase and therefore this last biomarker might be an important predictor of survival.

Another form of multimodality treatment is represented by intracavitary intraoperative hyperthermic chemotherapy, which can be conducted after either extrapleural pneumonectomy (64) or pleurectomy/decortication (65). A longer
follow-up would be necessary to determine whether new procedures have equal long-term results compared to the classical surgical techniques. Some improvement on prognosis seems to be achieved by photodynamic therapy of the pleura, which is an experimental treatment aimed at eradicating residual microscopic disease after macroscopic complete resection by means of intracavitary administration of non-toxic photosensitizing agent activated by laser (66). Photodynamic therapy can be combined with pleurectomy/decortication (67) as well as extrapleural pneumonectomy (68) and does not preclude other treatments such as adjuvant chemotherapy and/or radiation therapy.

**EORTC and CALGB prognostic scores**

The difficulty of individuating a unique prognostic predictor favored the development of clinical score systems with a survival finality.

The more widely used prognostic scoring system is the EORTC prognostic score, which identifies a high-risk subgroup in the presence of the following parameters: poor performance status (ECOG>0), serum leukocytosis (≥8.3*10⁹/L), male gender, probable diagnosis of mesothelioma and sarcomatous histotype. In the patients with a good prognosis one-year survival rate was 40%, against 12% in those patients predicted to have a poor prognosis (29). The EORTC prognostic score has been independently validated (30) and it is used for stratifying patients in clinical trials.

The other prognostic scoring system was developed by the CALGB. Patients were classified into six subgroups using an algorithm that maximized differences in the survival distribution calculated by the log-rank test. A stepwise analysis generated a regression model with successive stratification into groups according to prognostic factor with progressively decreasing risk ratio. Prognostic factors considered were pleural involvement, breathlessness as major symptom, elevated serum lactate dehydrogenase levels (>500 U/l), poor performance status (ECOG>0), chest pain, thrombocytosis (>400*10⁹/L), low hemoglobin levels (<14g/dL), nonepithelial histology, and elevated age (>75 years) (25). Survival curves were generated for subgroups defined by these supposed prognosticators.

**Biologic and genetic prognostic factors**

Several biological and genetic alterations involved in malignant pleural mesothelioma have been recently identified (17). These include serum biomarkers, oxidative stress, cell-life modulators, cyclooxygenase and metalloproteinase enzymes, growth factors and genetic abnormalities. A better knowledge of the biology and molecular pathways involved in malignant pleural mesothelioma may facilitate the identification of better-responder categories working as indirect predictor of prognosis.

**Serum biomarkers**

Ideal serum biomarker should allow early discovery of all histotypes of mesothelioma in risk-subjects, and correlate with the spread of disease in order to monitor treatment response and predict prognosis (16). Mesothelin is a differentiation antigen on mesothelial cells that is highly expressed in mesothelioma (69). High serum levels of the isoforms of serum mesothelin may reveal the presence or predict the future onset of a mesothelioma (70). Elevated serum mesothelin levels are a poor prognostic factor, as they correspond with a greater volume of disease (71). Magakaryocyte potentation factor is a soluble protein produced by proteolytic cleavage of the mesothelin precursor protein, is secreted by mesothelioma cell lines and may be useful in both diagnosis and therapy-response (72). Its prognostic role has been recently reported by Hellevoet et al (73).

Osteopontin is a glycoprotein involved in the cell adhesion and bone-matrix interactions. Serum levels are higher in mesothelioma patients with both the epithelioid and sarcomatoid subtypes and are correlated to the duration of asbestos exposure (74). Hollevoet et al proved that low baseline osteopontin levels were associated with favorable progression-free and overall survival (75).

The glycoprotein 90K was originally described as a tumor-secreted antigen interacting with an endogenous lectin, galecin-3, and playing a role in tumor metastasis through this interaction. The levels of glycoprotein 90K in patients with malignant pleural mesothelioma in sera greater than 7.3 microg/ml showed a correlation with a best prognosis (76).

**Oxidative stress**

It was hypothesized that carcinogenic effects of asbestos are determined both by direct action and by production of an induced-inflammatory environment containing reactive oxygen and nitrogen species (77). These substances stimulate
multiple cell signaling pathways suggesting the involvement of a myriad of transcription factors regulated from gene expression. The prevalent initial response to asbestos is characterized by lytic cell death resulting in compensatory proliferation of mesothelial cells. At this point viral infections, polycyclic aromatic hydrocarbons, hyperoxia and reactive oxygen species are able to shift the balance towards hyperproliferation and tumor onset (78).

Gene overexpressed in this cascade are the mitogen-activated protein kinase known also as extracellular signal-regulated kinases (ERK) (79), transcription factor activator protein-1, transcription nuclear factor k-light-chain-enhancer of activated B-cells (NF-kB) (80), protein kinase C: the activation of these genes may be associated to the onset of a mesothelioma and could represent a prognostic predictor.

**SV40 hypothesis**

The role of simian virus 40 (SV40) in the pathogenesis of mesothelioma is controversial (81). SV40 contaminated polio vaccines in many countries from 1954 to 1978 (82). Many laboratories detected SV40 in mesothelioma tumor samples and intrapleural injection of SV40 in hamsters often induces mesothelioma within 6 months. Interestingly, low asbestos levels may be sufficient to cause mesothelioma in the majority of hamsters infected the variant dl883 of SV40 (83). Thus, the determination of an infection with SV40 may be useful in predicting the likelihood of developing a mesothelioma in the presence of asbestos exposure. Whether the SV40 in polio vaccines resulted in any cancers, and whether SV40 acts as a co-carcinogen with asbestos, remain a matter of significant debate.

**Altered cell-life modulators**

The modern analytical gene chip technologies allowed the evaluation of gene expression. Gordon et al in 2003 (84) first identified by means of the reverse transcriptase-polymerase chain reaction technique a gene list capable to predict the 1-year survival in pleural mesothelioma. Since that time many publications added to this list other genes linked mainly in a network mainly associated with cell cycle regulation and death cell (17, 85).

The control of cell-cycle proteins has progressively become a wide and interesting investigation area in oncology (86). A key role is played by cell-cycle kinases, relatively small proteins regulated by the arrangement in a multimeric complex with larger proteins, called “cyclins” because of their cyclical expression and degradation during the cell-cycle. Cell-cycle kinase/cyclin complexes are negatively modulated by the interaction with a family of small proteins called cell-cycle kinase inhibitors, namely p21 and p27 (87) (Fig.1).

**Figure 1.** Main activation and inhibition pathways in malignant pleural mesothelioma.

The p53 tumor suppressor gene is also involved in cell-cycle checkpoints by virtue of its action as a transcription factor for several cell-cycle regulatory proteins, including the inhibition of p21 gene (88). Other proteins linked to p53
functions is p14ARF, which inhibits MDM2, a protein inactivating p53, thus promoting the tumor suppressor effects of the latter protein (89). Hopkins-Donaldson et al found that p53 is activated by DNA damage, and in this way it contributes to the apoptotic response induced by cisplatin (90). Furthermore, they also demonstrate that removal of downstream inhibitors such as survivin can enhance this response.

In 2001 Bongiovanni et al (91) described as p27kip1 positively correlated to the length of survival: all patients presenting with length of survival exceeding 24 months had high p27kip1 values. Baldi et al (92) investigated the expression of p21 and found a significant positive relationship between p21 expression level and the overall survival of the patients, which suggests that the p21 pathway is involved in the pathogenesis of mesothelioma. A greater reduction in p21 expression will cause greater aggression and result in a poorer prognosis. In a different study, Baldi et al analyzed the potential prognostic value of the immunohistochemical expression of p27 in 29 malignant mesotheliomas already screened for the expression of p21 and p53 (93). In this work they confirmed that low p21 and p27 expression were associated with a statistically significant decrease in survival.

The use of the monoclonal antibody MIB-1 represents one of the most simple and reliable techniques in assessing the proliferation rate of a neoplastic cell population. This antibody recognizes a nuclear protein, Ki67 antigen, expressed in proliferating but not resting cells, that can be used to assess the growth fraction (i.e., the number of cells in cell cycle) of normal, reactive and neoplastic tissues. A close correlation between MIB-1 staining and biological behaviour of malignant mesothelioma in long-term and short-term survivors was showed by Comin et al (94).

Apoptosis is a process characterized by programmed cell death in order to control the cell proliferation and destruction. In malignant tumors and namely in mesothelioma the expression of genes regulating the balance by activating apoptosis are altered (95). Apoptosis is normally induced by extrinsic receptor pathway activated by tumor necrosis factors (TNF) family ligands and intrinsic pathway activated by Bcl-2 gene families. These two ways converge to promote mitochondrial membrane to release caspase activators, which is an apoptotic executor (96) (Fig.1).

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) are proteins belonging to TNF family activating the apoptotic process. Mesothelioma cells have found to be resistant to TRAIL-induced apoptosis (97). Downregulation of TRAIL may have an impact on prognosis as well as the overexpression of the nuclear factor k-light-chain-enhancer of activated B-cells (NF-kB), which protects the cell from apoptosis. This complex is largely expressed in malignant mesothelioma (98). It is considered a constitutive survival factor in cell with asbestos-induced damages (80). Expressions of several genes promoting both pathways are altered in mesothelioma and those increases with the severity of the disease correlating with the prognosis (17, 85). High expressions on Bcl-xL, which is a Bcl-2 antiapoptotic protein have been detected in all mesothelioma cell lines (99). Conversely, reduced levels of Bcl-2-associated X (BAX) protein which is a tumor-suppressor and pro-apoptotic gene has been associated with a poor outcome in mesothelioma (100). Phosphatase and tensin homolog (PTEN) enzyme works as part of a chemical pathway that signals cells to stop dividing and causes cells to undergo programmed cell death (Fig.1). These functions prevent uncontrolled cell growth that can lead to the formation of tumors. Its high expression was found as a strong predictor of longer survival in mesothelioma patients independently from the histological subtype (101).

Another important intracellular signaling pathway originates from the phosphoinositide 3-kinase (PI3K) activating serine/threonine-protein kinase (AKT) that activates mammalian target of rapamycin (mTOR). This pathway enhances cell growth and obstacles apoptosis in malignant pleural mesothelioma (102). Another molecule correlated with outcome through an apoptotic process is the glucose transporter-1, which regulates glycolysis. An overexpression of glucose transporter-1 measured by immunohistochemical staining intensity in mesothelioma specimens proved correlated to shorter survival (103).

Cyclooxygenase enzymes

Significant progress has been made in understanding the molecular and cellular pathogenesis of neoplasms. Cyclooxygenase-2 (COX-2) is an isoenzyme form that converts arachidonic acid to prostaglandin-H2, becoming abundant in activated macrophages and activating inflammation. COX-2 has been implicated in carcinogenesis of several neoplasms and it over-expression has been noted in many solid tumours and has been correlated with a worse prognosis in non-small cell lung cancer, colorectal cancer and gastric cancer (104, 105). COX-2 is implicated in multiple events throughout the tumorigenic process producing highly reactive products that may alter cell growth, immunoresponse, apoptosis and angioneogenesis (106). Thus, high expression of COX-2 could be a prognostic marker in many tumors included mesothelioma (93).
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Edwards et al (106) demonstrated that COX-2 protein high levels were correlated with poor survival (P=0.01). High COX-2 expression (P=0.003) and non-epithelioid cell type (P=0.0007) were independent predictors of poor prognosis in multivariable analysis. High levels of COX-2 immunoreactivity indirectly contributed to both CALGB (P=0.001) (25) and EORTC (P=0.003) (30) prognostic indices.

Mineo et al (107) evaluated the impact on prognosis and the influence on therapeutic strategy of immunohistochemical expression of COX-2, p21 and p27 in specimens from patients treated for malignant pleural mesothelioma. The combination of high COX-2 and low p21 and p27 expression resulted at the Cox regression analysis the only negative. According to these results, COX-2 is a potential new therapeutic target in mesothelioma and justifies evaluation of the recently developed selective COX-2 inhibitors, such as celecoxib and rofecoxib, alone and in combination with existing treatment modalities (108).

Matrix metalloproteinases

Matrix metalloproteinases, in particular the gelatinases (metalloproteinase-2 and -9), play a role in tumor invasion and angiogenesis (109). The expression of matrix metalloproteinases may be reasonably elevated in malignant mesothelioma. Edwards et al (110) analyzed matrix metalloproteinase expression by immunohistochemistry in formalin-fixed, paraffin-embedded specimens. Increasing pro- and total matrix metalloproteinase-2 were associated with a poor survival on multivariate analysis (P = 0.03 and 0.04) and both indirectly contributed to the CALGB prognostic index (25). Inhibition of matrix metalloproteinase activity using selective synthetic matrix metalloproteinase inhibitors, particularly if administered into the pleural cavity (111), may provide a novel therapeutic approach in malignant pleural mesothelioma.

Growth signal pathways

Many growth factors appear to be highly expressed in malignant pleural mesothelioma (16, 17) and this may have a significance in prognosis.

Epidermal growth factor (EGF) when combines with its receptor (EGFR) induces epidermal cell proliferation. EGFR is a family of four structurally related receptor tyrosine kinases. EGFR is highly over-expressed in malignant pleural mesothelioma whereas it did not result an independent prognostic predictor (112). It is mainly associated with epithelial histotypes and this it could explain the relatively more favorable prognosis (113, 114). Asbestos fibers promote EGFR expression in SV-40 transformed human mesothelial cells (115). The following activations of phosphatidyl-inositol-3-kinase and of ERK pathways result in cell survival and proliferation. Inhibition of EGFR activity with specific tyrosine kinase inhibitors had resulted in vitro in control of tumor growth and inhibition of angiogenesis (116). EGFR could a potential target for future therapies in malignant pleural mesothelioma (16). Anyway EGFR mutations are uncommon in mesothelioma (117) and this may partly explain why both erlotinib and gefitinib have demonstrated minimal activity in phase II trials (118, 119).

Vascular endothelial growth factor (VEGF) is a growth factor related to angiogenesis and it is overexpressed in mesothelioma (120). Several studies recognized VEGF as a significant prognosticator in mesothelioma (121, 122). Elevated levels of VEGF were described in serum and pleural fluid of mesothelioma patients and they were associated with decreased survival (120). High VEGF expression correlates with high microvessel density, tumor necrosis thus implying a poorer survival (123). In theory, anti-VEGF drugs could be reasonably used with antitumoral purposes. Unfortunately, phase II studies with inhibitors of VEGF tyrosine kinase or VEGF receptor-2 have revealed only scant results (124, 125, 126).

Among VEGFs the placenta growth factor (PLGF) has recently revealed an inverse relationship with survival in mesothelioma after extrapleural pneumonectomy suggesting a pivotal role of this factor in the recurrence and progression (127, 128).

Kumar-Singh et al demonstrated that also an high expression of fibroblastic growth factor (FGF) associated with VEGF was correlated with a poor outcome (122). It is a common finding that an elevated platelet count represents a negative prognostic factor in malignant mesothelioma (16, 25). Overexpression of platelet-derived growth factor (PDGF) and of its receptor (PDGFR) is thought to play a role in this phenomenon (129). Antibodies against this receptor have been recently isolated from serum in mesothelioma patients thus representing an hypothetical novel serum biomarker of disease (130). PDGFR is another tyrosine kinase protein which can be inhibited like EGFR by selective inhibitors. However, there were no objective responses in the single-agent phase II trials of the PDGF inhibitor imatinib (131, 132).
Hepatocyte growth factor (HGF) is recognized as a multifunctional growth factor that induces cell proliferation. The receptor for HGF is synthesized by c-MET, a proto-oncogene with a tyrosine-kinase activity located in 7q31 chromosome (133). Overexpression of HGF and c-MET has been associated with angiogenesis and increased matrix metalloproteinases expression (134) HGF can be retrieved by immunohistochemistry from specimens of mesothelioma (135) and by ELISA from pleural effusion (136).

Other growth factors are related to mesothelioma development: transforming growth factor beta (TGF-β), which is implicated in tumor growth (122); and insulin-like growth factor (IGF), which promotes tumor proliferation and cell migration (137).

**Angiogenesis**

Blood supply is essential for tumor growth and formation of new vascular structures is mandatory step in carcinogenesis. Angiogenesis is triggered by the hypoxic environment following the chaotic growth of the tumor. According to these fundamentals angiogenesis may be have an important role in revealing the aggressiveness of the neoplasm and in predicting the prognosis. Microvessel density is universally used as an indirect measure of the intensity of angiogenesis. Therefore, it might have a prognostic relevance also in malignant mesothelioma (113, 122). Several growth factors are implicated in angiogenesis such as VEGF, EGF and PDGF (138, 139) already described in the previous section. Microvessel density measured with immunohistochemistry by anti-CD34 monoclonal antibodies resulted a poor prognostic factor in both univariate (P=0.01) and multivariate analysis (P=0.01) (140). Angiogenesis may be a potential therapeutic target in this disease (124).

**Tumor necrosis**

Many studies have demonstrated that the microscopical evidence of tumor necrosis may be a predictor of poor prognosis in solid tumors such as breast and non-small cell lung cancer (141). The role of tumor necrosis was also investigated in malignant pleural mesothelioma correlating its presence with clinic-pathological factors (i.e. low hemoglobin, high platelet count and high density microvessel count) (113). Tumor necrosis resulted inversely correlated with survival at uni- and multivariate analysis (123). Tumor necrosis was included as independent factor to both the CALGB (P=0.03)(25) and EORTC (P=0.03) (30) scoring systems.

As previously described for both breast and non-small cell lung cancer, tumor necrosis correlates is a poor prognostic factor also in malignant pleural mesothelioma. The correlation with high microvessel density indicates that tumor necrosis is associated with an aggressive tumor phenotype (123, 141).

**Thymidylate synthase**

The role of the expression of this enzyme has been already mentioned in the clinical prognostic predictors. It could be correlated to the prediction of responsiveness to pemetrexed, which is presently one of the most effective drugs in the treatment of malignant pleural mesothelioma (63). Thymidylate synthase mRNA expression levels were inversely correlated with pemetrexed activity. Subsequently high mRNA expression are associated with higher risk of progression disease and shorter survival (142).

**Chromosomal disbalance and gene mutation**

Disbalance is defined as the loss or gain of DNA sequences after deletions, duplications or amplifications. All these changes were identified as significant spies of mitotic processes alteration and as negative prognostic indices (143) All these variations can be assessed by comparative genomic hybridization also called chromosomal microarray analysis. In 2001 Yang et al (144) showed a p14ARF deficiency as a result of homozygous deletions of INK4a/ARF locus sited in chromosome 9p21. p14ARF deficiency fails to inhibit MDM2, a protein inactivating p53, thus limiting its tumor suppressor effect. In 2006 Hopkins-Donaldson et al (90) in contrast with this observation suggested that p53 is functional in malignant pleural mesothelioma even in the absence of p14ARF.

The same INK4a/ARF locus also encodes the protein p16INK4A, which regulates the retinoblastoma protein (pRB) phosphorylation and induces cell cycle arrest in G1 phase (145). This mutational event produces a strong limitation in tumor suppressor pathway and occurred in more than 70% cases of malignant pleural mesothelioma (146, 147). Deletion of p16 has been demonstrated as another chromosomal abnormality associated with a poor survival (148, 149). Lopez Rios et al (143) found a median survival in homozygous deletion of 10 months versus 34 months for non-deleted cases (P=0.001).

A few gene mutations have been recognized in malignant pleural mesothelioma (150, 151). Interestingly, a loss of heterozygosity of 22q12 was noted in the almost totality of cases with gene mutations (152). On this site is located
the gene 2 neurofibromatosis, which normally acts as a tumor suppressor gene and as a gatekeeper in asbestos-induced-mesothelioma (153, 154). Mutations of this gene was found in 40% of mesothelioma and are correlated to a shorter survival rate (155).

Four-gene signature comprising KIA A097, Guanosine 5Diphosphate-dissociation inhibitor 1, cytosolic thyroid hormone-binding protein and an expressed sequence tag similar to the L6 tumor antigen, correlated with a good and poor prognostic groups (83). Furthermore, the presence of an eleven-gene, oncogene-driven-pathway signature, is associated with a poor prognosis in mesothelioma patients (156). A large gene-expression analysis identified and validated aurora kinases as predictive of outcome. Aurora kinases are serine/threonine kinases that are essential for cell proliferation. The enzyme helps the dividing cell dispense its genetic materials to its daughter cells. More specifically, aurora kinases play a crucial role in cellular division by controlling chromatid segregation. Defects in this segregation can cause genetic instability, a condition which is highly associated with tumorigenesis (143).

Pass et al demonstrated that specific microRNAs can discriminate operated patients into good or bad prognosis categories (157). Increased expression of homo sapiens-microRNA (hsa-miR)-29c* predicted a more favorable prognosis, whereas overexpression of the microRNA in mesothelioma cell lines resulted in significantly decreased proliferation, migration, invasion, and colony formation. Hsa-miR-29c* high expression down-regulates DNA methyltransferases and upregulates demethylating genes.

Conclusions

At the end of this review we can certainly affirm that both clinical and biological variables may contribute to the prognostic evaluation of malignant mesothelioma. Many biological factors are currently under evaluation as potential targets for novel therapeutic agents and, despite initial disappointing results, they represent the most advanced prospective in the treatment of this dreadful and hopeless disease.

References


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Appendix
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