Vascular endothelial growth factor C complements the ability of positron emission tomography to predict nodal disease in lung cancer

Farhood Farjah, MD, MPH,[a,b] David K. Madtes, MD,[c,d] Douglas E. Wood, MD,[a] David R. Flum, MD, MPH,[b] Megan E. Zadworny, MHA,[b] Rachel Waworuntu, BS,[e] Billanna Hwang, MPH,[e] and Michael S. Mulligan, MD[a,e]

ABSTRACT

Objective: Vascular endothelial growth factors (VEGFs) C and D are biologically rational markers of nodal disease that could improve the accuracy of lung cancer staging. We hypothesized that these biomarkers would improve the ability of positron emission tomography (PET) to predict nodal disease among patients with suspected or confirmed non–small cell lung cancer (NSCLC).

Methods: A cross-sectional study (2010-2013) was performed of patients prospectively enrolled in a lung nodule biorepository, staged by computed tomography (CT) and PET, and who underwent pathologic nodal evaluation. Enzyme-linked immunosorbent assay was used to measure biomarker levels in plasma from blood drawn before anesthesia. Likelihood ratio testing was used to compare the following logistic regression prediction models: $Model_{PET}$, $Model_{PET/VEGF\text{-}C}$, $Model_{PET/VEGF\text{-}D}$, and $Model_{PET/VEGF\text{-}C/VEGF\text{-}D}$. To account for 5 planned pairwise comparisons, P values <.01 were considered significant.

Results: Among 62 patients (median age, 67 years; 48% men; 87% white; and 84% NSCLC), 58% had fluorodeoxyglucose uptake in hilar and/or mediastinal lymph nodes. The prevalence of pathologically confirmed lymph node metastases was 40%. Comparisons of prediction models revealed the following: $Model_{PET/VEGF\text{-}C}$ versus $Model_{PET}$ ($P = .0069$), $Model_{PET/VEGF\text{-}D}$ versus $Model_{PET}$ ($P = .1886$), $Model_{PET/VEGF\text{-}C/VEGF\text{-}D}$ versus $Model_{PET}$ ($P = .0146$), $Model_{PET/VEGF\text{-}C/VEGF\text{-}D}$ versus $Model_{PET/VEGF\text{-}C}$ ($P = .2818$), and $Model_{PET/VEGF\text{-}C/VEGF\text{-}D}$ versus $Model_{PET/VEGF\text{-}D}$ ($P = .0095$). In $Model_{PET/VEGF\text{-}C}$, higher VEGF-C levels were associated with an increased risk of nodal disease (odds ratio, 2.96; 95% confidence interval, 1.26-6.90).

Conclusions: Plasma levels of VEGF-C complemented the ability of PET to predict nodal disease among patients with suspected or confirmed NSCLC. VEGF-D did not improve prediction. (J Thorac Cardiovasc Surg 2015;150:796-803)

Comparison	Likelihood ratio test*
$Model_{PET/VEGF\text{-}C}$ versus $Model_{PET}$	p=0.0069
$Model_{PET/VEGF\text{-}D}$ versus $Model_{PET}$	p=0.1886
$Model_{PET/VEGF\text{-}C/VEGF\text{-}D}$ versus $Model_{PET}$	p=0.0146
$Model_{PET/VEGF\text{-}C/VEGF\text{-}D}$ versus $Model_{PET/VEGF\text{-}C}$	p=0.2818
$Model_{PET/VEGF\text{-}C/VEGF\text{-}D}$ versus $Model_{PET/VEGF\text{-}D}$	p=0.0095

Predicting nodal disease in lung cancer with and without lymphagiogenesis markers.

Central Message

Vascular-endothelial growth factor C complements positron emission tomography in predicting nodal disease for lung cancer staging.

Perspective

Our work justifies and motivates development and validation of a prediction model for nodal disease in lung cancer based on multiple radiographic predictors of nodal disease and vascular endothelial growth factor C. Prediction models may lead to better staging accuracy, fewer invasive staging procedures, and less provider-level variability in staging practices while ensuring personalized cancer care.

See Editorial Commentary page 804.

Supplemental material is available online.

Nodal staging is a key determinant of appropriate treatment selection and therefore outcomes among patients with nonmetastatic non–small cell lung cancer (NSCLC). Practice guidelines recommend the routine use of computed tomography (CT) and positron emission tomography (PET) to initially stage patients with suspected or confirmed NSCLC.[1,2] The diagnostic limitations of noninvasive imaging tests have been well characterized and have led to dependence on invasive staging modalities such as mediastinoscopy and endobronchial ultrasound-guided biopsy.[1] In the absence of novel imaging modalities, 2

From the [a]Division of Cardiothoracic Surgery, Department of Surgery, [b]Surgical Outcomes Research Center, and [c]Division of Pulmonary and Critical Care Medicine, University of Washington, Seattle, Wash; [d]Division of Pulmonary and Critical Care Medicine, Fred Hutchinson Cancer Research Center, Seattle, Wash; and [e]Center for Lung Biology, University of Washington, Seattle, Wash.

Funding information: This study was not externally funded.

Read at the 41st Annual Meeting of The Western Thoracic Surgical Association, Whistler, British Columbia, Canada, June 24-27, 2015.

Received for publication March 3, 2015; revisions received July 1, 2015; accepted for publication Aug 2, 2015; available ahead of print Aug 27, 2015.

Address for reprints: Farhood Farjah, MD, MPH, Division of Cardiothoracic Surgery, University of Washington, 1959 NE Pacific St, Box 356310, Seattle, WA 98195 (E-mail: ffarjah@uw.edu).

0022-5223/$36.00

http://dx.doi.org/10.1016/j.jtcvs.2015.08.001

Abbreviations and Acronyms

CT = computed tomography
EDTA = ethylenediaminetetraacetic acid
FDG = fluorodeoxyglucose
NPV = negative predictive value
NSCLC = non–small cell lung cancer
PET = positron emission tomography
SUV = standardized uptake value
VEGF = vascular endothelial growth factor

ways to improve lung cancer staging is through better use of the existing radiographic information and adoption of new risk factors for nodal disease. Prediction models may make better use of imaging information leading to higher accuracy and therefore better use of invasive staging procedures. Biomarkers measured in plasma and correlating with nodal disease may complement radiographic predictors of nodal involvement.

Advances in lymphangiogenesis research show that epithelial tumors—including NSCLC—metastasize to lymph nodes via the formation of de novo peritumoral lymphatic channels.[3] Experimental animal models demonstrate that expression of vascular endothelial growth factors (VEGF) C and D by tumor cells promote nodal metastases, and blocking the VEGF-C/VEGF-D receptor eliminates lymphatic metastases.[3-7] Studies in humans reveal higher tissue (both in primary tumor and lymph nodes) and plasma levels of VEGF-C and VEGF-D among node-positive NSCLC patients compared with node-negative patients, individuals with benign nodules, and healthy volunteers.[8-14] Markers of lymphangiogenesis appear to be biologically rational predictors of nodal disease.

The ultimate goal of our research is to develop and validate a prediction model based on multiple radiographic and molecular predictors of nodal disease. As a first step toward this goal, we sought to determine whether plasma levels of VEGF-C and/or VEGF-D improve the ability of PET to predict nodal disease in patients with suspected or confirmed nonmetastatic NSCLC. Five randomized trials show that PET improves the diagnostic accuracy of nodal staging.[15-19] A biomarker would ideally add diagnostic value to this dominant predictor of nodal disease. We hypothesized that VEGF-C and/or VEGF-D would improve the ability of PET to predict nodal disease.

MATERIALS AND METHODS

Patients and Study Design

The source of patient information for this study was a lung nodule biorepository maintained by the Fred Hutchinson Cancer Research Center. Since 2008, all patients referred to the Seattle Cancer Care Alliance Lung Cancer Early Detection and Prevention Clinic have been approached to be prospectively enrolled in the biorepository. The Fred Hutchinson Cancer Research Center institutional review board approved this biorepository (file #6663, protocol #2242). Patients are required to provide written consent. The biorepository is strictly observational. It was designed for multiple research purposes and is not associated with a parent study involving interventions. Therefore, patient selection and treatment reflect usual care. Study variables are ascertained from patient records by trained abstractors using standard definitions. A board-certified thoracic surgeon resolved uncertainties over study eligibility and variable classification, and verified the accuracy of data collection for all patients included in the analysis.

A retrospective cross-sectional study was conducted of patients consecutively enrolled in the biorepository between October 2010 and December 2013. The start date was chosen based on the availability of plasma specimens preserved in ethylenediaminetetraacetic acid (EDTA). Patients eligible for study included adults with suspected or confirmed primary NSCLC who had been staged with CT and PET, were without evidence of metastatic disease, and had undergone endobronchial ultrasound guided biopsy, mediastinoscopy, and/or intraoperative nodal assessment at the time of pulmonary resection.

Plasma Sample Preparation and Storage

Blood samples were collected before the administration of anesthesia using a standardized protocol—temporary storage at 2°C to 8°C, centrifuging at 4°C for 10 minutes at 1300 × g, preservation using EDTA, and long-term storage of plasma specimen aliquots in a −80°C freezer within 4 hours of blood draw.

Biomarker Measurement

Plasma levels of VEGF-C and VEGF-D were measured using a commercially available enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minn). The manufacturer reported sensitivity limit and range for VEGF-C was 48.4 pg/mL and 109 to 7000 pg/mL, respectively, and the sensitivity limit and range for VEGF-D was 31.3 pg/mL and 125 to 4000 pg/mL. Assay performance was validated in serum, heparinized plasma, saliva, cell culture supernates, EDTA-preserved plasma, and platelet-poor plasma. Measurements were performed in triplicate for each patient and the mean result was recorded for each individual. The coefficient of variation was calculated for each patient as a measure of within-subject intraassay variation.[20]

Analysis

STATA (special edition 12.1; Statacorp, College Station, Tex) was used to conduct all statistical analyses. Independent variables selected a priori included PET findings and plasma levels of VEGF-C and VEGF-D. A binary indicator of fluorodeoxyglucose (FDG) uptake within hilar and/or mediastinal lymph nodes was created to summarize PET findings. FDG uptake above background levels as reported by the radiologist was considered a positive PET result. Plasma levels of VEGF-C and VEGF-D were log-transformed to achieve a normal distribution and were modeled as continuous variables. The primary dependent variable was a binary indicator of nodal disease based on pathologic examination of tissue obtained by invasive staging procedures and/or intraoperatively. There were no missing data.

Logistic regression was used to determine whether VEGF-C and VEGF-D improve the predictive performance of PET. Four models were created using different sets of predictors to estimate the probability of nodal disease. For descriptive purposes, we also report associations (using odds ratios), discriminatory accuracy (using c-statistics), and calibration (using goodness-of-fit tests). The study was not powered to detect differences in these parameters. A likelihood ratio test was used to formally test the hypothesis that 1 or more biomarkers would improve the predictive performance of PET.[21] Our sample size was informed by a study indicating that a logistic regression analysis is reasonably powered by 5 to 10 events per covariate.[22] We estimated needing between 15 and 30 patients with nodal disease to have sufficient power to conduct our a priori-specified