Tissue Microarrays are Reliable Tools for the Clinicopathological Characterization of Lung Cancer Tissue

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Abstract. Background: The advantage of tissue microarray (TMA) is its ability to efficiently analyze large numbers of tissue specimens in a methodologically uniform way. The reliability of TMAs, especially with regard to clinicopathological characterizations, when compared to conventional immunohistochemistry (IHC) was evaluated. Materials and Methods: Seventy-two embedded tissue sections from lung cancer specimens were stained with monoclonal antibodies against the tumor-associated markers TA-MUC1 and Lewis Y. Three representative cores of every tumor were embedded in a paraffin array multiblock. The IHC was evaluated by the immunoreactive score (IRS). Results: The data for the TMA IHC and the conventional IHC were concordant (kappa ≥80%) for both markers. Likewise, discordance (McNemar’s test) was low, and sensitivity and specificity were above 80% for both markers. In the samples with high positive expression, the concordance increased (kappa ≥90%), discordance disappeared (McNemar p=1.0), and sensitivity and specificity increased above 90% for both markers. Using Cox regression models, all the clinicopathological dependencies were equivalent for both techniques and both markers. Conclusion: Immunohistochemistry with tissue microarrays is valid and provides results equivalent to conventional immunohistochemistry with respect to expression patterns and clinicopathological characterizations.

At present, lung cancer is the leading cause of cancer-related death in both sexes in the U.S. (22), with an average overall survival rate of less than 15% (35). Unfortunately, this situation is not expected to change within the next decades, although most cases could be avoided (1) as lung cancer pathogenesis is dramatically influenced by exposure to tobacco smoke (36). Given its poor prognosis and its high incidence, emphasis should be put on earlier diagnosis and improved therapeutic options. Referring to this, the use of the tissue microarray (TMA) technology for immunohistochemical (IHC) purposes appears to be an advantageous development, as it assures the homogeneous and synchronous analysis of different tissue samples and their gene expression patterns under equivalent test conditions (24, 30, 42). As shown by Kononen et al., this technique allows the simultaneous IHC analysis of up to 1,000 samples (24). To construct a multi-sample tissue block, core biopsy specimens are retrieved from well-defined regions of donor blocks by a biopsy needle. These cores can then be arrayed into a recipient paraffin array block (51). Due to the high number of different tissue cores embedded in the acceptor block, it is possible to efficiently process and determine the prognostic and diagnostic value of a variety of different antigens under equivalent laboratory conditions (33).

To our knowledge, the histochemical staining results obtained by conventional IHC and TMA IHC have not been compared systematically with respect to clinical, laboratory, radiological and pathological lung cancer patient data. Therefore, the present study aimed to examine the applicability of the TMA technique for lung cancer research with respect to clinicopathological parameters. Two recently developed specific antibodies against tumor associated MUC1 (CD227) (TA-MUC1) and Lewis Y (Le3, CD174) were chosen. The MUC1 transmembrane glycoprotein is an antigen which is expressed on both normal glandular epithelia and on epithelial tumors (46). However, TA-MUC1 differs from normal MUC1 by its aberrantly glycosylated and truncated glycan side-chains (5), which are caused by up-regulation of different...
N-acetylgalactosaminyltransferases (17). Furthermore, TA-MUC1 displays enhanced antibody binding, as it is unmasked, compared to normal MUC1 (6). Because of the increased expression of TA-MUC1 (14) and its aberrant structure (5), this antigen appears to be an ideal tumor marker and a potential target for immunotherapeutic approaches (2, 9, 15, 47). The second antigenetic structure used was Lewis Y, a terminal tetrasaccharide, which is structurally related to the ABH histo blood group antigens. Its structure is characterized by its fucosylated tetrasaccharid epitope (Fucα1-2Galβ1-4[Fucα1-3]GlcNAcβ1-), fashioned by a series of enzymes, such as glycosyltransferases and glycosidases, working in specific sequences, mainly in the ER-Golgi plasmalemma pathway (25). Due to its strong expression in solid tumors, such as of the colon (18, 53), lung (18, 26, 53), breast (18, 53) and ovary (18, 53), it plays a potential role as a tumor-associated antigen and might be of immunotherapeutic use (52).

As most IHC studies of lung cancer have been performed with full tissue samples, the use of TMAs is relatively new for lung tumor research. In order to assess the value of TMA, the staining results of conventionally embedded lung tumor specimens were compared to those of tissue cores, embedded in a multi-tissue sample block, with the focus on concordance, discordance and validity. Furthermore, both conventional staining results and TMA staining results were correlated to the clinicopathological data.

Materials and Methods

Samples. Tumor samples from approximately 100 patients, who had undergone a surgical resection of their lung tumor at the Johannes Gutenberg University Hospital (Mainz, Germany) from mid 1999 to mid 2003 were selected. Both paraffin blocks containing sufficient formalin-fixed tumor material for sampling three tissue cores and clinical data were available for 72 patients. The study population consisted of 68 non-small cell carcinomas (NSCLC, 94%) and four small cell carcinomas (SCLC, 6%) (Table I). The patients’ laboratory parameters (i.e. established lung tumor markers and hemoglobin), Eastern Cooperative Oncology Group (ECOG) performance status, pathological classification and clinical TNM status are shown in Table I. The clinical TNM staging (including clinical examination, CT scans, sonography, endoscopy, MRI, bone scan) was performed according to the International Union Contra le Cancer/American Joint Committee on Cancer (IUCC/AJCC) (3, 45). To determine a definite tumor stage, post-surgery pathological examination was included (Table I).

In 66 out of the 72 patients (92%), resection of the primary pulmonary lesion was the only therapy. One patient received primary chemotherapy and four patients (6%) received neo-adjuvant chemotherapy with a platinum-based regimen prior to lung surgery. All the patients had follow-up visits on a regular basis and on demand. Restaging included clinical examination, chest X-ray, abdominal ultrasound scan, blood tests and CT scans, especially if progression was suspected. Overall survival was calculated from the date of histological diagnosis to death, and the median survival time was 1,056 days (Table I).

<table>
<thead>
<tr>
<th>Table I. Baseline characteristics of the study population.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients evaluated (n=72)</td>
</tr>
<tr>
<td>---------------------------</td>
</tr>
<tr>
<td>Age(^b) (years)</td>
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<tr>
<td>Gender</td>
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<tr>
<td>Performance status</td>
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<tr>
<td>ECOG 0</td>
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<tr>
<td>ECOG I</td>
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<tr>
<td>ECOG II</td>
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<tr>
<td>pT</td>
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<tr>
<td>T1</td>
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<td>T3</td>
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<td>T4</td>
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<td>pN</td>
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<td>II</td>
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<td>III</td>
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<tr>
<td>IV</td>
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<tr>
<td>Histological tumor type</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td>Large cell carcinoma</td>
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<tr>
<td>Bronchoalveolar carcinoma</td>
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<tr>
<td>Adenosquamous carcinoma</td>
</tr>
<tr>
<td>Unspecified NSCLC</td>
</tr>
<tr>
<td>SCLC</td>
</tr>
<tr>
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<td>G1</td>
</tr>
<tr>
<td>G2</td>
</tr>
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<td>G3-4</td>
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<tr>
<td>Hemoglobin b (g/dl)</td>
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<tr>
<td>LDH(^b) (U/l)</td>
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<tr>
<td>NSE(^b) (ng/ml)</td>
</tr>
<tr>
<td>CEA(^b) (ng/ml)</td>
</tr>
<tr>
<td>Survival time(^c) (days)</td>
</tr>
</tbody>
</table>

\(^a\)% of non-missing values; \(^b\)Mean ± SD; \(^c\)Median. ECOG: Eastern Cooperative Oncology Group performance status, NSCLC: non-small cell lung cancer, SCLC: small cell lung cancer, LDH: lactate dehydrogenase, NSE: neuron-specific enolase, CEA: carcinoembryonic antigen.

Construction of the tissue microarray. The Multiblock\(^{\text{TM}}\)-system, first described by Wasielwski et al. (49) was used. This system requires the microscopic identification of tumor sections on hematoxylin and eosin (H&E)-stained slides and the subsequent marking of these sections on the corresponding paraffin block. Subsequently, three cores of morphologically representative, non-necrotic tumor areas were then punched out with a biopsy needle. A minimal distance of at least 1 mm between the three selected tissue cores of every original donor block was left, wherever possible. The three selected specimens each with a diameter of 1.35 mm were then embedded and melted within the paraffin acceptor.
block. A maximum of 60 punches and one control tissue were placed on one single block. Figure 1 shows the constructed multiblock with the selected tissue samples and the slide corresponding to the paraffin block. The multiblocks were routinely cut with a microtome, serial sections (up to 50 of each TMA) were mounted on capillary gap slides (Dako, Hamburg, Germany) and baked overnight.

**Immunostaining.** After the paraffin was removed in xylene, the TMA slides were dehydrated through a degraded alcohol series and finally washed in distilled water. After target retrieval at pH 9, all the slides were stained simultaneously using a computer-controlled autostainer (Dako TechMate 500) and the Dako-EnVision-System (Dako) (41). To avoid unspecific tissue peroxidase activity, the slides were incubated with Dako Peroxidase Blocking Reagent. The primary monoclonal antibodies used were PankoMab (9) (TA-MUC1, IgG1, κ; 1:10) and A70-C/C8 (8) (Lewis Y, IgM, κ; 1:10) (both from Nemod, Berlin, Germany). After incubation, the slides were processed with the detection reagent (ChemMate Dako EnVision/HRP, Rabbit/Mouse ENV), a dextran backbone coupled with a secondary anti-mouse/anti-rabbit-antibody molecules (raised in goats) and horseradish peroxidase molecules. The marked target antigens were then visualized by a brown deposition, consisting of concentrated diaminobenzidine solution (ChemMate DAB+ Chromogen) and hydrogen peroxide (ChemMate Substrate Buffer), counterstained with hematoxylin and covered with Entellan (Merck, Darmstadt, Germany). In addition to the automated staining process described above, some slides were also manually stained.

In all the experiments, internal controls were included. Every TMA slide contained an additional non-malignant non-lung tissue sample (always negative). Since the lung tissues core size was quite large (1.35×1.35 mm²) and due to the unpredictable three dimensional tissue structure, some of the cores contained partially non-malignant structures.

**Staining criteria.** After IHC staining, all the slides (TMA and conventional) were assessed blindly by three investigators (L.H.S., S.B. and R.W.) according to the immunoreactive score (IRS), first described by Remmle and Stegner (37). For the evaluation of the staining patterns only tumor cells were taken into consideration, excluding also potentially positively staining tumor cell debris. The percentage of positive cells (PP; 1 point: 0-10%, 2 points: 11-50%, 3 points: 51-80%, 4 points: 81-100%) and the staining intensity (SI; 1 point: weak, 2 points: moderate, 3 points: strong) were determined and multiplied. An IRS value ≥3 was considered as a positive and an IRS value ≥9 as a highly positive staining result. The IRS score for the staining evaluation ranges from 0 up to a maximum of 12.

**Statistical analysis.** Results of all the IHC analyses (TMA and conventional) were statistically analysed using SPSS 13.0 software (SPSS GmbH, München, Germany). The study population was described by standard descriptive statistics. In order to compare the IHC staining results from the original tumor blocks with the staining results achieved by the TMA technique, the concordance (Cohen’s kappa), discordance (p-value of McNemar’s test) and the validity (sensitivity, specificity; conventional IHC was considered as reference) were calculated (Table II). In addition to the comparative analysis of the staining results, the clinicopathological correlations and dependencies for antigenetic expressions regarding sex, age, performance status, pT, pN, metastasis, stage, laboratory values and histopathological criteria were assessed using Fisher’s exact test. The prognostic analysis was carried out by two multivariable analyses using a Cox proportional hazards regression (likelihood forward selection model; inclusion criterion: p-value of the score test ≤5%, exclusion criterion: p-value of the likelihood ratio test ≥10%), including sex (male (reference) vs. female), age (<70 (ref.) vs. ≥70 years), tumor size (T1 (ref.) vs. ≥T2), lymphatic spread (N0 (ref.) vs. ≥N1), metastasis (M0 (ref.) vs. M1), stage (I (ref.) vs. ≥II), performance status (ECOG 0 (ref.) vs. ECOG ≥1), grading (G1-2 (ref.) vs. G3-4), adenocarcinoma (no adenocarcinoma (ref.) vs. adenocarcinoma), squamous cell carcinoma (no squamous cell carcinoma (ref.) vs. squamous cell carcinoma), SCLC (no SCLC (ref.) vs. SCLC) and the staining results for TA-MUC1 (negative (ref.) vs. positive (IRS≥3)) and Lewis Y (negative (ref.) vs. positive (IRS≥3)). Two models were considered, one contained TMA-MUC1 and TMA-LeY as potential influencial variables in the first block together with all the other variables listed above except for the MUC1 and LeY results of conventional IHC, which were considered in a second block. In the second approach, the results of conventional IHC were put in the first block and TMA results in the second block.

**Results**

**TMA IHC and conventional IHC.** A total of 216 punches (3 from each of the 72 tumors) were embedded in the TMAs. Due to handling defects and the exhaustion of the Multiblock, 15 cores (7%) could not be taken into statistical account: in 7 cases (3%) , one of the three cores and in 4 cases, (2%) two of the three cores could not be evaluated due to insufficiency of tumor material. In no case were all the three cores of one tumor lost. The required minimal distance of 1 mm in between the three selected tissue probes could not be maintained for 11 of the 72 (15%) originally embedded tumors, as the donor block did not contain enough lung cancer tissue. The positive staining (IRS≥3) and strong staining (IRS≥9) results for TA-MUC1 and Lewis Y expression with the TMA and conventional IHC are shown in Figure 2 and in Table II.

<table>
<thead>
<tr>
<th>Tumor marker</th>
<th>IRS≥3</th>
<th>IRS≥9</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA-MUC1</td>
<td>43</td>
<td>37</td>
</tr>
<tr>
<td>Conv. IHC</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Lewis Y</td>
<td>30</td>
<td>27</td>
</tr>
<tr>
<td>Conv. IHC</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

IRS: Immunoreactive score.
Comparison of automated versus manual TMA staining results. The standard operating procedure (SOP) for automated staining obtained by Dako techmate500 was compared with the SOP for manual staining for both the markers. This comparison revealed an almost absolute agreement with both methods, as a very strong concordance was found for both markers (TA-MUC1: 97.1%, \( p < 0.001 \); Lewis Y: 100%, \( p < 0.001 \)). Mc Nemar's test did not disclose any discordance for this analysis (\( p \)-values for both TA-MUC1 and Lewis Y=1.0). The validity (sensitivity and specificity) of the staining results was \( \geq 97\% \). Thus, in our hands, the different staining procedures were unlikely to cause differences in staining patterns.

Comparison of the staining results for TMA IHC and for the conventional IHC. The TMA results were compared with the conventional IHC results from the original paraffin blocks.
For the positive results (IRS≥3), a concordance of about 80% (TA-MUC1: 83.2%, p<0.001; Lewis Y: 79.7% p<0.001) was calculated. In the strong staining pattern (IRS≥9) cases, the percentage further increased up to at least 90% (p<0.001, all comparisons) (Table III).

McNemar’s test disclosed almost no discordance for Lewis Y (p=0.453). In contrast, for TA-MUC1, the McNemar’s p test confirmed discordant results (p=0.031), as six cases (8%) were regarded as positive by the TMA in contrast to the conventional technique. There were virtually no differences when only the high positive staining patterns were considered (McNemar’s p=1.0, all comparisons) (Table III).

The sensitivity and specificity were ≥80% for a positive expression of both TA-MUC1 and Lewis Y. These rates rose to ≥90% for the high positive expression patterns of both the markers (Table III).

Since we constructed the tissue array with three cores of morphologically representative, non-necrotic tumor areas in a standardized fashion, all analyses presented in this paper were based on the combined results of the three cores. When the analysis of three cores was compared to that of one or two cores for TMA IHC in comparison with conventional IHC, as expected, the sensitivity, specificity, Cohen’s kappa and McNemar’s p increased for all the parameters from one...
Table III. Comparison of staining results. Statistical analysis of TMA IHC with conventional IHC for both markers regarding positive (IRS≥3) and strong positive (IRS≥9) staining patterns. Conventional IHC was considered as the reference (true positive/negative), with sensitivity = true positive / (true positive + false negative) and specificity = true negative / (true negative + false positive).

<table>
<thead>
<tr>
<th>Measure</th>
<th>Marker</th>
<th>IRS≥3</th>
<th>IRS≥9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohen’s kappa</td>
<td>TA-MUC1</td>
<td>83%</td>
<td>93%</td>
</tr>
<tr>
<td></td>
<td>Lewis Y</td>
<td>80%</td>
<td>90%</td>
</tr>
<tr>
<td>McNemar's p</td>
<td>TA-MUC1</td>
<td>0.031</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>Lewis Y</td>
<td>0.453</td>
<td>1.000</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>TA-MUC1</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Lewis Y</td>
<td>93%</td>
<td>92%</td>
</tr>
<tr>
<td>Specificity</td>
<td>TA-MUC1</td>
<td>83%</td>
<td>99%</td>
</tr>
<tr>
<td></td>
<td>Lewis Y</td>
<td>89%</td>
<td>98%</td>
</tr>
</tbody>
</table>

IRS: Immunoreactive score.

Table IV. Clinicopathological dependencies regarding immunohistochemistry. p-values according to Fisher’s exact test for both markers (TA-MUC1, Lewis Y) and both immunostaining techniques (TMA IHC and conventional IHC).

<table>
<thead>
<tr>
<th>Variable (categories)</th>
<th>TA-MUC1</th>
<th>Lewis Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (&lt;70 vs. ≥70 years)</td>
<td>0.803 0.806</td>
<td>0.623 0.316</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>0.009 0.012</td>
<td>0.002 0.002</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>0.014 0.002</td>
<td>0.152 0.082</td>
</tr>
<tr>
<td>Grading (G1-2 vs. G3-4)</td>
<td>0.344 0.477</td>
<td>0.636 0.625</td>
</tr>
<tr>
<td>pT (1 vs. ≥2)</td>
<td>0.089 0.473</td>
<td>0.330 0.808</td>
</tr>
<tr>
<td>pN (0 vs. ≥1)</td>
<td>0.582 1.000</td>
<td>1.000 0.269</td>
</tr>
<tr>
<td>Metastasis</td>
<td>0.438 1.000</td>
<td>0.694 1.000</td>
</tr>
<tr>
<td>Stage (I vs. ≥II)</td>
<td>0.453 0.811</td>
<td>0.806 0.804</td>
</tr>
<tr>
<td>ECOG (0 vs. ≥1)</td>
<td>0.474 0.238</td>
<td>0.811 1.000</td>
</tr>
<tr>
<td>Hemoglobin (&lt;12 vs. ≥12 g/dl)</td>
<td>0.642 0.674</td>
<td>1.000 0.646</td>
</tr>
<tr>
<td>LDH (&lt;260 vs. ≥260 U/l)</td>
<td>0.316 0.622</td>
<td>1.000 0.282</td>
</tr>
<tr>
<td>CEA (&lt;5 vs. ≥5 ng/ml)</td>
<td>0.393 0.148</td>
<td>0.772 1.000</td>
</tr>
<tr>
<td>NSE (&lt;17.5 vs. ≥17.5 ng/ml)</td>
<td>0.392 0.671</td>
<td>1.000 1.000</td>
</tr>
</tbody>
</table>


Clinicopathological correlations and dependencies for MUC1 and Lewis Y. A dependency for the expression of both TA-MUC1 and Lewis Y for adenocarcinomas (TA-MUC1: 83%, p=0.009; Lewis Y: 70%, p=0.002) was shown by Fisher’s exact test (Table IV). This coherence was also found for the conventional staining results (TA-MUC1: 74%, p=0.012; Lewis Y: 65%, p=0.002). Further dependencies were obtained for the expression of the TA-MUC1 antigen and squamous cell carcinomas for both staining techniques (p=0.014 and p=0.002, respectively). No further relationships were detected for all the other clinicopathological variables (Table IV).

Prognostic results for TMA IHC and conventional IHC. The similarity of IHC results was demonstrated with respect to multivariate analysis using the Cox proportional hazards model. For both IHC systems, patient survival improved with detectable expression of TA-MUC1 (p=0.001, TMA and p=0.019, conventional) (Table V) and no prognostic value was demonstrated for Lewis Y (p=0.955, TMA and p=0.259, conventional). Furthermore, SCLC was shown to be an independent prognostic parameter for both IHC systems for both markers (Table V), whereas sex was of borderline dependency. All the other variables included in the model, age, tumor size, lymphatic spread, metastasis and other histolopathological subtypes did not show any prognostic impact with either IHC systems, or either markers (Table V).

Discussion

The main finding of the study was the high concordance, low discordance and validity of TMA IHC when compared to conventional IHC with respect to the staining results (kappa ≥80%) and assessment of the clinicopathological parameters, which applied to both the tumor-associated antigens. Similar concordance of staining patterns between conventional IHC and TMA IHC were also found by other groups for breast tissue (7, 12), fibroblastic malignant tumors (20), colon cancer (19), stomach cancer (16) and lung cancer tissue (27). The discordance analysis displayed good results for positive staining of Lewis Y (p=0.453), however, a somewhat unexpected, but obvious difference for TA-MUC1 staining results (p=0.031) was found on the secondary diagonal, i.e. the TMA technique generated more positive results than the conventional method for this marker. In the original data of these cases, the IRS score was 2 (=negative) with conventional IHC compared to an IRS score 3 or 4 (=positive) with the TMA method. In addition to the possible technical distinctions, these cases, the IRS score was 2 (=negative) with conventional method for this marker. In the original data of these cases, the IRS score was 2 (=negative) with conventional IHC compared to an IRS score 3 or 4 (=positive) with the TMA method. In addition to the possible technical distinctions, these cases, the IRS score was 2 (=negative) with conventional method for this marker. In the original data of these cases, the IRS score was 2 (=negative) with conventional method for this marker. In the original data of these cases, the IRS score was 2 (=negative) with conventional method for this marker. In the original data of these cases, the IRS score was 2 (=negative) with conventional method for this marker.
Although semi-quantitative assessment schemes are commonly used for routine clinical diagnostics and research, they are highly dependent on the observer (13). As demonstrated by a large multicenter trial in Germany, among 172 participating laboratories for the detection of estrogen receptor, the kappa value for the interobserver reproducibility ranged between 41% and 72%, indicating an only moderate concordance (49). Not only is the evaluation critical, but also the expenditure of time is crucial and represents a limiting step in the evaluation process (50).

The sensitivity and specificity for the detection of positive and negative staining was higher than 80% for TA-MUC1 and Lewis Y. In the samples with high positive expression patterns (IRS ≥9), even higher values for concordance (kappa ≥90%), discordance (McNemar’s test: p=1.000), sensitivity (≥90%) and specificity (≥90%) were found for both markers. Precisely those tissues displaying high antigenetic expression patterns might be useful potential targets for immunotherapeutic approaches for lung cancer therapy and could therefore be of special interest for further investigation.

In answer to the question of whether TMAs could reliably be used for clinical studies, equivalent dependencies for all the clinicopathological variables for TMA IHC and conventional IHC and for both markers were demonstrated (Table IV). Of interest, the association of lung adenocarcinoma with the tested tumor antigens has been confirmed by current published results for TA-MUC1 (29,32) and Lewis Y (23), emphasizing the effective use of TMAs for diagnostic lung cancer studies. However, the small p-values for adenocarcinoma vs. marker and squamous cell carcinoma vs. marker could also be artifacts of multiple testing.

Similarly, equal results were also found with the Cox proportional hazards model evaluating eight parameters for their independent prognostic value (Table V). Thus we can recommend the TMA technique for all aspects of lung cancer research, especially when looking at clinicopathological data with regard to the evaluation of a potential prognostic impact of a given marker. However, its clinical use for routine diagnostics should not be recommended, because of the partial insight (12).

The selection of tumor cores from the original tumor block is crucial and should always involve a skilled pathologist. In the present study, due to the relatively large core size of 1.35 mm and the rather low number of 60 cores on one multi-tissue sample block, good orientation on the block and sufficient representation of the original tissue was guaranteed. Altogether, 7% of the tissue cores could not be taken into statistical account. However, this loss rate compared well with other reports in which 9%-37% of the punches were lost during the immunostaining process (10, 20, 27, 30, 31, 40, 42, 49), which was attributed to the transfer of tumor location from H&E stained slides to the tumor block (20, 30), or by the height and the exhaustion of the tissue samples (27). These findings emphasize the need for three selected tumor cores, as the loss of one core would not then automatically exclude one complete case. Additionally with three cores, it is possible to reflect the tumor heterogeneity reliably, as tumors generally display several regions with varying antigenetic patterns.

### Table V. Multivariate survival analysis (TMA IHC and conventional IHC) for the potentially prognostic variables in the Cox proportional hazards model.

<table>
<thead>
<tr>
<th></th>
<th>TMA IHC</th>
<th>Conv. IHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA-MUC1 expression</td>
<td>0.001 0.355 0.188 0.673</td>
<td>0.019 0.463 0.244 0.881</td>
</tr>
<tr>
<td>Lewis Y expression</td>
<td>0.955 - - -</td>
<td>0.259 - - -</td>
</tr>
<tr>
<td>SCLC</td>
<td>0.006 5.183 1.616 16.625</td>
<td>0.002 6.292 1.970 20.098</td>
</tr>
<tr>
<td>Sex</td>
<td>0.108 - - -</td>
<td>0.057 - - -</td>
</tr>
<tr>
<td>All others</td>
<td>&gt;0.1 - - -</td>
<td>&gt;0.1 - - -</td>
</tr>
<tr>
<td>Block 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA-MUC1 expression</td>
<td>0.871 - - -</td>
<td>n.a. e - - -</td>
</tr>
<tr>
<td>Lewis Y expression</td>
<td>0.588 - - -</td>
<td>n.a. e - - -</td>
</tr>
<tr>
<td>TA-MUC1 expression</td>
<td>(TMA IHC) n.a. e - - -</td>
<td>0.059 - - -</td>
</tr>
<tr>
<td>Lewis Y expression</td>
<td>(TMA IHC) n.a. e - - -</td>
<td>0.555 - - -</td>
</tr>
</tbody>
</table>

*P-value according to Cox regression analysis; HR: hazard ratio; CI: confidence interval; all other variables included in the model: age, pT, pN, metastasis, stage, ECOG, grading, adenocarcinoma, squamous cell carcinoma; n.a. e not applicable.
As its clinical use for routine diagnostics should not be recommended, because of the partial insight (12), emphasis should be placed on its beneficial use for research projects. For example, in a TMA-based study Fong et al. demonstrated that TROP2 overexpression was significantly associated with a reduced prognosis in squamous cell carcinoma of the oral cavity (11). Furthermore, the TMA technique can also be used for quality assurance for IHC and ISH (in situ hybridization) procedures, allowing the assessment of local differences in tissue fixation, processing, and staining, leading to the cost-effective assessment of interlaboratory variability in IHC staining (21, 48).

In the future, optical IHC analysis may be replaced by digital image analysis (50) and special data processing (28), enabling the data to be saved and archived in a standardized and uniform way (4) and leading to the production of TMA databases for the identification of gene products of clinical importance (43).

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References


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