Minimally invasive diagnostics and immunotherapy of lung cancer

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Chapter 3

Endosonography for lung cancer staging: predictors for false-negative outcomes

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ABSTRACT

Objectives

Non-small cell lung cancer (NSCLC) guidelines recommend endosonography (endobronchial [EBUS] and/or transesophageal ultrasound [EUS]) as the initial step for mediastinal tissue staging. Identifying predictors for false negative results could help establish which patients should undergo confirmatory surgical staging.

Materials and Methods

775 NSCLC patients staged negative by EBUS, EUS or combined EUS/EBUS were retrospectively analyzed. Predictors of false-negative outcomes were identified by logistic regression analysis.

Results and Conclusion

Three predictors for false-negative outcomes were identified: central location of the lung tumor (OR 3.7/4.5/3.6 for EBUS, EUS and EUS/EBUS respectively, $p<0.05$), nodal enlargement on CT (OR 3.2/2.5/4.9 for EBUS, EUS and EUS/EBUS respectively, $p<0.05$) and FDG-avidity of N2/N3 lymph node stations on PET (OR 4.2/4.0/7.5 for EBUS, EUS and EUS/EBUS respectively, $p<0.05$). One subgroup (peripheral lung tumor, nodal enlargement on CT without FDG-avidity for N2/N3) had a low predicted probability (7.8%) for false-negative EUS. For combined EUS/EBUS, two subgroups were identified: peripheral located tumor with nodal enlargement on CT but without FDG-avidity for N2/N3 (predicted probability 4.7%) and centrally located tumor without affected lymph nodes on CT or PET (predicted probability 3.4%). In conclusion, for specific well-defined subsets of NSCLC patients the low predicted probability of metastasis after negative endosonography might justify omitting confirmatory surgical staging.

INTRODUCTION

Lung cancer is the most common cause of cancer mortality in men in the developed world and one of the leading causes in women[1]. NSCLC comprises about 80% of all lung cancers[2]. Clinical TNM staging is pivotal because it forms the basis for treatment and has prognostic value[2]. Non-invasive staging methods such as computed tomography (CT) and positron emission tomography (PET) are valuable diagnostic tools that provide information about the size and location of the primary tumor as well as an indication of the presence of local and distant metastases. Tissue sampling of mediastinal lymph nodes is required for confirmation when imaging techniques such as CT and PET show signs of suspected nodal metastatic involvement since nodal status dictates treatment when distant metastases are absent[3].

Endosonography is a minimally invasive mediastinal tissue staging technique that allows sampling of intrathoracic lymph nodes under ultrasound guidance either by the airways (endobronchial ultrasound-guided transbronchial needle aspiration [EBUS-TBNA]) or the esophagus (transesophageal ultrasound-guided fine needle aspiration [EUS-FNA])[4]. Endosonography is advised as the test of choice for mediastinal nodal assessment in current guidelines[3,5,6] due to its high sensitivity of 83% (EUS) and 88% (EBUS)[7,8]. Additionally, combined endosonography (EUS-FNA and EBUS-TBNA) has been shown to improve sensitivity and predictive value compared to EUS or EBUS alone[3,9,10]. In the ASTER randomized clinical trial, combined EUS/EBUS was compared with surgical staging, which was previously considered as the gold standard for mediastinal tissue staging. Combined endosonography showed comparable sensitivity to surgical staging (85% vs 79%, p=0.47) but led to fewer unnecessary thoracotomies (18% vs 7%, p=0.02). Furthermore, it was shown that eleven patients needed to undergo mediastinoscopy in order to detect one single patient with N2 disease missed by combined EUS/EBUS[11].

An important question is whether patients should routinely undergo confirmatory surgical staging after negative endosonography or proceed directly to surgical resection of the primary tumor with systematic lymph node dissection. False-negative endosonography findings lead to suboptimal staging and treatment. However, unnecessary mediastinoscopies are associated with morbidity, treatment delay and higher health care costs[12]. In this study, we aim to identify patient-, tumor- and procedure-related predictors for false-negative endosonography results. Subsequently, we identify specific subgroups of NSCLC patients at risk of having a false-negative endosonography outcome (thus justifying confirmatory surgical staging) as well as subsets of patients for whom additional surgical staging has limited value.

MATERIALS AND METHODS

After examination of patient records from 1999-2013 by reviewing Endosonography databases from the Leiden University Medical Center (LUMC, The Netherlands), Leeuwarden Medical Center (MCL, The Netherlands) and Ghent University Hospital (GUH, Belgium), patients were retrospectively included based on the following criteria:

- intrapulmonary lesion suspected of or histologically confirmed as primary NSCLC
- endoscopic staging procedures (EUS, EBUS or both) performed without detection of mediastinal nodal metastases (cN0/N1)
- availability of a surgical mediastinal nodal reference standard
- final diagnosis of NSCLC.

Of all patients, gender and age was noted. Based on computed tomography (CT) and positron emission tomography (PET) reports, several tumor characteristics were recorded, such as tumor location, nodal enlargement on CT (short axis > 10mm) and FDG-avidity of mediastinal lymph nodes (N2/N3) on PET scans. Furthermore, it was determined whether the tumor was located centrally (inner third of the thorax) or at the periphery of the lung. When available, a histological diagnosis before endosonography procedures was determined based on patients' pathology reports.

Procedures

EUS-FNA, EBUS-TBNA or combined procedures were performed in three hospitals (LUMC, MCL, GUH) with linear echo-endoscopes using 22-gauge needles, as previously described[11,13,14]. The mediastinum was assessed in a standardized fashion [15]. From all endosonography procedures, the number of needle passes and the short axis of the largest lymph node were noted. Furthermore, it was recorded whether mediastinal lymph nodes with echographic features suggestive of malignancy (either short axis >10 mm, round shape, sharp demarcation, or a diffusely hypoechoic ultrasound pattern) were detected.

Surgical staging was performed by mediastinoscopy with systematic assessment of left and right high and lower paratracheal and subcarinal nodes. Parasternal mediastinotomy (MT) or video assisted thoracoscopic surgery (VATS) was used in case of a suspected lymph node metastasis in lymph node station 5 or 6. Thoracotomy was performed by (bi)lobectomy or pneumectomy with dissection of regional lymph node stations according to current guidelines[3,16]. Based on pathology reports, a final diagnosis of NSCLC was determined.

Data analysis

The outcome measure of this study was either the presence or absence of false negative endosonography (EUS, EBUS or combined EUS/EBUS) findings. False-negative findings

(N2/N3) were defined as mediastinal nodal metastases missed by endosonography that were found during either surgical staging (mediastinoscopy, parasternal mediastinotomy or VATS) or surgical resection with systematic lymph node dissection. False-negatives were classified as either detection errors (lymph node metastasis not detected by endosonography) or sampling errors (a missed metastasis despite lymph node sampling during endosonography). True negative endosonography findings (N0/N1) were defined as negative endosonography results that were confirmed by surgical sampling of mediastinal lymph nodes during surgical staging procedures, thoracotomy or both.

Since the outcome measure is binary, all potential predictors were assessed by multivariable logistic regression, thereby adjusting for potentially confounding variables. Subsequently, we performed an automatic variable selection procedure with a p-value of >0.10 based on the log likelihood ratio test (with backward selection). Hereby, the model was reduced which led to the strongest predictors that remained in the final model. Results were reported as odds ratios (ORs) with 95% confidence intervals (95% CI). Based on the predicted probabilities of the final model, an area under the ROC curve (c-statistic) was calculated for each dataset (EUS, EBUS, EUS/EBUS combined) to assess the discriminative ability of the model. A Hosmer–Lemeshow test was done to determine goodness-of-fit of the final logistic regression model. All analyses were conducted with SPSS Statistics for Windows, Version 20.0 (SPSS Inc., Chicago, IL).

RESULTS

Data were retrieved from NSCLC patients who underwent EBUS (n=182), EUS (n=471), or a combined EUS/EBUS procedure (n=122) for diagnostic or staging purposes. A summary of patient, tumor and procedure-related characteristics of all three cohorts is displayed in **Table 1**.

EBUS-TBNA

From 2004 to 2013, a total of 182 patients were identified in whom EBUS-TBNA did not demonstrate nodal metastases and who underwent subsequent surgical verification of mediastinal nodal status. 109 patients underwent surgical staging by mediastinoscopy (n=101), parasternal mediastinotomy (n=5) or VATS (n=3). These procedures showed histologically proven mediastinal metastases (N2/N3) in 26 patients (23.9%). In 83 patients, no mediastinal metastases were detected, of whom 80 patients subsequently underwent thoracotomy. Surgical resection of the tumor with lymph node dissection showed the presence of metastases in mediastinal lymph node stations (N2/N3) in 15 patients (18.8%), whereas 65 patients were confirmed to be free of mediastinal metastases (N0/

Table 1. Patient characteristics

Study characteristics	EBUS (n= 182)	EUS (n= 471)	EUS/EBUS (n= 122)
Hospital			
Leiden University Medical Center	130	296	94
Ghent University Hospital	52	39	28
Leeuwarden Medical Center	0	136	0
Age, mean (SD), y	66 (9)	65 (10)	65 (9)
Sex, No. (%)			
Male	117 (64)	330 (70)	85 (70)
Female	65 (36)	141 (30)	37 (30)
Indication for staging, No. (%)			
Suspected NSCLC	76 (42)	132 (28)	30 (25)
Staging NSCLC			
Adenocarcinoma	33 (18)	81 (17)	33 (27)
Squamous cell carcinoma	45 (25)	153 (33)	32 (26)
Large cell carcinoma	7 (4)	24 (5)	9 (7)
NSCLC NOS	19 (11)	81 (17)	18 (15)
Tumor localization, No. (%)			
Left lower lobe	19 (10)	88 (19)	20 (16)
Left upper lobe	27 (15)	183 (39)	31 (25)
Right upper lobe	86 (47)	111 (24)	44 (36)
Middle lobe	7 (4)	15 (3)	4 (3)
Right lower lobe	43 (24)	72 (15)	23 (19)
Central right	0 (0)	2 (0)	0 (0)
Central tumor on CT, No. (%)			
Yes	76 (42)	223 (47)	43 (35)
No	104 (57)	242 (52)	79 (65)
Unknown	2 (1)	5 (1)	0 (0)
Nodal status PET, No. (%)			
N0/N1	72 (39)	140 (30)	61 (50)
N2/N3	70 (39)	162 (34)	58 (48)
No PET	40 (22)	169 (36)	3 (3)
Nodal enlargement on CT, No. (%)			
Yes	77 (42)	191 (41)	52 (43)
No	105 (58)	280 (59)	70 (57)
Short axis of largest LN on CT, mean (SD), mm	11 (6)	12 (5)	11 (4)
Lymph node suspect during E(B)US			
Yes	67 (37)	155 (33)	49 (40)
No	91 (50)	308 (65)	71 (58)
Missing	24 (13)	8 (2)	2 (2)
Number of needle passes, mean (range), No.	3 (0-8)	3 (0-9)	5 (0-14)

Table 1. Patient characteristics (continued)

Study characteristics	EBUS (n= 182)	EUS (n= 471)	EUS/EBUS (n= 122)
Short axis of largest LN during E(B)US, mean (SD), mm	11 (7)	8 (6)	11 (5)
Final diagnosis, No. (%)			
Adenocarcinoma	72 (40)	155 (33)	46 (38)
Squamous cell carcinoma	62 (34)	233 (50)	46 (38)
Adenosquamous	3 (2)	11 (2)	2 (2)
Large cell carcinoma	7 (4)	16 (3)	6 (5)
Neuroendocrine	7 (4)	3 (1)	1 (1)
Carcinoid	3 (2)	1 (0)	0 (0)
NSCLC NOS	28 (15)	52 (11)	21 (17)

Abbreviations: CT computed tomography, EBUS endobronchial ultrasound-guided transbronchial needle aspiration, EUS transesophageal ultrasound-guided fine needle aspiration, LN lymph node, NOS not otherwise specified, NSCLC non-small cell lung cancer, SD standard deviation

N1). For 73 patients, thoracotomy was the next step after negative EBUS findings. Mediastinal metastases (N2/N3) were found in 11 patients (15.1%), whereas 62 patients did not have mediastinal metastases (N0/N1). A flowchart of this cohort is presented in **online supplementary Figure 1**.

A total of 52 false-negatives occurred in this cohort (**Table 2**). False-negative findings were classified as either detection errors (n=24) or sampling errors (n=28). Lymph node stations 2R, 4R, 2L, 4L and 7 are considered to be within the diagnostic reach of EBUS. Nine false-negative outcomes (17%) occurred in lymph node stations outside the reach of EBUS. Lymph node stations 4R (n=15) or multiple stations (n=18) were most frequently affected. In the latter group of multiple affected lymph node stations, only two cases involved stations outside the reach of EBUS (stations 5, 6, 8 and 9).

All potential predictive variables (age, sex, central location, nodal enlargement on CT, FDG-avidity for N2/N3 on PET, tumor location, tumor histology, number of needle passes, enlarged LN during EUS, suspect LN during EUS) were included in the multivariable logistic regression analysis. After reduction of the model by backward selection, seven predictors (age, sex, tumor location, tumor histology, number of needle passes, enlarged LN during EUS, suspect LN during EUS) failed to reach statistical significance ($p > 0.05$). Three variables remained strongly associated with false-negative EBUS outcomes (**Table 3**): central location of the lung tumor (OR 3.7, CI 95% 1.5-8.9, p= 0.004), nodal enlargement on CT (OR 3.2, CI 95% 1.3-7.8, p= 0.009) and FDG-avidity for N2/N3 on PET (OR 4.2, CI 95% 1.6-10.7, p=0.003). The c-statistic (area under the ROC curve), based on the predicted probabilities of the final model, was 0.782, indicating good discriminative ability of this model. The Hosmer-Lemeshow test was non-significant (p=0.488), indicating good calibration of this model.

Table 2. False-negative endosonography outcomes. Stations 2R, 4R, 2L, 4L and 7 are considered within the reach of EBUS. Stations 2L, 4l, 7, 8 and 9 are considered within the reach of EUS.

	EBUS (n= 52)	EUS (n= 112)	EUS/EBUS (n= 18)
Number of FN, No. (%)			
Detection error	24 (46)	70 (63)	7 (39)
Sampling error	28 (54)	42 (37)	11 (61)
Detected by MS/MT/VATS	26 (50)	51 (46)	9 (50)
Detected by Thoracotomy	26 (50)	61 (54)	9 (50)
FN within reach of test (%)	43 (83)	53 (47)	13 (72)
FN outside reach of test (%)	9 (17)	59 (53)	5 (28)
FN stations			
2R	1	3	1
2L	0	1	0
3	0	0	1
4R	15	21	5
4L	4	9	2
5 and/or 6	6	35	5
7	7	23	0
8R	0	0	2
8L	0	2	1
9	1	4	0
Multiple stations	18	14	1

Detection error: tissue-proven mediastinal nodal metastasis not detected by endosonography.
Sampling error: a tissue-proven mediastinal nodal metastasis missed by endosonography despite sampling
Abbreviations: EUS transesophageal ultrasound-guided fine needle aspiration, EBUS endobronchial ultrasound-guided transbronchial needle aspiration, FN False negative, MS mediastinoscopy, MT parasternal mediastinotomy; VATS video assisted thoracoscopic surgery

EUS- FNA

From 1999 to 2013, a total of 471 patients were identified who had undergone EUS-FNA for mediastinal staging of (suspected) NSCLC without detection of mediastinal metastases and in whom a surgical reference standard was available. 289 patients proceeded to surgical staging by cervical mediastinoscopy (n=276), parasternal mediastinotomy (n=7) or VATS (n=5). Surgical staging showed histologically proven mediastinal metastases (N2/N3) in 51 patients (17.6%). In 238 patients, no mediastinal metastases were found, of whom 225 patients subsequently underwent surgical resection of the tumor. Lymph node sampling during thoracotomy showed 34 patients (15.1 %) with metastases in mediastinal lymph node stations (N2/N3), whereas 191 patients were confirmed to be free of mediastinal metastases (N0/N1). For 182 patients, the clinical decision was made to

Table 3. Predictors of false-negative EBUS, EUS and combined EUS/EBUS results.

EBUS			
Variable	***Odds Ratio***	***95% CI***	***P value***
Central location of lung tumor	3.7	1.5 - 8.9	0.004
Nodal enlargement on CT	3.2	1.3 - 7.8	0.009
FDG-avidity for N2/N3 on PET	4.2	1.6 - 10.7	0.003
EUS			
Variable	***Odds Ratio***	***95% CI***	***P value***
Central location of lung tumor	4.5	2.4 - 8.6	0.000
Nodal enlargement on CT	2.5	1.4 - 4.8	0.004
FDG-avidity for N2/N3 on PET	4.0	2.0 - 8.2	0.000
EUS/ EBUS			
Variable	***Odds Ratio***	***95% CI***	***P value***
Central location of lung tumor	3.6	1.1 - 11.6	0.036
Nodal enlargement on CT	4.9	1.4 - 17.6	0.015
FDG-avidity for N2/N3 on PET	7.5	1.5 - 36.5	0.013

Abbreviations: CI Confidence Interval, CT computed tomography, EBUS endobronchial ultrasound-guided transbronchial needle aspiration, EUS transesophageal ultrasound-guided fine needle aspiration, PET positron emission tomography

proceed to direct thoracotomy after negative EUS-FNA findings. In 27 patients (14.8 %), mediastinal metastases (N2/N3) were found during thoracotomy, whereas 155 patients were free of mediastinal metastases (N0/N1). A flowchart of this cohort is presented in **online supplementary Figure 2**.

A total of 112 false-negative EUS results occurred (**Table 2**). False-negative findings were classified as either detection errors (n=70) or sampling errors (n=42). Lymph node stations 2L, 4L, 7, 8 and 9 are considered to be within the diagnostic reach of EUS. 59 false-negative outcomes (53%) occurred in lymph node stations outside the reach of EUS, of which stations 5/6 (n=35) and 4R (n=21) were affected in the majority of cases.

All potential predictors were evaluated by multivariable logistic regression analysis. After reduction of the model by backward selection, seven predictors (age, sex, tumor location, tumor histology, number of needle passes, enlarged LN during EUS, suspect LN during EUS) failed to reach statistical significance ($p > 0.05$). Three variables remained strongly associated with false-negative EUS outcomes (**Table 3**): central location of the lung tumor (OR 4.5, CI 95% 2.4-8.6, $p < 0.001$), nodal enlargement on CT (OR 2.5, CI 95%

1.4-4.8, p= 0.004) and FDG-avidity for N2/N3 on PET (OR 4.0, CI 95% 2.0-8.2, p< 0.001). The c-statistic (area under the ROC curve) was 0.773, indicating good discriminative power of this model. The Hosmer-Lemeshow test was non-significant (p=0.989), indicating good calibration of this model.

Combined EUS/EBUS

From 2005 to 2013, a total of 122 patients underwent a combined EUS/EBUS procedure for mediastinal staging of (suspected) NSCLC without detection of mediastinal metastases. 84 patients underwent additional surgical staging by mediastinoscopy (n=79), parasternal mediastinotomy (n=2) or VATS (n=3). These procedures showed histologically proven mediastinal metastases (N2/N3) in 9 patients (10.7%). In 75 patients, no mediastinal metastases were detected, of whom 69 patients subsequently underwent thoracotomy. Surgical resection of the tumor with lymph node dissection showed the presence of metastases in mediastinal lymph node stations (N2/N3) in 7 patients (10.1 %), whereas 62 patients were confirmed to be free of mediastinal metastases (N0/N1). 38 patients proceeded directly to surgical resection after negative EUS/EBUS outcomes. Mediastinal metastases (N2/N3) were found in 2 patients (5.3%), whereas 36 patients did not have mediastinal metastases (N0/N1). A flowchart of this cohort is presented in **online supplementary Figure 3**.

A total of 18 false-negatives occurred in this cohort (**Table 2**). False-negative findings were classified as either detection errors (n=7) or sampling errors (n=11). 5 false-negative EUS/EBUS outcomes (28%) occurred in lymph node stations 5/6, which are difficult to reach even when combining both procedures.

All potential predictive variables were included in the multivariable logistic regression analysis (**Table 3**). Again seven predictors failed to reach statistical significance (p> 0.05), whereas the same three variables as in the previous cohorts remained strongly associated with false-negative EUS/EBUS outcomes: central location of the lung tumor (OR 3.6, CI 95% 1.1-11.6, p= 0.036), nodal enlargement on CT (OR 4.9, CI 95% 1.4-17.6, p= 0.015) and FDG-avidity for N2/N3 on PET (OR 7.5, CI 95% 1.5-36.5, p=0.013). The c-statistic (area under the ROC curve) was 0.832, indicating good discriminative power of this model. The Hosmer-Lemeshow test was non-significant (p=0.923), indicating good calibration of this model.

Predicted probabilities of logistic regression model after backward selection

In all three cohorts (EUS, EBUS and combined EUS/EBUS) the same three variables reached statistical significance (p<0.05) and remained in the logistic regression model after backward selection: central location of the lung tumor, nodal enlargement on CT and FDG-avidity for N2/N3 on PET. This reduced model was used to predict the occurrence of false negative findings for EUS, EBUS and EUS/EBUS combined. **Table 4** displays

the predicted probabilities of false negative occurrence generated by this model for all three cohorts when combining the three main variables.

Table 4. Predicted probabilities of false negative endosonography results in all three cohorts (EBUS, EUS, EUS+EBUS combined). Probabilities were generated based on the logistic regression model using the combination of the three main variables that reached statistical significance (p<0.05). Patient subsets with predicted post-test probability of <5% are indicated in bold.

	EBUS	EUS	EUS/EBUS
Peripheral tumor, no nodal enlargement on CT, PET N0/N1	**0.04253**	**0.032**	**0.00987**
Peripheral tumor, nodal enlargement on CT, PET N0/N1	0.12542	0.078	**0.04656**
Peripheral tumor, no nodal enlargement on CT, PET N2/N3	0.15611	0.119	0.06959
Central tumor, no nodal enlargement on CT, PET N0/N1	0.14059	0.131	**0.03423**
Peripheral tumor, nodal enlargement on CT, PET N2/N3	0.37393	0.255	0.26823
Central tumor, nodal enlargement on CT, PET N0/N1	0.34562	0.277	0.14798
Central tumor, no nodal enlargement on CT, PET N2/N3	0.40523	0.377	0.21012
Central tumor, nodal enlargement on CT, PET N2/N3	0.68748	0.606	0.56589

Abbreviations: CT computed tomography, EBUS endobronchial ultrasound-guided transbronchial needle aspiration, EUS transesophageal ultrasound-guided fine needle aspiration, PET positron emission tomography

DISCUSSION

We retrospectively identified 775 NSCLC patients who were staged N0/N1 by either EBUS (n=182), EUS (n=471) or combined EUS/EBUS (n=122), and underwent subsequent surgical evaluation of the mediastinum. In these three cohorts 52 (28.6%), 112 (23.8%) and 18 (14.7%) false-negative cases occurred respectively, which supports the assertion that endosonography has limitations in excluding mediastinal metastatic disease and that complete mediastinal staging by combined EUS/EBUS has superior test characteristics in comparison to EUS and EBUS alone[9-11,17-20]. The relatively high prevalence of false-negative cases can in part be explained by the diagnostic reach of the techniques. In the EUS cohort 59 false negatives (52.6%) occurred outside the diagnostic reach of this technique (**Table 2**). 24 of these false-negatives were located in right sided paratracheal lymph nodes (stations 2R/4R) within the reach of EBUS, indicating that a combined approach could have reduced the number of false-negatives. When adjusted for the diagnostic reach of these techniques the false-negative rates for EBUS (23.6%), EUS (11.3%) and combined EUS/EBUS (10.7%) are well within the previously reported range[3].

Our study focused on identifying determinants associated with false-negative endosonography outcomes by multivariable logistic regression analysis. In all three cohorts, the same three variables stood out: central location of the lung tumor (OR 4.5/ 3.7/3.6 for EUS, EBUS and EUS/EBUS respectively, p<0.05), nodal enlargement on CT (OR 2.5/3.2/4.9

for EUS, EBUS and EUS/EBUS respectively, p<0.05) and FDG-avidity of N2/N3 lymph node stations on PET (OR 4.0/4.2/7.5) for EUS, EBUS and EUS/EBUS respectively, p<0.05). Several other clinical determinants such as tumor location (left vs right sided lung tumors) failed to reach statistical significance.

The reduced logistic regression model, containing the three main variables (central location of the lung tumor, nodal enlargement on CT and FDG-avidity for N2/N3) was used to predict the occurrence of mediastinal metastases (N2/N3) after negative EUS, EBUS and combined EUS/EBUS outcomes (**Table 4)**. A post-test probability of nodal metastasis of 5% or less was proposed as an acceptable cut-off point for clinicians[21].

For EUS, the subgroup of patients with a peripheral tumor and nodal enlargement on CT without FDG-avidity has a low predicted probability (7.8%) of providing a false-negative result. This might imply that this subgroup has very limited chance of benefiting from additional surgical staging. For EBUS, such a subgroup is difficult to identify. Patients with a peripheral tumor and a normal mediastinum on CT and PET have a low probability (4.2%) of having a false-negative outcome, but since the pre-test probability of a mediastinal nodal metastasis is very low in these patients anyway, they generally proceed directly to surgical resection without further tissue staging[3,22]. For combined EUS/EBUS, two subgroups with low post-test probability of false-negative outcome can be identified. Patients with a peripheral tumor with nodal enlargement on CT but without FDG-avidity have a probability of 4.7% to be false-negative, whereas patients with a central lung tumor but without affected lymph nodes on CT or PET have a post-test probability of 3.4% to be false-negative. The constructed model also predicts low to moderate probability (7.0%) of false-negative outcomes in patients with a peripheral located lung tumor, no nodal enlargement on CT but with FDG-avidity of N2/N3 stations. Clinicians should consider whether additional mediastinoscopy is really beneficial to patients in these subgroups considering that a recent randomized trial established the need for 11 mediastinoscopies to detect one patient with N2 disease missed by combined EUS/EBUS[11]. The current data underlie the importance of combined EBUS/EUS staging which can be achieved by only using the EBUS scope[17].

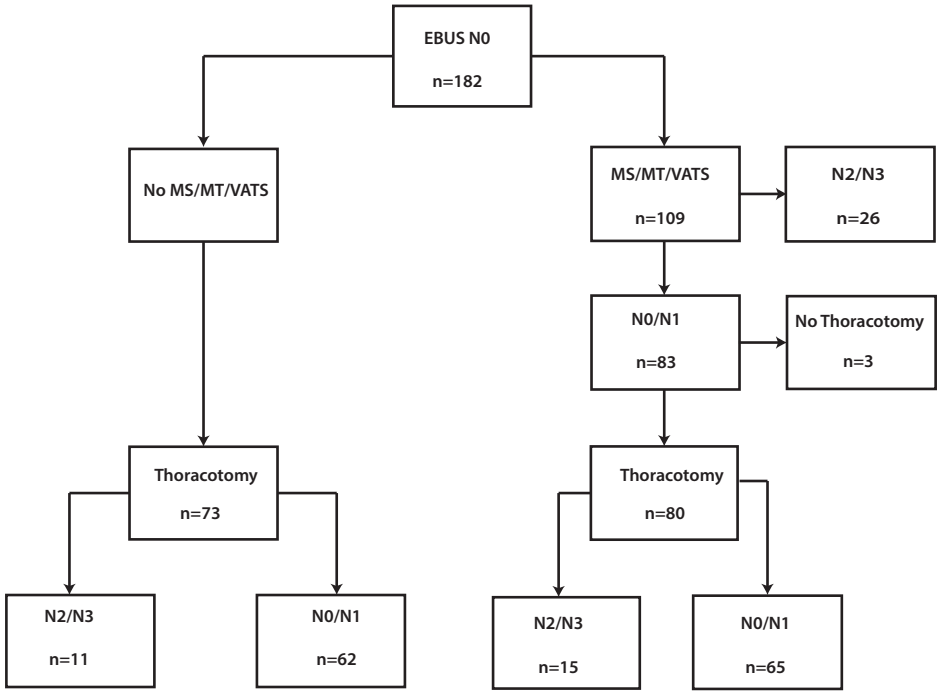
Several limitations apply to this study. Its retrospective design has inherent drawbacks. In a considerable subset of patients, mainly in the EUS cohort, PET results were not available since these scans were only recently implemented in the routine preoperative staging of NSCLC. Also, a selection bias might be present as from one center (MCL) only EUS results were available and from another center (GUH) data on a surgical reference standard was difficult to retrieve from referring hospitals, which accounts for its relatively modest contribution to sample size. Finally the logistic regression model should, despite its good calibration and discriminative ability, be validated on a prospective cohort of NSCLC patients in order to establish its external validity.

In conclusion, these data show that three variables (central location of the lung tumor, nodal enlargement on CT and FDG-avidity for N2/N3 on PET) are associated with false-negative EUS/EBUS outcomes. By combining these variables in a logistic regression model, we were able to identify subgroups of patients who have a low probability of false-negative endosonography outcomes, which might imply that these patients have limited benefit from additional surgical staging and can proceed directly to surgical resection. However, prospective studies should confirm these data in order to establish external validity of this model.

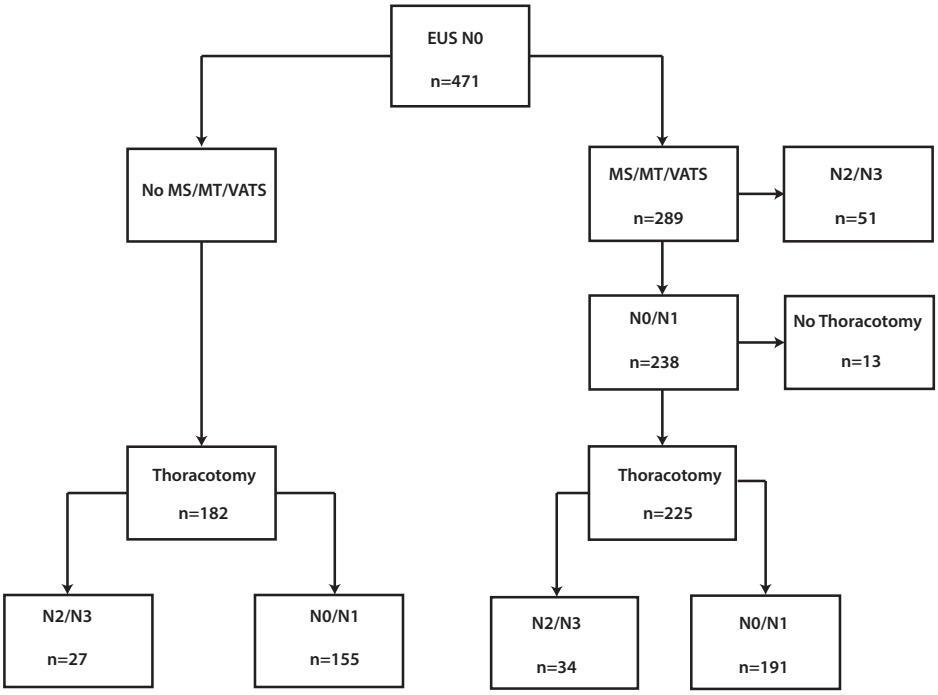
REFERENCE LIST

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin 2013;63:11-30.
2. Herbst RS, Heymach JV, Lippman SM. Lung cancer. N Engl J Med 2008;359:1367-80.
3. Silvestri GA, Gonzalez AV, Jantz MA, et al. Methods for staging non-small cell lung cancer: Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest 2013;143:e211S-e50S.
4. Annema JT, Rabe KF. State of the art lecture: EUS and EBUS in pulmonary medicine. Endoscopy 2006;38 Suppl 1:S118-S22.
5. De Leyn P, Dooms C, Kuzdzal J, et al. Revised ESTS guidelines for preoperative mediastinal lymph node staging for non-small-cell lung cancer. Eur J Cardiothorac Surg 2014;45:787-98.
6. Vansteenkiste J, De RD, Eberhardt WE, et al. Early and locally advanced non-small-cell lung cancer (NSCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2013;24 Suppl 6:vi89-vi98.
7. Adams K, Shah PL, Edmonds L, Lim E. Test performance of endobronchial ultrasound and transbronchial needle aspiration biopsy for mediastinal staging in patients with lung cancer: systematic review and meta-analysis. Thorax 2009;64:757-62.
8. Micames CG, McCrory DC, Pavey DA, Jowell PS, Gress FG. Endoscopic ultrasound-guided fine-needle aspiration for non-small cell lung cancer staging: A systematic review and metaanalysis. Chest 2007;131:539-48.
9. Zhang R, Ying K, Shi L, Zhang L, Zhou L. Combined endobronchial and endoscopic ultrasound-guided fine needle aspiration for mediastinal lymph node staging of lung cancer: a meta-analysis. Eur J Cancer 2013;49:1860-7.
10. Kang HJ, Hwangbo B, Lee GK, et al. EBUS-centred versus EUS-centred mediastinal staging in lung cancer: a randomised controlled trial. Thorax 2013.
11. Annema JT, van Meerbeeck JP, Rintoul RC, et al. Mediastinoscopy vs endosonography for mediastinal nodal staging of lung cancer: a randomized trial. JAMA 2010;304:2245-52.
12. Rintoul RC, Glover MJ, Jackson C, et al. Cost effectiveness of endosonography versus surgical staging in potentially resectable lung cancer: a health economics analysis of the ASTER trial from a European perspective. Thorax 2013.
13. Annema JT, Veselic M, Rabe KF. Endoscopic ultrasound-guided fine-needle aspiration for the diagnosis of sarcoidosis. Eur Respir J 2005;25:405-9.
14. Tournoy KG, Rintoul RC, van Meerbeeck JP, et al. EBUS-TBNA for the diagnosis of central parenchymal lung lesions not visible at routine bronchoscopy. Lung Cancer 2009;63:45-9.
15. Tournoy KG, Annema JT, Krasnik M, Herth FJ, van Meerbeeck JP. Endoscopic and endobronchial ultrasonography according to the proposed lymph node map definition in the seventh edition of the tumor, node, metastasis classification for lung cancer. J Thorac Oncol 2009;4:1576-84.
16. P. DL, Lardinois D, Van Schil PE, et al. ESTS guidelines for preoperative lymph node staging for non-small cell lung cancer. Eur J Cardiothorac Surg 2007;32:1-8.
17. Annema JT. Complete endosonographic staging of lung cancer. Thorax 2014.
18. Ohnishi R, Yasuda I, Kato T, et al. Combined endobronchial and endoscopic ultrasound-guided fine needle aspiration for mediastinal nodal staging of lung cancer. Endoscopy 2011;43:1082-9.
19. Rintoul RC, Skwarski KM, Murchison JT, Wallace WA, Walker WS, Penman ID. Endobronchial and endoscopic ultrasound-guided real-time fine-needle aspiration for mediastinal staging. Eur Respir J 2005;25:416-21.

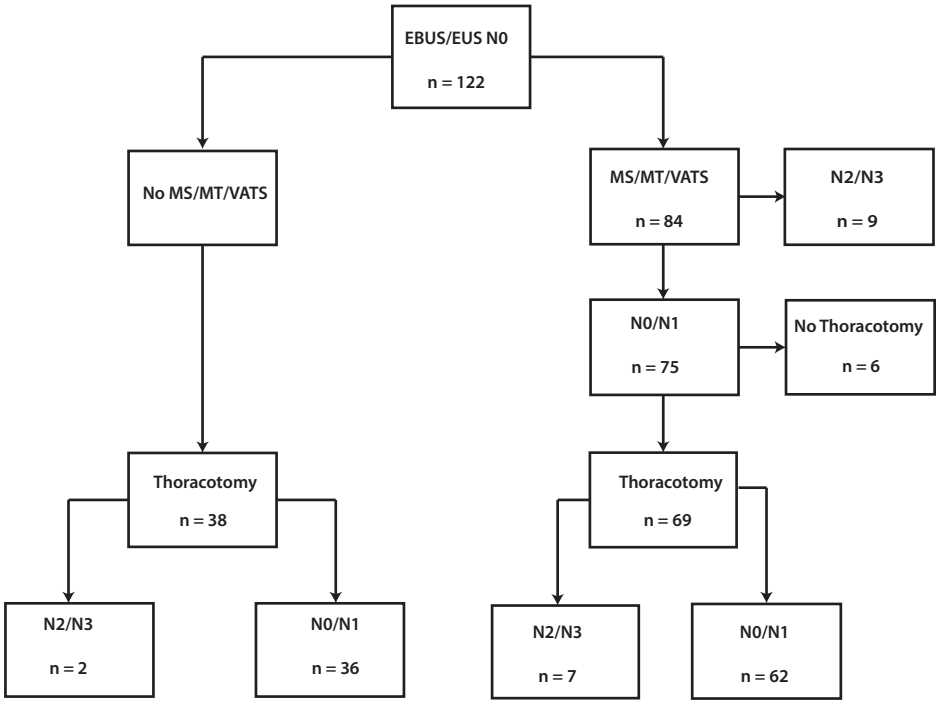
20. Vilmann P, Krasnik M, Larsen SS, Jacobsen GK, Clementsen P. Transesophageal endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) and endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) biopsy: a combined approach in the evaluation of mediastinal lesions. Endoscopy 2005;37:833-9.
21. Tournoy KG, Keller SM, Annema JT. Mediastinal staging of lung cancer: novel concepts. Lancet Oncol 2012;13:e221-e9.
22. Cerfolio RJ, Bryant AS, Eloubeidi MA. Routine mediastinoscopy and esophageal ultrasound fine-needle aspiration in patients with non-small cell lung cancer who are clinically N2 negative: a prospective study. Chest 2006;130:1791-5.



Online supplementary Figure 1. EBUS-TBNA study cohort
Abbreviations: EBUS endobronchial ultrasound-guided transbronchial needle aspiration, MS cervical mediastinoscopy, MT parasternal mediastinotomy, VATS video assisted thoracoscopic surgery



Online supplementary Figure 2. EUS-FNA study cohort
Abbreviations: EUS transesophageal ultrasound-guided fine needle aspiration, MS cervical mediastinoscopy, MT parasternal mediastinotomy, VATS video assisted thoracoscopic surgery



Online supplementary Figure 3. EUS/EBUS-TBNA study cohort
Abbreviations: EUS, transesophageal ultrasound-guided fine needle aspiration, EBUS, endobronchial ultrasound-guided transbronchial needle aspiration, MS cervical mediastinoscopy, MT parasternal mediastinotomy, VATS video assisted thoracoscopic surgery