Light microscopical morphometry, immunocytochemistry, and clinical correlations of pituitary adenomas at various stages of oncocyty transformation

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Abstract. In a series of 124 pituitary adenomas displaying oncocyty alterations, we studied the degree of oncocyty transformation by light microscopical and morphometrical means for semiquantitative analysis. We established three groups with different percentages of oncocytyically transformed cells for comparison of clinical and immunocytochemical data. Of the patients, 32.3% exposed adenomas with less than 50% oncocyty alterations (group I), whereas 22.6% showed tumours with oncocyty alterations between 51% and 75% (group II). Oncocyty parts consisting of more than 75% of the tumour cells were found in 41.1% of the patients (group III). All three groups differed in the rate of immunocytochemically positive cases, but not in sex distribution, tumour size, and rate of recurrency. Immunocytochemical analyses for PRL and GH (81 vs 78 adenomas) showed a decline of immunohistochemically positive adenomas with increasing proportions of oncocyty transformation for both hormones. Whereas in group I 38% of the adenomas were PRL-positive and 15% GH-positive, group III displayed only 9% PRL-positive and 3% GH-positive adenomas. The results display the correlation between the increasing volume of oncocyty transformation and its effect on decreasing hormone content in pituitary adenomas.

The identification of oncocytyically transformed pituitary adenomas by light microscopy has been described by several authors (Hamperl 1936, 1962; Kovacs et al. 1974; Landolt & Oswald 1973; Landolt et al. 1978; Saeger 1975). Immunocytochemical methods and electron microscopy for ultrastructural differentiation have increased our knowledge of the structure and hormonal activity of these pituitary alterations (Bauersanm et al. 1976; Horvath & Kovacs 1976; Kalyanaraman et al. 1980; Kovacs & Horvath 1973; Kovacs et al. 1974, 1981; Landolt & Oswald 1973; Landolt 1975, 1978; Paiz & Henninger 1970; Saeger 1975, 1981). Even so, the criteria of the diagnosis of oncocytyoma are rather discrepant.

Some authors use the term oncocytyoma only when increased fine granular cytoplasmic change is evident in nearly every adenoma cell (Kovacs & Horvath 1985). We refer to the definition that more than 50% of the tumour cells must show oncocyty transformation for classification as an oncocytyoma (Saeger 1975; Landolt et al. 1978). In addition to this, adenomas with less than 50% oncocyty parts are defined as adenomas with oncocyty parts (Saeger 1975).

Further examinations of pituitary adenomas revealed that oncocytyomas are much more frequent than previously supposed (Horvath & Kovacs 1976; Kovacs & Horvath 1985; Saeger 1981).

Additional results made obvious that oncocyty adenomas are mostly endocrinologically inactive (Horvath & Kovacs 1976; Kovacs et al. 1981; Landolt 1979). Very few have induced acromegaly (Saeger 1975), hyperprolactinaemia (Kalyanaraman et al. 1980; Rollet et al. 1975) or Cushing's disease (Gjerris et al. 1978).
Therefore, the aim of our present work was to differentiate oncocytomas and adenomas with oncocytyic parts in an extensive series of adenomas by morphometry, and to compare the different groups of oncocytyically transformed pituitary adenomas as to their frequency, immunohistochemical findings and clinical data for a revision of this established classification.

**Material and Methods**

Between September 1975 and December 1984, 571 patients underwent transsphenoidal or transnasal extirpation of a pituitary adenoma in the Department of Neurosurgery, University of Hamburg.

This study is based on 156 adenomas, each of which was pre-classified either as an oncocytoma (more than 50% oncocytos) or as an adenoma with oncocytyic parts (less than 50% oncocytos) by one pathologist (W. Saeger). Adenomas with less than 25% oncocytos or extended necrotic areas were excluded. Endocrine disturbances are listed in Table 1. Intra-operatively, the size and extension of the tumours were measured. In all patients, the sella was definitely enlarged. For comparative reasons, we divided the tumours according to the extracellular extension into three different groups:

- **Size I**: Parasellar extension: up to 1 cm. Suprasellar extension: without compression of the third ventricle.
- **Size II**: Parasellar extension: up to 1 cm. Suprasellar extension: with compression of the third ventricle.
- **Size III**: Parasellar extension: > 1 cm. Suprasellar extension: with compression of the third ventricle.

For light microscopy, the specimens were fixed in glutaraldehyde with cacodylate-buffer and osmium tetoxide and embedded in Epon 812. The tumours were classified on toluidine-blue stained sections of 1 μm thickness.

For morphometry, the slices were photographed with identical primary magnification (400 x) in a Leitz Varioorthomat. Final photographic magnification was 820 x (size of the photographs was 18 x 24 cm). On the basis of 6–8 photographs taken at random from each adenoma, the analysis of the relative amount of oncocytos per adenoma was done under single-blind conditions by one observer.

Cells with more than 50% of fine granular structures in their cytoplasm were defined as oncocytos, cells with 30–50% cytoplasmatic alterations were considered preoncocytos. To determine the relative amount of oncocytos in each adenoma, we computed the non-oncocytic cells of each and calculated the proportional part of oncocytos for every adenoma.

For immunocytochemistry, other parts of the specimens were fixed in Bouin’s solution and embedded in

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Chromophobe adenoma (N3/83) with 35% oncocytyic parts: Oncocytos with granular or vacuolar structures of cytoplasm (↓). Many necrotic cells with shrunken nuclei. Epon semithin section; toluidine blue; magnification 440 x.

![Fig. 1. Chromophobe adenoma (N3/83) with 35% oncocytyic parts: Oncocytos with granular or vacuolar structures of cytoplasm (↓). Many necrotic cells with shrunken nuclei. Epon semithin section; toluidine blue; magnification 440 x.](image-url)
Fig. 2.
Oncocytic adenoma (N9/79) with 73% oncocytic parts: Most cells with granular or cloudy cytoplasm. Epon semithin section; toluidine blue; magnification 440×.

Fig. 3.
Oncocytic adenoma (N28/83) with 95% oncocytic parts: Nearly all adenoma cells with typical oncocytic structure. Epon semithin section; toluidine blue; magnification 440×.
paraffin. We applied the peroxidase-antiperoxidase technique of Sternberger et al. (1970) and used commercial antisera: Anti PRL (Serono, Freiburg, FRG, 1:250) and anti STH (Kabi, Munich, FRG, 1:100).

In order to demonstrate the significance of differences, we used several statistical tests (Student's t-test; comparison of two relativities; Chi² multiple-field-test) and established a probability of error of $P < 0.005$ for all tests (Sachs 1976).

Results

In our collection, 27.3% of all adenomas showed oncocytyc transformation of various degree (156 out of 571). Of these 156 adenomas, 124 (80%) were suitable for morphological and clinical analysis.

For morphometrical comparison, the adenomas were classified as follows: Adenomas with oncocytyc parts between 26% and 50% were defined as adenomas with oncocytyc parts (group I). Tumours with oncocytyc alterations between 51% and 75% (group II) and those with more than 75% oncocytes (group III) were defined as oncocytyomas (Table 1).

Sex and age

Oncocytyc transformation of the adenomas did not depend on the age of the patients in our study. There were 93 (75%) men, 51 (25%) women. Male patients showed a three times higher frequency of oncocytycally transformed pituitary tumours than female patients. This difference was significant for all three groups (Table 2).

An age difference within the two sexes could be confirmed, too, in oncocytycally transformed adenomas.

At the time of operation, the women were on the average 6 years older than the men. The average age was 53 years for the men and 59 years for the women. These data were statistically significant ($t_i = 2.94 > t_{(0.05;59)}$).

Tumour size

The comparison between tumour size and intensity of oncocytyc transformation disclosed no significant differences (Table 3).

<table>
<thead>
<tr>
<th>Group</th>
<th>Relative amount of oncocytically transformed cells</th>
<th>Adenomas</th>
<th>Immunohistology</th>
<th>Clinical hyperfunction</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>26–50%</td>
<td>40</td>
<td>PRL + 11/29 (37.9%)</td>
<td>17 patients with hyperprolactinaemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GH + 4/26 (15.4%)</td>
<td>4 patients with acromegaly</td>
</tr>
<tr>
<td>II</td>
<td>51–75%</td>
<td>28</td>
<td>PRL + 6/17 (35.3%)</td>
<td>17 patients with hyperprolactinaemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GH + 1/17 (5.9%)</td>
<td>no patient with acromegaly</td>
</tr>
<tr>
<td>III</td>
<td>76–100%</td>
<td>56</td>
<td>PRL + 3/35 (8.6%)</td>
<td>14 patients with hyperprolactinaemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GH + 1/34 (2.9%)</td>
<td>1 patient with acromegaly</td>
</tr>
<tr>
<td>I–III</td>
<td>26–100%</td>
<td>124</td>
<td>PRL + 20/81 (24.7%)</td>
<td>48 patients with hyperprolactinaemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GH + 6/77 (7.8%)</td>
<td>5 patients with acromegaly</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>N</th>
<th>%</th>
<th>Significance*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Male</td>
<td>28</td>
<td>70</td>
<td>$z_i = 3.58$</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>12</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Male</td>
<td>22</td>
<td>79</td>
<td>$z_i = 4.27$</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>6</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Male</td>
<td>43</td>
<td>77</td>
<td>$z_i = 5.56$</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>13</td>
<td>23</td>
<td></td>
</tr>
</tbody>
</table>

* $z_i$ values of the statistical difference (a value of $z_{(0.05)} > 1.96$ is interpreted as significant).
Medium adenomas classified as tumour size II showed frequencies varying between 57.9% and 69.2%. Large adenomas (size III) were comparatively rare (between 11.3% and 15.4%). The frequency of small adenomas (size I) varied between 15.4% and 28.9%, depending on our classification. At the time of surgery, a suprasellar extension was demonstrable in 89.5% of all adenomas without any statistical differences between the three groups.

A correlation between sex and tumour extension could be established and was statistically proved (Chi² = 9.14 > Chi²(0.05;2)). Adenomas of size III had a four times higher frequency in the men; tumours in the men were on the average larger than those in the women. Correlations to the age of the patients were not determinable.

Recurrent adenomas were found in all three groups. Rate of recurrence was 21.8% for all patients and did not depend on the rate of oncocytic transformation in our study.

### Table 3.
Comparison of tumour size and oncocytic transformation**.

<table>
<thead>
<tr>
<th>Group</th>
<th>Size I</th>
<th>Size II</th>
<th>Size III</th>
<th>Significance*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>I</td>
<td>11</td>
<td>28.9</td>
<td>22</td>
<td>57.9</td>
</tr>
<tr>
<td>II</td>
<td>4</td>
<td>15.4</td>
<td>18</td>
<td>69.2</td>
</tr>
<tr>
<td>III</td>
<td>15</td>
<td>28.3</td>
<td>12</td>
<td>60.4</td>
</tr>
</tbody>
</table>

* Chi² values of the statistical difference (a value of Chi²(0.05;4) > 9.49 is interpreted as significant).

** 7 patients without description of the sella alterations had to be excluded.

### Immunocytochemical findings
A comparison between the rate of oncocytic transformation and immunocytochemical PRL-content is shown in Table 4.

Adenomas of group I are PRL-positive in 38%, adenomas of group II reveal PRL-positive results in 35%, and the almost totally oncocytic transformed adenomas of group III are PRL-positive in only 9% of our cases. Therefore, the rate of immunocytochemical PRL-positive adenomas declines with increasing part of oncocytic transformation in the adenomas. Immunocytochemical PRL-negative adenomas were more often found in group III (91%) than in group II and I (65% vs 62%).

Similar results were found when investigating the immunocytochemical GH-content and oncocytic transformation (Table 5). The tendency is for the number of immunocytochemical GH-positive adenomas to decrease with a growing degree of oncocytic transformation. GH-positive adeno-

### Table 4.
Comparison of immunocytochemical PRL content and oncocytic transformation.

<table>
<thead>
<tr>
<th>Group</th>
<th>PRL-positive adenomas</th>
<th>PRL-negative adenomas</th>
<th>Significance*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N %</td>
<td>N %</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>11 37.9</td>
<td>18 62.1</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>6 35.3</td>
<td>11 64.7</td>
<td>Chi² = 10.03</td>
</tr>
<tr>
<td>III</td>
<td>3 8.6</td>
<td>32 91.4</td>
<td></td>
</tr>
</tbody>
</table>

* Chi² values of the statistical difference (a value of Chi²(0.05;2) > 5.99 is interpreted as significant).

### Table 5.
Comparison of immunocytochemical GH content and oncocytic transformation.

<table>
<thead>
<tr>
<th>Group</th>
<th>GH-positive adenomas</th>
<th>GH-negative adenomas</th>
<th>Significance*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N %</td>
<td>N %</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>4 15.4</td>
<td>22 84.6</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>1 5.8</td>
<td>16 94.2</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>1 2.9</td>
<td>34 97.1</td>
<td></td>
</tr>
</tbody>
</table>

* Number of adenomas too small for specific statistical test – results may be looked upon as tendencies.
mas were most frequent in group I (15%) and less frequent in group II and III (6% vs 3%) depending on the rate of oncocyty transformation.

Discussion

The classic and so far accepted description of oncocytytically transformed cells in the anterior lobe of the pituitary gland is based on light microscopical findings (Hamperl 1936, 1962). Some authors studied the finely granular or finely vacuolar cytoplasmic alterations with paraffin-embedded and conventionally stained tumour sections, but confirmed that this method does not allow a reliable differentiation between eosinophilic adenomas and adenomas with oncocyty transformation (Kovacs et al. 1974; Landolt & Oswald 1973; Landolt 1975; Saeger 1975; Trouillas et al. 1975).

The granular structure of the cytoplasm in the light microscope is seen as densely arranged mitochondria in the electron microscope (Bausermann et al. 1978; Horvath & Kovacs 1976; Kalyanaraman et al. 1980; Kovacs & Horvath 1973, 1985; Kovacs et al. 1974; Landolt 1975), but electron microscopy is too time-consuming for routine diagnostic. Therefore, we preferred the examination of Epon-embedded toluidine-blue stained semi-thin sections, allowing the identification of oncocyes by their cloudy granular cytoplasmic structure which can be easily differentiated from secretory granules (Kovacs et al. 1974; Landolt et al. 1978; Saeger 1975).

In our study, a broad continuous spectrum of oncocytyt transformation could be seen, but no natural gap was visible. Riedel et al. (1985a) described the use of an artificial subdivision for morphometry when examining the granular density of pituitary adenomas causing acromegaly, and Saeger (1975) classified oncocytes and adenomas with oncocyty parts, depending on a limit of 50% of oncocytytically transformed cells. With reference to these two studies, we formed three different groups of oncocytytically transformed pituitary adenomas to show clinical and immuno-cytochemical correlations to the degree of oncocyty transformation.

The reported rate of pituitary tumours with oncocyty alterations varies between 11.5% (Trouillas et al. 1975) and 37.5% (Kovacs et al. 1974). These inconsistent rates are partly explained by the lack of consistent definitions and probably are due to small numbers of cases. In accordance with the definition of Saeger (1975), who described a rate of 28.7% for oncocyty transformation in pituitary adenomas, the frequency in our series was 27.3%.

As to the patients with oncocytyt pituitary adenomas, our investigations established a men to female ratio of 3 to 1. Similar results were reported by Landolt (1975). In other examinations, the number of reported cases was too small for reliable correlations (Landolt 1978; Trouillas et al. 1975).

Oncocytomas and adenomas with oncocyty parts usually occur in older patients and are rarely diagnosed in patients under 40 years (Kovacs & Horvath 1985). The average age of patients in our series was 56.2 years. In other reports of patients with oncocyty adenomas, the average age varies between 50.5 years (Landolt 1975) and 66 years (Trouillas et al. 1975), but these rates are not comparable with out data, because the number of adenomas was much smaller.

To our knowledge, sex differences with regard to the average age (men 53 years; women 59.4 years) or to the tumour size (men 15.7% tumours of size III; women 3.6% tumours of size III) as found in our series have not been described for oncocyty adenomas until now. They have, however, been mentioned in studies of adenomas inducing hyperprolactinaemia or acromegaly (Riedel et al. 1985a,b). The average rate of tumour recurrency of 21.8% in our study corresponds with the data of Landolt (1975), who described recurrences in 25% of the patients. There were no other correlations between the data and the rate of oncocyty transformation. We, therefore, assume that these variables do not depend on the oncocyty transformation, but need further explanation.

Oncocytytically transformed pituitary cells are predominantly considered as hormonally inactive owing to a degeneration of metabolic function of the mitochondria which possibly induces a cessation of hormone production (Landolt et al. 1978, 1979; Kovacs et al. 1981; Saeger 1981). Endocrinologically active adenomas have rarely been found in series of oncocyty adenomas (Gjerris et al. 1978; Riedel et al. 1985b; Rollet et al. 1975). In some cases, hyperprolactinaemia with PRL plasma...
levels up to 130 µg/l can be found in oncocytically transformed pituitary adenomas owing to an alteration of pituitary inhibiting factor and a decreased suppression of para-adenomous PRL cells (Riedel et al. 1985b). Therefore, immunocytochemistry can lead to clear results in differentiation between active and inactive adenomas in hyperprolactinaemia. Acromegaly is very rare in series of patients with oncocytic adenomas (Saeger 1975). Whether hormone production takes place in the non-oncocytic areas or whether oncocytically transformed pituitary cells are able to produce hormones is not definitely explored (Saeger 1975). Declining rates of immunocytochemically hormone-positive adenomas with increasing parts of oncocytic transformation as established in our series, suggest the greater possibility of the former view (Saeger 1975).

Conclusions

The oncocytically transformed pituitary adenomas should be considered as a separate tumour type, displaying a broad spectrum of continuous oncocytic transformation and, in distinction to other adenohypophysial adenomas, shows the lowest rates of endocrinologic hyperfunction. Morphometrical analysis of the intensity of oncocytic transformation reveals correlations with some clinical and immunocytochemical data, but is not always necessary for differentiation of oncocytomas and adenomas with oncocytic parts. Light microscopical examination of plastic-embedded sections of this particular tumour type is absolutely sufficient for identifying the oncocytic transformation in pathological routine work.

References


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