Serum Biomarkers May Prognosticate Recurrence in Node-Negative, Non-Small Cell Lung Cancers Less Than 4 Centimeters

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Background. **A significant proportion of patients who undergo lung resection for less than 4 cm non-small cell lung cancer (NSCLC) will die of disease recurrence within 5 years. The ability to identify patients at greatest risk for recurrence may help individualize treatment and surveillance regimens and improve outcomes. We hypothesized that a serum-based biomarker panel could help risk stratify patients with node-negative NSCLC less than 4 cm for recurrence after lung resection.**

Methods. **An institutional biorepository of more than 1,800 cases was used to identify patients with resected, node-negative NSCLC less than 4 cm in size. Clinical and radiographic data were collected. Preoperative serum specimens were evaluated in a blinded manner for 47 biomarkers that sampled biological processes associated with metastatic progression, including angiogenesis, energy metabolism, apoptosis, and inflammation. Receiver-operating characteristics curves and log rank tests were used to evaluate individual biomarkers with respect to recurrence, followed by random forest analysis to generate and cross validate a multiple-analyte panel to risk stratify patients for recurrence.**

Results. **The cohort included 123 patients with a median follow-up of 58.2 months; 23 patients had recurrences. A seven-analyte panel consisting of human epididymis protein 4, insulinlike growth factor–binding protein 1, beta-human chorionic gonadotropin, follistatin, prolactin, angiopoietin-2, and hepatocyte growth factor optimally identified patients with disease recurrence with a cross-validated specificity of 91%, sensitivity of 22%, negative predictive value of 83%, positive predictive value of 36%, and accuracy of 78%, providing an area under the receiver-operating characteristics curve of 0.70.**

Conclusions. **Serum-based biomarkers may be useful for risk stratifying patients with node-negative NSCLC less than 4 cm for recurrence after lung resection.**

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Lung cancer is the leading cause of cancer death in the United States, with approximately 220,000 new diagnoses and 160,000 deaths observed in 2016 [1]. Based on the findings of the National Lung Screening Trial, the National Comprehensive Cancer Network recently recommended lung cancer screening with low-dose computed tomography scans for appropriately selected high-risk patients [2]. Likewise, the Centers for Medicare and Medicaid Services have approved payment for lung cancer screening in these patients [3]. Widespread implementation of lung cancer screening programs will almost certainly result in the identification of more early stage lung cancers than ever before. Unfortunately, approximately 1 in 5 patients with pathologic stage IA non-small cell lung cancer will die of disease recurrence within 5 years of resection [4]. That suggests that occult metastases were present at the time of surgery but went undetected by current clinical and pathology staging methods [5, 6].

Although level I data suggest an overall survival benefit with adjuvant chemotherapy in patients with stage I to IIIA NSCLC, no benefit has been demonstrated in patients with node-negative, NSCLC less than 4 cm [7, 8]. In 2008, the results of Cancer and Leukemia Group B 9633 were reported, in which 344 stage IB patients were randomly assigned to adjuvant cisplatin-based chemotherapy or control [9]. With a median follow-up of 74 months, no survival advantage was demonstrated;

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Abbreviations and Acronyms

EGF = epidermal growth factor
HCG = human chorionic gonadotropin
HE = human epididymis protein
HGF = hepatocyte growth factor
IGF = insulinlike growth factor
IGFBP = insulinlike growth factor–binding protein
NSCLC = non-small cell lung cancer
VEGF = vascular endothelial growth factor

however, a post hoc analysis showed an overall survival advantage for tumors 4 cm or greater.

Meta-analyses attempting to identify a benefit associated with adjuvant chemotherapy in early stage NSCLC have also shown a survival advantage for patients with stage IB and stage II disease [7, 10]. The Lung Adjuvant Cisplatin Evaluation meta-analysis reported a 5.4% overall survival benefit at 5 years with adjuvant chemotherapy compared with observation; however, the observed benefit was primarily among stage II and III patients [7]. Although only three trials were included, a trend toward benefit was shown in another meta-analysis for patients with stage IA disease [10].

Taken together, these data support the hypothesis that a benefit could be provided by adjuvant chemotherapy, if appropriate stage I NSCLC with occult micrometastatic disease could be selected. The concept of identifying a subset of stage I NSCLC patients who may benefit from adjuvant therapy becomes particularly attractive with the emergence of targeted molecular therapies. The objective of this study was to investigate whether serum-based biomarkers are capable of risk stratifying patients with node-negative NSCLC less than 4 cm for recurrence after lung resection.

Patients and Methods

Patient Cohorts

Between 2004 and 2015, serum and tissue specimens from more than 1,800 patients who underwent pulmonary resection for suspected lung cancer at Rush University Medical Center were collected in an institutional lung tumor biorepository. Inclusion criteria for the current study included patients with pathologically proven, node-negative NSCLC less than 4 cm with serum available in the Rush University Cancer Center Biorepository. Patients with tumors 2 cm to 3.9 cm were included if they underwent lobectomy or pneumonectomy with mediastinal lymph node dissection or sampling, whereas patients with tumors less than 2 cm were included if they received lobar or sublobar resection with mediastinal lymph node dissection or sampling. Exclusion criteria included age less than 18 years, neuroendocrine histology, absence of mediastinal lymph node dissection, lack of an R0 resection, administration of neaodjuvant or adjuvant chemotherapy or radiotherapy, or survival less than 30 days.

Computed tomography scans and positron emission tomography–computed tomography scans were routinely used in the clinical staging of lung cancer patients during the study period. Endobronchial ultrasound-guided fine needle aspiration and mediastinoscopy were selectively used, as clinically indicated. The type of resection performed was based on patient and tumor characteristics, as well as on surgeon judgment. A wide parenchymal margin was routinely sought when sublobar resections were performed. All patients were followed with a history, physical examination, and noncontrast computed tomography scan every 6 months for the first 2 years, then yearly for life. If findings concerning for recurrence were identified, further investigation was conducted, as required. Recurrence was defined as that occurring between 3 and 60 months from the date of surgery. Locoregional recurrence was defined as that occurring in the ipsilateral hilum, mediastinum, or ipsilateral lung; all other recurrences were classified as distant. All patients were evaluated in a multidisciplinary fashion to determine whether new lesions represented disease recurrence or a second primary lesion. Overall survival was calculated as time from date of surgery to death or last documented follow-up. Disease-free survival was calculated from the date of surgery to the date of first recurrence, defined as the last date of negative radiographic imaging and clinical examination. If there was no recurrence, and the patient died, the disease-free survival was based on the date of death. This study was conducted in compliance with the Institutional Review Board at Rush University Medical Center. Clinical follow-up was completed through September 21, 2015.

Measurement of Serum Biomarker Concentrations

All serum samples were assayed simultaneously by a blinded technician for serum biomarkers hypothesized to have value for risk stratifying patients for early recurrence. These biomarkers were chosen based on our previous work and sampled biological processes associated with metastatic progression, including angiogenesis, energy metabolism, apoptosis, and inflammation [11–21]. Pretreatment serum specimens were prepared using standard techniques, supplemented with 25 μL/mL mammalian protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO), and archived at −80°C in aliquots with no specimen subjected to more than two freeze-thaw cycles, as previously described [12, 18].

The 47 biomarkers evaluated in this study included insulinlike growth factor (IGF)-I (Milliplex MAP human IGF-I single plex; EMD Millipore Corp, Billerica, MA); IGF-II (Milliplex MAP cancer biomarker panel); insulinlike growth factor–binding protein (IGFBP)-1, IGFBP-2, IGFBP-3, IGFBP-4, IGFBP-5, IGFBP-6, and IGFBP-7 (Milliplex MAP human IGF binding protein panel); soluble tumor necrosis factor I (Milliplex MAP human soluble cytokine receptor panel); macrophage inflammatory protein (MIP)-1α, interferon-gamma (Milliplex MAP human cytokine/chemokine panel I); human chorionic