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CHAPTER 8

CLASSIFICATION OF LOW-GRADE NEUROENDOCRINE TUMORS OF MIDGUT AND UNKNOWN ORIGIN

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Classification of low-grade neuroendocrine tumors

Abstract
Metastasized neuroendocrine tumors of the gastrointestinal tract and of unknown origin show a highly variable clinical course. Within this group, low-grade and high-grade malignant tumors can be recognized based on the 'revised classification of neuroendocrine tumours of the lung, pancreas and gut' by Capella et al in 1995. The present study investigated whether fine-tuning the prediction of prognosis was possible by dividing the group of low-grade malignant tumors of midgut and of unknown origin into typical and atypical carcinoids by grading them according to the World Health Organisation classification (WHO) criteria for neuroendocrine tumors of the lung. Moreover, the prognostic value of immunohistochemical stainings and clinical parameters was evaluated. The study group comprised patients diagnosed between 1983 and 1999 with liver metastases of a neuroendocrine tumor of the midgut (n= 40) or of unknown origin (n= 16). As a control for the consistency of grading, 10 patients with metastasized neuroendocrine tumors of the lung were evaluated as well. Immunohistochemical stainings for chromogranin A, synaptophysin, Leu 7/CD57, NCAM/CD56, cytokeratin 8, bcl 2, p53, Ki67 and HER2/neu were performed. The clinical parameters age, gender, urinary 5-HIAA level and presence or absence of the carcinoid syndrome were evaluated.
Tumors of the midgut and of unknown origin were evaluated together, because they were clinically similar. In this group of 56 patients, both the Capella- and the WHO-classification systems recognized the high-grade malignant tumors with a bad prognosis. When the low-grade malignant tumors (Capella) were divided into typical and atypical carcinoids (WHO) no difference in survival was observed, but when the dichotomy into typical and atypical was based on mitotic count alone, the difference became borderline significant (p= 0.072). Of the immunohistochemical stainings used, synaptophysin, cytokeratin 8 and Ki67 had limited prognostic value. Age above 60 was the only clinical parameter of unfavorable prognostic significance.
We conclude that high-grade malignant neuroendocrine tumors of the midgut and of unknown origin are recognized by both the Capella classification and by the WHO classification of neuroendocrine tumors of the lung. Further subdividing low-grade malignant tumors at this location appears of less value than in the lung, but assessing the mitotic activity of these tumors might be of prognostic value.

Introduction
The term 'Karzinoid' was introduced in 1904 by Oberndorfer, who described malignant intestinal tumors with distinct morphologic characteristics that behaved less aggressively than conventional adenocarcinomas in this site. The neuroendocrine origin of carcinoids was proposed by Ciaccio in 1906 and further established by Gosset and Masson during the following decades. Today it is well known that neuroendocrine tumors do not only arise throughout the gastrointestinal tract, but also in the lung, pancreas and, less frequently, in the medi-
Although Oberndorfer already knew that most gastrointestinal neuroendocrine tumors have a better prognosis than gastrointestinal adenocarcinomas, accurate prediction of survival in patients with gastrointestinal neuroendocrine neoplasms remains a difficult task for both clinicians and pathologists. Even in a group of patients with metastatic disease, the clinical course is highly variable. Therefore, more insight into the clinical and histologic features associated with prognosis is needed to optimize patient management. This insight can help the clinician choose the optimal pharmacologic treatment and make a better selection of patients who would benefit from extensive surgical procedures, such as valvular replacement to treat carcinoid heart disease.

Probably the most useful classification for predicting prognosis of neuroendocrine tumors of the gastrointestinal tract is the revised classification of neuroendocrine tumours of the lung, pancreas and gut published by Capella et al in 1995. This classification system evaluates not only the histopathologic criteria of tumor size, angioinvasion, and infiltrative growth, but also the tumor site and whether or not the tumor is functionally active (i.e., causes a clinical syndrome by hormone production). Based on these criteria, in each site neuroendocrine tumors are graded as benign, borderline malignant, low-grade malignant or high-grade malignant. Many patients with gastrointestinal neuroendocrine tumors present with metastatic disease and most of their tumors would be low-grade malignant according to the Capella classification system. The same holds true for patients with neuroendocrine tumors of unknown origin. In these cases of metastatic disease, a disadvantage the Capella classification system is, that in this group of low-grade malignant tumors, it is not possible to distinguish patients with significant differences in survival, although this would be desirable from a clinical standpoint. For neuroendocrine tumors of the lung, a histologic classification system that shows a clear correlation with prognosis was proposed by Travis et al and subsequently adopted by the World Health Organization (WHO). This classification system is now used in daily practice. Based on cellular atypia, mitotic activity and presence or absence of necrosis, pulmonary neuroendocrine tumors are divided into 4 categories: typical carcinoid, atypical carcinoid and high-grade neuroendocrine carcinoma, large-cell type or small-cell type. Until now, no studies have been published investigating whether the WHO classification for neuroendocrine tumors of the lung would be applicable on neuroendocrine tumors of other sites and would be of more prognostic value than other classifications. Thus the present study is the first to examine whether grading metastasized neuroendocrine tumors of the gastrointestinal tract or of unknown origin according to the criteria used for pulmonary neuroendocrine tumors provides a more accurate prediction of survival than the Capella classification. As a control for the consistency of grading, a group of patients with a neuroendocrine tumor of the lung was evaluated similarly. Also evaluated is the prognostic value of immunohistochemical stainings for
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Material and methods

Patients

The study included 56 patients referred to The Netherlands Cancer Institute between 1983 and 1999 with liver metastasis of a neuroendocrine tumor of the midgut or of unknown origin. To establish the site of the primary tumor, CT scans and octreotide- and/or meta-iodobenzylguanide (MIBG) scans were performed in all patients. In all cases the diagnosis of neuroendocrine tumor had been confirmed by histologic examination. The diagnosis of liver metastasis was made on radiologic imaging in all patients, 29 of whom had liver biopsies included in this study. Patients with a second malignancy and patients with mixed tumors were excluded. Applying these criteria, 40 patients with a neuroendocrine tumor of the midgut and 16 patients with a neuroendocrine tumor of unknown origin were included in the study. Ten patients with a metastasized neuroendocrine tumor of the lung served as a control group to validate tumor grading. Clinical data of each patient - sex, age at diagnosis of liver metastasis, site of the primary tumor, level of urinary 5-HIAA, signs and symptoms consistent with the carcinoid syndrome and follow up - were retrieved from the medical records. Survival of the patients was calculated from the date of diagnosis of the liver metastases. The study end point was the patient’s death. Observation time was from 1983 to June 2001.

Histology

For each patient, a tissue sample was available either from the primary tumor or from 1 or more metastases or from the primary tumor and 1 or more metastases (table 8.1).

<table>
<thead>
<tr>
<th></th>
<th>P</th>
<th>M</th>
<th>P+ M</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midgut</td>
<td>(n=40)</td>
<td>7</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td>Unknown</td>
<td>(n=16)</td>
<td>-</td>
<td>16</td>
<td>-</td>
</tr>
<tr>
<td>Lung</td>
<td>(n=10)</td>
<td>5</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 8.1 Number of patients in each group, of whom tissue is available from the primary tumor (P), one or more metastases (M) or the primary tumor and one or more metastases (P+ M). Total=the total number of tissue blocks examined in each group.
Table 8.2 The number of patients in each group of whom slides were available for immunohistochemical staining. M = midgut, U = of unknown origin, L = lung. Chr. A = chromogranin A, Syn = synaptophysin, CK 8 = cytokeratin 8, HER 2 = HER2/neu.

A total of 98 specimens of 66 patients were evaluated, with multiple specimens obtained from 27 patients.

Histologic evaluation was performed on 4μm routinely hematoxylin and eosin-stained slides from formalin-fixed and paraffin-embedded tissue. The slides of both primary tumors and metastases were graded according to the Capella classification and also, regardless of their site, according to the WHO classification of neuroendocrine tumors of the lung. The presence of necrosis was also evaluated separately.

Immunohistochemistry

When unstained slides of the paraffine-embedded tissue were available, immunohistochemical stains for chromogranin A, synaptophysin, CD57/Leu7, CD56/NCAM, cytokeratin 8, p53, bcl2, HER2/neu and Ki67 were performed on tumor sections from all sites in each case (table 8.2).

The stainings were performed according to routinely used methods with the antibodies and pretreatment listed in table 8.3.

The Ki67 staining was evaluated by counting the number of positive tumor cells per 1 mm². For the other stainings tumor cell positivity was divided into 6 categories: 0%, <5%, 5 to 25%, 25 to 50%, 50 to 75% and 75 to 100%. Because the results of most stainings fell in either the highest or lowest categories, for practical purposes staining of <25% of the tumor cells was considered negative, and staining of >25% of the tumor cells was considered positive. Only membrane staining was considered positive for CD56/NCAM and HER2/neu; cytoplasmic staining was regarded as background.

If stains from more than 1 site in 1 patient were available, then the site with the highest histologic grade was used for statistical analysis. If the sites did not differ in histologic grade, the ‘most unfavorable’ result was used, that is, the highest value.
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<table>
<thead>
<tr>
<th>Antibody</th>
<th>Dilution</th>
<th>Source</th>
<th>Pretreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromogranin A</td>
<td>1:2000</td>
<td>NeoMarkers, Freemont, CA</td>
<td>None</td>
</tr>
<tr>
<td>Synaptophysin</td>
<td>1:400</td>
<td>DAKO, Glostrup, Denmark</td>
<td>Pronase</td>
</tr>
<tr>
<td>CD57/Leu7</td>
<td>1:100</td>
<td>Becton Dickinson, Franklin Lakes, NJ</td>
<td>Retrieval</td>
</tr>
<tr>
<td>CD56/NCAM</td>
<td>1:1600</td>
<td>NeoMarkers, Freemont, CA</td>
<td>Retrieval</td>
</tr>
<tr>
<td>Cytokeratin 8</td>
<td>1:250</td>
<td>Becton Dickinson, Franklin Lakes, NJ</td>
<td>Pronase</td>
</tr>
<tr>
<td>P53</td>
<td>1:8000</td>
<td>DAKO, Glostrup, Denmark</td>
<td>Retrieval</td>
</tr>
<tr>
<td>Bcl 2</td>
<td>1:100</td>
<td>DAKO, Glostrup, Denmark</td>
<td>Retrieval</td>
</tr>
<tr>
<td>HER2/neu</td>
<td>1:10000</td>
<td>NeoMarkers, Freemont, CA</td>
<td>Retrieval</td>
</tr>
<tr>
<td>Ki67</td>
<td>1:1200</td>
<td>DAKO, Glostrup, Denmark</td>
<td>Retrieval</td>
</tr>
</tbody>
</table>

**Table 8.3** Immunohistochemical materials and methods. Mouse M. = mouse monoclonal antibody, Rabbit P. = rabbit anti-human polyclonal antibody.

for p53, bcl2, HER2/neu and Ki67 and the lowest value for chromogranin A, synaptophysin, Leu7/CD57 and cytokeratin 8. Because the literature provides inconsistent data on the relationship between prognosis and CD56/NCAM expression, analyses with both the highest and the lowest value of CD56/NCAM were performed.
Statistical analysis

SPSS 10.0 (SPSS, Chicago, IL) was used for all statistical analyses. Survival was calculated using the Kaplan-Meier method. Univariate analysis of clinical and histologic data as prognostic variables was performed using the log-rank test. Cox’s proportional hazards regression model was used to identify independent prognostic variables. The significance of categorical differences between groups was calculated using Fisher’s exact test. Pearson’s correlation test was used to determine the correlation between the number of Ki67-positive cells and mitotic activity. P-values ≤ 0.05 were considered statistically significant.

Results

Clinical parameters

The clinical parameters of the patients in each group are shown in table 8.4. The groups show a similar sex and age distribution and generally the expected correlation between normal or elevated levels of 5-HIAA and the absence or presence of the carcinoid syndrome. Only 5 patients with tumors of the midgut and 2 patients with tumors of unknown origin had elevated 5-HIAA levels without the carcinoid syndrome and 1 patient with a tumor of unknown origin suffered from the carcinoid syndrome, but had a normal 5-HIAA level. Because the patients with neuroendocrine tumors of the midgut and the patients with neuroendocrine tumors of unknown origin did not show statistically significant differences in the aforementioned clinical characteristics, these groups have been evaluated together as 1 group of 56 patients in the analyses described here. Median follow up in this group was 44 months. Of the 56 patients, 42 died of tumor-related causes.

<table>
<thead>
<tr>
<th></th>
<th>Midgut (n=40)</th>
<th>Unknown (n=16)</th>
<th>Lung (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>16</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Female</td>
<td>24</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Age</td>
<td>40-83 (median 62)</td>
<td>37-79 (median 62)</td>
<td>37-83 (median 61)</td>
</tr>
<tr>
<td>Elevated 5-HIAA</td>
<td>35/39</td>
<td>13/16</td>
<td>5/9</td>
</tr>
<tr>
<td>Carcinoid syndrome</td>
<td>29/40</td>
<td>12/16</td>
<td>5/9</td>
</tr>
</tbody>
</table>

Table 8.4 The clinical characteristics of the patients in each group.
Classification of low-grade neuroendocrine tumors

<table>
<thead>
<tr>
<th></th>
<th>Midgut/Unknown origin (n=56)</th>
<th>Lung (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capella, low grade</td>
<td>54</td>
<td>8</td>
</tr>
<tr>
<td>Capella, high grade</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>WHO, typical carcinoid</td>
<td>37</td>
<td>4</td>
</tr>
<tr>
<td>WHO, atypical carcinoid</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>WHO, high grade</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 8.5 Neuroendocrine tumors of midgut and unknown origin and pulmonary neuroendocrine tumors graded according to the Capella classification and according to the WHO classification of neuroendocrine tumors of the lung.

Median follow up in the group of patients with pulmonary tumors was 33 months. During this period, 8 patients died of tumor-related causes. Statistical analysis did not show significant differences in the clinical parameters between the group of 56 patients with tumors of the midgut and of unknown origin and the group of 10 patients with tumors of the lung. Moreover, univariate analysis of the clinical parameters showed that age above 60 years was the only clinical parameter of prognostic significance in both groups (p=0.02 and p=0.03 respectively); older patients had a significantly shorter survival.

Histology

The results of grading all tumors according to the Capella classification and according to the WHO classification of neuroendocrine tumors of the lung are summarized in Table 8.5.

To evaluate the grading methods, the tumors of the 10 patients with metastasized neuroendocrine tumors of the lung were analyzed first. In this group no discrepancies in grade were found by either the Capella or the WHO classification, when more than 1 site in 1 patient was examined. Both classifications recognized the same 2 tumors as high-grade malignant. In the lung, high-grade malignant tumors had a statistically significant worse prognosis than tumors of low-grade malignancy (Capella) or typical and atypical carcinoids (WHO) (p=0.0100 in both classifications). The difference in survival between typical carcinoids and atypical carcinoids showed a trend toward significance (p=0.085). The 4 patients with atypical carcinoids all died with a mean survival of 29 months, whereas 2 of the 4 patients with typical carcinoids survived, with a mean follow-up of 53 months.
In the group of 56 patients with tumors of the midgut or of unknown origin, discrepancies in grade between different sites in 1 patient were found in 1 case using the Capella classification (1 metastasis of low-grade malignancy and 1 metastasis of high-grade malignancy) and in 5 cases using the WHO classification (2 typical carcinoid primaries with atypical carcinoid metastases, 2 atypical carcinoid primaries with typical carcinoid metastases and 1 unknown primary with an atypical carcinoid metastasis and a high-grade malignant metastasis). In these patients, the highest grade was used for statistical analysis. By applying the Capella classification, 2 of the 56 tumors were graded as high-grade malignant, whereas the WHO classification recognized 3 tumors as high-grade in this group.

Analysis of grade related to survival showed that both classifications recognized the high-grade malignant tumors with a bad prognosis on 1 side and the tumors with better survival rates - that is, of low-grade malignancy (Capella) or typical and atypical carcinoids (WHO) - on the other side (p = 0 and p = 0.0039 respectively). Fine-tuning of predicting survival in the group of patients with metastatic low-grade malignant disease by grading according to the WHO classification of neuroendocrine lung tumors seemed impossible; the difference in
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survival between patients with metastasized typical carcinoid and patients with metastasized atypical carcinoid was not statistically significant (p=0.2). However, the WHO classification is based on 2 parameters: mitotic activity and necrosis. If the low-grade malignant tumors were divided into 2 groups, based on mitotic activity alone, using a threshold of 2 mitoses/2 mm², then the difference in survival showed a trend toward significance (p=0.072, Fig 8.1). In addition, the prognostic value of the presence or absence of necrosis was analyzed separately. All high-grade tumors and 5 low-grade tumors of midgut or unknown origin showed necrosis, but this parameter had no prognostic significance.

**Immunohistochemistry**

The results of the immunohistochemical stainings are shown in table 8.6. The neuroendocrine markers chromogranin A, synaptophysin and CD57/Leu7 are positive in 95 to 100% of both pulmonary tumors and tumors of the midgut.

<table>
<thead>
<tr>
<th></th>
<th>M/U TC</th>
<th>M/U AC</th>
<th>M/U High grade</th>
<th>Lung TC</th>
<th>Lung AC</th>
<th>Lung High grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chr.A</td>
<td>29/31</td>
<td>14/16</td>
<td>2/3</td>
<td>4/4</td>
<td>3/4</td>
<td>0/2</td>
</tr>
<tr>
<td>Syn</td>
<td>27/31</td>
<td>12/15</td>
<td>2/3</td>
<td>4/4</td>
<td>2/4</td>
<td>2/2</td>
</tr>
<tr>
<td>CD57/Leu7</td>
<td>28/31</td>
<td>14/15</td>
<td>1/3</td>
<td>2/4</td>
<td>2/4</td>
<td>0/2</td>
</tr>
<tr>
<td>CD56 low</td>
<td>13/24</td>
<td>8/15</td>
<td>2/3</td>
<td>2/3</td>
<td>3/4</td>
<td>2/2</td>
</tr>
<tr>
<td>CD56 high</td>
<td>15/24</td>
<td>9/15</td>
<td>2/3</td>
<td>2/3</td>
<td>3/4</td>
<td>2/2</td>
</tr>
<tr>
<td>Cytokeratin 8</td>
<td>28/30</td>
<td>14/16</td>
<td>2/3</td>
<td>3/3</td>
<td>3/4</td>
<td>1/2</td>
</tr>
<tr>
<td>P53</td>
<td>0/31</td>
<td>0/15</td>
<td>1/3</td>
<td>0/3</td>
<td>1/4</td>
<td>2/2</td>
</tr>
<tr>
<td>Bcl 2</td>
<td>0/29</td>
<td>1/15</td>
<td>2/3</td>
<td>1/4</td>
<td>3/4</td>
<td>2/2</td>
</tr>
<tr>
<td>HER2/neu</td>
<td>0/24</td>
<td>0/15</td>
<td>0/3</td>
<td>0/3</td>
<td>0/4</td>
<td>0/2</td>
</tr>
<tr>
<td>Ki67</td>
<td>0/31</td>
<td>0/15</td>
<td>2/3</td>
<td>0/4</td>
<td>0/4</td>
<td>2/2</td>
</tr>
</tbody>
</table>

**Table 8.6** Results of the immunohistochemical stainings according to grade and primary site. The number of positives per number of cases stained, are indicated. M/U=tumors of midgut or unknown origin, TC=typical carcinoid, AC=atypical carcinoid, Chr.A=chromogranin A, Syn=synaptophysin. When more than 1 site was stained for CD56/NCAM and discrepancies were found, the analysis was done with the lowest score (CD56 low) as well as with the highest score (CD56 high).
CHAPTER 8

or unknown origin. Bcl2 is only occasionally positive in the latter group. In both pulmonary tumors and tumors of midgut and unknown origin, the number of Ki67-positive cells correlates with the mitotic activity of the tumor. Pearson's coefficient is 0.88 when low- and high-grade malignant tumors are analyzed together and 0.55 when only low-grade tumors are analyzed. In both groups, high-grade tumors are recognized in the Ki67 staining, when a threshold of 1000 positive nuclei per mm$^2$ is used. The number of cases showing positivity for p53 was too small (midgut or unknown origin, n=1; lung, n=3), to allow reliable statistical analysis. Low or absent expression of cytokeratin 8 or synaptophysin in tumors of midgut or unknown origin turns out to be an unfavorable prognostic factor in both the group of low-grade tumors and in the group as a whole (cytokeratin 8: all tumors of midgut or unknown origin, p = 0.001; low-grade tumors of midgut or unknown origin, p = 0.006; synaptophysin: all tumors of midgut or unknown origin, p = 0.009; low-grade tumors of midgut or unknown origin, p = 0.03).

Discussion

This study investigated the prognostic value of histologic grading, immunohistochemical stainings, and clinical parameters in a group of 56 patients. The goal was to find a way to fine-tune the prediction of prognosis and thereby optimize patient management in metastasized neuroendocrine tumors of the gastrointestinal tract and of unknown origin. This is the first study in nonbronchial neuroendocrine tumors comparing the prognostic value of 2 histological classifications: the revised classification of neuroendocrine tumors of the lung, pancreas and gut by Capella et al. and the WHO classification of neuroendocrine tumors of the lung. The latter might provide a basis for a more accurate prediction of survival, because it distinguishes 2 categories (typical and atypical carcinoid) in a group of metastasized tumors that would all be graded as low-grade malignant according to the Capella classification system. As a control for the consistency of grading, a group of 10 patients with a metastasized neuroendocrine tumor of the lung was evaluated.

In the group of 56 patients with tumors of the midgut and of unknown origin the tumors graded as high-grade malignant according to the Capella (2 tumors) and the WHO classification (3 tumors), had a significantly worse prognosis (p = 0 and p = 0.0039) than the other tumors in this group. However, when these other tumors (all low-grade malignant according to the Capella classification system) were graded according to the WHO classification system, no significant difference in survival was found between patients with typical and atypical carcinoids (p = 0.2). When only mitotic activity (threshold 2/2 mm$^2$) and not the absence or presence of necrosis was used as a criterium to identify 2 prognostically different groups within the group of low-grade malignant tumors, the difference in survival showed a trend toward significance (p = 0.072).
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In the group of lung tumors both the Capella and the WHO classification systems recognized the high-grade malignant neoplasms with a significantly worse prognosis than the other tumors in the group (p=0.0001). When the low grade malignant tumors were divided into typical and atypical carcinoids, differences in survival showed a trend toward significance (p=0.085). Because the number of patients is limited and all patients have metastasized tumors which influences prognosis regardless of histologic grade, this trend toward significance appears to be in concordance with the expectations based on the literature and indicates that the criteria for grading the tumors were applied correctly. When interpreting these results, it is important to realize that the study group of nonbronchial tumors is a selected group comprising patients with metastatic disease. Finding differences in survival in a group of patients with an unfavorable prognostic factor (i.e., metastases) might require a larger group of patients than was available in the present study to reach statistical significance.

Moreover, it may well be that neuroendocrine tumors of midgut and unknown origin are fundamentally different from neuroendocrine tumors of the lung. Important findings in this respect were recently published by Zhao et al. These authors examined neuroendocrine tumors of the lung and gastrointestinal tract by comparative genomic hybridization and found deletions of chromosome 11q exclusively in 36% of the bronchial tumors and losses of chromosome 18q and 18p in 36% and 33%, respectively, of only the gastrointestinal tumors.

These differences in genetic alterations indicate that neuroendocrine tumors of the lung are histogenetically different from neuroendocrine tumors of the gastrointestinal tract and thus might behave differently. Therefore, a classification developed for grading neuroendocrine tumors of the lung is not necessarily applicable on neuroendocrine tumors of other sites.

A remarkable finding was that in the study group, in contrast to pulmonary tumors, necrosis could not be used as a criterium to distinguish typical and atypical carcinoids. This possibly may be due to the effect of the hormones secreted by gastrointestinal tumors. These often cause (mesenterial) fibrosis and ischemia. Necrosis in these tumors might be ischemic (secondary) necrosis, which does not negatively influence prognosis, instead of true tumor necrosis, which might be a prognostically adverse characteristic of the neoplasm itself.

Of the investigated immunohistochemical stainings investigated, Ki67, cytokeratin 8 and synaptophysin had at least some prognostic value. Bel 2 was not correlated to survival, but was more often expressed in the pulmonary tumors. This is in concordance with the results of other studies. Positivity for p53 and bcl 2 is mainly, although not exclusively, reported in neuroendocrine tumors of the lung, especially in small cell carcinomas. Their role in neuroendocrine tumors of the gastrointestinal tract seems limited.

A promising study has been published on the amplification and immunohistochemical expression of the epidermal growth factor receptor HER2/neu in neuroendocrine tumors of the gastrointestinal tract, predominantly in metastasized...
tumors. Surprisingly, none of the tumors in the present study - all metastasized by definition - showed any positivity. Although the possibility that this is due to technical differences and to different methods of scoring the immunohistochemical stainings can not be ruled out, the present study does not provide evidence for a role of HER2/neu in neuroendocrine tumors.

Conflicting data on the prognostic significance of NCAM expression have been published, This study found no prognostic value of this marker, but the number of tumors stained was limited. It would be interesting to investigate whether metastases of neuroendocrine tumors lose expression of this cellular adhesion molecule in comparison with the primary tumor.

Several studies have reported the prognostic value of Ki67 positivity in neuroendocrine tumors. However, the correlation between Ki67 positivity and mitotic activity is not taken into account, although this seems to be essential in determining the additional value of a marker for cell proliferation. This relationship appears to depend on tumor type and differentiation. In this study, as expected, the number of Ki67 positive cells correlated better with mitotic activity when high-grade and low-grade malignant tumors were evaluated together than it did in the group of low-grade malignant tumors alone. No additional value of Ki67 staining was seen; high-grade malignant tumors can be easily recognized by counting mitotic figures in an hematoxylin and eosin staining, whereas Ki67 positivity does not show significant differences within the group of low-grade malignant tumors.

In this study the only clinical parameter with (unfavorable) prognostic value was age greater than 60 years. This result is in concordance with other studies in which age turned out to be a significant prognostic factor. Prognostic value of elevated urinary 5-HIAA and the presence of carcinoid syndrome has been described. However, these parameters might not be independent, but might be related to metastatic disease, which is prognostically unfavorable anyway.

The results of the present study support this notion; all patients had metastases, but urinary 5-HIAA level and presence or absence of carcinoid syndrome were not of prognostic significance.

In conclusion, the present study shows that high-grade and low-grade neuroendocrine tumors of the gastrointestinal tract and of unknown origin can be recognized by 2 different classifications. Moreover, mitotic activity of ≥2/2 mm² may recognize tumors with an unfavorable prognosis within the group of low-grade malignant tumors, although more research in this area is needed. A better understanding of the molecular biology and pathogenesis of neuroendocrine tumors might be of crucial importance in further exploring this area.

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