Role of Synaptophysin, Chromogranin and CD56 in adenocarcinoma and squamous cell carcinoma of the lung lacking morphological features of neuroendocrine differentiation: a retrospective large-scale study on 1170 tissue samples

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Abstract

Background: Synaptophysin, chromogranin and CD56 are recommended markers to identify pulmonary tumors with neuroendocrine differentiation. Whether the expression of these markers in pulmonary adenocarcinoma and pulmonary squamous cell carcinoma is a prognostic factor has been a matter of debate. Therefore, we investigated retrospectively a large cohort to expand the data on the role of synaptophysin, chromogranin and CD56 in non-small cell lung cancer lacking morphological features of neuroendocrine differentiation.

Methods: A cohort of 627 pulmonary adenocarcinomas (ADC) and 543 squamous cell carcinomas (SqCC) lacking morphological features of neuroendocrine differentiation was assembled and a tissue microarray was constructed. All cases were stained with synaptophysin, chromogranin and CD56. Positivity was defined as > 1% positive tumor cells. Data was correlated with clinico-pathological features including overall survival and disease free survival.

Results: 110 (18%) ADC and 80 (15%) SqCC were positive for either synaptophysin, chromogranin, CD56 or a combination. The most commonly positive single marker was synaptophysin. The least common positive marker was chromogranin. A combination of ≤2 neuroendocrine markers was positive in 2–3% of ADC and 0–1% of SqCC. There was no significant difference in overall survival in tumors with positivity of neuroendocrine markers neither in ADC (univariate: \( P = 0.4 \); hazard ratio [HR] = 0.867; multivariate: \( P = 0.5 \); HR = 0.876) nor in SqCC (univariate: \( P = 0.1 \); HR = 0.694; multivariate: \( P = 0.1 \), HR = 0.697). Likewise, there was no significant difference in disease free survival.

(Continued on next page)
Conclusions: We report on a cohort of 1170 cases that synaptophysin, chromogranin and CD56 are commonly expressed in ADC and SqCC and that their expression has no impact on survival, supporting the current best practice guidelines.

Keywords: Synaptophysin, Chromogranin, CD56, Immunohistochemistry, Non-small cell lung cancer

Background
Synaptophysin, chromogranin and CD56 are recommended markers to identify pulmonary tumors with neuroendocrine differentiation [1]. These markers are frequently used to confirm a diagnosis of typical carcinoid, atypical carcinoid, small cell lung cancer and large cell neuroendocrine carcinoma (LCNEC). In the routine diagnostic setting, particularly the differentiation of LCNEC and pulmonary adenocarcinoma (ADC) with solid growth pattern or non-keratinizing squamous cell carcinoma (SqCC) might be challenging. According to current guidelines only non-small cell carcinomas (NSCLC) that exhibit morphological features of neuroendocrine differentiation should be stained with neuroendocrine markers. In case of a negative result these tumors should be labelled NSCLC with neuroendocrine morphology in biopsy specimens with a comment that the tumor is suspected to exhibit neuroendocrine differentiation that could not be confirmed by immunobiological staining. On the other hand, ADC and SqCC may show the expression of neuroendocrine markers despite the lack of neuroendocrine morphology. The clinical significance in this constellation has been investigated in previous studies [2–11]. While some of the studies suggested an impact of neuroendocrine marker expression on survival [4, 7, 12–16] most of the studies reported no prediction of survival [2, 10, 11]. In this study we investigated over 1000 patient samples to expand the data on the role of synaptophysin, chromogranin and CD56 in NSCLC lacking morphological features of neuroendocrine differentiation.

Methods
Patient cohort
Formalin fixed and paraffin embedded NSCLC specimens resected from 2002 to 2010 in the Thoracic Hospital Heidelberg at Heidelberg University were extracted from the archive of the Institute of Pathology, Heidelberg University, with the support of the tissue bank of the National Center for Tumour Diseases. Tissues were used in accordance with the ethical regulations of the NCT tissue bank defined by the local ethics committee (#S315–2020, NCT#2603). Diagnoses were made according to the recommendations of the 2015 world health classification of tumours of the lung, thymus and heart [1]. One thousand one hundred seventy patients with NSCLC including ADC and SqCC were selected. Tissue microarrays were constructed as described previously [17, 18].

Immunohistochemistry
Immunohistochemical (IHC) staining was performed as previously described [18, 19]. In brief, slides were deparaffinized, pretreated with an antigen retrieval buffer and stained using an automated device. Immunohistochemical stainings were performed on a Ventana Benchmark Ultra (Roche, Switzerland). The antibody and staining conditions are shown in Table 1. The evaluation was carried out by an experienced pathologist (MK). Synaptophysin and chromogranin were considered when located in the cytoplasm, CD56 was evaluated when located on the membrane. Positivity of a marker was defined as > 1% positive tumor cells, as in previous studies [2]. Typical examples of positive and negative staining results of ADC and SqCC are shown in Figs. 1 and 2. The results from the conventional NSCLC markers TTF-1 and p40 were published previously [20, 21].

Molecular data
Molecular data included results for KRAS, EGFR, BRAF, ROS1 and ALK testing were available for ADC from a previous investigation [22]. In brief, cases were analyzed

| Antibody | Company | Clone | Pretreatment | Buffer incubation time (min) | Antibody incubation time (min) | Dilution |
|----------|---------|-------|--------------|-----------------------------|-----------------------------|----------|
| p40      | Ventana | BC28  | Tris/Borat/ EDTA, pH 8.4 | 48                          | 24                          | RTU      |
| TTF-1    | Novocastra | SPT24 | Tris/Borat/ EDTA, pH 8.4 | 56                          | 24                          | 1:100    |
| Synaptophysin | Cell Marque | MRQ-40 | Tris/Borat/ EDTA, pH 8.4 | 48                          | 24                          | RTU      |
| Chromogranin A | Dako | polyclonal | Tris/Borat/ EDTA, pH 8.4 | 32                          | 24                          | 1:400    |
| CD56     | Ventana | MRQ-42 | Tris/Borat/ EDTA, pH 8.4 | 40                          | 24                          | RTU      |

CD cluster of differentiation, TTF-1 thyroid transcription factor 1
Fig. 1 Example of a pulmonary adenocarcinoma positive for neuroendocrine markers. The typical acinar growth pattern of pulmonary adenocarcinoma is seen (a, HE, 200x). Synaptophysin shows homogenous moderate to strong positivity (b, Synaptophysin, 200x). Chromogranin is negative (c, Chromogranin, 200x). CD56 shows focal moderate positivity (d, CD56, 200x).

Fig. 2 Example of a pulmonary squamous cell carcinoma positive for neuroendocrine markers. Typical morphological features of squamous cell carcinoma with local dyskeratosis is seen (a, HE, 200x). Synaptophysin shows focal moderate positivity (b, Synaptophysin, 200x). Chromogranin and CD56 are negative in this example (c, Chromogranin, d, CD56, 200x).
by Sanger sequencing for KRAS (exon 1), EGFR (exons 18–21) and BRAF (exon 15). Cases tested for ROS1 and ALK were prescreen using IHC, results were subsequently validated by fluorescence in situ hybridization (FISH) using a break-apart probe. Only cases with FISH confirmation were considered positive.

Data analysis
Statistical analyses were performed using R-Statistical Software (www.r-project.org, v.4.0.0, Free Software Foundation), R-Studio (v. 1.2.5042, Affero General Public License, USA), or Excel 2019 (Microsoft, USA). Correlation of the immunohistochemical stains with clinicopathological characteristics was by the unpaired t-test for numerical and by the Fisher-Freeman-Halton test for categorical variables. Analysis of overall survival (OS), disease-free survival (DFS) and Kaplan-Meier plots were done with the survival and the survminer package in R. In the multivariate Cox regression model no model selection procedures were applied as we aimed to fit a model with all, from the clinical/diagnostic point of few, main effects and also show the missing impact of statistically not significant variables. P-values < 0.05 were considered significant.

Results
Patient characteristics
Overall, 1170 NSCLC including 627 ADC and 543 SqCC were analyzed. 816 (70%) patients were male, 354 (30%) were female. Median age was 64 years (min-max: 30–89 years). Most patients underwent surgery with pT2 tumors and negative lymph-node status.

Expression of p40, TTF-1, Synaptophysin, Chromogranin and CD56
548 (87%) ADC were positive with antibodies against TTF-1. Only 8 (1%) ADC showed positivity against p40.

These cases also exhibited positivity for TTF-1 in the same tumor cells and showed a typical growth pattern of adenocarcinoma. The vast majority of ADC were negative for p40 (99%). 511 (94%) SqCC were positive with antibodies against p40. Only 6 (1%) SqCC exhibited focal weak TTF-1 positivity. These tumors showed keratinization and intercellular bridges and were therefore classified as SqCC. The majority of SqCC were negative for TTF-1 (99%). None of the ADC and SqCC

| Table 2 | IHC staining characteristics of ADC and SqCC tumors |
|---------|-----------------------------------------------|
|         | ADC  | SqCC |
| Patients, n | 627  | 100  | 543  | 100  |
| General NSCLC markers |     |      |      |      |
| TTF1    |      |      |      |      |
| Positivity | 548  | 87   | 6    | 1    |
| Negativity | 79   | 13   | 537  | 99   |
| p40     |      |      |      |      |
| Positivity | 8    | 1    | 511  | 94   |
| Negativity | 619  | 99   | 32   | 6    |
| Positivity for neuroendocrine marker |     |      |      |      |
| Overall |     |      |      |      |
| Synaptophysin | 84  | 13   | 20   | 4    |
| Chromogranin A | 16  | 3    | 4    | 1    |
| CD56 |      |      |      |      |
| Synaptophysin / Chromogranin A | 12  | 2    | 1    | 0    |
| Synaptophysin / CD56 | 19  | 3    | 4    | 1    |
| Chromogranin A / CD56 | 10  | 2    | 2    | 0    |
| Synaptophysin / Chromogranin A / CD56 | 10  | 2    | 0    | 0    |

ADC adenocarcinoma, IHC immunohistochemistry, NSCLC non-small cell lung carcinoma, SqCC squamous cell carcinoma
*Overall positivity was defined as positivity for ≥1 neuroendocrine marker

Fig. 3 Upset plots indicating the proportion of neuroendocrine marker positivity in ADC and SqCC. a ADC, adenocarcinoma; b SqCC, squamous cell carcinoma
showed morphological features of neuroendocrine differentiation. Overall, 110 (18%) ADC and 80 (15%) SqCC were positive for either synaptophysin, chromogranin, CD56 or a combination of these. The most commonly positive single marker was synaptophysin in ADC (13%) and SqCC (4%). The least common positive marker was chromogranin in ADC (3%) and CD56 in SqCC (1%). A combination of either two or three neuroendocrine markers was positive in 2–3% of ADC and 0–1% of SqCC. A summary of the expression of p40, TTF-1 and the neuroendocrine markers is provided in Table 2 and Fig. 3. No significant difference of gender, age, T- and N-categories as well as clinical stage were observed between ADC and SqCC with and without expression of neuroendocrine markers (Tables 3 and 4).

### Table 3 ADC patient characteristics and stratification by neuroendocrine marker

| ADC overall cohort | ADC neuroendocrine marker positive | ADC neuroendocrine marker negative | p value |
|--------------------|-----------------------------------|-----------------------------------|---------|
| n                  | %                                 | n                                 | %       | n       | %       |         |
| Patients           | 627 100                           | 110 100                           | 517 100 |         |
| Gender             |                                   |                                   |         |         |
| Male               | 365 58                            | 67 61                             | 298 58  | 0.528   |
| Female             | 262 42                            | 43 39                             | 219 42  |         |
| Age, median y (range) | 63 (30–89)                      | 63 (41–84)                       | 63 (30–89) | 0.373   |
| TNM                |                                   |                                   |         |         |
| pT                 |                                   |                                   |         |         |
| pT1                | 127 20                            | 25 23                             | 102 20  | 0.535   |
| pT2                | 388 62                            | 63 57                             | 325 63  |         |
| pT3                | 94 15                             | 17 15                             | 77 15   |         |
| pT4                | 18 3                              | 5 5                               | 13 3    |         |
| pN                 |                                   |                                   |         |         |
| pN0                | 314 50                            | 63 57                             | 251 49  | 0.068a  |
| pN1                | 94 15                             | 14 13                             | 80 15   |         |
| pN2                | 192 31                            | 28 25                             | 164 32  |         |
| pN3                | 5 1                               | 0 0                              | 5 1     |         |
| pNX                | 22 4                             | 5 5                               | 17 3    |         |
| pM                 |                                   |                                   |         |         |
| pM1                | 26 4                             | 2 2                               | 24 5    |         |
| pMX                | 601 96                            | 108 98                            | 493 95  |         |
| Stage              |                                   |                                   |         |         |
| I                  | 254 41                            | 46 42                             | 208 40  | 0.153b  |
| II                 | 130 21                            | 29 26                             | 101 20  |         |
| III                | 217 35                            | 33 30                             | 184 36  |         |
| IV                 | 26 4                             | 2 2                               | 24 5    |         |
| Genetic aberrations|                                   |                                   |         |         |
| KRAS               | 147c 36                           | 29d 36                           | 118e 36 | 0.732f  |
| EGFR               | 64c 16                            | 10f 8                            | 52 16   |         |
| BRAF               | 14c 3                             | 3f 3                             | 12 4    |         |
| ROS1               | 5e 1                             | 1e 1                             | 4 1     |         |
| ALK                | 5e 1                             | 0f 0                             | 5 2     |         |

ADC adenocarcinoma, M metastases, N nodal stage, T tumor size, y year

*aN0 versus pN1/pN2/pN3; pNX not included
*bStage I versus II versus III/IV
*cAvailable for 405 cases
*dAvailable for 80 cases
*eAvailable for 327 cases

Survival analysis

OS was analyzed in patients with ADC and SqCC with respect to the expression of neuroendocrine markers. Although survival in ADC and SqCC with expression of neuroendocrine marker expression was better, but no significant difference was detected in univariate OS analysis in ADC (P = 0.4; hazard ratio [HR] = 0.867; 95% confidence interval [CI95 = 0.622–1.207]) and SqCC (P = 0.1; HR = 0.694 [CI95 = 0.462–1.042]. Likewise, no
significant difference was detected in univariate DFS in ADC (P = 0.4; HR = 1.136; CI95 = 0.832–1.136) and SqCC (P = 0.3; CI95 = 0.448–1.260). Kaplan-Meier plots are shown in Figs. 4 and 5.

Multivariate Cox-proportional hazard analysis for OS showed a significant impact of clinical stage and gender in ADC, but only of clinical stage in SqCC. No significance of neuroendocrine marker expression was detected for ADC and SqCC regarding OS in multivariate analysis (Tables 5 and 6).

**Discussion**

In the present study we investigated the impact of the expression of synaptophysin, chromogranin and CD56 in ADC and SqCC without neuroendocrine morphology on overall survival in a large study including more than

### Table 4 SqCC patient characteristics and stratification by neuroendocrine marker

| SqCC overall cohort | SqCC neuroendocrine marker positive | SqCC neuroendocrine marker negative | p value |
|---------------------|------------------------------------|------------------------------------|---------|
| Patients            | n = 543 | n = 80 | n = 463 |          |
| Gender              |         |        |        |          |
| Male                | 451 (83) | 67 (84) | 384 (83) | 0.858   |
| Female              | 92 (17)  | 13 (16) | 79 (17)  |          |
| Age, median y (range) | 65 (38–83) | 64 (40–82) | 65 (38–83) | 0.428   |
| TNM                 |         |        |        |          |
| pT                  |         |        |        |          |
| pT1                 | 106 (20) | 16 (20) | 90 (19)  | 0.645   |
| pT2                 | 324 (60) | 48 (60) | 276 (60) |          |
| pT3                 | 93 (17)  | 15 (19) | 78 (17)  |          |
| pT4                 | 20 (4)   | 1 (1)   | 19 (4)   |          |
| pN                  |         |        |        |          |
| pN0                 | 255 (47) | 35 (44) | 220 (48) | 0.570   |
| pN1                 | 179 (33) | 23 (29)  | 156 (34) |          |
| pN2                 | 98 (18)  | 20 (25)  | 78 (17)  |          |
| pN3                 | 1 (0)    | 0 (0)    | 1 (0)    |          |
| pNX                 | 10 (2)   | 2 (3)    | 8 (2)    |          |
| pM                  |         |        |        |          |
| pM1                 | 8 (1)    | 2 (3)    | 6 (1)    |          |
| pMX                 | 535 (99) | 78 (98)  | 457 (99) |          |
| Stage               |         |        |        |          |
| I                   | 185 (34) | 28 (35)  | 157 (34) | 0.437   |
| II                  | 208 (38) | 26 (33)  | 182 (39) |          |
| III                 | 142 (26) | 24 (30)  | 118 (25) |          |
| IV                  | 8 (1)    | 2 (3)    | 6 (1)    |          |

*M* metastases, N nodal stage, *SqCC* squamous cell carcinoma, *T* tumor size, *y* year

*pN0 versus pN1/pN2/pN3; pNX not included

*Stage I versus II versus III/IV*

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![Fig. 4](image-url) Univariate OS and DFS analysis of ADC cases with regard to positivity and negativity of neuroendocrine marker. Overall positivity was defined as positivity for ≥1 neuroendocrine marker. NM, neuroendocrine marker; OS, overall survival
1000 patients. This is the largest cohort reported on this topic to date. We found that neuroendocrine marker expression is common and is not associated with OS and DFS.

Staining a combination of synaptophysin, chromogranin and CD56 is currently advised to establish evidence of neuroendocrine differentiation in thoracic tumors [23]. However, staining should be restricted to NSCLC exhibiting neuroendocrine differentiation, as it has been shown that ADC and SqCC may exhibit positive staining in 10–30% in most studies [2, 6]. Studies reporting a higher positivity rate were commonly done on whole slides [3] and not on tissue micro-arrays [2, 4, 11], with one exception reporting neuroendocrine marker expression in up to 90% of tumors [5]. Thus, our results are in line with the literature [3, 6, 10]. The differences of the reported positivity rates might also be explained by different cut-offs for the definition of positivity and the application of different antibody clones [5, 10, 11]. We investigated only one cut-off value for positivity and choose a cut-off of > 1% positive tumor cells. This cut-off has been used in other previous studies but is somewhat arbitrary [2, 3]. We decided to use this cut-off as single cell positivity is a physiologic finding in lung tissue and single neuroendocrine cells overgrown by tumor cells and unspecific background staining might not be reliably distinguished from positive tumor cells [24]. Moreover, cut-off values above 1% are rarely helpful in the routine diagnostic setting. Ionescu et al. reported CD56 to be most commonly expressed closely followed by synaptophysin [2], while Sterlacci et al. reported synaptophysin to be the most commonly detected positive marker in ADC and SqCC, as in our study [11]. In line with these large-scale investigations, chromogranin was least commonly expressed in our study.

The impact of neuroendocrine marker expression on survival of patients with ADC and SqCC is controversially discussed. While most investigations found no impact on prognosis, some more recent studies challenged this finding [4, 25, 26]. Feng et al. investigated the impact of neuroendocrine marker expression on OS and DFS in one of the largest cohorts including a total of 451 patients and found a significantly worse survival in patients with tumors expressing neuroendocrine markers [4]. However, another large study including more than 200 ADC and SqCC did not find any prognostic impact neither on OS nor DFS, in line with the findings of our study [2].

Another marker of neuroendocrine differentiation, Insulinoma-associated Protein 1 (INSM1), has been reported to support the diagnosis of neuroendocrine differentiation in thoracic tumors and has the potential to complement the currently recommended neuroendocrine markers [27, 28]. Interestingly, INSM1 has been reported to be more sensitive and specific as compared to the single markers Synaptophysin, Chromogranin and CD56 and was therefore advocated as a first-line stand alone marker or in combination with CD56 to detect neuroendocrine differentiation [28–31]. INSM1 marker expression

### Table 5

Multivariate Cox proportional hazard analysis for OS in ADC

| Variable                             | HR (CI95)     | p value |
|--------------------------------------|---------------|---------|
| Stage II                             | 2.76          | < 0.001*|
|                                      | (1.576–3.581) |         |
| Stage III                            | 4.649         | < 0.001*|
|                                      | (3.276–6.597) |         |
| Stage IV                             | 6.729         | < 0.001*|
|                                      | (3.726–12.155)|         |
| Age (> 59 versus < 59 years)         | 1.036         | 0.809   |
|                                      | (0.776–1.384) |         |
| Gender (female versus male)          | 0.564         | < 0.001 |
|                                      | (0.420–0.757) |         |
| Neuroendocrine marker (positivity versus negativity) | 0.876 | 0.463 |
|                                      | (0.616–1.247) |         |

*n = 617

*as compared to Stage I

OS overall survival
has been suggested to be prognostic in high-grade neuroendocrine neoplasms, but if INSM1 expression has a prognostic impact in ADC or SqCC remains to be investigated [32]. Moreover, we could not detect any differences in the rate of common genetic aberrations in pulmonary ADC when we compared tumors with and without expression of neuroendocrine markers. Although we analyzed a large cohort, these data must be interpreted with caution, because the respective patient subsets were small.

Our study has several limitations: first, the retrospective design of the investigation. Prospective large-scale studies are not available to the best of our knowledge. Second, we used a tissue microarray as a surrogate for the biopsy situation. As only two cores from the whole tumor were investigated, it is not entirely clear if other parts of tumors that were judged negative in our study exhibit neuroendocrine immunoreactivity. This problem is also highlighted by the fact that previous studies on whole slides reported higher rates of neuroendocrine positivity [3]. On the other hand numerous studies comparing the results of tissue microarray studies with the findings from conventional large sections using other biomarkers have shown that all well-established associations between molecular markers and tumor phenotype or patient prognosis can be reproduced with tissue microarrays [33].

Conclusion
In summary, we show that synaptophysin, chromogranin and CD56 are commonly expressed in ADC and SqCC and that their expression as no impact on OS and DFS supporting the current best practice guidelines.

Table 6 Multivariate Cox proportional hazard analysis for OS in SqCC

| Variable | HR (95% CI) | p value |
|----------|------------|---------|
| Stage II | 1.657 (1.135–2.419) | 0.009* |
| Stage III | 2.889 (1.954–4.274) | 0.001* |
| Stage IV | 4.205 (1.298–13.624) | 0.017* |
| Age (> 59 versus < 59 years) | 1.282 (0.900–1.826) | 0.168 |
| Gender (female versus male) | 0.790 (0.504–1.239) | 0.305 |
| Neuroendocrine marker (positivity versus negativity) | 0.697 (0.436–1.113) | 0.131 |

n = 533
OS overall survival
*a as compared to Stage I

Abbreviations
ADC: Adenocarcinoma; CD: Cluster of differentiation; DFS: Disease-free survival; FISH: Fluorescence in situ hybridization; IHC: Immunohistochemistry; INSM1: Insulinoma-associated Protein 1; LCNEC: Large cell neuroendocrine carcinoma; NSCLC: Non-small cell lung cancer; OS: Overall survival; SqCC: Squamous cell carcinoma

Acknowledgements
None.

Authors’ contributions
Design of the study: KK, MK, AW. Construction of TMA: CZ, TM, HW, AW. Contribution of clinical data: TM, CP, MT, HW, ME, FE, FH. Evaluation of IHC staining: MK. Diagnosis: MW, EH, BG, AS, AW, MK. Data analysis: KK, MK. Draft: KK, MK. Review of the manuscript: all authors. The author(s) read and approved the final manuscript.

Funding
The study has been self-funded. Open Access funding enabled and organized by Projekt DEAL.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations
Ethics approval and consent to participate
Ethics approval has been obtained from the ethics committee of the University Heidelberg (#S315–2020). Consent to participate has been given by all patients.

Consent for publication
Not applicable.

Competing interests
There are no competing interests.

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Received: 7 June 2020 Accepted: 2 April 2021
Published online: 01 May 2021

References
1. Travis WD, Brambilla E, Nicholson AG, Yatabe Y, Austin JHM, Beasley MB, et al. The 2015 World Health Organization Classification of lung tumors: impact of genetic, clinical and radiologic advances since the 2004 classification. J Thorac Oncol. 2015;10(9):1243–60. https://doi.org/10.1097/JTO.0000000000000630.
2. Ionescu DN, Treaba D, Gilks CB, Leung S, Renouf D, Laskin J, et al. Nonsmall cell lung carcinoma with neuroendocrine differentiation—an entity of no clinical or prognostic significance. Am J Surg Pathol. 2007;31(1):26–32. https://doi.org/10.1097/01.pas.0000231319.64919.97.
3. Howe MC, Chapman A, Kerr K, Douglas M, Anderson H, Hasleton PS. Neuroendocrine differentiation in non-small cell lung cancer and its relation to prognosis and therapy. Histopathology. 2005;46(2):195–201. https://doi.org/10.1111/j.1365-2559.2005.02047.x.
4. Feng J, Sheng H, Zhu C, Qian X, Wan D, Su D, et al. Correlation of neuroendocrine features with prognosis of non-small cell lung cancer.
16. Gajra A, Tatum AH, Newman N, Gamble GP, Lichtenstein S, Rooney MT, Kriegsmann M, Muley T, Harms A, Tavernar L, Goldmann T, Dienemann H, Kazdal D, Endris V, Allgauer M, Kriegsmann M, Leichsenring J, Volckmar AL, Kowalski DM, Krzakowski M, Jaskiewicz P, Olszewski W, Janowicz-Zebrowska K, Cremer M, Zgorzelski C, Harms A, Muley T, Winter H, et al. Large-scale comparative analyses of immunomarkers for diagnostic subtyping of non-small-cell lung cancer biopsies. Histopathology. 2012;61(6):1017–25. https://doi.org/10.1111/j.1365-2553.2012.04308.x.

21. Kriegsmann K, Cremer M, Zgorzelski C, Harms A, Muley T, Winter H, et al. Agreement of CK5/6, p40, and p63 immunoreactivity in non-small cell lung cancer. Pathology. 2015;47(5):240–5. https://doi.org/10.1016/j.pathol.2015.11.009.

22. Warth A, Penzel R, Lindenmaier H, Brandt R, Stenzinger A, Herpel E, et al. EGFR, KRAS, BRAF and ALK gene alterations in lung adenoscarcinomas: patient outcome, interplay with morphology and immunophenotype. Eur Respir J. 2011;37(1):21–33. https://doi.org/10.1183/09031936.0018013.

23. Yatabe Y, Dacic S, Borczuk AC, Warth A, Russell PA, Lantejouls S, et al. Best practices recommendations for diagnostic immunohistochemistry in lung cancer. J Thorac Oncol. 2019;14(3):377–407. https://doi.org/10.1016/j.jtho.2018.12.005.

24. Cutz E. Neuroendocrine cells of the lung. An overview of morphologic characteristics and development. Exp Lung Res. 1982;3(3):41–85. https://doi.org/10.1080/10647938209547047.

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