Lymphovascular Invasion as a Prognostic Indicator in Stage I Non-Small Cell Lung Cancer: A Systematic Review and Meta-Analysis

Nathan M. Mollberg, DO, Carrie Bennette, MPH, Eric Howell, MD, Leah Backhus, MD, Beth Devine, PhD, PharmD, and Mark K. Ferguson, MD

Department of Cardiothoracic Surgery and Pharmaceutical Outcomes Research and Policy Program, University of Washington, Seattle, Washington; and Department of Surgery, University of Chicago, Chicago, Illinois

Background. Lymphovascular invasion (LVI) is considered a high-risk pathologic feature in resected non-small cell carcinoma (NSCLC). The ability to stratify stage I patients into risk groups may permit refinement of adjuvant treatment recommendations. We performed a systematic review and meta-analysis to evaluate whether the presence of LVI is associated with disease outcome in stage I NSCLC patients.

Methods. A systematic search of the literature was performed (1990 to December 2012 in MEDLINE/EMBASE). Two reviewers independently assessed the quality of the articles and extracted data. Pooled hazard ratios (HRs) and 95% confidence intervals (CI) were estimated with a random effects model. Two end points were independently analyzed: recurrence-free survival (RFS) and overall survival (OS). We analyzed unadjusted and adjusted effect estimates, resulting in four separate meta-analyses.

Results. We identified 20 published studies that reported the comparative survival of stage I patients with and without LVI. The unadjusted pooled effect of LVI was significantly associated with worse RFS (HR, 3.63; 95% CI, 1.62 to 8.14) and OS (HR, 2.38; 95% CI, 1.72 to 3.30). Adjusting for potential confounders yielded similar results, with RFS (HR, 2.52; 95% CI, 1.73 to 3.65) and OS (HR, 1.81; 95% CI, 1.53 to 2.14) both significantly worse for patients exhibiting LVI.

Conclusions. The present study indicates that LVI is a strong prognostic indicator for poor outcome for patients with surgically managed stage I lung cancer. Future prospective lung cancer trials with well-defined methods for evaluating LVI are necessary to validate these results.

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Prognosis for patients with resectable non-small cell lung cancer (NSCLC) is currently assessed by the tumor-node-metastasis (TNM) staging system, with surgical resection offering the best chance for cure for early-stage NSCLC. However, 5-year survival rates for stage I patients range widely, from 50% to 90% [1, 2]. Important within-stage variations in prognosis are related to a variety of histopathologic, surgical, biological, and patient factors. Lymphovascular invasion (LVI) has been demonstrated to be a negative prognostic factor for recurrence-free (RFS) and overall survival (OS) in NSCLC [3–8].

Although how the presence of LVI would change adjuvant treatment recommendations for more advanced-staged tumors is unclear, the ability to stratify stage I patients into risk groups may permit a more individualized approach to adjuvant treatment recommendations. The presence of LVI in NSCLC pathology specimens varies widely, from 5% to 40% [9, 10]; therefore, the effect of LVI on RFS or OS in an individual study may be determined by a small number of patients. With the use of a meta-analytic approach, the aim of the present study was to clarify whether LVI represents a prognostic factor for patients with stage I NSCLC.

Patients and Methods

Two authors (N. M. and E. H.) searched the PubMed (National Library of Medicine, Bethesda, MD) and EMBASE (Elsevier, Amsterdam, the Netherlands) databases for published articles (1990 to 2012) reporting the prognostic role of LVI in NSCLC.

Search Strategy

The literature was first searched for existing systematic reviews and meta-analyses on the prognostic role of LVI, including blood vessels or lymph vessels, in NSCLC. One meta-analysis evaluated the prognostic significance of lymphatic vessel invasion alone, and one meta-analysis evaluated the prognostic significance of blood vessel invasion alone [11, 12]. We used the following free text terms to identify studies for inclusion in this meta-analysis: “non-small-cell lung
cancer,” “NSCLC,” “lymph vessel invasion,” “blood vessel invasion,” “vascular invasion,” “angiolympathic invasion,” “lymphovascular invasion,” “relapse,” “recurrence,” “survival,” “prognostic,” and “prognosis.” The titles and abstracts of the search findings were screened for potentially eligible studies. Full articles were obtained for detailed evaluation, and eligible studies were included. The reference lists of relevant articles were manually searched to find other potentially eligible studies.

Selection Criteria for Considering Studies Eligible for This Review

Abstracts, reviews, and case reports were excluded because of insufficient data for meta-analysis. Non-English language articles were not included in the review. We noted the author names and institutions to avoid duplication of patient data. Only the most recently reported data or complete data were used if more than one publication reported the same population data. To be eligible, studies had to report the association between LVI with prognosis (RFS or OS, or both). We excluded editorials and letters, had to report the same population data. To be eligible, studies or complete data were used if more than one publication noted the author names and institutions to avoid duplication of patient data. Only the most recently reported data or complete data were used if more than one publication reported the same population data. To be eligible, studies had to report the association between LVI with prognosis (RFS or OS, or both). We excluded editorials and letters, had to report the association between LVI with prognosis (RFS or OS, or both). We excluded editorials and letters, had to report the association between LVI with prognosis (RFS or OS, or both). We excluded editorials and letters, had to report the association between LVI with prognosis (RFS or OS, or both). We excluded editorials and letters, had to report the association between LVI with prognosis (RFS or OS, or both). We excluded editorials and letters, had to report the association between LVI with prognosis (RFS or OS, or both). We excluded editorials and letters, had to report the association between LVI with prognosis (RFS or OS, or both).

Initial Review of Studies

Citations were screened, without blinding, by review of the title and abstract to capture relevant studies for inclusion based on the previously identified criteria. The initial database created from the electronic searches was compiled, and all duplicate citations were eliminated. Only after assessment of the full-text articles by 2 authors (N.M. and E.H.) were the studies selected for inclusion in the review. Any disagreement between the 2 authors was arbitrated by a third author (M.F.) after reviewing the original article.

Data Abstraction and Assessment of Study Quality

Data from the selected studies were recorded independently by 2 authors (N.M. and E.H.). The following items were extracted: publication details (title, authors, study institution, study country of origin), type of study (prospective or retrospective), clinicopathologic data (demographics, study size, histology, staging, and percentage of patients with lymphovascular invasion), results of statistical analysis, and methods used to diagnose LVI. We used the European Lung Cancer Working Party scale [13] to assess the quality of each study.

Statistical Analysis

We used four random-effects models to examine the relationship between LVI and survival outcomes in observational studies conducted in stage I NSCLC patients [14]. The results from each study in the meta-analyses were combined using inverse-variance weighting. We assessed the adjusted and unadjusted pooled effect of LVI on each survival outcome (RFS and OS) by aggregating HR estimates and their corresponding 95% confidence intervals (CIs) or by reestimating these parameters from available data. All but one study that reported adjusted estimates of survival reported HR and CIs (or standard errors) directly; the one remaining study reported an HR and p value [6], which we used to reconstruct the CI. Several studies reported unadjusted results for RFS (n = 4) [6, 10, 15, 16] and OS (n = 6) [4–6, 10, 17, 18] only as graphic representations (Kaplan-Meier curves) of the survival distributions. To calculate HR estimates from these curves, we first used the Engauge digitizer to extract the survival probabilities at specified times (every 3 months) and then reconstructed the HR estimate and its variance using the methods of Parmar and colleagues [19], with the assumption that censoring occurred at a constant rate across follow-up time and was noninformative.

We assessed heterogeneity using the I² statistic (using p < 0.1 as a conservative threshold to indicate heterogeneity). We prespecified sensitivity analyses stratified by one of three clinical factors: pathologic stage (T1a vs T1b), region of origin (Asian vs North American), and histology (adenocarcinoma vs squamous cell). Lastly, we examined funnel plot asymmetry to assess publication bias using the Egger test [20]. All analyses were conducted using STATA 12 software (StataCorp LP, College Station, TX).

Results

Study Selection and Characteristics

Our electronic data search retrieved 138 references (Fig 1). The overall meta-analysis did not include 101 studies because they investigated lymphatic vessel invasion or blood vessel invasion (rather than both) and outcome in...
NSCLC patients. Thirteen studies investigated the relationship between LVI and outcome in NSCLC patients but did not include separate outcome data on stage I patients. An additional four studies were excluded due to lack of sufficient data to calculate survival.

We identified 20 studies that reported comparative survival outcomes of patients with and without LVI (Table 1) [3–10, 15–18, 21–28]. The total number of patients was 8,032 (median, 272; range, 47–1,477). In all, evaluating LVI as defined by the authors of the studies, 23.4% of tumors exhibited LVI (range, 4.9% to 48.6%).

Seven studies identified LVI as a significant predictor for poor RFS on univariate analysis [6, 10, 15, 16, 21, 24, 27], whereas 2 studies did not [3, 26]. Seven studies reported LVI as a significant predictor for poor RFS by multivariate analysis [6, 15, 16, 21, 24, 27], whereas 1 study did not [26]. Thirteen studies identified LVI as a significant prognostic factor for OS in univariate analysis [3–7, 10, 16–18, 22, 23, 25, 26, 28], but LVI was not a statistically significant prognostic factor in 1 study [8]. In multivariate analyses, LVI was a significant predictor of OS in 9 studies [3–6, 16, 17, 23, 25, 28] compared with 3 studies [7, 8, 22] with nonsignificant results. The median global quality assessment score, expressed as a percentage, was 59.2% (range, 43.2% to 72.7%).

Meta-Analysis of the Effect of LVI on RFS for Overall Population

In the univariate analysis, LVI significantly increased the risk for cancer recurrence (Fig 2), with a combined HR of 3.63 (95% CI, 1.62 to 8.14; 4 studies; 1,147 patients) [6, 10, 15, 16]. Six studies provided p values only and, therefore, were not included in the random effects model. Four of these studies identified LVI as a significant prognostic indicator for RFS [16, 21, 24, 27], whereas 2 identified it as not significant [3, 26]. There was significant interstudy heterogeneity ($I^2 = 82.2\%$, $p = 0.001$) among these studies. Multivariate analyses showed patients with LVI were 2.52 times (95% CI, 1.73 to 3.65) more likely to relapse (Fig 3) compared with those without LVI (6 studies; 2,488 patients) [15, 16, 21, 24, 26, 27]. Significant heterogeneity was identified among these studies ($I^2 = 58.1\%$, $p = 0.036$).

Meta-Analysis of the Effect of LVI on OS for the Overall Population

We next analyzed the association between LVI and OS in NSCLC patients by univariate (8 studies, 2,355 patients) [4–6, 10, 16–18, 25] and multivariate analysis (12 studies, 4,530 patients) [3–5, 7, 8, 16, 17, 22, 23, 25, 26, 28]. The pooled HR estimate was 2.38 (95% CI, 1.72 to 3.3) by univariate analysis (Fig 4), with significant heterogeneity ($I^2 = 65.0\%$, $p = 0.006$). There was a significant increased risk for poor OS (HR, 1.81; 95% CI, 1.53 to 2.14) by multivariate analysis (Fig 5) in LVI-positive patients compared with LVI-negative patients. There was some evidence of heterogeneity in the adjusted OS that did not reach statistical significance ($I^2 = 37.5\%$, $p = 0.084$) but did reach our prespecified threshold of $p < 0.1$ to indicate heterogeneity. Furthermore, significant...
heterogeneity was observed when both estimates from Zhang and colleagues [28] were excluded from the analysis of adjusted OS ($I^2 = 47.0\%$, $p = 0.042$). The funnel plots for each of the four meta-analyses were symmetric, indicating minimal publication bias.

**Subgroup Analyses**

There were too few studies to conduct sensitivity analyses using published subgroup analyses of patients by histology and pathologic stage for each end point. However, 4 studies (2,519 patients) provided information on adjusted overall survival for stage IA patients [17, 23, 25, 28]. The pooled HR estimate was 1.81 (95% CI, 1.54 to 2.13) without significant heterogeneity ($I^2 = 0\%$, $p = 0.9$). In addition, 2 studies have been published since the end of our analysis that demonstrated LVI was a significant predictor of poor RFS and OS in stage IA patients [29, 30].

Meta-regression analyses that controlled for the proportion of patients with adenocarcinoma or the stage of patients (IA, IB, or all stage I) in each study sample found overall, that neither the proportion of patients with adenocarcinoma nor the stage of patients was significantly associated with the heterogeneity in OS or RFS ($p > 0.16$ for all). Two post hoc meta-regression analyses were performed by the percentage of the study population with LVI and year of publication. The percentage of the study population with LVI explained a significant proportion of the heterogeneity in unadjusted OS (residual $I^2 = 29\%$; $p = 0.043$) and nonsignificant amount in unadjusted RFS (residual $I^2 = 2.2\%$; $p = 0.064$). We did not observe a significant association with LVI in adjusted analysis ($p > 0.4$ for both) or with year of publication in adjusted or unadjusted analyses ($p > 0.14$ for all).

**Comment**

LVI has been shown to be in an independent predictor of poor outcome in numerous solid organ tumors [31–33].
Currently, however, only endometrial/cervical and head and neck cancers consider the presence of LVI as indication for further adjuvant therapy. Although numerous studies have demonstrated LVI is an independent predictor for poor RFS and OS in lung cancer, most included cohorts that already had indications for adjuvant chemotherapy. We studied stage I NSCLC patients in the current meta-analysis to determine the prognostic significance of LVI in patients treated with surgical intervention alone. We hoped to determine whether outcomes in this relatively favorable group could be stratified, thus helping to identify subgroups of patients at risk who might benefit from adjuvant therapy.

There are a number of challenges in differentiating among arterial, venous, or lymph vessel invasion in pathologic specimens. More recently, immunostaining with lymph endothelium-specific marker D2-40 and the panendothelial marker CD34 has been used to distinguish between lymph and blood vessel invasion in breast cancer specimens [34]. However, current routine

### Table 1: Forest plot showing unadjusted association between lymphovascular invasion and overall survival in stage I non-small cell lung cancer patients

<table>
<thead>
<tr>
<th>Author</th>
<th>Publication year</th>
<th>Number of patients</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Han</td>
<td>2001</td>
<td>85</td>
<td>4.15 (1.64, 10.49)</td>
</tr>
<tr>
<td>Hsu</td>
<td>2009</td>
<td>272</td>
<td>3.33 (1.98, 5.59)</td>
</tr>
<tr>
<td>Miyoshi</td>
<td>2009</td>
<td>258</td>
<td>2.01 (0.87, 4.65)</td>
</tr>
<tr>
<td>Varlotti</td>
<td>2010</td>
<td>306</td>
<td>3.70 (2.12, 6.44)</td>
</tr>
<tr>
<td>Ruffini</td>
<td>2011</td>
<td>393</td>
<td>1.54 (1.15, 2.07)</td>
</tr>
<tr>
<td>Schuchert</td>
<td>2011</td>
<td>524</td>
<td>1.50 (1.15, 1.95)</td>
</tr>
<tr>
<td>Kato</td>
<td>2012</td>
<td>195</td>
<td>2.47 (0.79, 7.71)</td>
</tr>
<tr>
<td>Tsuchiya</td>
<td>2007</td>
<td>322</td>
<td>3.50 (1.51, 8.14)</td>
</tr>
<tr>
<td>Overall (I-squared=65.0%, p=0.006)</td>
<td></td>
<td></td>
<td>2.38 (1.72, 3.30)</td>
</tr>
</tbody>
</table>

**NOTE:** Weights are from random effects analysis.

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**Fig 4.** Forest plot shows the unadjusted association between lymphovascular invasion and overall survival in stage I non-small cell lung cancer patients. Study by Kato and colleagues [6] used disease-specific survival. The solid squares denote mean difference, the horizontal lines represent the 95% confidence intervals (CI), and the diamond denotes the weighted mean differences. (HR = hazard ratio.)

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**Fig 5.** Forest plot shows the association between lymphovascular invasion and overall survival in stage I non-small cell lung cancer patients, adjusted for confounders. The solid squares denote mean difference, the horizontal lines represent the 95% confidence intervals (CI), and the diamond denotes the weighted mean differences. (HR = hazard ratio.)
pathologic evaluation does not use immunostaining. In addition, none of the eligible studies in the current meta-analysis used immunostaining to evaluate the presence of LVI.

LVI was defined as the presence of neoplastic cells within an arterial, venous, or lymphatic lumen during routine histologic evaluation with hematoxylin and eosin (H&E) stains. Pathologists usually differentiate blood vessels from lymphatic vessels according to the evidence of blood cells in endothelium-lined channels. However, the distinction among an arterial, venous, or lymphatic lumen is difficult. Elastic stains are used along with H&E staining in some institutions as a routine examination for blood and pleural invasion because lymphatic vessels do not contain elastic fibers. This staining method is reliable for the detection of tumor cells in vessels; however, once again, the ability to differentiate between lymphatic vessel invasion and blood vessel invasion is limited. There is evidence that the predictive ability of blood vessel invasion may depend on the distinction between venous or arterial invasion, which H&E combined with elastic staining is unable to differentiate [33]. In addition, 20% to 53% of specimens have been shown to harbor both lymph and blood vessel invasion [9, 17], and which is of greater importance is unclear [11, 12]. Therefore, the current study excluded studies that reported separate estimates of lymph or blood vessel invasion as prognostic indicators because the results might have been confounded by overlapping populations.

Our results demonstrate that LVI is a strong predictor of unfavorable prognosis in patients with stage I NSCLC. The presence of LVI has been shown to correlate with KRAS gene amplification and mutation [36], as well as size [25, 28], stage [10], and preoperative carcinoembryonic antigen levels [17, 25]. It may be that LVI represents a histopathologic correlate to aggressive tumor biology.

The current study has a number of limitations. The results are based on low-level evidence from retrospective studies. There was no accounting for the type of pathologic review at each institution, and some studies detected LVI by staining with H&E alone or combined with elastic van Gieson stain. The percentage of the study population with LVI varied widely across studies, which explained a significant proportion of the heterogeneity in our unadjusted overall survival meta-analysis. Therefore, standards for evaluating and reporting LVI need to be established.

Importantly, a significant amount of heterogeneity in most of our meta-analyses remained unexplained. The heterogeneity was reduced in adjusted models, likely indicating that different patient populations explain some of the heterogeneity. There were a number of potential biases that could have affected our results as well. Publication bias has to be considered, because studies with positive results are more likely to be accepted for publication; however, we found no evidence of this.

Some HR estimates were derived from survival curves, which involved extrapolation and assumptions about censoring patterns; however, these assumptions were applied to patient groups with and without LVI and were therefore unlikely to bias the HR estimates away from the null.

In conclusion, patients whose tumors demonstrated LVI were at significantly increased risk for recurrence and death than patients whose tumors did not. Adjuvant therapy may be indicated in patients with stage I NSCLC at increased risk for poor outcome due to the presence of LVI. Large prospective studies comparing the reliability of standard H&E staining in determining the presence of LVI with that of immunostaining need to be performed to standardize pathologic reporting. Subsequent studies comparing outcomes for patients with and without LVI will need to be performed to determine if LVI can provide further prognostic information over TNM stage alone.

References

INVITED COMMENTARY

A number of retrospective reports have suggested that lymphovascular invasion (LVI) represents a high-risk pathologic feature in patients with resected non-small cell lung cancer (NSCLC), and negatively affects their survival after surgical resection. Mollberg and associates [1] conducted a systematic review and metaanalysis of 20 published studies that reported the comparative survival with and without LVI among patients with resected stage I NSCLC to investigate the association of LVI with the recurrence-free survival (RFS) and overall survival (OS). In total, 8,032 patients were examined for LVI and survival data, and 23.4% of the cases of completely resected stage I NSCLC were proven to exhibit LVI (range, 4.9% to 48.6%). Importantly, the meta-analysis demonstrated that, after adjusting for potential confounders, LVI was significantly associated with a worse RFS (hazard ratio 2.52, 95% confidence interval: 1.73 to 3.65) and OS (hazard ratio 1.81, 95% confidence interval: 1.53 to 2.14). Such data can potentially identify patients at an increased risk of recurrence, and the descriptor might also warrant consideration of inclusion in future revisions of the TNM of lung cancer after a more detailed assessment of even larger patient cohorts. In addition, the researchers suggested that resected stage I NSCLC patients harboring LVI might benefit from adjuvant chemotherapy.

Although this study metaanalytically revealed the significance of LVI in patients with resected NSCLC, the definition of LVI was not clearly described in this article. Mollberg and colleagues [1] examined retrospective studies that evaluated the presence of lymphatic or blood vessel invasion, or both, as a single covariate, whereas the studies that used blood vessel invasion or lymphatic vessel invasion as two different covariates were excluded. However, can these two factors be regarded as one factor, namely, LVI? As the researchers discuss in the Comment section, it is true that differentiating blood vessel invasion from lymphatic vessel invasion is difficult, but the clinical