

A Histochemical Approach to the Diagnosis of Visceral Pleural Infiltration by Non-small Cell Lung Cancer

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Abstract Introduction: Although invasion of the visceral pleura (VPI) by non-small cell lung cancer (NSCLC) is a TNM-relevant diagnostic criterion and is known to affect the patients' prognoses, until recently there were no standardized or internationally accepted guidelines. This resulted in a diagnostic ambiguity leading to different tumor staging systems and to hardly comparable patient collectives in research studies world wide. The major problem in this issue is to exactly define what constitutes for the diagnosis of VPI with respect to anatomical landmarks. Methods: In order to address this problem we investigated the pleural infiltration depth of 173 NSCLC specimens without lymph node metastases and proven tumor-related death using elastic stains and a scoring system referring to prominent pleural elastic layers, the lamina elastica externa and interna, as anatomical landmarks. Results: Performing comparative Kaplan-Meier survival analyses for each patient collective we could not find any significant difference in the patients' survival. This indicates that a differential evaluation of the tumor infiltration depth according to the elastic layers is not practicable.

Conclusions: Our findings support the consequent application of the recently proposed, pragmatic approach of the international staging committee for lung cancer (IASLC) to define an internationally accepted and standardized staging system for VPI.

Keywords Visceral pleural infiltration (VPI) · Staging · Pathology · Elastic layers · Lung cancer · NSCLC · Pleura · Infiltration · Elastic layer · Staining

Introduction

According to the current TNM staging system (6th edition), invasion of the visceral pleura increases the T category of non-small cell lung carcinomas (NSCLCs) that otherwise only meet pT1 criteria to pT2 [1]. However, there are no internationally accepted guidelines on what constitutes visceral pleura infiltration (VPI) although this diagnosis is considered as an important and independent prognostic factor in patients who undergo complete resection for NSCLCs [2–8]. The need for broadly accepted, standardized criteria defining VPI was recently demonstrated by Butnor and colleagues [9] who performed an online quiz consisting of 15 NSCLCs adjacent to or involving the visceral pleura. One hundred three participants including 42.7% specialized in pulmonary pathology took part in the quiz. Interestingly, the interobserver agreement whether VPI was present, absent, or indeterminate ranged from 36.9% to 93.2% (mean 73.0) [9]. These results impressively underline the current diagnostic ambiguity of VPI. Furthermore, the lack of consensus concerning VPI diagnosis lead to diverse tumor staging systems world wide [10]. Any studies concerning NSCLCs, especially on novel therapies need careful evaluation how VPI was defined to avoid

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comparison of different patient collectives due to varying staging criteria. Unfortunately, not all authors specify how they define VPI as present or absent in their publications. Whereas some, in accordance to the recommendation of The Japan Lung Cancer Society criteria [11], consider only tumors extending to the visceral pleural surface as positive for VPI, others define VPI as positive when NSCLCs extend to within a specific distance of the visceral pleural surface [3]. Some pathologists even categorize tumors as “VPI present” when the tumor abuts the visceral pleura with no intervening alveoli on H&E staining [9].

The microscopic anatomy of the visceral pleura consists classically of five layers, although there is some variation in the number and composition of the tissue layers. There is a thin mesothelial layer, followed by a layer of connective tissues and elastic fibers (Fig. 1). An important anatomical landmark is the elastic layer, consisting of a broader and stronger layer (lamina elastica externa; LEE) and a much thinner inner layer (lamina elastica interna; LEI), which represents the most prominent anatomical landmark close to the lung parenchyma [12]. In between these elastic layers there are blood vessels, lymph vessels, and connective tissue. Especially in non-pathologic tissue these two elastic bands tend to fuse and appear as a single band. In NSCLCs there is often a prominent elastic reduplication of the layers due to the tumor-induced pleural puckering, making it difficult or sometimes even impossible to clearly separate the layers. In addition to routine stains elastic stains are essential to identify the layers [13]. Since recent studies found that a complete tumor infiltration through the elastic layer is of prognostic significance, most pathologists consider only the extension of tumors beyond the LEE as VPI present [9]. None of the current studies investigating the significance of VPI analyzed the difference between tumors extending to the LEI and tumors extending beyond the LEI without breaking through the LEE [9], most probably due to the difficulties in separating LEE and LEI. Furthermore, although the currently published studies precisely described their definition of “VPI present”, the diagnostic criteria for “VPI absent” remain uncertain. Does “VPI absent” include tumor extension beyond LEI or not? Therefore, the zone between LEI and LEE is currently a diagnostic “grey area”. In order to narrow-

in to an anatomical landmark which might be suitable for a more precise definition of VPI we evaluated 173 NSCLCs extending close to the visceral pleura, beyond the LEI, and beyond the LEE without reaching the pleural surface using consecutive tissue slices with hematoxylin-eosin (HE) and elastic stains. The patient survival for each group was evaluated performing Kaplan-Meier analyses.

Material and Methods

Patients

We reviewed all patients with stage I NSCLCs (pT1 or pT2) who underwent surgical treatment from 2002 to 2005 for tumors located close to the visceral pleura and for which survival data were available. Patients with lymph node or other metastases were excluded. All tumors were removed in the Department of Thoracic Surgery, Thoraxklinik at the University Hospital Heidelberg, Germany and were diagnosed in the Institute for Pathology, University Hospital Heidelberg, Germany. We finally obtained a total of 173 patients.

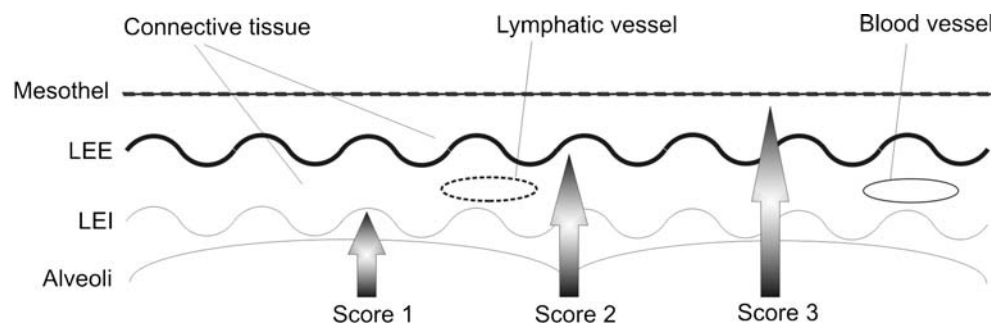
Histologic Evaluation and Scoring

All cases were reviewed for tissue slices with direct tumor association to the pleura. From all corresponding paraffin blocks we performed two consecutive slices, one for a hematoxylin-eosin (H&E) staining and one for an elastic staining according to standard protocols. Tumor extension in relation to the elastic layer was scored according to the following system (Figs. 1 and 2):

- Score 1: Tumor extension directly to the LEI without penetration
- Score 2: Tumor extension beyond the LEI without penetration beyond the LEE
- Score 3: Tumor extension beyond the LEE without exposure to the pleural surface

Tumors not adjacent to the LEI or cases with tumor exposure to the pleural surface were excluded. Furthermore, cases comprising an interlobar fissure were excluded.

Fig. 1 Schematic illustration of the scoring system to define visceral pleural infiltration depth according to the morphology of the elastic layers. LEE = Lamina elastica externa, LEI = Lamina elastica interna



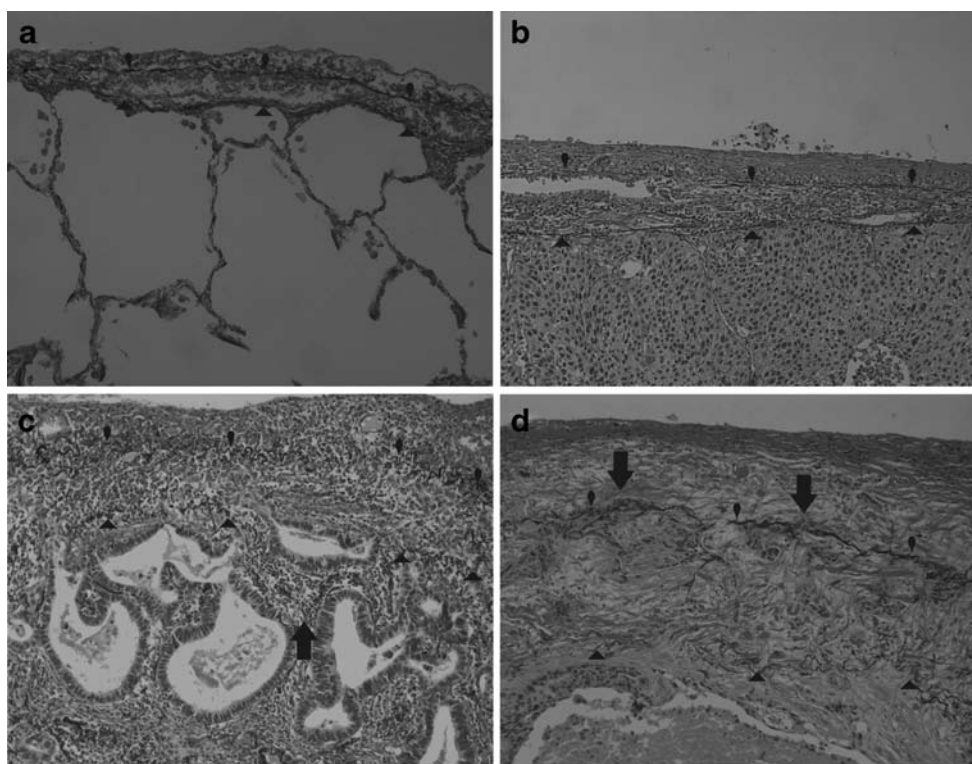


Fig. 2 Histologies of the applied score on EVG stained slides. Round arrows = Lamina elastica externa (LEE); triangles = Lamina elastica interna (LEI). **a:** Pleura of a normal lung with stronger LEE and a scant LEI close to the alveolar walls. Within the elastic layers blood and lymph vessels are visible (see also B). **b:** Squamous cell carcinoma extending close to the LEI without penetration (Score 1). Note the pleural thickening in b–d compared to a. **c:** Adenocarcinoma

extending to and invading the LEI, which is only left in small, discontinuous fragments. In central areas (large arrow) there is obvious tumor penetration beyond the fragmented LEI but not beyond the LEE (Score 2). **d:** Adenocarcinoma with invasion and fragmentation of the LEI and tumor extension beyond the LEE (large arrows) without tumor invasion to the pleural surface (Score 3)

Follow-up and Statistical Analyses

Following our case review, we obtained a collective of 173 patients. None of the patients had lymph node or distant metastases. The mean age of the patients was 64.7 (38–85) years, the median follow-up 40.6 months (95%CI: 35–46 months). Overall death was 24.5%, proven tumor-related death was 17.3%. Gender ration (m/f) was 113/60. 89 patients (51.4%) had an adenocarcinoma, 60 a squamous cell carcinoma (34.7%), and 24 patients (13.9%) NSCLCs of other histological types. Median survival after 1, 3, and, 4 years was 90%, 77.8%, and 71.1%, respectively. 89% received only operation as primary therapy, 8.7% adjuvant chemotherapy, 1.2% additionally radiation and 1.2% additionally chemotherapy and radiation. For statistical and Kaplan-Meier analyses we used SPSS 15.0, SPSS Inc. Chicago, Illinois, USA.

Results

Following the described scoring system we obtained the following three patient collectives: Score 1 = 60 patients;

Score 2 = 49 patients; Score 3 = 64 patients. Performing comparative Kaplan-Meier survival analyses according to these scores, we could not find any difference in the patients' survival (Fig. 3). A grouping of the score collectives, e.g. score 1 plus score 2 vs. score 3, also did not result in significant differences (not shown). In addition, we further analyzed each patient collective according to the scores with the criteria NSCLC (all patients), tumor entities (adenocarcinoma, squamous cell carcinoma, others), and tumor diameter compared to the survival in order to find possible relations to these factors. However, this also did not result in significant differences (not shown).

Discussion

Visceral pleural infiltration (VPI) of NSCLC is an important prognostic factor in stage I and stage II NSCLCs [6, 7, 10, 14] and a pT2 criterion in the current TNM system (6th edition) for staging of lung cancer [1]. Furthermore, it has been recognized that VPI is associated with extensive mediastinal and especially N2 lymph node metastases [6, 15], most

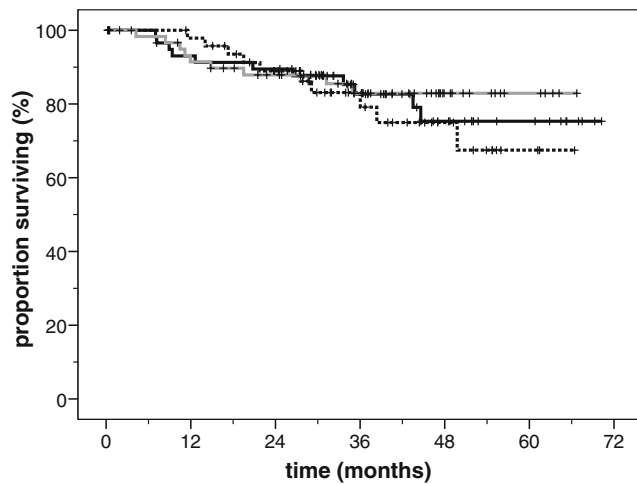


Fig. 3 Kaplan-Meier survival analysis according to the established scoring system. Black solid: Score 1, Black dotted: Score 2, Grey solid: Score 3

probably by drainage of tumor cells through the pleural lymphatics which are located between the two elastic layers LEI and LEE. Although some studies specifically addressed the problem of VPI, no study investigated the area between LEI and LEE in detail, even though the LEI is the most prominent anatomical landmark between the pleura and the lung parenchyma.

Both The Japan Lung Cancer Society (JLCS) [11] and others [8, 10] suggested modified classifications for visceral pleural infiltration (VPI) by NSCLC according to outcome-based analyses (Table 1). However, these classifications did not clearly define VPI concerning LEI and LEE involvement, since only the term “elastic layer” is used. The JLCS classification belongs to a detailed system for classifying VPI by Hammar et al. [16] into the following categories (p0–p2): p0: no VPI; p1: tumor penetration through the elastic layer but not to the visceral pleural surface; p2: penetration to the visceral pleural surface without involvement of the parietal pleura. A classification based on outcome assessment was suggested by Shimizu and co-workers [8] which is similar to the proposal of Butnor et al. [10] (Table 1). To shed more light on the remaining “grey zone” of all these classifications between LEI and LEE, we used a new scoring system to define the tumor infiltration depth according to both elastic layers. We found no prognostic differences in regard to

patient survival. One possibility is the need for larger collectives to obtain significant differences. However, our data most likely reflect the difficulties or even the impracticability in clearly classifying the tumor infiltration depth on histological slides when small and sometimes hardly visible structures like the LEI are used for a classification system. A novel classification should therefore clearly define a histological parameter which is detectable for all pathologists in daily practice and should reflect the patient’s prognosis as well. The international staging committee for lung cancer (IASLC) recently published a proposal to assess VPI for the forthcoming 7th edition of the TNM system for lung cancer [17]. In this proposal, the use of elastic stains is recommended. Furthermore, it is suggested that VPI should be defined as invasion beyond the elastic layer [17] according to a slightly modified system as suggested by Hammar et al. [16] previously. To distinguish PL0 (VPI absent) from PL1 (VPI present) the authors proposed a pragmatic approach: The thickest elastic layer should be identified in elastic stains, regardless if it is closer to the pleural surface (LEE) or closer to the lung parenchyma (LEI). Only tumor invasion beyond this layer is regarded as PL1, whereas tumor invasion into connective tissues adjacent to the lung parenchyma or tumor cells intermingled with elastic fibres without penetration beyond the thickest elastic layer remains PL0. In cases where the layers are not clearly discernable or when there is prominent reduplication (elastosis) of the elastic fibres, one has to assign the lower TNM category [17]. Nevertheless, validation of the proposed staging system needs to be performed, especially concerning the patients’ outcome, and first applications of the system already indicate that changes may be necessary in forthcoming revision, e.g. concerning the influence of the tumor size [18].

In summary, a more sophisticated analysis of tumor invasion of visceral pleural elastic layers does not provide a suitable landmark to specify VPI by NSCLC. Our data support the consequent application of the recently published proposal of the IASLC which provides a detailed guideline on how to assess VPI. In our own experience, this pragmatic approach is the most suitable one in daily practice and its application will hopefully provide a basis for generally accepted and internationally standardized diagnostic criteria and thereby may contribute to internationally comparable study data.

Table 1 Summary of modified classification systems for VPI

Classification	<3 cm without VPI	<3 cm with VPI	>3 cm without VPI	>3 cm with VPI
TNM (WHO) [1]	T1	T2	T2	T2
JLCS [11]	T1 (p0)	T1 (p1) T2 (p2)	T2 (p0)	T2 (p1/2)
Butnor et al. [10]	T1	T2	T2	T3
Shimizu et al. 2004 [8]	T1	T2	T2	T3

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