Utility of Osteopontin and Serum Mesothelin in Malignant Pleural Mesothelioma Diagnosis and Prognosis Assessment

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Abstract

Purpose: Malignant mesothelioma is a highly aggressive tumor and is often diagnosed too late for a curative treatment. We compared diagnostic and prognostic values of mesothelin and osteopontin in 172 patients suspected of malignant pleural mesothelioma (MPM) and in a control group of 112 asymptomatic asbestos-exposed subjects.

Experimental Design: Osteopontin and mesothelin were assayed with commercial ELISA kits in a series of 43 patients with pleural metastases of various carcinomas, 33 patients with benign pleural lesions associated with asbestos exposure, 96 patients with MPMs, and 112 asbestos-exposed healthy subjects. Results were correlated with patient's diagnosis and survival.

Results: Serum osteopontin level was higher in MPM patients compared with healthy asbestos-exposed subjects and had a good capability to distinguish between these two populations. However, osteopontin was unable to distinguish between MPM and pleural metastatic carcinoma or benign pleural lesions associated with asbestos exposure. Neither plasma nor pleural fluid osteopontin were more powerful in this respect. Serum mesothelin had a good ability for diagnosing MPM but was unable to identify patients with nonepithelioid mesothelioma subtypes. Survival analysis identified tumor histologic subtype along with serum osteopontin and serum mesothelin as independent prognostic factors in mesothelioma patients.

Conclusions: Osteopontin has a lower diagnostic accuracy than mesothelin in patients suspected of MPM. Insufficient specificity limits osteopontin utility as diagnostic marker. Both molecules have a potential value as prognostic markers.

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The incidence of malignant pleural mesothelioma (MPM) has raised continuously in the last decades due to previous exposure to asbestos, the most important factor involved in MPM pathogenesis (1). Mesothelioma is an extremely aggressive tumor, highly resistant to chemotherapy and to radiotherapy. Thus, significant advances in treatment of MPM will imply an early diagnosis to select candidates for therapy with curative intent (2). The discovery of a marker that would permit an earlier diagnosis could lead to an increase of the proportion of patients diagnosed with early-stage mesothelioma, in which a multimodal treatment, including surgery and radiotherapy/chemotherapy, would eventually result in a better outcome (2). To date, there is no recognized marker for the diagnosis of mesothelioma or for screening of at-risk asbestos-exposed individuals. Recent reports have raised interest on soluble mesothelin-related peptides (3–5) and osteopontin (6) as possible markers for diagnosing MPM. Osteopontin seems especially interesting as a potential early diagnostic marker because it has been shown to differentiate asbestos-exposed patients from stage I mesothelioma patients (6). However, questions were raised about the clinical utility of this marker because this first report did not include other pleural malignancies and nonmalignant asbestos-induced pleural diseases as controls (7, 8). Indeed, osteopontin is expressed

in various nonpleural malignant diseases (8–10) and elevated serum osteopontin levels have been reported in ovarian, colon, breast, prostate, and lung cancers (11, 12) or in tuberculosis (13). Osteopontin is an extracellular cell adhesion protein involved in nonmineral bone matrix formation, but is also a key cytokine in mediating type I immune responses (14), and was implicated in regulation of metastatic spread of tumor cells (15). In fact, osteopontin was first described as being secreted by transformed malignant epithelioid cells (16).

Mesothelin is a physiologically expressed, membrane-bound peptide on the surface of normal mesothelial cells and is also found expressed in various cancers, including malignant mesothelioma (17), pancreatic (18) or ovarian carcinoma (17), sarcomas (18), and in some gastrointestinal (19) or pulmonary carcinomas (19–21). A soluble form, released from the membrane from the membrane-bound mesothelin (22, 23), can be detected in sera from mesothelioma patients. Thus, we preferred to use the term of soluble mesothelin instead of soluble mesothelin-related peptides, which was initially proposed. However, the mechanism of release of mesothelin from the cell surface into the blood is unknown. Serum mesothelin level is low in healthy subjects exposed to asbestos (3, 4).

The goal of our study was to evaluate the diagnostic value of osteopontin measured both in blood and in pleural fluid and to compare it with mesothelin, in a series of patients suspected of MPM. We also evaluated prognostic value of both markers.

Materials and Methods

Patients. Starting May 2003, we recruited all consecutive patients suspected of, or recently diagnosed with, MPM from 20 different pulmonary or thoracic surgery departments from the north and west of France. All these patients had clinical symptoms consistent with a diagnosis of MPM (i.e., at least chest pain or dyspnea associated with pleural thickening and/or pleural effusion on the thoracic computed tomography scan). Exclusion criteria were any concomitant infectious disease and previous therapy against MPM. Pathologic diagnosis was obtained on pleural biopsies following recent international guidelines (24).

Final diagnosis, based on pleural histology, divided the 172 recruited patients into three groups: 96 with confirmed MPM, 33 patients with benign pleural lesions associated with asbestos exposure (BPLAE group), and 43 patients with pleural metastasis of various carcinomas (Mets group; see details in Table 1). In patients with MPM, staging was done according to International Mesothelioma Interest Group classification (25). A complete staging was available in 83 (86.4%) MPM patients. In 13 cases, some data were missing, mainly the N status. Eleven (13.3%) patients were in stage I, 21 (25.3%) patients were in stage II, 32 (38.6%) patients were in stage III, and 19 (22.9%) patients were in stage IV. The primary tumor in the Mets group was bronchopulmonary adenocarcinoma in 22 (51.1%) patients, breast adenocarcinoma in 10 (23.2%), digestive adenocarcinomas in 3 (7%), ovarian adenocarcinoma in 2 (4.7%), adenocarcinoma of unknown origin in 3 (7%), and other carcinomas in 3 (7%) cases. A subset of the 172 recruited patients (119 cases, 63 with MPM, 31 with Mets, and 25 with BPLAE) were already included in our previous report assessing the value of mesothelin in MPM diagnosis (4).

From the 96 MPM patients, 73 (76.1%) were of epithelioid subtype, 10 (10.4%) were sarcomatoid, and 13 (13.5%) were biphasic mesotheliomas. Therapy assigned to MPM patients was decided by a medical staff, including surgeons, medical oncologists, radiotherapists, and pneumologists. Irradiation of chest wall drainage points (with 21 Gray in 7 fractions) was done for all MPM patients, including those under supportive care. Seventy (72.9%) patients received chemotherapy with cisplatin and pemetrexed, 5 (5.2%) received an association of cisplatin and gemcitabine, 5 (5.2%) received other combinations of cisplatin-based chemotherapy, and 16 (16.7%) received supportive care only. Only 10 (10.4%) patients were subjected to surgery, 9 undergoing an extrapleural pneumonectomy and 1 patient a pleural decortication.

We also recruited between December 2001 and June 2004 a cohort of 112 subjects who were occupationally exposed to asbestos (AE). They were working in a single facility involved in textile and friction material processing. They were subsequently followed in the Occupational Medicine Department for a median time of 26 months [interquartile range (IQR), 7-30 months]. These subjects had no clinical complaint, and none of them developed a MPM or another malignancy during their follow-up. Only serum was available in these patients.

A standard operating procedure about patients sampling and data retrieval was set in place. Serum and EDTA-anticoagulated plasma samples, as well as pleural fluid samples retrieved without anticoagulant, if available, were collected from each patient and stored at -80°C

Table 1. Demographic data of recruited patients

	Mets (n = 43)	BPLAE (n = 33)	MPM (n = 96)	AE (n = 112)
Age, y (mean ± SD)	66.1 ± 11.2	62.2 ± 10.5	65 ± 9.7	63 ± 6.8
Male gender, n (%)	24 (56)	32 (97)	78 (81)	95 (84)
Mode of diagnosis, n (%)				
Blind pleural biopsy	2 (4.7)	—	1 (1)	NA
Thoracoscopy	29 (67.4)	22 (66.7)	60 (62.5)	
Surgery (VATS or open surgery)	8 (18.6)	11 (33.3)	31 (32.3)	
Guided biopsy (CT or US)	4 (9.3)	—	4 (4.1)	
Confirmed asbestos exposure, n (%)	15 (34.9)	28 (84.8)	82 (85.4)	112 (100)
Survival, median (95% CI) mos*	9 (6-12)	—	14 (10-18)	NA
Staging (IMIG classification)†				
Stage I	NA	NA	11	NA
Stage II			21	
Stage III			32	
Stage IV			19	

Abbreviations: VATS, video-assisted thoracoscopic surgery; CT, chest computed tomography scan; US, ultrasound; IMIG, International Mesothelioma Interest Group; NA, not applicable.

*Median survival was not calculated for the BPLAE group; only three (9.1%) deaths were noted in this group, one due to one bronchial adenocarcinoma and two nonneoplastic, non-asbestos–related deaths.

† Complete tumor-node-metastasis staging was available for 83 patients.

in aliquots until analyzed. Clinical data and outcome of the patients were also collected. Study protocol has been approved by the local ethics committee. All patients were informed about inclusion in this study and none refused.

ELISA assays. All assays were done in a single laboratory (Institut National de la Sante et de la Recherche Medicale U774). Assays for osteopontin were done in serum, plasma, and pleural fluid using the human osteopontin kit from Immunobiological Laboratories. Serum and pleural levels of soluble mesothelin-related peptide were assayed with a commercial ELISA kit (Mesomark, CISBio International) according to the manufacturer's instructions and results were expressed in nanomoles per liter.

Statistical analysis. All data are reported as median and IQR as well as mean with SD. Comparisons between groups were done using both Kruskall-Wallis test and a nonparametric ANOVA after rank transformation as suggested by Conover and Iman (26). The Bonferroni correction was applied for multiple comparisons in post hoc tests. Areas under receiver operating characteristic (ROC) curves (AUC) are reported with their 95% confidence intervals (95% CI). Comparisons of AUC were done as suggested by Hanley and McNeil (27) using values available for both variables. Unavailable data were coded as missing. A discriminant analysis was done to seek combinations of different assay results, which would result in a better classification of the patients.

A Cox proportional hazard model was used to examine associations between the various variables and survival. Survival was defined as the number of weeks from the date of pathologic diagnosis until the date of death if the patient died or until the date of last follow-up visit. Patients still alive at last follow-up were considered censored. The cutoff values for serum assays, which best differentiate survivors and nonsurvivors, were determined by an algorithm of maximization of hazard ratio (28, 29). In the multivariate analysis, mesothelin and osteopontin were introduced either as continuous variables or as binary values (high versus low) using the determined cutoffs. The log-rank test statistic was used to compare differences in survival between groups. For all tests, a two-sided P value of <0.05 was considered significant. Statistical calculations were done with the SPSS statistical package (version 12.0F, SPSS) and the SAS system (version 8.2).

Results

Diagnostic value of serum osteopontin. We recruited 112 healthy subjects previously exposed to asbestos (AE group) and 43 patients with pleural metastasis of adenocarcinomas (Mets group), 33 patients with benign pleural lesions associated with asbestos exposure (BPLAE group), and 96 patients with MPM (Table 1). As expected, serum osteopontin values were low in the AE group with a median of 34.7 ng/mL (IQR, 21.8-43.5 ng/mL). Higher serum osteopontin levels were found in patients with BPLAE (median, 50.4 ng/mL; IQR, 29.6-118.8 ng/mL; $P = 0.006$), MPM (median, 69.9 ng/mL; IQR, 29.9-145.1 ng/mL; $P = 0.001$), and Mets (median, 89.8 ng/mL; IQR, 40.5-412.7 ng/mL; $P = 0.001$; Fig. 1A). Interestingly, we found no statistically significant difference in serum osteopontin between the three groups of patients (MPM, Mets, and BPLAE; $P > 0.38$ for all comparisons). Serum osteopontin has a good ability to distinguish between patients with MPM and asbestos-exposed healthy subjects (AE) with an AUC of 0.724 (95% CI, 0.650-0.798; Fig. 1C).

Serum osteopontin had also a significant ability to distinguish patients with any pleural involvement (MPM, Mets, or BPLAE) and healthy asbestos-exposed subjects (AE; AUC, 0.735; 95% CI, 0.677-0.793) and patients with malignant pleural involvement (MPM or Mets) from patients with a benign pleural involvement (Fig. 2; Table 2).

Osteopontin is cleaved by thrombin after blood coagulation and serum values are much lower than corresponding plasma values (30, 31). We therefore investigated if plasma values could be more useful for diagnosing MPM on a subset of 131 patients from which plasma was available. Serum and plasma osteopontin values were highly correlated (Pearson $r^2 = 0.732$; $P < 0.001$). Interestingly, higher plasma osteopontin levels were found in the Mets group (median, 1,176 ng/mL; IQR, 569.4-2719) compared with MPM patients (median, 682.6; IQR, 423.7-1,063) and to BPLAE patients (median, 420.5 ng/mL; IQR, 320-687; $P < 0.02$ for all comparisons). ROC curve analysis showed similar values for AUC when differentiating MPM from Mets (AUC, 0.689; 95% CI, 0.579-0.798, with higher values for Mets group) or BPLAE (AUC, 0.677; 95% CI, 0.562-0.792). Plasma osteopontin had a significant ability to discriminate between neoplastic pleural lesions (MPM or Mets) and BPLAE (AUC, 0.729; 95% CI, 0.631-0.827). This value was similar to that of serum osteopontin.

Diagnostic value of serum mesothelin. We investigated serum level of mesothelin to compare its diagnostic value with osteopontin. Low serum levels were found for mesothelin in both AE and BPLAE groups and slightly higher values in Mets patients (Fig. 1B). MPM patients had significantly higher values of mesothelin (median, 1.94 nmol/L; IQR, 1.03-4.07 nmol/L) than Mets, BPLAE, or AE group ($P < 0.001$ for all comparisons). Median serum mesothelin values were low in patients with a sarcomatoid or biphasic subtype of mesothelioma (data not shown). A statistically significant correlation between serum mesothelin and blood (serum or plasma) osteopontin values were found ($P < 0.02$) in all subgroups of patients (MPM, BPLAE, or Mets). However, the magnitudes of these correlations were low, with a maximum Pearson r^2 of 0.45 between serum mesothelin and plasma osteopontin in the subgroup of MPM patients.

Contrary to osteopontin, serum mesothelin had a good capability to distinguish not only between MPM patients and AE subjects (AUC, 0.866; 95% CI, 0.811-0.920) but also between MPM and BPLAE (AUC, 0.834; 95% CI, 0.755-0.912) or Mets patients (AUC, 0.719; 95% CI, 0.624-0.814).

Serum mesothelin could also distinguish patients with any pleural involvement (MPM, Mets, or BPLAE) from asbestos-exposed patients (AUC, 0.741; 95% CI, 0.684-0.799) and malignant pleural lesions (MPM and Mets) and from benign (BPLAE and AE) pleural involvement (AUC, 0.784; 95% CI, 0.727-0.842; Table 2).

Pleural values of osteopontin and mesothelin. Pleural osteopontin levels were slightly higher in the MPM group than in patients with metastatic carcinoma (Mets) or benign pleural lesions (BPLAE) but the differences did not reach statistically significance (Fig. 3A). Consequently, pleural osteopontin had no value for differentiating those types of pleural lesions. No correlation was found between pleural osteopontin and serum or plasma osteopontin ($r < 0.038$; $P > 0.74$ for both tests).

Pleural mesothelin value was significantly higher in patients with MPM than either BPLAE or Mets patients ($P = 0.001$ for both comparisons). The AUC for pleural mesothelin differentiating MPM and BPLAE patients was 0.829 (95 CI, 0.740-0.918) and was lower when differentiating MPM and Mets (AUC, 0.758; 95% CI, 0.664-0.853). Pleural mesothelin had no better diagnostic value than serum mesothelin because AUCs for serum and pleural mesothelin were similar irrespective of the comparison made.

Combined value osteopontin and mesothelin for diagnosing malignant mesothelioma. Because our results indicated that osteopontin is a high sensitivity marker but with poor specificity and mesothelin had good specificity but with a lower sensitivity, we wondered if a combination of the two markers could improve patient classification. A discriminant analysis was done and showed that patient classification was not improved using a combination of the two markers than using mesothelin alone.

Osteopontin and mesothelin as prognostic factors. We also investigated if mesothelin and osteopontin could be related to patients' outcome. Only five patients with MPM were lost at follow-up after the initial inclusion visit. Thus, 91 MPM patients were included in the analysis. Survival data from all the 43 patients with Mets and 33 patients with BPLAE were available (Table 3). Median survival was lower in Mets than in MPM patients.

In the subgroup of Mets patients, neither age, sex, primary tumor localization, mesothelin (serum or pleural), or osteopontin (serum, plasma, or pleural) could predict the survival of patients.

In the MPM group, neither age, sex, nor pleural values of mesothelin and osteopontin were also related to survival. According to stage, median survival in stage I MPM patients exceeded 20 months, in stage II patients was 17 months (95% CI, 11-23), in stage III was 12 months (95% CI, 5-19), and was only 8 months in stage IV (95% CI, 3-17). Due to the low number of patients, the log-rank test resulted in a P of only 0.058. Patients with sarcomatoid mesothelioma subtype had a significantly shorter survival (median, 5 months; 95% CI, 3-7 months) than patients with epithelioid (median, 17 months; 95% CI, 11-23 months) or biphasic subtype (median, 17 months; 95% CI, 6-28; P = 0.002, log-rank test; Fig. 4A; Table 3). In MPM patients, a significant relationship with survival was found for serum mesothelin as well as serum and plasma osteopontin. Serum osteopontin had a higher hazard ratio than plasma osteopontin, so only the serum value was subsequently introduced in the multivariate model. In the multivariate model, serum mesothelin and osteopontin were introduced either as continuous values or as binary values (high versus low) after calculating for each marker a cutoff that best differentiate longer survivors. Cutoff values of 3.5 nmol/L for serum mesothelin and 350 ng/mL for serum osteopontin were established using an algorithm of maximization of hazard ratio (28, 29). Patients with a high mesothelin level (>3.5 nmol/L) had a median survival of 7 months (95% CI, 3-11 months) compared with 19 months (95% CI, 13-25) for the low mesothelin level group (P = 0.003, log-rank test; Fig. 4B).

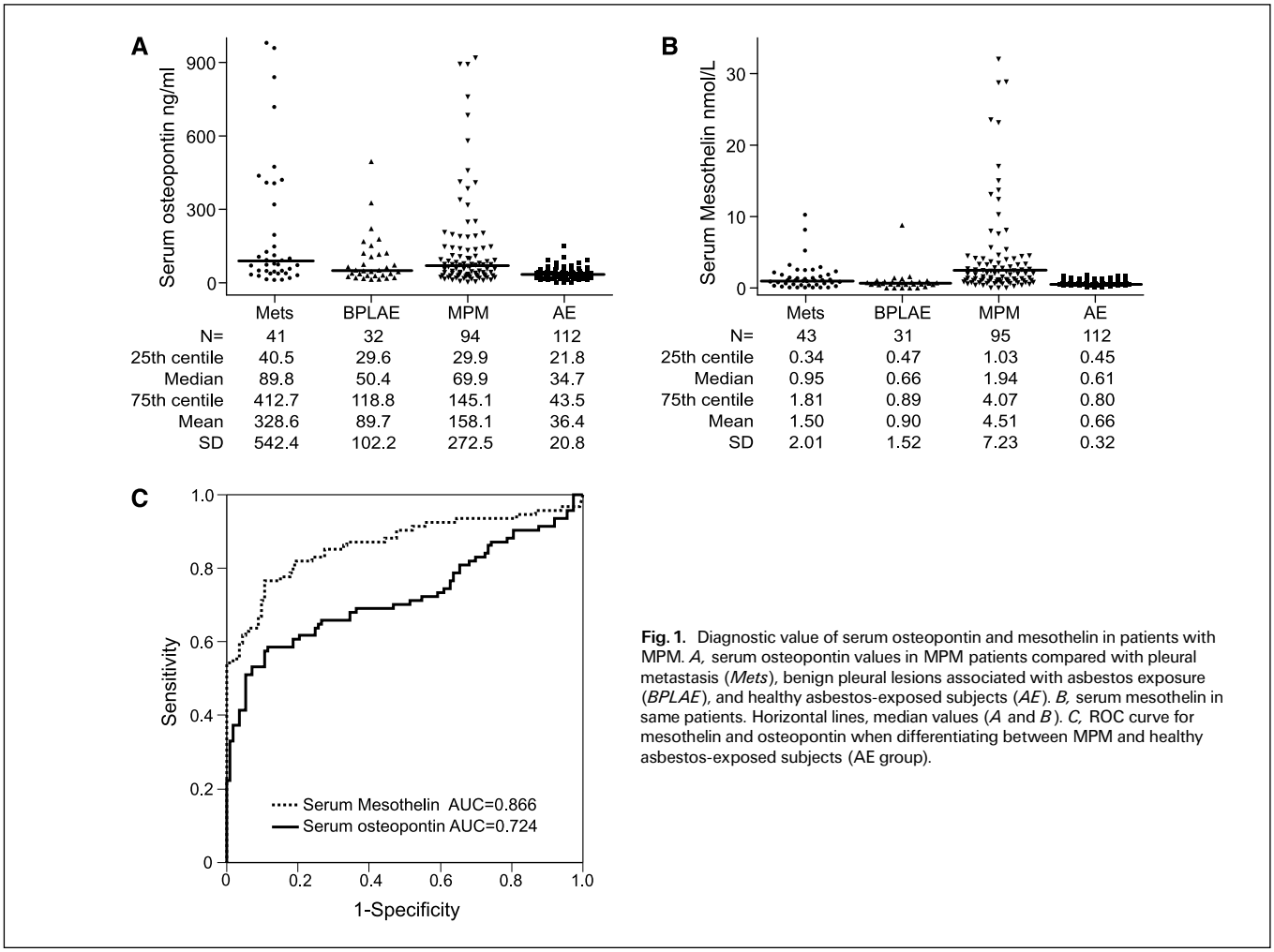

Fig. 1. Diagnostic value of serum osteopontin and mesothelin in patients with MPM. *A,* serum osteopontin values in MPM patients compared with pleural metastasis (*Mets*), benign pleural lesions associated with asbestos exposure (*BPLAE*), and healthy asbestos-exposed subjects (*AE*). *B,* serum mesothelin in same patients. Horizontal lines, median values (*A* and *B*). *C,* ROC curve for mesothelin and osteopontin when differentiating between MPM and healthy asbestos-exposed subjects (AE group).

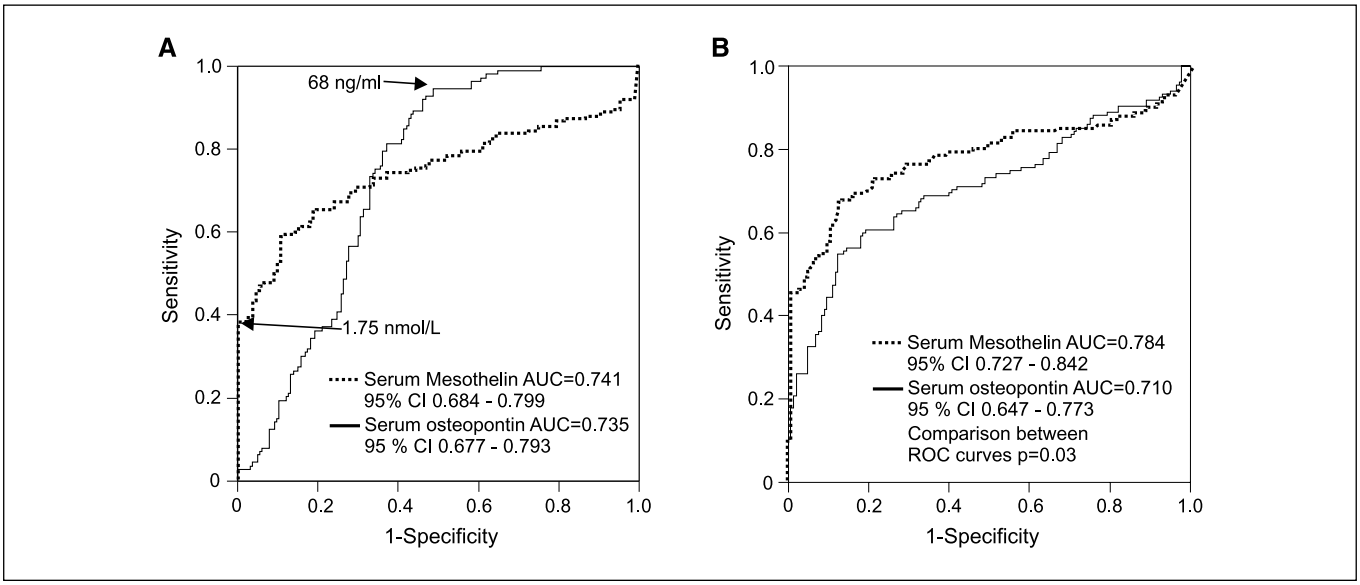

Fig. 2. ROC curve for serum mesothelin and osteopontin to distinguish patients with any pleural involvement (MPM, BPLAE, or Mets) and healthy asbestos-exposed patients (AE; *A*) and patients with malignant (MPM and Mets) and benign pleural involvement (BPLAE and AE; *B*).

Similarly, patients with a high serum osteopontin (i.e., >350 ng/mL) had a significantly shorter survival (median, 5 months; 95% CI, 2-8 months) than patients with low serum osteopontin level (median, 15 months; 95% CI, 11-19 months; Fig. 4C).

In the multivariate survival analysis, mesothelioma subtype and serum values of mesothelin and osteopontin retained statistical significance (Table 3) but tumor stage did not. Thus, mesothelioma histologic subtype and both serum values of mesothelin and osteopontin are independent prognostic factor in MPM patients and had a higher influence on prognostic than tumor stage.

Discussion

Mesothelioma is a highly aggressive cancer, which is often diagnosed up to 30 to 40 years after asbestos exposure. Because tumor growth is generally insidious and the usual clinical signs (dyspnea, cough, and chest pain) are unspecific, MPM diagnosis is generally obtained too late for a curative treatment. Modern combined chemotherapy has only a limited effect on disease progression, even if it offers a small, but significant longer survival (32). Thus, disease markers have been searched in an attempt to help early diagnosis, but none has been proven to be reliable in clinical practice yet. Recently, osteopontin has been proposed as an early marker for MPM diagnosis (6). In this first report, osteopontin serum level was related to duration of asbestos exposure and had a good ability to discriminate between asymptomatic asbestos-exposed individuals and early-stage mesothelioma patients. However, this study did not include patients with diffuse pleural thickening and/or a benign pleural effusion related to asbestos nor patients with pleural metastasis of carcinoma, two common clinical situations from which MPM need to be differentiated. In addition, osteopontin expression and elevated circulating osteopontin levels have been shown in other pulmonary diseases (30) and a variety of tumoral localizations, including lung cancer (33–35). Thus, doubt has been raised about the potential utility of this marker (8). When measuring serum osteopontin (with the same ELISA assay), we found similar results as Pass et al. (6) who reported a mean osteopontin value of 30 ng/mL (SE, 3 ng/mL) in exposed

Table 2. AUCs for different comparisons

	Any pleural disease vs AE* AUC (95% CI)	Malignant vs benign pleural disease† AUC (95% CI)
Serum osteopontin	0.735 (0.677-0.793)	0.710 (0.647-0.773)
Plasma osteopontin	—	0.729 (0.631-0.827)‡
Pleural osteopontin	—	0.478 (0.332-0.624)‡
Serum mesothelin	0.741 (0.684-0.799)	0.784 (0.727-0.842)
Pleural mesothelin	—	0.646 (0.542-0.749)‡

*Pleural disease includes MPM, Mets, and BPLAE.
†Malignant pleural diseases include MPM and Mets, and benign pleural diseases include BPLAE and AE.
‡These comparisons include only patients with BPLAE in the benign pleural disease group.

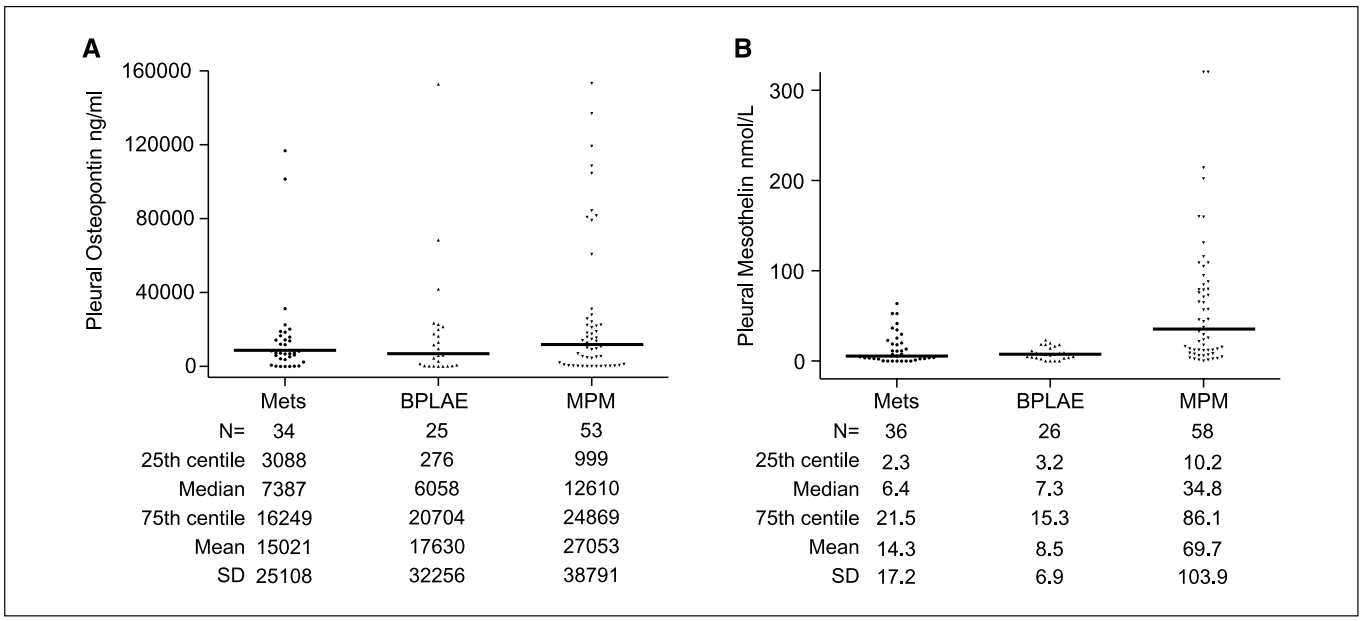

Fig. 3. Pleural values of osteopontin (*A*) and mesothelin (*B*) in patients with MPM, pleural metastasis of carcinoma (*Mets*), and benign pleural lesions associated with asbestos exposure (*BPLAE*). Horizontal lines, median values.

patients and 133 ng/mL (SE, 10 ng/mL) in patients with MPM. However, contrary to this previous report, we found a significant number of mesothelioma patients with very low serum osteopontin values (Fig. 1A). Thus, serum osteopontin has a good ability to discriminate between patients with MPM and asbestos-exposed healthy subjects as already suggested by Pass et al. (6). Moreover, osteopontin was unable to discriminate patients with MPM from BPLAE or Mets patients.

Osteopontin and mesothelin had similar AUC when differentiating patients with any pleural involvement and asbestos-exposed patients but the shape of the ROC curves were different: whereas osteopontin could offer a good sensitivity, mesothelin had a much better specificity. However, if we try to identify only patients with malignant pleural involvement, mesothelin had a higher AUC than osteopontin (Fig. 2B).

The use of osteopontin as an MPM screening marker is expected to be difficult because incidence of the disease is very low, even in the asbestos-exposed population, and, consequently, most patients with a positive test will be in fact false positive. Thus, doing a thoracoscopy with multiple pleural biopsies (the gold standard for MPM diagnosis) in all osteopontin-positive patients seems unrealistic and inadvisable. At best, osteopontin could be used as a first step selection marker combined with a more specific assay in the subgroup of osteopontin-positive patients. However, it should be taken into account that osteopontin is a complex molecule and there are several distinct forms/proteolytic fragments of osteopontin (36) and results depend on the choice of the assay used. Therefore, in the future, quantification of other soluble isoforms/proteolytic fragments of osteopontin could possibly give better results. The same also applies for mesothelin (37, 38).

Serum mesothelin did better than osteopontin as a MPM diagnostic marker in our series. However, mesothelin levels are low in nonepithelioid MPM (3, 4). Therefore, despite a good specificity, the poor sensitivity of mesothelin makes it

Table 3. Survival analysis in MPM patients

Variables	Categories	Univariate analysis		Multivariate analysis	
		HR (95% CI)	*P*	HR (95% CI)	*P*
Tumor stage*			0.058	—	—
Histologic subtype	Epithelioid	—	—	—	—
	Biphasic	0.91 (0.37-2.24)	0.848	0.943 (0.350-2.539)	0.908
	Sarcomatoid	4.08 (1.72-9.66)	0.001	6.683 (2.525- 17.683)	0.001
Serum mesothelin	>3.5 nmol/L	2.39 (1.247-4.583)	0.009	2.785 (1.384-5.607)	0.004
Pleural mesothelin	Continuous	0.994 (0.988-1.001)	0.118	ND	—
Serum osteopontin	>350 ng/mL	5.263 (1.875-14.777)	0.002	3.459 (1.098-10.895)	0.034
Plasma osteopontin	Continuous	1.001 (1.000-1.001)	0.001	ND	—
Pleural osteopontin	Continuous	1.000	0.259	ND	—

Abbreviation: ND, not done.
*Tumor stage was not retained as an independent prognostic factor in the multivariate analysis.

insufficient for use as a unique screening marker. Combining both osteopontin a first step selection followed by assessment of serum mesothelin did not result in a better classification of patients than using mesothelin alone in our series.

The most interesting result of our report is the potential usefulness of both serum osteopontin and serum mesothelin as prognostic markers in MPM. Tumor stage in this series was only marginally significant as a prognostic factor perhaps due to an insufficient number of patients. The International Mesothelioma Interest Group classification is the best available staging method available and has been proven its utility in large series of surgical patients. Even if the International Mesothelioma Interest Group criteria are certainly the best we have right now for MPM, it is commonly admitted that this staging method is hard to assess without complete surgical investigation and this explains the missing complete classification in 13 (13.5%) patients in our series. As reported previously, we also found that the pathologic subtype of MPM is a prognostic factor (2). Patients with sarcomatoid mesothelioma had a worse prognosis than patients with either epithelioid or biphasic subtype.

Our patients had been treated following commonly accepted practices according to the initial staging of the patients, and the global survival in our series is comparable with those reported in recent trials (32). Therefore, there is little possibility that survival analysis results have been biased by treatment allocation.

We can speculate that serum mesothelin is a prognostic factor because it is directly produced by the tumor itself and could be a mirror of tumor burden as already suggested by Robinson et al. (3) who found higher serum mesothelin levels in patients having larger tumors. However, in our series, we did not find any correlation between tumor stage and serum mesothelin levels (data not shown). In contrast, the link between higher blood osteopontin level and patient's shorter survival is more elusive. Osteopontin is also a cytokine and has been involved in a broad range of biological processes as cellular immune responses, tissue remodeling, cell survival, and cancer progression and metastasis (39). Elevated osteopontin level could stimulate tumor growth and spread and thus explain a shorter survival. Similar correlations between high osteopontin

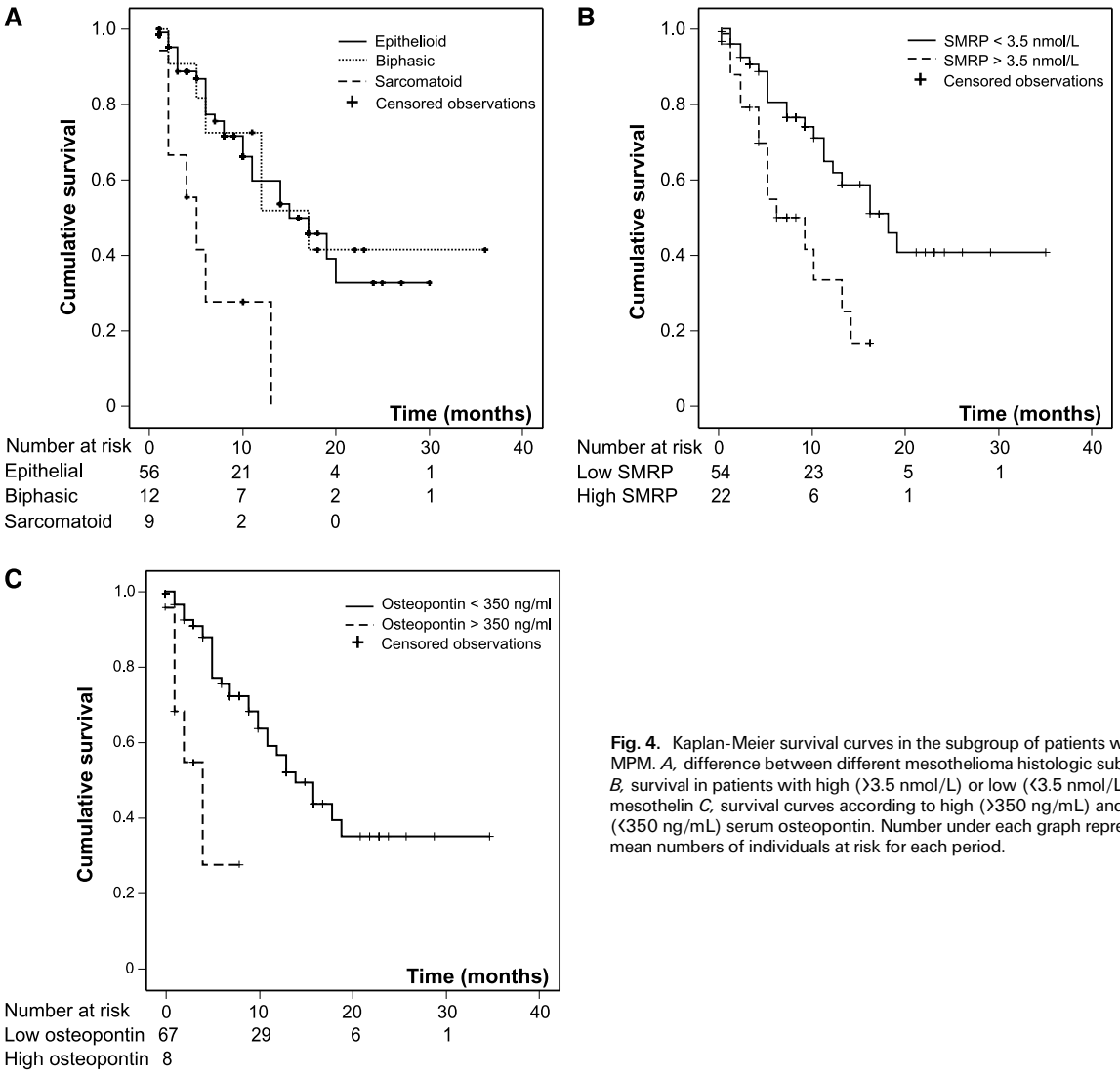

Fig. 4. Kaplan-Meier survival curves in the subgroup of patients with MPM. *A,* difference between different mesothelioma histologic subtypes. *B,* survival in patients with high (>3.5 nmol/L) or low (<3.5 nmol/L) serum mesothelin *C,* survival curves according to high (>350 ng/mL) and low (<350 ng/mL) serum osteopontin. Number under each graph represents mean numbers of individuals at risk for each period.

expression and a shorter survival have been described in various tumoral localizations as breast (40), prostate (41), colon (42), pancreatic (43), esophageal (44), lung (10), or soft tissue tumors (45).

Mesothelioma histologic subtype and blood levels of mesothelin and osteopontin were independent prognostic factors for survival because they probably reflect different aspects of tumor biology. This is also sustained by the absence of tight correlations between values of osteopontin and mesothelin. Further studies are needed to explain how mesothelin and osteopontin are produced, secreted, and involved in mesothelioma tumor progression. Kinetic studies will also be important to assess the value of these markers in monitoring patient response to therapy.

In conclusion, we confirmed that MPM patients have higher levels of serum osteopontin than asbestos-exposed individual as it has been suggested by Pass et al. (6), but this finding is of little diagnostic value because osteopontin cannot differentiate between MPM, pleural metastatic carcinoma, or even benign pleural lesions associated with asbestos exposure. The utility of osteopontin as a screening marker is hampered by an insufficient specificity, which would result in a very high number of false-positive tests. Serum mesothelin alone has probably insufficient specificity and sensitivity for MPM screening too, but serum mesothelin retains a significant diagnostic value even if it detects only the epithelioid subtype of mesothelioma. The most important finding is that both serum mesothelin and osteopontin levels are correlated with survival in patients with MPM.

References

1. Robinson BW, Musk AW, Lake RA. Malignant mesothelioma. Lancet 2005;366:397–408.
2. Sugarbaker DJ, Flores RM, Jaklitsch MT, et al. Resection margins, extrapleural nodal status, and cell type determine postoperative long-term survival in trimodality therapy of malignant pleural mesothelioma: results in 183 patients. J Thorac Cardiovasc Surg 1999;117:54–63; discussion 63–5.
3. Robinson BW, Creaney J, Lake R, et al. Mesothelin-family proteins and diagnosis of mesothelioma. Lancet 2003;362:1612–6.
4. Scherpereel A, Grigoriu BD, Conti M, et al. Soluble mesothelin-related protein in the diagnosis of malignant pleural mesothelioma. Am J Respir Crit Care Med 2006;173:1155–60.
5. Hassan R, Remaley AT, Sampson ML, et al. Detection and quantitation of serum mesothelin, a tumor marker for patients with mesothelioma and ovarian cancer. Clin Cancer Res 2006;12:447–53.
6. Pass HI, Lott D, Lonardo F, et al. Asbestos exposure, pleural mesothelioma, and serum osteopontin levels. N Engl J Med 2005;353:1564–73.
7. Cullen MR. Serum osteopontin levels—is it time to screen asbestos-exposed workers for pleural mesothelioma? N Engl J Med 2005;353:1617–8.
8. O'Regan AW, Serlin D, Berman JS. Asbestos exposure and serum osteopontin. N Engl J Med 2006; 354:304–5.
9. Brown LF, Papadopoulos-Sergiou A, Berse B, et al. Osteopontin expression and distribution in human carcinomas. Am J Pathol 1994;145:610–23.
10. Chambers AF, Wilson SM, Kerkvliet N, et al. Osteopontin expression in lung cancer. Lung Cancer 1996; 15:311–23.
11. Kim JH, Skates SJ, Uede T, et al. Osteopontin as a potential diagnostic biomarker for ovarian cancer. JAMA 2002;287:1671–9.
12. Fedarko NS, Jain A, Karadag A, Van Eman MR, Fisher LW. Elevated serum bone sialoprotein and osteopontin in colon, breast, prostate, and lung cancer. Clin Cancer Res 2001;7:4060–6.
13. Koguchi Y, Kawakami K, Uezu K, et al. High plasma osteopontin level and its relationship with interleukin-12-mediated type 1 T helper cell response in tuberculosis. Am J Respir Crit Care Med 2003;167:1355–9.
14. Ashkar S, Weber GF, Panoutsakopoulou V, et al. Eta-1 (osteopontin): an early component of type-1 (cell-mediated) immunity. Science 2000;287:860–4.
15. Wai PY, Kuo PC. The role of osteopontin in tumor metastasis. J Surg Res 2004;121:228–41.
16. Senger DR, Wirth DF, Hynes RO. Transformed mammalian cells secrete specific proteins and phosphoproteins. Cell 1979;16:885–93.
17. Chang K, Pastan I. Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers. Proc Natl Acad Sci U S A 1996;93:136–40.
18. Ordonez NG. Application of mesothelin immunostaining in tumor diagnosis. Am J Surg Pathol 2003; 27:1418–28.
19. Scholler N, Fu N, Yang Y, et al. Soluble member(s) of the mesothelin/megakaryocyte potentiating factor family are detectable in sera from patients with ovarian carcinoma. Proc Natl Acad Sci U S A 1999;96:11531–6.
20. Bhattacharjee A, Richards WG, Staunton J, et al. Classification of human lung carcinomas by mRNA expression profiling reveals distinct adenocarcinoma subclasses. Proc Natl Acad Sci U S A 2001;98: 13790–5.
21. Ordonez NG. The immunohistochemical diagnosis of mesothelioma: a comparative study of epithelioid mesothelioma and lung adenocarcinoma. Am J Surg Pathol 2003;27:1031–51.
22. Ho M, Onda M, Wang QC, et al. Mesothelin is shed from tumor cells. Cancer Epidemiol Biomarkers Prev 2006;15:1751.
23. Hellstrom I, Raycraft J, Kanan S, et al. Mesothelin variant 1 is released from tumor cells as a diagnostic marker. Cancer Epidemiol Biomarkers Prev 2006;15: 1014–20.
24. Galateau-Sallé FE. Pathology of malignant mesothelioma. 1st ed. New York: Springer-Verlag; 2006. p. 204.
25. Rusch VW; from the International Mesothelioma Interest Group. A proposed new international TNM staging system for malignant pleural mesothelioma. Chest 1995;108:1122–8.
26. Conover WJ, Iman RL. Analysis of covariance using the rank transformation. Biometrics 1982;38:715–24.
27. Hanley JA, McNeil BJ. A method of comparing the areas under receiver operating characteristic curves derived from the same cases. Radiology 1983;148: 839–43.
28. Contal C, O'Quigley J. An application of change-point methods in studying the effect of age on survival in breast cancer. Comput Stat Data Anal 1999;30: 253–70.
29. Mandrekar JN, Mandrekar SJ, Cha SS. Cutpoint determination methods in survival analysis using SAS®. In: SAS User Group 28, Seattle, Washington; March 30-April 2, 2003 1999. p. Paper 261–28.
30. O'Regan A. The role of osteopontin in lung disease. Cytokine Growth Factor Rev 2003;14:479–88.
31. Bautista DS, Saad Z, Chambers AF, et al. Quantification of osteopontin in human plasma with an ELISA: basal levels in pre- and postmenopausal women. Clin Biochem 1996;29:231–9.
32. Vogelzang NJ, Rusthoven JJ, Symanowski J, et al. Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. J Clin Oncol 2003;21: 2636–44.
33. Boldrini L, Donati V, Dell'Omodarme M, et al. Prognostic significance of osteopontin expression in early-stage non-small-cell lung cancer. Br J Cancer 2005; 93:453–7.
34. Donati V, Boldrini L, Dell'Omodarme M, et al. Osteopontin expression and prognostic significance in non-small cell lung cancer. Clin Cancer Res 2005;11: 6459–65.
35. Hu Z, Lin D, Yuan J, et al. Overexpression of osteopontin is associated with more aggressive phenotypes in human non-small cell lung cancer. Clin Cancer Res 2005;11:4646–52.
36. O'Regan A, Berman JS. Osteopontin: a key cytokine in cell-mediated and granulomatous inflammation. Int J Exp Pathol 2000;81:373–90.
37. Onda M, Nagata S, Ho M, et al. Megakaryocyte potentiation factor cleaved from mesothelin precursor is a useful tumor marker in the serum of patients with mesothelioma. Clin Cancer Res 2006;12:4225–31.
38. Shiomi K, Miyamoto H, Segawa T, et al. Novel ELISA system for detection of N-ERC/mesothelin in the sera of mesothelioma patients. Cancer Sci 2006; 97:928–32.
39. Rittling SR, Chambers AF. Role of osteopontin in tumour progression. Br J Cancer 2004;90:1877–81.
40. de Silva Rudland S, Martin L, Roshanlall C, et al. Association of S100A4 and osteopontin with specific prognostic factors and survival of patients with minimally invasive breast cancer. Clin Cancer Res 2006; 12:1192–200.
41. Thalmann GN, Sikes RA, Devoll RE, et al. Osteopontin: possible role in prostate cancer progression. Clin Cancer Res 1999;5:2271–7.
42. Agrawal D, Chen T, Irby R, et al. Osteopontin identified as lead marker of colon cancer progression, using pooled sample expression profiling. J Natl Cancer Inst 2002;94:513–21.
43. Kolb A, Kleeff J, Guweidhi A, et al. Osteopontin influences the invasiveness of pancreatic cancer cells and is increased in neoplastic and inflammatory conditions. Cancer Biol Ther 2005;4:740–6.
44. Shimada Y, Watanabe G, Kawamura J, et al. Clinical significance of osteopontin in esophageal squamous cell carcinoma: comparison with common tumor markers. Oncology 2005;68:285–92.
45. Bramwell VH, Tuck AB, Wilson SM, et al. Expression of osteopontin and HGF/Met in adult soft tissue tumors. Cancer Biol Ther 2005;4:1336–41.

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