May cyclooxygenase-2 (COX-2), p21 and p27 expression affect prognosis and therapeutic strategy of patients with malignant pleural mesothelioma?

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Abstract

Objectives: The expression of cyclooxygenase-2 (COX-2) and cell-cycle proteins (p21 and p27) proves useful in predicting prognosis and orientating therapy in many malignant tumours. Malignant pleural mesothelioma is an uncommon and lethal cancer for which there are limited treatment options. In this study, we 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. Methods: We retrospectively reviewed immunohistochemical expression of COX-2, p21 and p27 dichotomised into high and low expression from specimens of 77 consecutive patients undergoing biopsy-plus-pleurodesis (n = 6), pleurectomy-decortication (n = 44) or extrapleural pneumonectomy (n = 27) operations for malignant pleural mesothelioma between 1987 and 2007. Histology was of epithelioid (n = 50), biphasic (n = 17) and sarcomatoid (n = 10) subtypes. Tumour node metastasis (TNM)-stage was I (n = 21), II (n = 36) and III (n = 20). Therapies used were sole adjuvant radiotherapy (n = 17), adjuvant radio-chemotherapy (n = 56) and neo-adjuvant chemotherapy plus adjuvant radiotherapy (n = 4). From 2005 on, preoperative maximal standard uptake value (SUV$_{\text{MAX}}$) was also measured by fluorodeoxyglucose positron-emission-tomography. Significance was investigated by Kaplan—Meier survival and Cox regression analysis. Results: The median survival was 10 months. Survival was negatively influenced by histology (epithelioid vs biphasic vs sarcomatoid) (p = 0.009), positive macroscopic resection margins (p = 0.016), metastatic mediastinal lymph nodes (p = 0.016), high COX-2 (p = 0.0001) expression, low p21 (p = 0.001) expression and low p27 (p = 0.001) expression. Conversely, neither the type of surgery (biopsy-plus-pleurodesis vs pleurectomy-decortication vs extrapleural pneumonectomy), nor preoperative SUV$_{\text{MAX}}$ (>6.0 vs ≤6.0), or combined therapies (sole radiotherapy vs adjuvant radio-chemotherapy vs neo-adjuvant chemotherapy plus adjuvant radiotherapy) reached a significant level of difference. Cox regression analysis showed that only immunohistochemical triple combination of high COX-2 and low p21 and p27 expression influenced survival (p = 0.0001, hazard ratio 4.7, 95% confidence intervals 3—11) regardless of type of treatment. Conclusions: At Cox regression analysis, a combination of high COX-2 and low p21 and p27 expression resulted in the only negative prognosticator of malignant pleural mesothelioma. With this combination, less aggressive surgical options might be preferred.

Keywords: Malignant pleural mesothelioma; Cyclooxygenase-2 (COX-2); p21; p27; Thoracic surgery

1. Introduction

Malignant pleural mesothelioma is a rare, highly aggressive tumour which accounts for less than 1% of all cancer deaths [1]. Despite recent advances in oncological therapy, the prognosis for this neoplasm continues to be very poor. Chemo- or radiotherapy by itself provides only marginal results. Surgery is often overdue, ineffective and restricted to a very exiguous number of subjects [1]. Some results have been achieved in selected patients at earlier stages with a multimodal approach combining extrapleural pneumonectomy with chemotherapy and radiotherapy [2,3]. However, some patients did better than others, and this is not justifiable only with the stage or the histology, which are two of the main credited prognostic factors.

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-H$_2$, becoming abundant in multiple events throughout the tumourigenic process. With this combination, less aggressive surgical options might be preferred.

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The control of cell-cycle proteins has progressively become a wide and interesting area of investigation in oncology [6]. 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 interaction with a family of small proteins called cell-cycle kinase inhibitors, namely p21 and p27 [6]. The p53 tumour 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 p21 gene [7].

In this study, we 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.

2. Methods

We retrospectively reviewed the specimens of 77 ‘consecutive’ patients, 63 men and 14 women (mean age 61.3 ± 10 years, range 27–82 years), undergoing biopsy-plus-pleurodesis (n = 6), pleurectomy—decortication (n = 44) or extrapleural pneumonectomy (n = 27) operations for malignant pleural mesothelioma between 1987 and 2007. A vast majority of the patients undergoing simple biopsy (n = 55) in the same time frame were ruled out from the study due to lack of specimens of adequate size for immunohistochemical studies and/or scant information regarding survival.

All clinical, laboratory and histological data were retrospectively reviewed. Histology was of epithelioid (n = 50), biphasic (n = 17) and sarcomatoid (n = 10) subtypes. The patients’ stage was reviewed according to the tumour node metastasis (TNM)-staging system developed by the International Mesothelioma Interest Group [8] based on the clinical data available at the time of surgery, and 21 patients were staged as I, 36 as II and 20 as III.

The decision of performing one surgical procedure instead of another was taken on the basis of preoperative work-up, including total body computed tomography (CT), arterial blood gases, plethysmography, timed spirometry, single breath diffusing lung capacity for carbon monoxide and pulmonary perfusion scan. Exercise tolerance was tested by the 6-min walk test and the Bruce protocol. Cardiac functional assessment always included an echocardiographic evaluation of the ejection fraction and right heart function. Hepatic and renal functions were also assessed. Performance status was evaluated by the Karnofsky index (score 0–100): the lower the score, the worse the survival. In many instances, a contrast-enhanced magnetic resonance imaging was performed to better evaluate the depth of the invasion within the chest wall, the diaphragm or the mediastinum. From 2005, all patients also underwent a positron-emission-tomography (PET) scanner and activity was evaluated by preoperative maximal standard uptake value (SUV_MAX). Preoperative cervical mediastinoscopy was performed whenever CT evidenced enlarged (a maximum diameter >15 mm) upper mediastinal lymph nodes.

Surgical resection was considered whenever the tumour appeared radiologically confined to one hemithorax, with the absence of mediastinal organ or full-thickness pericardial—myocardial involvement. Diffused or multifocal chest wall disease, extension through the diaphragmatic and/or spread directly into the spine were also considered as exclusion criteria.

Patients initially considered eligible for extrapleural pneumonectomy were those with a confirmed preoperative diagnosis of malignant pleural mesothelioma, without radiological evidence of N2-disease, with a Karnofsky index equal or greater than 90% and without major organ dysfunction and adequate post-pneumonectomy respiratory reserve. According to the last condition, the criteria for exclusion for pneumonectomy were the following: expected postoperative forced expiratory volume in 1 s less than 40% of the predicted value or anyway inferior to 1 l; evidence of controlateral hypoperfused lung (<55% of the right or 45% on the left); air room arterial pCO2 greater than 45 mmHg or pO2 less than 65 mmHg; estimated cardiac ejection fraction less than 45%; and right ventricular dilatation. Patients with these conditions were scheduled for pleurectomy—decortication. Other indications for pleurectomy—decortication were the presence of major co-morbidity or N2-status. In some instances, a planned extrapleural pneumonectomy was intra-operatively limited to a pleurectomy—decortication given the surgical impossibility of accomplishing an adequate resection.

Thoracotomies were performed through the sixth intercostal space and when extrapleural pneumonectomy became feasible, a counter-incision in the eighth one was added to accomplish the diaphragmatic step of the operation. The diaphragm was preferentially resected and replaced by prosthetic material. The pericardium was always reconstructed. In patients who were found to have a multicentric macroscopic chest wall or mediastinal invasion, the surgical procedure was reduced to a pleurectomy—decortication aiming at removing nearly all neo-plastic tissue and marking the unresectable tumour with metal clips to target radiotherapy. All accessible mediastinal lymph nodes were resected to allow an accurate surgical staging of the disease.

For patients of the biopsy-plus-pleurodesis group, multiple and large specimens were retrieved through video-assisted thoracoscopic surgery followed by talc pleurodesis.

Non-surgical therapy has changed considerably over the years due to the drugs used or the quantity of radiation or due to the sequential pattern between radio- and chemotherapy. Due to the fragmentation and inconsistency of each therapeutic subset, we preferred to stratify the study sample according to three major groups: sole adjuvant radiotherapy (n = 17), adjuvant radio-chemotherapy (n = 56) and neo-adjuvant chemotherapy plus adjuvant radiotherapy (n = 4). All strategies were planned each time after a panel discussion with oncologists and radiotherapists.

At the beginning of our experience, adjuvant therapy consisted mainly of sole radiation therapy addressed to reduce residual bulky disease. After the introduction of chemotherapy, radiotherapy was used as a concomitant treatment, but for some patients it still remained the unique therapeutic instrument. External beam radiotherapy was
delivered with an energy ranging from 4 to 15 MV. The total radiation doses to the hemithorax and mediastinum were normally 30 Gy and 40 Gy, respectively, fractioned in 1.5-Gy doses. A boost dose (14 Gy in 2-Gy fractions) was always delivered to areas of gross residual disease or positive resection margins, metastatic lymph nodes, sites of thoracic and chest drain. The total dose varied according to the status of the resection margins and was significantly higher after extrapleural pneumonectomy.

The adjuvant chemotherapy regimen consisted of four to six cycles of cisplatin (100 mg m$^{-2}$) given at day 15, and etoposide (120 mg m$^{-2}$) administered on days 1, 2 and 3 or, since 1996, gemcitabine (1 g m$^{-2}$) administered on days 1, 8 and 15. Treatment was interrupted or delayed in case of disease progression or intolerable toxicity. Chemotherapy began between 4 and 10 weeks after surgery, depending on the postoperative general health status.

Concurrent radiation therapy started after the first cycles of chemotherapy, with different dosages chosen individually, and alternated with further chemotherapeutic cycles.

From 2006, we started a new therapeutic protocol including neo-adjuvant chemotherapy plus adjuvant radiotherapy. Neo-adjuvant chemotherapy consisted of three cycles of cisplatin and gemcitabine, given every 28 days at the same dosages indicated for adjuvant therapy. Dose reduction was performed according to evidence of renal and haematologic toxicity. Response was assessed by measuring pleural thickness using CT. In this period, adjuvant radiotherapy was delivered according to a three-dimensional conformational radiotherapy. Patients received a radiation dose of 30 Gy to the ipsilateral hemithorax and a boost dose of 15 Gy delivered to the area of highest risk of recurrence. The fractionation schedule used was 1.8—2 Gy per day, five times per week.

2.1. Clinical follow-up

Whenever possible, clinical outcome was directly assessed during a dedicated session of follow-up outpatient clinic. As an alternative, basic information was retrieved by medical records or from general practitioner or patients’ interviews by telephone. Cross-sectional contact for all surviving patients was performed in March 2009. Given that clinical symptoms and radiographic studies available during the beginning of the study period were not sensitive enough to accurately diagnose early recurrence, the disease-free interval was not evaluated. Therefore, survival was the major end point of this study. The survival duration was measured from the date of surgery until the date of the patient’s last follow-up contact or death. The terminal event was death attributable to cancer.

2.2. Immunohistochemical studies

All cases were assessed by immunohistochemistry for the presence of COX-2, p27, p21 and p53. Five-micrometre sections were cut from each specimen, mounted on glass and dried overnight at 37°C. All sections were then deparaffinised in xylene, rehydrated through a graded alcohol series and washed in phosphate-buffered saline, which was used for all subsequent washes and for antibody dilution. Endogenous peroxidase activity was blocked by 5% hydrogen peroxide. We used goat polyclonal antibodies (sc-1745, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) specific for COX-2, mouse monoclonal antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA 95060, USA) specific for p27 (mouse monoclonal; sc-1641) and p21 (mouse monoclonal sc-6246) applied at room temperature for 1 h at a dilution of 1:100. A monoclonal antibody specific for p53 (D01; Dako Corporation, Carpinteria, CA, USA) was used at a 1:500 dilution. The optimal working dilution was defined on the basis of titration experiments. The sections were then immunostained with the streptavidin—biotin system (Dako Corporation, Carpinteria, CA, USA) using dianminobenzidine as the final chromogen and haematoxylin as the nuclear counterstain. Negative controls for each tissue section were prepared by omitting the primary antibodies. A suitable positive control was run with each set of slides. All samples were processed under the same conditions.

A dichotomised scoring system was used as follows: p21, p27 and p53 expression in more than 5% of tumour cell nuclei was defined as high expression [9]. COX-2 expression was scored positive whenever cytoplasmatic staining was observed in more than 10% of malignant cells [10].

2.3. Statistical analysis

Fisher’s exact test was used to assess the relationship between level of expression of COX-2, p27, p21 and clinicopathological variables. Univariate survival analysis for each prognostic variable on overall survival was estimated according to the Kaplan—Meier method. Variables included age greater or less than 65 years, side of tumour, sex, smoking history, asbestos exposure, chest pain, dyspnoea, cough, side of tumour, preoperative Karnofsky index, histology (epithelial vs biphasic vs sarcomatous), involved resection margins, N-stage (N0—N1 vs N2), COX-2, p21, p27 and p53 expression (high vs low). The $p$ values <0.05 were regarded as statistically significant in two-tailed tests. Significance was evaluated with the log-rank test and a value less than 0.05 was considered the threshold value. Significant variables at univariate analysis were entered into Cox regression analysis to select the most predictive one; variables were also associated to study the more predictive combination. SPSS software version 10.00 (SPSS, Chicago, IL, USA) was used for statistical analysis.

3. Results

In the extrapleural pneumonectomy group, we experienced only one 30-day perioperative mortality caused by pulmonary embolism. This patient was excluded from the survival analysis. In the other surgical groups, there were no 30-day perioperative deaths. Major morbidity was observed in the extrapleural pneumonectomy group 33% (9/27) and included cardiac arrhythmias ($n = 4$), bleeding requiring re-operation ($n = 2$), vocal cord palsy ($n = 1$), deep venous thrombosis ($n = 1$) and broncho-pleural fistula ($n = 2$) followed by chronic empyema; some patients had more than one cause of morbidity. In the pleurectomy/decortication group, major morbidity rate was less than 14% (6/44) and
included prolonged bleeding (n = 4) and deep venous thrombosis (n = 2).

The mean follow-up interval was 11.3 ± 9 months (range: 0.2–44 months) with a median survival of 10 months. Sixty-four patients (83%) died due to causes directly or indirectly related to the disease: local invasion of vital thoracic or abdominal organs in 40 (62%), sepsis of a broncho-pleural fistula in two patients (3%), respiratory failure in nine (14%) or distant metastases in 13 (20%). Demographics that were not significantly associated with the duration of survival included age ≥65 years, cigarette use, asbestos exposure, chest pain, dyspnoea, cough, preoperative performance status and side of tumour (Table 1). Univariate analysis identified three significant variables associated with poorer prognosis: histology (epithelioid vs biphasic vs sarcomatoid) (**p** = 0.009), positive macroscopic resection margins (**p** = 0.016) and mediastinal lymph nodes with metastasis (**p** = 0.016).

On the other hand, neither the type of surgery (biopsy-plus-pleurodesis vs pleurectomy-decortication vs extrapleural pneumonectomy, **p** = 0.11) nor preoperative SUV_MAX (≥6.0 vs <6.0, **p** = 0.08), or combined therapies (sole radiotherapy vs adjuvant radio-chemotherapy vs neo-adjuvant chemotherapy plus adjuvant radiotherapy, **p** = 0.75) reached a significant level of difference.

Immunohistochemical staining did not reveal significant interdependence between classic prognostic factors and COX-2 and cell-cycle protein expression (Table 2).

At univariate survival analysis, high COX-2 (**p** = 0.0001) (Fig. 1) expression, low p21 (**p** = 0.0001) expression (Fig. 2) and low p27 (**p** = 0.001) expression (Fig. 3) were the significant predictors of a negative prognosis, while no significance was demonstrated for high expression of p53. Only three (10%) patients with high expression COX-2 had a survival longer than 2 years, and all them died in a short time later. Similarly, only six patients with low expressions of p21

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**Table 1**
Univariate analysis of main clinicopathologic variables.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Frequency</th>
<th>Median survival (months)</th>
<th><strong>p</strong>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (≥65 vs &lt;65)</td>
<td>35 versus 42</td>
<td>9 versus 11</td>
<td>0.4</td>
</tr>
<tr>
<td>Sex (male vs female)</td>
<td>63 versus 14</td>
<td>10 versus 10</td>
<td>0.9</td>
</tr>
<tr>
<td>Performance status (90 vs 100)</td>
<td>37 versus 40</td>
<td>8 versus 11</td>
<td>0.1</td>
</tr>
<tr>
<td>Cigarette use (yes vs no)</td>
<td>57 versus 20</td>
<td>10 versus 10</td>
<td>0.8</td>
</tr>
<tr>
<td>Asbestos exposure (yes vs no)</td>
<td>59 versus 28</td>
<td>8 versus 11</td>
<td>0.08</td>
</tr>
<tr>
<td>Chest pain (yes vs no)</td>
<td>57 versus 20</td>
<td>8 versus 11</td>
<td>0.07</td>
</tr>
<tr>
<td>Dyspnoea (yes vs no)</td>
<td>45 versus 32</td>
<td>9 versus 10</td>
<td>0.5</td>
</tr>
<tr>
<td>Cough (yes vs no)</td>
<td>28 versus 39</td>
<td>10 versus 10</td>
<td>0.8</td>
</tr>
<tr>
<td>Side (right vs left)</td>
<td>43 versus 34</td>
<td>10 versus 10</td>
<td>0.9</td>
</tr>
<tr>
<td>Final histology (sarcomatoid vs biphasic vs epithelial)</td>
<td>10 versus 17 versus 50</td>
<td>4 versus 9 versus 15</td>
<td>0.009</td>
</tr>
<tr>
<td>Preoperative maximal standard uptake value^a^ (≥6.0 vs &lt;6.0)</td>
<td>10 versus 6</td>
<td>8 versus 11</td>
<td>0.07</td>
</tr>
<tr>
<td>Resection (biopsy vs pleurectomy/decortication vs extrapleural pneumonectomy)</td>
<td>6 versus 44 versus 27</td>
<td>7 versus 10 versus 11</td>
<td>0.07</td>
</tr>
<tr>
<td>Concomitant therapy (radiotherapy vs adjchemo-radio vs neochemo-adjradio)</td>
<td>17 versus 36 versus 4</td>
<td>9 versus 10 versus 10</td>
<td>0.8</td>
</tr>
<tr>
<td>Pathologic stage (III vs vs II vs I)</td>
<td>21 versus 36 versus 20</td>
<td>6 versus 10 versus 13</td>
<td>0.06</td>
</tr>
<tr>
<td>N-disease (N2 vs N1 and N0)</td>
<td>19 versus 58</td>
<td>7 versus 13</td>
<td>0.008</td>
</tr>
<tr>
<td>Resection margins (positive vs negative)</td>
<td>12 versus 65</td>
<td>8 versus 12</td>
<td>0.016</td>
</tr>
</tbody>
</table>

^a^ 16 patients.

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**Table 2**
Distribution pattern of COX-2, p-21, and p-27 expression according to main clinical features.

<table>
<thead>
<tr>
<th>Variable</th>
<th>COX-2 expression</th>
<th>p-value</th>
<th>p-21 expression</th>
<th>p-value</th>
<th>p-27 expression</th>
<th>p-value</th>
<th>p-53 expression</th>
<th>p-value</th>
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<td>pTNM stage</td>
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<td></td>
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<td></td>
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<tr>
<td>I (n = 20)</td>
<td>High n = 31</td>
<td>Low n = 46</td>
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<td></td>
<td>8</td>
<td>12</td>
<td>0.6</td>
<td>9</td>
<td>11</td>
<td>0.4</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>II (n = 36)</td>
<td>13</td>
<td>23</td>
<td>0.05</td>
<td>14</td>
<td>36</td>
<td>0.1</td>
<td>13</td>
<td>37</td>
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<tr>
<td>III (n = 21)</td>
<td>10</td>
<td>11</td>
<td>0.8</td>
<td>13</td>
<td>7</td>
<td>0.14</td>
<td>7</td>
<td>14</td>
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<td>Histology</td>
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<tr>
<td>Epithelioid (n = 50)</td>
<td>15</td>
<td>35</td>
<td>0.05</td>
<td>14</td>
<td>36</td>
<td>0.1</td>
<td>13</td>
<td>37</td>
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<tr>
<td>Biphasic (n = 17)</td>
<td>10</td>
<td>7</td>
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<td>10</td>
<td>7</td>
<td>0.10</td>
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<td>10</td>
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<tr>
<td>Sarcomatoid (n = 10)</td>
<td>6</td>
<td>4</td>
<td>6</td>
<td>4</td>
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<tr>
<td>Biopsy-plus-pleurodesis (n = 6)</td>
<td>2</td>
<td>4</td>
<td>0.6</td>
<td>1</td>
<td>5</td>
<td>0.1</td>
<td>1</td>
<td>5</td>
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<tr>
<td>Pleurectomy-decortication (n = 44)</td>
<td>20</td>
<td>24</td>
<td>0.05</td>
<td>13</td>
<td>31</td>
<td>0.1</td>
<td>17</td>
<td>27</td>
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<td>Extrapleural pneumonectomy (n = 27)</td>
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<td>18</td>
<td>0.14</td>
<td>13</td>
<td>14</td>
<td>0.21</td>
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<tr>
<td>Therapy</td>
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<tr>
<td>Radiotherapy (n = 17)</td>
<td>6</td>
<td>11</td>
<td>0.7</td>
<td>8</td>
<td>9</td>
<td>0.6</td>
<td>9</td>
<td>8</td>
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<tr>
<td>Adjuvant chemo + radiotherapy (n = 56)</td>
<td>23</td>
<td>33</td>
<td>0.18</td>
<td>18</td>
<td>38</td>
<td>0.36</td>
<td>16</td>
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<tr>
<td>Neo-adjuvant chemo + adjuvant radiotherapy (n = 4)</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>1</td>
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</table>
and p27 survived for a period longer than 2 years. Finally, triple combination of high COX-2, low p21 and low p27 expression disclosed a high significance ($p = 0.0001$) with no patients surviving more than 10 months (Fig. 4).

At Cox regression analysis, none of the single investigated factors significant at univariate analysis reached the required threshold of significance. Only the triple combination of high COX-2 and low p21 and p27 expression resulted in...
significantly influencing survival ($p = 0.0001$, hazard ratio 4.7, 95% confidence intervals 3—11).

4. Discussion

The discovery of prognostic factors is a fundamental step in a rational and modern approach to any neoplastic disease. Prognostic factors may allow the choice of the most appropriate form of treatment and address the following therapeutic phases and observational strategies. In the case of malignant pleural mesothelioma, the role of prognostic factors seems to have a minor value, due to the severity of disease rapidly leading to death despite early discovery and aggressive treatment.

Histological classification is usually considered one of the most practical and effective criteria to identify neoplasms with a favourable prognosis: the epithelioid type is usually deemed less aggressive than the biphasic and sarcomatoid types. On these bases, it was suggested that the latter histotypes should be less suitable for extensive resection and they should be preferably addressed to minor operations or non-surgical treatment [1].

The spread of the disease is another classic standpoint logically influencing the prognosis and the therapeutic strategy. Several staging systems have been proposed so far for malignant pleural mesothelioma. The last TNM-staging system developed by the International Mesothelioma Interest Group [8] represented a significant attempt at unifying all previous systems. However, the surgical staging and, namely, a reliable assessment of N-stage is not applicable in the event of advanced disease. In the case of malignant pleural mesothelioma, it is difficult to preoperatively differentiate direct mediastinal invasion from lymph nodal involvement. This may hinder the routine introduction of cervical mediastinoscopy, which should be an indispensable staging device [11]. Nevertheless, TNM-staging evidenced some failures in predicting survival also in patients undergoing intentionally radical surgery in a multimodality treatment [2].

All these considerations, together with the increasing evidence of the importance of the biologic features of the tumour, have recently favoured the elaboration of new prognostic factors based on biological variables [12].

COX-2 is an isoenzymatic form found only at the sites of inflammation and overexpressed in many solid tumours, including malignant pleural mesothelioma [13]. The role of COX-2 in carcinogenesis is based on many roles played by the enzyme such as inhibition of apoptosis, induction of neoangiogenesis, down-regulation of cellular immunity and production of carcinogenic metabolites such as malondialdehyde [4,5]. In 2002, Edwards and colleagues first reported that COX-2 expression is a strong prognosticator in malignant pleural mesothelioma, affecting long-term survival independently from the other clinical and histopathological factors [13]. Since that time, several reports confirmed the high expression of COX-2 as an independent indicator of a shorter survival. O’Kane and colleagues have recently demonstrated that COX-2 inhibitors enhance the activity of new chemotherapy drugs outlining a possibility for new drugs in the therapy of mesothelioma [14].

The role of other biological factors in malignant pleural mesothelioma has been investigated. The expression of matrix metalloproteinases and, in particular, metalloproteinase-2 was assessed by immunohistochemistry; it demonstrated a significant association with poor survival on multivariate analysis [12]. The role of epidermal growth factor receptor (EGFR) expression as a positive prognosticator was documented in a univariate analysis proving its significant correlation with the epithelioid cell type [15]. More recently, a study from our group evidenced that placenta growth factor can be overexpressed in malignant pleural mesothelioma and inversely correlated with survival after extrapleural pneumonectomy, suggesting a pivotal function of this factor in the progression of the disease and targeted therapy [16].

In 2001, Bongiovanni and colleagues [17] first evidenced the role of high values of p27kip1 in predicting long-term survival. Indeed, p27kip1 is an inhibitor of the transition from G1- to S-phase; therefore, it can work as a tumour suppressor gene [6]. The same authors also confirmed the role of the Ki-67 as a negative indicator and showed that the combination of the two proteins may reinforce the reliability in the prediction of survival.

Baldi and colleagues [18] evidenced a significant positive relationship between p21 expression level and the overall survival of the patients: a greater reduction in p21 expression will cause greater aggression and result in a poorer prognosis. The same authors, in an analysis of the potential prognostic value of p27 and COX-2 in 29 malignant mesotheliomas [9], found that a high level of COX-2 and low levels of p21 and p27 were associated with a statistically significant decrease in survival. It has also been proposed that COX-2 exerts its influence on mesangial cell proliferation in vitro by a novel mechanism involving the tumour suppressor p53 and the cell-cycle inhibitors p21 and p27 [19].

In our study, biological factors proved to be not correlated with classic prognostic factors, whereas they were revealed as strong independent prognosticators. Biological factors are assuming an increasingly significant role in determining the prognosis and in selecting the appropriate therapeutic strategy in pleural mesothelioma. In fact, optimal therapeutic options are still controversial: chemotherapy alone (platinum-based) offers a median survival of 11—12 months [20] and equivalent results are reported with video-assisted thorascoscopic debulking surgery [21]. Extrapleural pneumonectomy, followed by adjuvant chemotherapy [2], can achieve a median survival of 19 months and significant improvements in quality of life [22]. Nearly equivalent results are documented with pleurectomy—decortication [23,24], especially in the presence of N2-disease [21].

More recently, the introduction of neo-adjuvant chemotherapy followed by extrapleural pneumonectomy and radiotherapy has shown promising results with a median survival of up to 20 months [3]. Notwithstanding, there are still studies favourable to pleurectomy—decortication followed by new chemoradiotherapeutic protocols such as pemetrexed and intensity-modulated radiotherapy, in which median survival is reported to be equivalent to or longer than after extrapleural pneumonectomy [24].

On the basis of our results and in the absence of a procedure or multimodality therapy that is clearly superior to
another, we may suggest that surgical therapy, whatever the approach, could be more indicated to a greater extent in patients with the most favourable biologic pattern, thus avoiding too aggressive resections with scant prognostic results. At Cox regression analysis, a combination of high COX-2 and low p21 and p27 expression resulted in the only negative prognosticator of malignant pleural mesothelioma. In the presence of these combinations, therapeutic strategy should preferably consider only minor operations. The best treatment should be tailored according to several classic and biologic factors including also the general performance status.

Tumour activity can now be reliably quantified by SUVMAX, which revealed a significant negative correlation with survival [25]. In our small series, we could not find any significant association with a worse prognosis or with stronger biologic prognosticators but only a marginal trend, due to the limited number of cases available.

We acknowledge some limitations in our study. The most evident relies on the retrospective nature and the relative limited sample size. However, we must take into account the fact that immunohistochemistry reassessment of all specimens was performed during a limited period of time with the same technique and by the same readers. Moreover, although the study covers a 20-year period and multimodality treatments are slightly different due to the long time span, the surgical procedure was performed in the same centre according to the same guideline-based therapy protocols. The scant number of biopsy-plus-pleurodesis and non-consecutive pattern of their selection may represent a potential bias in outcome interpretation. However, this does not affect the main comparison between pleurectomy—decortication and extrapleural pneumonectomy.

In conclusion, biologic prognosticators, and namely expression of COX-2, p21 and p27, represent an interesting field of research, which can provide useful information on the prognosis and on the choice of the most appropriate treatment. Further advances would allow to recognise a subgroup of patients with significantly improved survival, in which it could be possible to achieve better response to therapy, hopefully introducing new molecular targeted treatments. The fight against mesothelioma has just begun. Despite the present unsatisfactory results, in our mind there is the hope that a better knowledge in biologic and molecular mechanisms will accomplish a consistent leap forward.

References


Appendix A. Conference discussion

Dr T. Treasure (London, United Kingdom): There is a matter we need to be clear about, the difference between a prognostic feature and a predictive feature. A feature may be prognostic in the sense of determining a differential natural history in the disease itself. Patients with good prognostic features will live longer whether you operate on them or not. On the other hand, R0 versus R1 may be predictive of better outcome. I would make that distinction. So I would put it to you that interesting though the results are, and important in avoiding unavailing surgery in patients who are going to die quite soon whatever you do, that you have done an analysis of prognostic factors, which are prognostic irrespective of whether you operate or not. It isn’t that you shouldn’t use that information but you should be very clear in your own mind exactly what you have found. I admire the study, it’s very thorough, and the more we know about this disease, the better we’re going to be able to treat it.

Dr Pompeo: Your comments are very important, and, of course, they underline the main limit of this study. I perfectly agree with your considerations. Maybe these factors might better be considered as predictive rather than prognostic factors because the main limit of this study is its retrospective nature. The hope is that in the future these kinds of prognosticators can help to avoid aggressive surgery in patients in whom it is unnecessary.

Dr M. Alam (Dublin, Ireland): I have just one quick question. You identified a high level of concentration of COX-2. Do you see a role for highly selective COX-2 inhibitors in the future management or treatment of mesothelioma?

Dr Pompeo: Yes. I think one important result is the association between all these factors. There has already been more than one study emphasizing the possible interdependence of all 3 of these factors in revealing the negative or positive effect on the natural history of solid tumours, and I think we will continue to assess all these factors together.

Dr W. Weder (Zurich, Switzerland): I have one question. From the study you have done now and the data, how does this influence your next steps?

Dr Pompeo: It’s difficult to answer. One thing might be to assess expression of these factors before surgery and to see in which patients COX-2 is very high and the contrary for the other factors, and then avoid extrapleural pneumonectomy in patients with a predictable poor result, but I think the most important thing is to consider this as a first step to better understand pathophysiologic mechanisms of mesothelioma progression and continue to try to find some new therapeutic agents that will be able to help us with this disease.

Editorial comment

May cyclooxygenase-2 (COX-2), p21 and p27 expression affect prognosis and therapeutic strategy of patients with malignant pleural mesothelioma?

Keywords: Malignant pleural mesothelioma; COX-2; p21; p27

Mineo and colleagues from the Department of Thoracic Surgery of the University of Rome retrospectively investigated the immunohistochemical expression of cyclooxygenase-2 (COX-2), p21 and p27 in a cohort of 77 consecutive mesothelioma patients [1]. The triple-combination of high COX-2 and low p21 and p27 expression was found to be the only independent prognosticator for shorter overall survival for mesothelioma patients in the whole panel of factors analysed (beside stage, histology or therapy).

This kind of research—analysis of different markers for the prognosis of several tumours—recently came under some criticism, finding ‘another marker amongst thousands’. In my opinion, it is still important research to be performed, especially in the context of mesothelioma research. Heterogeneous results concerning the outcome of patients with sarcomatoid histology or involved mediastinal lymph nodes after induction chemotherapy followed by surgery cannot be only explained by different patient groups [2–4]. There must be differences in the biological features of mesothelioma patients that are responsible for these differing survival outcomes! Therefore, investigation of markers and correlation with clinical outcome may provide new knowledge regarding the biology of this aggressive tumour. Furthermore, subgroups of patients benefitting from aggressive treatment regimens can be defined.

COX-2 is an inducible enzyme, which catalyses the conversion of arachidonic acid to prostaglandins in response to pro-inflammatory or mitogenic signals; it is overexpressed in many solid tumours; and in vitro experiments, by using specific COX-2 inhibitors, have shown that COX-2 may be a potential target for novel cancer therapies [5]. Therefore, this marker is a very promising one as it can be used not only for prognostic reasons but also as a therapeutic target. As mentioned in the discussion, several authors have already confirmed the role of COX-2 as a prognosticator in malignant pleural mesothelioma (MPM) [6,7]. The two cyclin-dependent kinase-inhibitors, p21 and p27, are cell-cycle regulators that are implicated in the regulation of the molecular mechanism of cell division. High expression of p27 was correlated to prolonged overall survival of MPM patients [8,9] but, in the underlying analysis, only the triple combination of all markers independently predicted longer overall survival.

The correlation between the expression of the different markers and several clinico-pathological markers was evaluated; it would have been interesting if the authors had provided additional information about the relationship between the different markers. We have shown that p27 immunostaining correlates in a cohort of 352 patients with the expression of p21 [8]. The same was observed by Baldi and colleagues and, in addition, a negative correlation between COX-2 expression and both p27 and p21 was shown [7].

In Table 1 of Mineo et al.’s paper, univariate analysis of the main clinico-pathological variables is illustrated and shows that out of 27 extrapleural pneumonectomies and 44 pleurectomy/decortications performed, 65 patients