Original Study

Elevated Circulating Activin A Levels in Patients With Malignant Pleural Mesothelioma Are Related to Cancer Cachexia and Reduced Response to Platinum-based Chemotherapy

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Abstract

Activin A has previously been associated with cancer cachexia and in vitro resistance to platinum-based chemotherapy. We studied circulating activin A concentrations as well as activin B and their antagonists' follistatin/follistatin-like 3 in presurgical patients with non–small-cell lung cancer and malignant pleural mesothelioma. We found that circulating activing A levels were elevated in malignant pleural mesothelioma and associated with cancer cachexia and poor response to platinum-based chemotherapy. Circulating activing A separated non–small-cell lung cancer from benign lung lesion.

Background: Previous preclinical studies have shown that activin A is overexpressed in malignant pleural mesothelioma (MPM), associates with cancer cachexia, and is observed in in vitro resistance to platinum-based chemotherapy. We evaluated circulating activin levels and their endogenous antagonists' follistatin/follistatin-like 3 in intrathoracic tumors. **Materials and Methods:** Patients suspected of thoracic malignancy were recruited prior to surgery. Serum samples were collected from 21 patients with MPM, 59 patients with non–small-cell lung cancer (NSCLC), and 22 patients with benign lung lesions. Circulating activin/follistatin levels were measured using enzyme-linked immunosorbent assay and compared with clinicopathologic parameters. **Results:** Circulating activin A levels were elevated in patients with MPM when compared with patients with NSCLC or benign lung lesion samples ($P < .0001$). Also, follistatin and follistatin-like 3 levels were the highest in MPM, although with less difference compared with activin A. Receiver operating characteristic analysis for activin A for separating NSCLC from benign lung lesion showed an area under the curve of 0.856 (95% confidence interval, 0.77-0.94). Activin A levels were higher in patients with cachexia ($P < .001$). In patients with MPM, activin A levels correlated positively with computed tomography-based baseline tumor size ($R = 0.549$; $P = .010$) and the change in tumor size after chemotherapy ($R = 0.743$; $P = .0006$). Patients with partial response or stable disease had lower circulating activin A levels than the ones with progressive disease ($P = .028$). **Conclusion:** Activin A serum level could be used as a biomarker in differentiating malignant and benign lung tumors. Circulating activin A levels were elevated in MPM and associates with cancer cachexia and reduced chemotherapy response.

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Keywords: Activin A, Biomarker, Cancer cachexia, Mesothelioma, NSCLC

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Introduction

Malignant pleural mesothelioma (MPM) is a primary tumor of the pleura with an estimated median survival of 8 to 14 months.[1] The diagnosis of MPM can be challenging, and it is usually diagnosed in advanced stage.[2] Many non-invasive biomarkers have been evaluated for diagnosis and prognosis, but none have been adopted into clinical diagnostic use or treatment response monitoring.[1,3] Therefore, novel biomarkers that could aid the diagnosis or estimate prognosis would be highly beneficial.

Activins belong to the transforming growth factor-β (TGF-β) superfamily and control cell proliferation and differentiation, embryogenesis, immune responses, wound repair, and various endocrine activities.[4] Like certain other TGF-β family proteins, activins signal through 1 of 2 activin type II transmembrane serine/threonine kinase receptors (ActRIIA or ActRIIB).[5] Activin signaling is regulated by numerous intra- and extracellular mechanisms, including follistatin (FS) and follistatin-like 3 (FSTL3; formerly known as FSRP or FLRG), that bind and neutralize activins.[6]

Activin signaling has multiple roles in carcinogenesis. In vitro studies have demonstrated its antiproliferative effect on breast, prostate, and liver cancer cells.[7,8] In turn, activin A was overexpressed in lung adenocarcinoma cells, and circulating activin A correlated with advanced stage and poor survival in patients with lung adenocarcinoma.[9,10] Similarly, activin A and B are expressed in mesothelioma tumor tissue and cells, and this overexpression is associated with a more aggressive behavior.[11,12] A previous study found that elevated circulating activin A levels are higher in patients with MPM than in healthy controls and associated with a poor prognosis in the epithelioid form.[13] Also, most mesothelioma cell lines express high FSTL3 levels in contrast to low FS levels.[11] Furthermore, the activin pathway is related to cancer cachexia, cancer-related bone loss, and resistance to platinum-based chemotherapy.[14-16]

In this study, we investigated circulating activin A and B, FS, and FSTL3 in thoracic malignancy with a focus on MPM. Our aim was to find out whether any of these known regulators of cancer cell growth could be used to estimate tumor type and to confirm previously reported associations. A prospective setting using biobank samples and a well-characterized patient population was chosen.

Materials and Methods

Study Design and Population

Patients were prospectively recruited from June 2016 to January 2018 in the Helsinki University Hospital region. The recruited patients provided a written Biobank consent, and the study was approved by the Scientific Steering Committee of the Helsinki Biobank. Research permission was granted by the Institutional Review Board of the Helsinki University Hospital. A statement has been received from the Helsinki University Hospital ethical board. The study was conducted in accordance with the Declaration of Helsinki.

The inclusion criterion to the study was that a serum sample was collected prior to a diagnostic or therapeutic surgical procedure. All patients with a suspected intrathoracic tumor were evaluated for the study. The initial screening resulted in 149 patients. Forty-three (29%) patients were excluded from the study: 25 (17%) had a postoperative blood sample, 3 (2%) had non-thoracic metastases (1 melanoma, 1 colorectal, 1 gingival), and 15 (10%) had an uncertain diagnosis.

Patient information was collected from the hospital electronic medical records. The recorded baseline characteristics included the date and site of the diagnosis, age, gender, smoking history, performance status defined by the Eastern Cooperative Oncology Group (ECOG), and Charlson comorbidity index (CCI). C-reactive protein (CRP) with a cutoff of 3 mg/L and hemoglobin (Hb) levels were measured at the time of the biomarker collection as part of the routine preoperative workup. Body mass index (BMI) and body weight changes during the previous 6 months were obtained from the electronic medical records. Cancer cachexia was defined according to a previously published international consensus report: $\geq$ 5% weight loss over the past 6 months, BMI $<$ 20 kg/m^2 and weight loss $>$ 2%, or skeletal muscle index (SMI) consistent with sarcopenia and weight loss $>$ 2%.[17] SMI in MPM was evaluated from pretreatment computerized tomography (CT) images by one of the authors (T.J.) according to a previously published method.[18] Namely, the cross-sectional area of the psoas, quadratus lumborum, paraspinal, transverse abdominal, external oblique, internal oblique, and rectus abdominis muscles at the level of L3 vertebra were identified and quantified with Osirix version 33 (32-bit Pixmeo, Sarl, Switzerland). The individual SMI was calculated correcting the skeletal muscle area for height (m^2), and patients were dichotomized into either sarcopenic or non-sarcopenic (cutoff, 55 cm^2/m^2 for men and 39 cm^2/m^2 for women) as previously described.[17]

The diagnosis was based on histopathologic findings and if needed, evaluated in a multidisciplinary team. All patients with non–small-cell lung carcinoma (NSCLC) and MPM were staged according to the TNM eighth edition American Joint Committee on Cancer/Union for International Cancer Control staging system.[19] The diagnostic and post-chemotherapy CT images for patients with MPM were re-evaluated by a thoracic radiologist (H.L.) The tumor size (TS) was approximated using our previously published method, where the maximal tumor thickness on axial images is multiplied by the tumor extent.[20] The radiologic response to chemotherapy was evaluated using change in TS.

Serum Biomarkers

Blood samples were obtained at the diagnosis prior to any interventions or treatments. Serum was stored after centrifugation at −80° C until utilization. Duplicate biomarker levels were measured blinded to clinical data using enzyme-linked immunosorbent assay immunoassays (AnshLabs LLC, Webster, TX) according to the manufacturer's guidelines. The immunoassays used in this study measure the total levels of the biomarkers in question with no significant in vitro cross-reactions with other related molecules.

Statistical Analyses

Categorical variables are expressed by percentages and were compared using a χ^2 test. Continuous data are presented as median and interquartile range (IQR). Statistical differences between the groups or subgroups were tested by nonparametric Mann-Whitney *U* test or Kruskal-Wallis test as appropriate. If

Table 1 Baseline Characteristics (n = 102)

	NSCLC, n (%)	Mesothelioma, n (%)	Benign Lung Lesion, n (%)	*P* Value
No. cases	59 (58)	21 (21)	22 (21)	
Median age (range), y	69.0 (41-81)	71.0 (63-77)	57 (24-79)	<.05
Male gender	36 (61)	20 (95)	7 (32)	<.05
ECOG performance status				.542
0	40 (68)	13 (62)	17 (77)	
1	19 (32)	8 (38)	5 (23)	
Mean CCI (SD)	1.4 (1.1)	0.8 (1.2)	0.6 (0.8)	.002
Mean BMI (SD), kg/m^2	27.2 (4.4)	26.5 (4.1)	27.0 (6.4)	.739
Smoking status				.011
Former/current	49 (83)	10 (48)	14 (64)	
Never	10 (17)	11 (52)	8 (36)	
Median pack-years (range)	30 (0-70)	0 (0-50)	10 (0-43)	.003
Stage				
I	31 (53)	3 (14)		
II	11 (19)	2 (10)		
III	15 (25)	14 (66)		
IV	2 (3)	2 (10)		
Histologic subtype				
Adenocarcinoma	46 (78)			
Squamous cell carcinoma	12 (20)			
Epithelioid MPM		14 (67)		
Biphasic MPM		3 (14)		
Sarcomatoid MPM		4 (19)		
Hamartoma			13 (60)	
Unspecified/miscellaneous	1 (2)		9 (40)	

Abbreviations: BMI = body mass index; CCI = Charlson comorbidity index; ECOG = Eastern Cooperative Oncology Group; MPM = malignant pleural mesothelioma; NSCLC = non–small-cell lung carcinoma; SD = standard deviation; Stage = pathologic stage for NSCLC, clinical stage for mesothelioma.

multiple comparisons were made, the *P*-values were adjusted by the Bonferroni correction for multiple tests. The Spearman correlation coefficient was calculated to assess the relationship of biomarkers and clinicopathologic factors. Multivariate logistic or linear regression analysis was used to determine independent influence of activin A on cachexia or chemotherapy response. Receiver operating characteristics curve analysis was used to assess the accuracy of biomarkers to diagnose lung cancer. The Youden index was calculated to determine the optimal cutoff values. Statistical analyses were performed using SPSS version 25.0 (IBM SPSS Statistics, Chigaco, IL). A *P*-value < .05 was considered significant.

Results

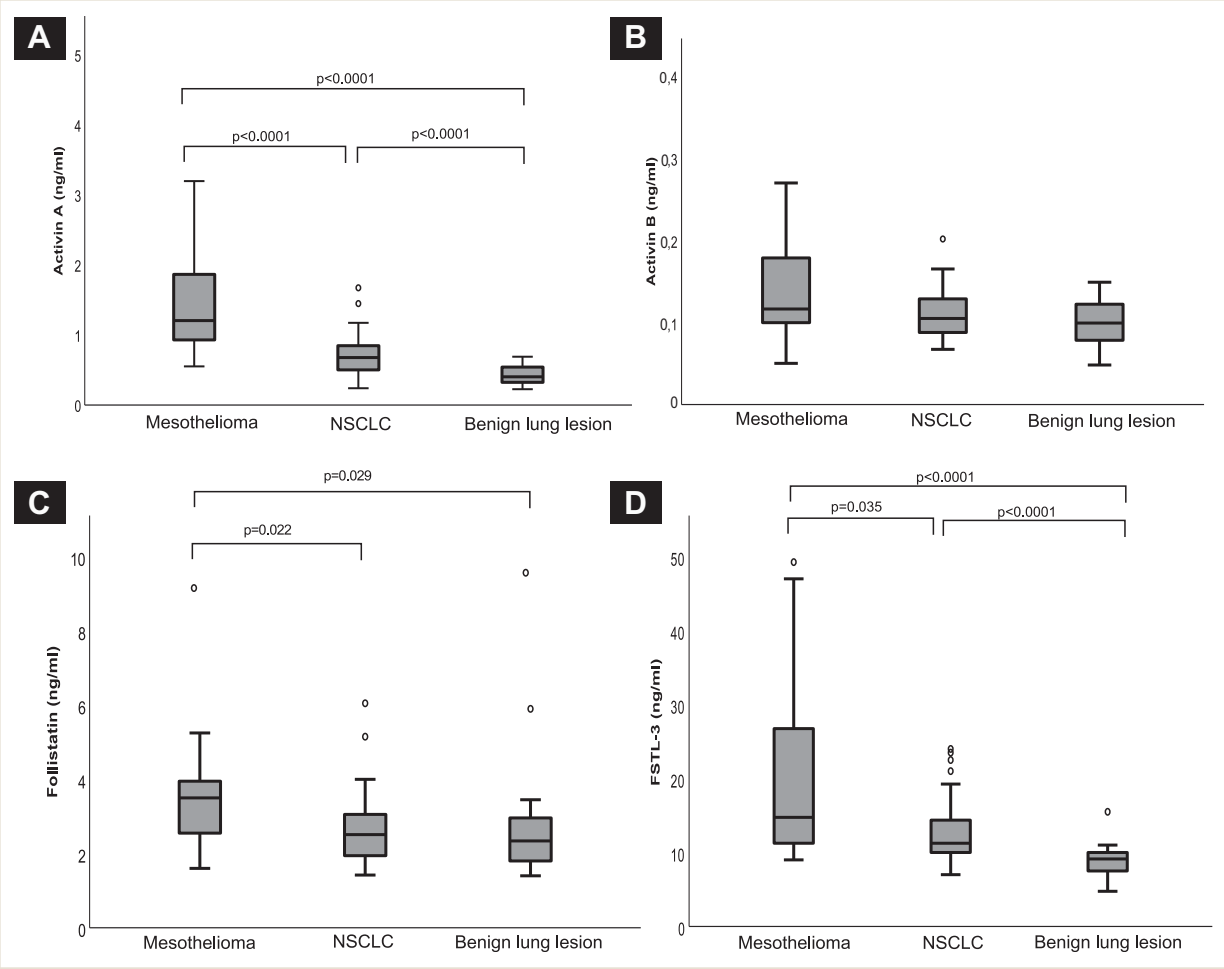
Baseline Characteristics

A total of 106 patients were included in the study. The largest disease groups were NSCLC, benign lung lesion, and MPM (Table 1). There were also 4 (3%) patients with non-specific benign pleuritis. The majority of patients with NSCLC had stage I disease (n = 31; 53%), whereas stage III disease (n = 14; 68%) was the most prevalent group in patients with MPM.

Circulating Biomarker Levels in Thoracic Tumors — Levels of Activin A are Elevated in Patients With MPM and Benign Pleuritis

Patients with MPM showed overall the highest levels of all the measured biomarkers when compared with patients with either NSCLC or benign lung lesion (Figure 1). The ratio of activins and FS/FSTL3 was higher in patients with MPM than benign controls ($P = .012$), but no differences were found between patients with MPM and NSCLC ($P = .325$) or patients with NSCLC and controls ($P = .179$). All biomarker levels were also increased in patients with benign pleuritis (n = 4) (see Supplemental Table 1 in the online version). Circulating activin A levels were observed to differentiate the main groups. Receiver operating characteristics curve analysis was used to assess the diagnostic accuracy of circulating biomarkers to discriminate patients with NSCLC from patients with a benign lung lesion. Activin A had an area under the curve of 0.856 (95% confidence interval [CI], 0.77-0.94; $P < .001$). With a cutoff level of 0.488 ng/mL, the sensitivity was 76% and specificity 73% (Figure 2). FSTL3 also performed well, with an area under the curve of 0.808 (95% CI, 0.69-0.92; $P < .001$), whereas activin B and FS were not useful.

Figure 1 Boxplot Showing Circulating Activin A (A), Activin B (B), Follistatin (C), and Follistatin-like 3 (D) Levels (ng/mL) in Patients With Malignant Pleural Mesothelioma (n = 21), Non–small-cell Lung Cancer (n = 59), and Benign Lung Lesion (n = 22). *P*-values Are Adjusted With Bonferroni Correction for Multiple Tests



Abbreviations: FSTL-3 = follistatin-like 3; NSCLC = non–small-cell lung cancer.

We found no differences between activin A levels for patients with squamous cell carcinoma (median, 0.736 ng/mL; interquartile range [IQR], 0.648-0.818 ng/mL) and patients with adenocarcinoma (median, 0.636 ng/mL; IQR, 0.472-0.872 ng/mL) ($P = .376$). Activin A levels were also similar in MPM histologic subgroups ($P = .152$)

Circulating Activin A Levels Correlate With Cachexia, Inflammation, and Age

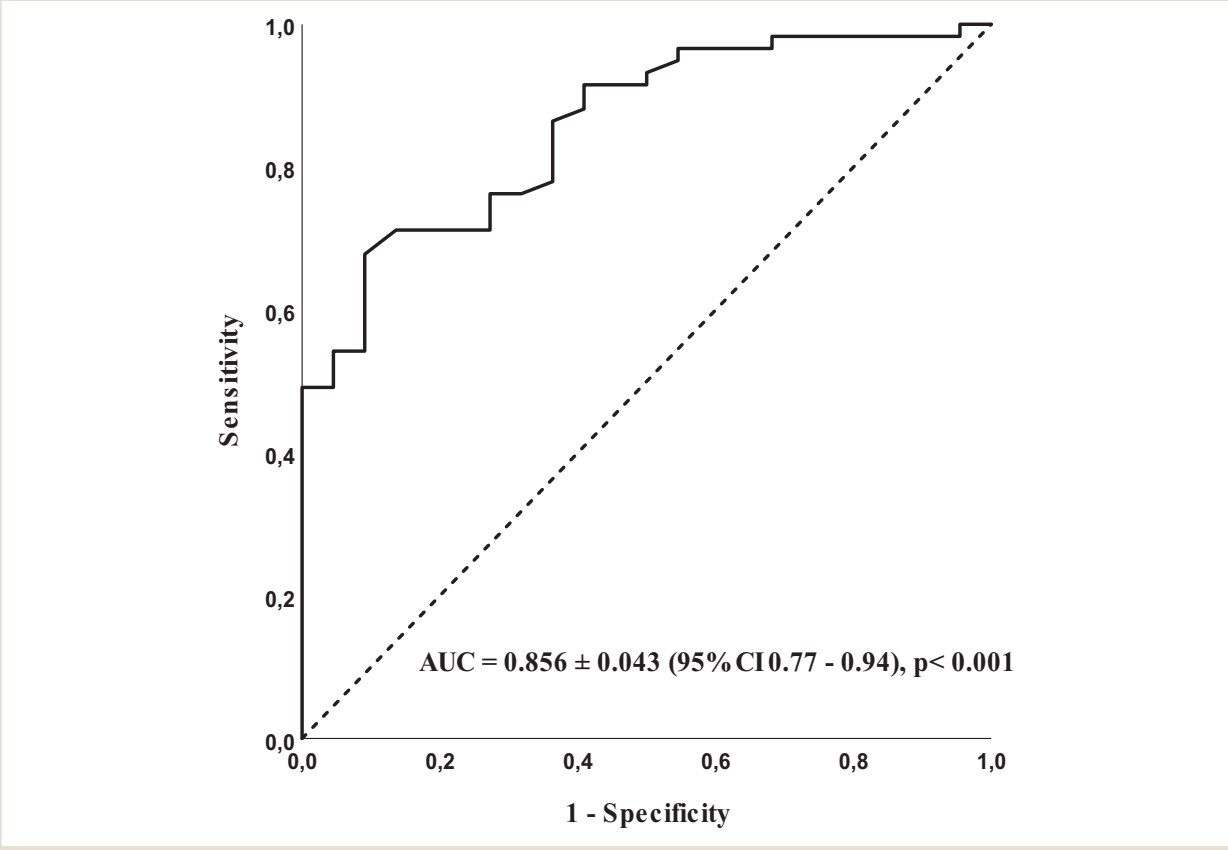
Patients with MPM (n = 12; 57%) were more cachectic than patients with NSCLC (n = 7; 12%) ($P < .001$). Activin A levels were higher in patients with cachexia (median, 1.179 ng/mL vs. 0.634 ng/mL; $P < .001$). Circulating activin A levels correlated positively with cachexia, age, and CRP (Table 2). Similarly, cachexia also correlated weakly with CRP (R = 0.375; $P < .001$) but not with activin B, FS, or FSTL3 in these patients. The association with cachexia remained significant for activin A after adjusting for age, gender, and CRP ($P = .047$).

Ancillary Exploratory Analysis for Activin A in Patients With MPM – An Association With Tumor Size and Response to Chemotherapy

After observing that circulating activin A levels were highest in patients with MPM, we investigated the relevance of this finding to clinically meaningful parameters in mesothelioma. Seven (33%) patients with MPM had palliative surgery, and 12 (57%) received chemotherapy for first-line treatment. All patients had diagnostic CT scans available for radiologic re-evaluation. There was a positive correlation between circulating activin A levels and pre-treatment tumor size (R = 0.549; $P = .010$) but no relation with clinical TNM stage (R = 0.141; $P = .542$). Thirteen (62%) of the patients with MPM were evaluated as sarcopenic according to SMI. We found no correlation with SMI and activin A levels (R = −0.261; $P = .266$).

Because activin A is linked to in vitro platinum-based chemotherapy resistance in lung adenocarcinoma,[16] we tested this association with chemotherapy action in MPM in our clinical setting. To approximate tumor response for platinum-based chemotherapy, 12

Figure 2 Receiver Operating Characteristics Curve for Circulating Activin A to Discriminate Patients Non–small-cell Lung Cancer (n = 59) From Benign Lung Lesions (n = 22). The Area Under the Curve Was 0.856 (95% CI, 0.77-0.94)



Abbreviations: AUC = area under the curve; CI = confidence interval.

post-chemotherapy CT scans were analyzed. We found a strong correlation with circulating activin A levels and the change in TS between the pre- and post-chemotherapy CT scans (R = 0.743; $P = .0006$) (Figure 3A). This correlation remained significant after adjusting for tumor size, CRP, gender, and age ($P = .008$). Patients with partial response or stable TS had lower circulating activin A levels than the ones with progressive disease (median, 1.206 ng/mL vs. 3.086 ng/mL; $P = .028$, Mann-Whitney *U* test) (Figure 3B). Other biomarkers showed no association with TS or response to chemotherapy.

Discussion

In this study, we evaluated circulating activin A and B levels and their biological antagonists FS and FSTL3 in the clinical setting of intrathoracic tumors. Consistent with previous preclinical studies and a clinical trial on patients with MPM, we found that circulating levels of activin A were higher in patients with MPM than in patients with NSCLC or benign controls.[11,13] Although FS and FSTL3 levels were also elevated in patients with MPM and to a lesser extent in patients with NSCLC, activin A was the most promising circulating biomarker. In contrast with previous findings, we did not observe any differences in activin A levels within histologic subgroups of MPM or NSCLC. This may be explained by the small amount of non-epithelioid mesotheliomas in our study.

Although FS is linked to a variety of solid tumors (including lung cancer), the role of FSTL3 has been established mainly in breast cancer.[21,22] In addition to being increased in MPM, we found that FSTL3 could also differentiate benign and malignant lung tumors. However, in contrast to previous reports, the difference between NSCLC and benign tumors was modest and insignificant for FS.[23] Because FS and FSTL3 are antagonists of activins, we looked at their ratio as an independent marker. We found no relevant addition to single markers; even if the ratio was higher in patients with MPM compared with benign controls, there were no other associations that could have a clinical benefit over activin A.

Consistent with a previous study on activin A, we found that benign pleuritis increased markedly all measured biomarkers.[13] The reason for this is unknown, but we assume that normal mesothelial cells may produce small amounts of activins that are increased by inflammation. Indeed, the association of activin A and inflammatory responses is well-characterized in previous preclinical studies, and circulating levels of activin A are elevated in patients with septicemia and other inflammatory diseases.[24,25] In addition to inflammation, circulating activin A levels have been found to increase with age, chronic renal failure, cardiovascular disorders, hyperthyroidism, liver cirrhosis, and pregnancy.[26,27] Thus, even if activin A has relatively good diagnostic accuracy for differentiating malignant and benign lung tumors, we believe that these numerous confounding factors would reduce its utility in diagnostic settings.

Table 2 Spearman Correlation Coefficient Between Circulating Biomarkers and Clinical Markers

	Activin A	Activin B	Follistatin	FSTL3
Age, y	0.414[b]	0.124	0.234[a]	0.482[b]
C-reactive protein	0.468[b]	0.231[a]	0.350[b]	0.410[b]
Hemoglobin	−0.136	−0.098	−0.097	−0.239[a]
Charlson comorbidity index	0.095	0.101	0.186	0.340[b]
ECOG performance status	0.068	0.178	0.256[b]	0.209[a]
pTumor size	0.343[a]	0.001	0.021	0.039
pStage, NSCLC	0.112	−0.107	−0.216	−0.237
cStage, MPM	0.141	0.114	0.208	0.408
Cachexia	0.412[b]	0.153	0.200[a]	0.227[a]
Body mass index	−0.048	0.089	0.124	0.186

Two-tailed significant *P*-values are marked with [a]$P < .05$ or [b]$P < .01$.
Abbreviations: cStage = clinical TNM stage; ECOG = Eastern Cooperative Oncology Group; MPM = malignant pleural mesothelioma; NSCLC = non–small-cell lung cancer; pStage = pathologic TNM stage; pTumor size = pathologic tumor size.

Cancer cachexia is characterized as a multifactorial syndrome with loss of muscle mass with or without loss of fat mass.[17] It has been recognized as a frequent and independent factor of poor prognosis in various cancer types. The negative effect of cachexia on thoracic cancers is well-established, but little is known of its prevalence in MPM.[15,28] Here, we report that cachexia was more prevalent in MPM than NSCLC, which may be explained in part by the different disease stage in patients with NSCLC in our cohort. Although the pathophysiology of cancer cachexia is complex, multiple preclinical studies have suggested that activins along with myostatin regulate negatively muscle growth via ActRII.[14,29] In addition, in clinical trials with patients with colorectal and lung cancer, high circulating activin A levels was associated with cancer cachexia and had an independent negative prognostic impact.[15,30] Here, we also observed that activin A levels were higher in patients with cachexia regardless of the underlying diagnosis. In turn, sarcopenia or SMI calculated from CT scans did not correlate with activin A levels. The reason for this could be explained by different hormonal mechanisms between these overlapping conditions.[31]

One possible link between activin A and cachexia is systemic inflammation. Many inflammatory markers are established as key components of cancer cachexia, although cachexia can be present in the absence of systemic inflammation.[32] Animal models in cancer cachexia suggest that blocking the activin pathway can reverse muscle loss without affecting inflammatory cytokines.[14] In addition, clinical studies have shown that blocking ActRII increased muscle mass, whereas the results for anti-inflammatory therapy (TNF-α or IL-6) has been variable in cancer cachexia.[33-36] We found a correlation with activin A and CRP, but after adjustments, only activin A proved to be an independent marker for cachexia. This suggests that even if activin signaling is partly controlled by inflammation, the activin pathway is a dominant regulator in muscle mass changes.

Owing to the unique rind-like growth pattern of mesothelioma, radiologic evaluation is problematic. The current standard method for response assessment is the modified Response Evaluation Criteria in Solid Tumors (RECIST) criteria, where tumor thickness perpendicular to the chest wall at 3 separate levels is measured.[37] However, this method has been criticized for interobserver variability. Other approaches, such as volumetric tumor evaluation, have been proposed.[38,39] In our previous study, we showed that a novel TS evaluation had independent prognostic impact with excellent intraclass correlation.[20] In this study, we used the same approach to assess MPM and found that activin A correlated with TS but not with TNM stage at baseline.

Despite recent improvements, platinum-antifolate combination chemotherapy remains as the current standard first-line treatment in MPM.[40] Chemotherapy has only a limited effect on disease progression at a reported response rate of 40%.[41] Therefore, new strategies to increase tumor response to chemotherapy are needed in MPM. Numerous mechanisms that explain platinum resistance in various solid tumors have been identified.[42] Using whole-genome screening, a recent preclinical publication of lung adenocarcinoma cells revealed that activin A signaling was one of the key mediators for platinum resistance, and that inhibition of the signaling could reverse that resistance in vitro.[16] In a clinical setting, we showed a similar association with high circulating activin A levels to reduced response to chemotherapy in MPM.

There are several limitations to our study. Although patients were prospectively collected, their clinical data was collected retrospectively, which may have increased bias. Because the patients were identified from thoracic surgery lists, the proportion of patients with benign pleuritis and metastatic NSCLC was small, which prevents us from reliably studying biomarker diagnostic accuracy in MPM. Nonetheless, this cohort represents a real-world population. Also, this study was not powered to assess the prognostic implications of the biomarkers in MPM.

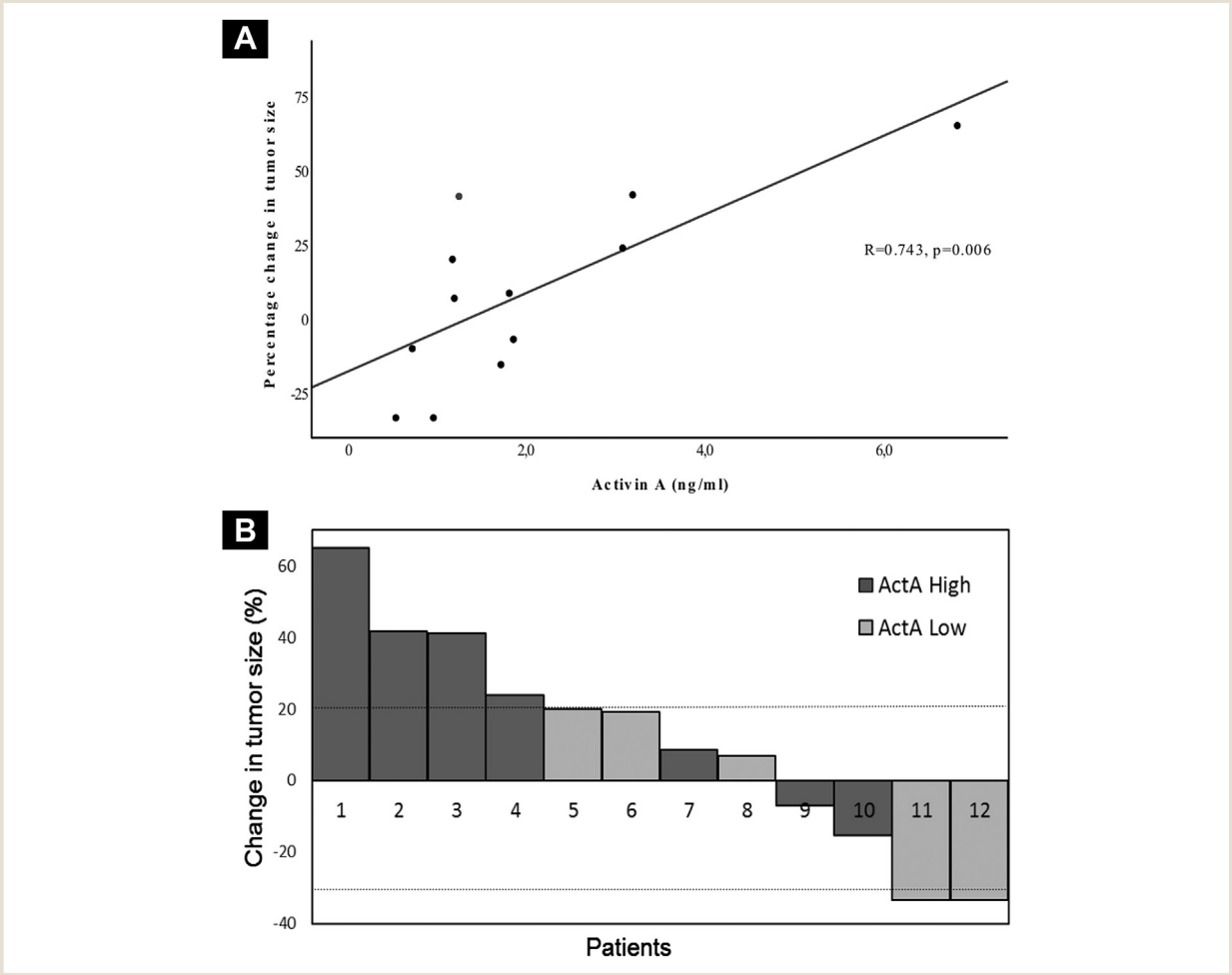
Conclusions

In conclusion, we observed that circulating activin A is elevated in patients with MPM and to a lesser extent in patients with NSCLC than in those with benign lung lesions. Activin A could be used as a biomarker in differentiating malignant and benign lung tumors, although numerous confounding factors do exist. Activin A was associated with cancer cachexia and reduced chemotherapy response in patients with MPM. These results should be tested in a clinical trial to see if activin pathway inhibition could benefit patients with thoracic cancer.

Clinical Practice Points

- Previous studies have shown that activin A is associated with cancer cachexia. We could confirm that association with patients with NSCLC and MPM. Over one-half of the patients with MPM were characterized as cachectic, which might be driven by high levels of activin A.

Figure 3 A, The Tumor Size Was Evaluated at Baseline and After First-line Platinum-based Chemotherapy in 12 Patients With Malignant Pleural Mesothelioma. The Change in Tumor Size Estimation Correlated With Circulating Activin A Levels (Spearman r = 0.743; *P* = .006). B, Individual Percentage Changes in Tumor Size From Baseline to First Response Evaluation in Patients With Malignant Pleural Mesothelioma (n = 12). Patients Are Stratified by Median Circulating Activin A Levels (Low < 1.2 ng/mL, High > 1.2 ng/mL). Patients With Partial Response or Stable Tumor Size Had Lower Circulating Activing A Levels than the Ones With Progressive Disease (*P* = .028, Mann-Whitney *U* Test). Horizontal Dashed Line at −30% Shows a Cutoff for Partial Response and Horizontal Dashed Line at 20% Shows a Cutoff for Progressive Disease



Abbreviation: ActA = activin A.

- Two-thirds of the patients with MPM were sarcopenic. There were no associations between CT-based skeletal muscle mass or circulating biomarkers.
- Activin A has been linked to in vitro platinum resistance in adenocarcinoma cells. We found that high activin A levels were related to poor response to platinum-based chemotherapy in patients with MPM.
- These findings should be further tested to confirm in a larger patient population. Activin overexpression could be potentially reversed by blocking ActRII to overcome cancer cachexia and platinum resistance.

Acknowledgments

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Disclosure

The authors have stated that they have no conflicts of interest.

Supplemental Data

Supplemental table accompanying this article can be found in the online version at https://doi.org/10.1016/j.cllc.2019.10.013.

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Supplemental Table 1 The Circulating Biomarker Levels in Study Subgroups

	Activin A, ng/mL	Activin B, ng/mL	Follistatin, ng/mL	FSTL3, ng/mL
NSCLC (n = 59)	0.678 (0.50-0.85)	0.105 (0.09-0.13)	2.510 (1.94-3.06)	11.250 (10.00-14.50)
Adenocarcinoma (n = 46)	0.636 (0.47-0.87)	0.102 (0.09-0.12)	2.265 (1.85-3.00)	11.000 (9.75-14.50)
Squamous cell ca (n = 12)	0.736 (0.65-0.82)	0.123 (0.10-0.16)	2.953 (2.06-3.90)	11.875 (10.50-14.13)
Miscellaneous (n = 1)	0.958 (NA)	0.119 (NA)	2.650 (NA)	19.250 (NA)
MPM (n = 21)	1.206 (0.90-2.02)	0.116 (0.10-0.18)	3.500 (2.48-3.98)	14.750 (11.13-35.13)
Epithelioid (n = 14)	1.197 (0.73-1.94)	0.113 (0.10-0.15)	3.475 (2.51-3.96)	14.125 (11.19-24.63)
Biphasic (n = 3)	3.198 (1.29-NA)	0.115 (0.08-NA)	2.700 (2.00-NA)	14.750 (10.50-NA)
Sarcomatoid (n = 4)	1.186 (0.99-1.20)	0.157 (0.09-0.19)	4.600 (2.19-8.98)	20.750 (11.94-41.94)
Benign lung lesion (n = 22)	0.403 (0.32-0.54)	0.098 (0.08-0.12)	2.340 (1.76-3.00)	9.125 (7.31-10.06)
Hamartoma (n = 13)	0.387 (0.32-0.47)	0.106 (0.09-0.13)	2.465 (1.86-3.18)	8.125 (5.81-9.81)
Unspecified (n = 9)	0.439 (0.32-0.55)	0.087 (0.07-0.12)	2.325 (1.60-2.97)	9.375 (8.44-12.13)
Benign pleuritis (n = 4)	1.268 (0.69-5.37)	0.149 (0.09-0.17)	3.300 (2.51-7.61)	11.750 (11.13-44.43)

The biomarkers are presented as a median (interquartile range).
Abbreviations: FSTL-3 = follistatin-like 3; MPM = malignant pleural mesothelioma; NA = not available; NSCLC = non—small-cell lung carcinoma.