A critical reappraisal of MIB-1 labelling index significance in a large series of pituitary tumours: secreting versus non-secreting adenomas

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Abstract

Pituitary tumours are usually benign neoplasia, but may have a locally aggressive or malignant evolution. This study aimed to identify factors which mostly influence their proliferative activity, in order to clarify its value for clinical and research purposes. The proliferative index was determined in a prospective series of 132 pituitary tumours as the percentage of monoclonal antibody MIB-1-immunopositive cells and referred to as the MIB-1 labelling index (LI). Its distribution was analysed according to both univariate and multivariate models. A life-threatening pituitary tumour is presented separately. The mean LI was 1.24 ± 1.59%, with significant differences between clinically secreting (CS) and clinically non-secreting (CNS) adenomas. In CS adenomas (n = 65), LI was highly variable and markedly influenced by pre-operative pharmacological treatment (0.80 ± 1.03 vs 2.06 ± 2.39% in treated vs untreated cases, P = 0.009); it decreased with patient’s age (P = 0.025, r = 0.28) and increased with tumour volume and invasiveness. The influence of pre-operative treatment and macroscopic features on LI in this group was confirmed by multivariate analysis. In CNS adenomas (n = 67), LI distribution was less variable than in CS adenomas (P < 0.0001), it was age-independent and correlations with tumour volume, invasiveness or recurrence did not reach significance. In a rapidly growing parasellar tumour, the mean LI was 24% at first surgery and exceeded 50% at second surgery performed 4 months later. LI should be interpreted according to hormone secretion and pre-operative treatment. Unusually high LI values deserve particular attention.

Introduction

Although pituitary tumours are usually benign and slowly growing neoplasms, their evolution is highly variable, with a broad clinical spectrum ranging from apparently steady microadenomas to grossly invasive or exceptionally malignant metastatic tumours. Their management is based on a multidisciplinary approach which includes surgery, radiotherapy and pharmacological therapy with either somatostatin analogues or dopamine agonist (DA) drugs for growth hormone (GH)- and/or prolactin (PRL)-secreting adenomas. Whereas DAs exert a direct anti-mitotic effect on PRL-secreting cells (Bevan et al. 1992), the cytostatic effect of
somatostatin (SMS) analogues is still controversial (Lamberts et al. 1996, Thapar et al. 1997b). With the exception of prolactinomas, surgery remains a first-line therapeutic choice for most pituitary adenomas, the indications for postoperative radiotherapy being still under debate (Jaffrain-Rea et al. 1993, Plowman 1995). Since few correlations have been observed between clinical evolution and histopathological features, highly aggressive or malignant tumours may initially present as common macroadenomas, and reliable markers of cell proliferation are expected to be a useful tool for their therapeutic management.

The pathogenesis of pituitary tumours is currently under extensive investigation, and molecular events closely interact with endogenous/exogenous permissive factors to determine the expansion of monoclonal cell populations (Melmed 1999). Markers of cell proliferation are also attractive for research purposes, by helping to identify factors associated with regulation of tumour growth and/or kinetic activity (Thapar et al. 1997b, Shimon et al. 1998, Tampanaru-Sarmesiu et al. 1998, Yokoyama et al. 1998, Jaffrain-Rea et al. 1999, Nishi et al. 1999, Turner et al. 2000). However, possible pitfalls in its interpretation should be carefully considered.

The MIB-1 antibody recognises the Ki-67 antigen, which is expressed throughout the cell cycle, and the percentage of immunopositive cells represents a reliable proliferation index for the study of human tumours, usually referred to as the MIB-1 labelling index (LI). Because of its applicability to routinely fixed paraffin-embedded sections, the MIB-1 antibody has now replaced the previous anti-Ki-67 antibodies (Cattoretti et al. 1992). The first reports on the use of Ki-67 and MIB-1 antibodies in pituitary tumours were published in 1986 (Landolt et al. 1987) and 1995 (Ekramullah et al. 1995, Gandour-Edwards et al. 1995, Kawamoto et al. 1995) respectively. Yet, despite a wide literature accumulating on this topic, the interpretation of LI in pituitary tumours remains surprisingly conflicting under many aspects (for reviews see Losa et al. 1998, Turner & Wass 1999). This might be explained by various factors, among which the most important may be the following: (i) most papers have focused on a few specific characteristics of the studied tumours, some of them quite heterogeneous, often leading to apparently different conclusions concerning LI variations according to tumour volume/invasivity, recurrence or secreting activity, (ii) the possible influence of age or pre-operative pharmacological treatment was generally not considered, and (iii) only LI mean values were considered, regardless of LI variability, which represents another important feature of LI distribution.

Thus, the aim of the present study was to better define the distribution and biological significance of LI in pituitary tumours by taking into account all the parameters cited hitherto in a large prospective series, using univariate models to compare data with previous studies and multivariate models to critically analyse these results and explain current discrepancies.

Materials and methods

Patients

A hundred and thirty-two consecutive pituitary tumours were operated on over a 4 year period and prospectively studied. Surgery was performed by either a transphenoidal or a transcranial route, 12 tumours being re-operated on during the study period because of tumour regrowth. Detailed clinical, hormonal and radiological information were obtained in all cases before surgery. Clinically secreting (CS) tumours were diagnosed according to currently accepted basal and dynamic hormonal criteria, the lack of bioclinical feature of hormone hypersecretion defining clinically non-secreting (CNS) tumours. Sixty-eight tumours were classified as CNS, whereas 42 presented with acromegaly, six with Cushing’s disease, 15 were PRL-secreting and one was thyrotrophin (TSH)-secreting. At the time of surgery, 31 patients were on medical treatment for CS tumours: 15 were receiving DA drugs for PRL-secreting (n = 9) and/or GH-secreting (n = 6) tumours and 20 were receiving s.c. octreotide (OCT) for active acromegaly (in association with DA drugs in four cases). Only two patients with recurrent CNS tumours had received radiotherapy, but seven patients with CNS had received a pre-operative course of DA drugs because of associated hyperprolactinaemia. Pre-operative NMR imaging was available in all cases. Microadenomas, defined by maximal tumour diameter ≤10 mm, were present in only 14 cases (eight with acromegaly, five with Cushing’s disease and one CNS). Macroscopic features of invasiveness were defined according to pre-operative radiological criteria and intra-operative findings, including macroscopic evidence for dural infiltration. According to such criteria, 48 adenomas were non-invasive and 84 were invasive. Tumours were also classified according to their suprasellar extension (SSE) using Wilson’s criteria (Wilson 1984): no SSE (grade O; n = 28), moderate SSE (grade A/B; n = 68), huge SSE (grade C/D; n = 36). Invasion of the cavernous sinus (grade E according to Wilson) and of the sphenoid sinuses were recorded separately.

Archive material from five patients (four with CNS and one with acromegaly) who were operated on for tumour recurrence during the study period but had previously undergone surgery at the same Neurosurgery Unit was also collected for retrospective determination of the LI. These cases were used only for the study of recurrences. During the prospective period of study, a 33-year-old male patient was also operated on for a parasellar mass which was finally diagnosed as a probable follicle-stimulating hormone (FSH)-secreting pituitary carcinoma. Data are presented as a separate case report and excluded from the statistical analysis.
Tumours

Immunohistochemical determination of pituitary tumour hormonal content was performed on paraffin-embedded tissue sections with polyclonal rabbit antibodies (anti-PRL, anti-GH, anti-FSH, anti-LH, anti-TSH, anti-ACTH) obtained from Dako LSABZ System, HRP, Dako Corporation, Carpinteria, CA, USA), using the streptavidin–biotin detection system. According to such analysis, CNS tumours were mainly represented by null cell adenomas (n = 41) and gonadotroph adenomas (n = 17), the others showing silent ACTH secretion (n = 5) or scattered GH-secreting cells (n = 5). Out of the 42 tumours associated with acromegaly, 21 were pure GH-secreting, 12 were mixed GH/PRL-secreting and seven were mixed GH/glycoprotein-secreting adenomas. Multihormonal adenomas were observed in two tumours associated with acromegaly, the one associated with TSH-dependent hyperthyroidism and one prolactinoma. The remaining prolactinomas and all those associated with Cushing’s disease displayed pure PRL- and ACTH-immunopositivity, respectively.

Evaluation of cell proliferation

The mouse monoclonal antibody (MoAb) MIB-1 (Diagnostic Brokers Associated, Milan, Italy) was used after microwave pretreatment, according to the manufacturer’s instructions. For each sample, immunopositivity was counted on 1000 randomly selected cells by a single observer who was unaware of the bioclinical characteristics of the tumours, and the results were expressed as a percentage of positive cells.

In a representative samples of 40 primary tumours (15 CNS, 25 GH- and/or PRL-secreting adenomas, including 13 treated and 12 untreated), morphological evaluation of nuclear atypia and mitotic index (MI) was also carefully performed on haematoxylin–eosin stained sections. Nuclear atypia were classified as absent, mild, moderate or marked and the MI was determined on 1000 randomly selected cells and expressed as a percentage.

Statistical analysis

Unless otherwise specified, all values are indicated as means ± s.d. The Mann–Whitney U-test and Kruskall–Wallis test were used as appropriate for univariate analysis of unpaired data, and the Wilcoxon ranked test was used for paired comparisons in the study of regrowing tumours. Variations in LI distribution among subgroups were studied by the F-test. Multivariate analysis were performed by MANCOVA. The chi-square test was used to compare percentages. Correlations between the LI and the age of the patients were studied by linear and multiple regression. P < 0.05 was considered significant.

Results

Preliminary results

In order to identify possible bias in the interpretation of LI in this series, a careful preliminary study was performed. Special attention was paid to the comparison of CS and CNS adenomas (Table 1), which showed that patients with CS adenomas were younger (P<0.0001) and presented less voluminous and invasive tumours than those with CNS adenomas (P<0.001 in both cases). In multivariate analysis, tumour volume and invasiveness were consistently found to be strongly linked parameters, but independent from patient’s age and/or pre-operative treatment on the whole series (data not shown).

General results

The mean LI in the whole series was 1.24 ± 1.59%, with a marked variability (see Fig. 1 and Table 2). Pre-operative treatment with either DA or SMS drugs was found to significantly influence mean LI values (0.73 ± 0.97 vs 1.45 ± 1.74% in treated vs untreated tumours respectively, P=0.013) and to markedly reduce its variability (P<0.0001). Accordingly, LI distribution was significantly influenced by the functional characteristics of the tumours and differed between CNS, treated and untreated CS (P=0.016) (Fig. 2). An inverse relationship was also found between LI and the age of the patients in the whole series (P=0.021, r = 0.20, Fig. 3), a similar trend being observed in untreated cases only (P=0.050, r = 0.20).

Factors influencing LI distribution have thus been first evaluated by univariate analysis in untreated pituitary adenomas only (n = 94) (Table 3). Mean LI values tended to increase with tumour volume, but only its variability was significantly higher in macroadenomas and especially in huge tumours. Mean LI values were higher in invasive than in non-invasive adenomas (P=0.04), a similar difference being found between tumours invading the cavernous sinus compared with those which did not (P=0.035), whereas invasion of the sphenoid sinus had no influence on this parameter. No significant difference was found between recurrent and primary tumours. Mean LI values tended to be higher in CS compared with CNS adenomas (P=0.092) and their variability was markedly greater in the former group (P<0.0001). Significant differences were found among the different hormone-secreting groups (P=0.023), the highest values being observed in PRL-secreting adenomas (Fig. 4).

In a subsequent multivariate analysis taking into account both age and pre-operative pharmacological treatment, LI was confirmed to be higher in macro- than in microadenomas (P=0.013), in invasive than in non-invasive adenomas (P=0.023), especially in those invading the cavernous sinus (P=0.006), and to increase with the grade of SSE (P=0.001). It was also confirmed to be correlated with patient’s...
Table 1 Clinical characteristics of clinically secreting (CS) and non-secreting (CNS) pituitary adenomas

<table>
<thead>
<tr>
<th>Pituitary adenomas</th>
<th>n</th>
<th>M/F ratio</th>
<th>Age (years) (means ± s.d.)</th>
<th>Macro-adenomas (%)</th>
<th>Invasive sinus invasion (%)</th>
<th>Cavernous sinus invasion (%)</th>
<th>Sphenoidal sinus invasion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNS adenomas</td>
<td>67</td>
<td>1.50</td>
<td>54.6 ± 12.2</td>
<td>98.6</td>
<td>76.1</td>
<td>58.2</td>
<td>22.3</td>
</tr>
<tr>
<td>CS adenomas</td>
<td>65</td>
<td>0.91</td>
<td>43.5 ± 15.2</td>
<td>78.4</td>
<td>50.8</td>
<td>36.9</td>
<td>10.7</td>
</tr>
<tr>
<td>P (chi-square value)</td>
<td>0.22</td>
<td>1.46</td>
<td>&lt;0.0001</td>
<td>0.0003 (13.2)</td>
<td>0.0025 (9.16)</td>
<td>0.027 (4.90)</td>
<td>0.067 (3.36)</td>
</tr>
</tbody>
</table>

Significant P values are underlined.

Table 2 MIB-1 labelling index (LI) distribution in a series of 132 human pituitary adenomas. LI is expressed as a percentage of immunopositive cells

<table>
<thead>
<tr>
<th>Pituitary adenomas</th>
<th>n</th>
<th>Means ± s.d.</th>
<th>Distribution in percentiles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>10°</td>
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<tr>
<td>Whole series</td>
<td>132</td>
<td>1.24 ± 1.59</td>
<td>0.00</td>
</tr>
<tr>
<td>Untreated</td>
<td>94</td>
<td>1.45 ± 1.74</td>
<td>0.00</td>
</tr>
<tr>
<td>Treated</td>
<td>38</td>
<td>0.73 ± 0.97*</td>
<td>0.00</td>
</tr>
<tr>
<td>Secreting adenomas</td>
<td>65</td>
<td>1.44 ± 1.95</td>
<td>0.00</td>
</tr>
<tr>
<td>Untreated</td>
<td>33</td>
<td>2.06 ± 2.39</td>
<td>0.08</td>
</tr>
<tr>
<td>Treated</td>
<td>32</td>
<td>0.80 ± 1.03**</td>
<td>0.00</td>
</tr>
<tr>
<td>CNS adenomas</td>
<td>67</td>
<td>1.05 ± 1.12</td>
<td>0.00</td>
</tr>
</tbody>
</table>

*P < 0.0001 by F-test vs untreated patients, **P = 0.013 and ***P = 0.09 by Mann–Whitney vs untreated patients.

Figure 1 Labelling index (LI) in 132 consecutive pituitary adenomas.

Figure 2 LI in pituitary adenomas according to their functional characteristics. CNS: clinically non-secreting adenomas; CS(t): clinically secreting adenomas, pre-operative treatment with pharmacological drugs; CS(ut): clinically secreting adenomas, untreated. Mean ± s.d. P < 0.02 by Kruskall-Wallis. *Significant difference between CNS, CS(t) and CS(ut).

Figure 3 Age-related distribution of the LI in 132 consecutive pituitary adenomas.
Table 3 Factors influencing LI values in a series of 94 untreated pituitary adenomas. The number of cases is indicated within brackets for each subgroup. SSE = grade of suprasellar extension according to Wilson (1984).

<table>
<thead>
<tr>
<th></th>
<th>LI (%)</th>
<th>Mann–Whitney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(means ± s.d.)</td>
<td>(+)</td>
</tr>
<tr>
<td>Gender, M (53) vs F (41)</td>
<td>1.58 ± 1.86 vs 1.29 ± 1.58</td>
<td>0.354</td>
</tr>
<tr>
<td>Volume</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Micro- (8) vs macroadenomas (86)</td>
<td>0.60 ± 0.70 vs 1.53 ± 1.79°°</td>
<td>0.108</td>
</tr>
<tr>
<td>SSE grade: O (14) vs A/B (48) vs C/D (30)</td>
<td>0.89 ± 0.90 vs 1.14 ± 1.36 vs 2.12 ± 2.18§,*</td>
<td>0.077</td>
</tr>
<tr>
<td>Invasivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Invasive (65) vs non-invasive (29) adenomas</td>
<td>1.66 ± 1.79 vs 1.00 ± 1.57</td>
<td>0.040</td>
</tr>
<tr>
<td>Cavernous sinus invasion + (49) vs − (45)</td>
<td>1.74 ± 1.87 vs 1.00 ± 1.38°</td>
<td>0.035</td>
</tr>
<tr>
<td>Sphenoidal sinus invasion + (17) vs − (77)</td>
<td>1.37 ± 1.15 vs 1.41 ± 1.65</td>
<td>0.515</td>
</tr>
<tr>
<td>Hormone secretion, CS (33) vs CNS (61) adenomas</td>
<td>2.06 ± 2.40 vs 1.13 ± 1.15°°°</td>
<td>0.092</td>
</tr>
<tr>
<td>Recurrences, recurrent (15) vs primary (79) adenomas</td>
<td>1.62 ± 1.59 vs 1.42 ± 1.78</td>
<td>0.516</td>
</tr>
</tbody>
</table>

Mean values were compared by Mann–Whitney univariate analysis (+: Kruskall–Wallis for SSE) and significant P values are underlined. The variability among subgroups was evaluated by F-test and significant P values are indicated by °P < 0.05, °°P < 0.01 and °°°P < 0.0001, and, for analysis according to SSE grade §P < 0.001 vs grade O, *P < 0.01 vs grade A/B.

Figure 4 LI in 94 untreated pituitary adenomas according to hormone secretion (means ± s.d.).

Possible relationships between LI and regrowth of CNS tumours were studied in two ways, leading to similar results: (i) considering prospective data only, LI was not significantly higher in recurrent than in primary tumours (1.45 ± 1.59 vs 0.95 ± 0.96%, P = 0.32), although its variability was greater in the former group (P = 0.008); (ii) including the analysis of archive material, paired analysis of data obtained in patients who were re-operated on for tumour regrowth failed to disclose significant differences between the two events (0.91 ± 1.05% for primary tumours vs 1.27 ± 1.52% for subsequent recurrences respectively, P = 0.48).

CNS adenomas

No age-related LI variations was observed in this group (P = 0.63, r = 0.06). LI was not significantly higher in invasive than in non-invasive tumours (1.19 ± 1.19 vs 0.62 ± 0.73%, P = 0.11) but its variability was greater in the former group (P = 0.026). Similarly, invasion of either cavernous or sphenoid sinus was not found to individually influence LI and no significant difference could be found according to patient’s gender, to the presence of visual field defects or to the grade of SSE (data not shown). The mean LI was similar in null cell and FSH/LH-secreting adenomas (0.95 ± 1.04 vs 1.00 ± 1.06 respectively, P = NS).

CS adenomas

Pre-operative treatment with either DA or SMS drugs was found to significantly reduce LI values (0.83 ± 1.04 vs age (P < 0.01 in all cases) and to decrease with pre-operative treatment (P < 0.025 in all cases).
Figure 5 A case of huge GH/PRL-secreting adenomas observed in a 21-year-old female patient, operated on before and after a 6 month pharmacological treatment with octreotide (OCT) and cabergoline. The LI decreased from 6% (a) to 2.7% (b) between the first and the second operation. LSABZ method. MoAb MIB-1, ×250.

2.15 ± 2.41% in treated vs in untreated tumours, \( P = 0.009 \) and its variability \( (P < 0.0001) \). An example of LI variations after pharmacological treatment with OCT and cabergoline in a young patient with a huge GH/PRL-secreting adenoma is shown in Fig. 5. A significant negative linear correlation was observed between LI values and the age of the patients \( (P = 0.025, r = 0.28) \).

Univariate analysis performed in untreated CS adenomas \( (n = 34) \) (Table 4) indicated that mean LI values were higher in macro- than in microadenomas \( (P = 0.013) \), and increased with the grade of ESS \( (P = 0.015) \) and invasiveness \( (P = 0.048) \), including invasion of the cavernous sinus \( (P = 0.039) \). LI variability was also higher in macro- than in microadenomas \( (P = 0.003) \) and in male than in female tumours \( (P = 0.048) \).

According to a multivariate analysis taking into account both age and pre-operative treatment, LI was confirmed to be higher in macro- than in microadenomas \( (P = 0.006) \), to increase with tumour invasiveness \( (P = 0.021) \), especially with invasion of the cavernous sinus \( (P = 0.012) \), and with the degree of SSE \( (P = 0.002) \). The difference between treated and untreated patients remained significant when corrected for age, tumour volume and invasiveness \( (P < 0.015 \) in all cases), whereas the correlation with age disappeared, indicating age as a less important parameter in determining LI values.

Analysis of CS subgroups
In order to further analyse the influence of pre-operative pharmacological treatment in GH- and PRL-secreting adenomas, each subgroup was evaluated separately by univariate analysis (Table 5).

Pre-operative plasma GH, insulin-like growth factor-I and PRL values were lower in acromegalic patients who

| Table 4 Factors influencing LI values in a series of 33 untreated CS pituitary adenomas. The number of cases is indicated within brackets for each subgroup. SSE grade: suprasellar extension (Wilson 1984) |
|----------------------------------|------------------|------------------|
| Gender, M (15) vs F (18)          | 2.88 ± 2.76 vs 1.38 ± 1.87*  | 0.048            |
| Invasivity                       | 2.86 ± 2.52 vs 1.22 ± 2.00*  | 0.048            |
| Invasive (16) vs non-invasive (13)| 2.96 ± 2.65 vs 1.20 ± 1.88*  | 0.038            |
| Cavernous sinus invasion + (13) vs – (16) | 0.43 ± 0.29 vs 1.99 ± 2.13 | 0.256            |
| Sphenoidal sinus invasion + (3) vs – (26) | 4.14 ± 2.73§,* | 0.013            |
| Volume                           | 0.54 ± 0.74 vs 2.47 ± 2.53** | 0.015            |
| Micro- (7) vs macroadenomas (22) | 0.88 ± 0.94 vs 1.59 ± 2.26 vs | 0.013            |
| SSE grade: O (10) vs A/B (17) vs C/D (2) | 4.14 ± 2.73§,* | 0.001            |

Mean values were compared by Mann–Whitney univariate analysis (+: Kruskal–Wallis for SSE grade) and significant P values are underlined. The variability among subgroup was evaluated by F-test and the corresponding significant P values are indicated by *P < 0.05 and **P < 0.005, and for SSE grade: $P = 0.001$ vs O and $^*P = 0.009$ vs A/B.
received pre-operative pharmacological treatment compared with those who did not (P = 0.0007, P = 0.003 and P = 0.006 respectively), including those treated by OCT alone (P = 0.0001, P = 0.004 and P = 0.018 respectively). The mean LI and LI variability were significantly higher in untreated GH-secreting tumours compared with treated tumours (P = 0.004 and P = 0.001 respectively), including those treated by OCT alone (P = 0.0009 and P = 0.003 respectively).

Similarly, despite a marked variability, prolactinoma patients who received pre-operative treatment with DA drugs had lower pre-operative plasma PRL compared with those who did not (P = 0.01). Accordingly, mean LI values and LI variability were lower in treated than in untreated prolactinomas (P = 0.025 and P = 0.026 respectively).

Correlations between histological features and MIB-1 index

Mitosis was observed in 8 of the 40 primary tumours studied (20%). The mean MI was extremely low (0.1±0.2%), but strongly correlated with LI by linear regression (P < 0.0001, r = 0.61). In contrast, no significant difference in either MI or LI could be found between tumours presenting with mild/absent atypia (n = 23) compared with those with moderate/marked atypia (n = 17). Similar to data obtained in the whole series, the LI was significantly higher in untreated CS (3.40±3.21%) compared with either treated CS (0.64±0.81%, P = 0.0006) or CNS adenomas (0.97±0.83, P = 0.001) in this subgroup of tumours. Mitosis could be detected only in CS tumours and the MI was significantly higher in untreated CS compared with treated CS (0.15±0.14 vs 0.05±0.08%, P = 0.038). Moderate/marked nuclear atypias were present mostly in CS tumours (60 vs 13.3%, P = 0.002) but were not significantly influenced by pre-operative medical treatment.

A case of rapidly growing parasellar FSH-secreting tumour

A 33-year-old male patient was admitted at the Neurosurgery Unit with a 6 month history of worsening headache with recent diplopia and palpebral ptosis in his right eye, NMR imaging being strongly suggestive for a voluminous cavernous sinus meningioma. After incomplete fronto-temporal surgical resection, histological examination concluded that there was an FSH-secreting pituitary tumour with marked cellular atypia and uncommonly elevated mitotic activity (mean LI = 24%) (Fig. 6a). Three months later, the patient was readmitted because of rapidly evolutive headache, vomiting, complete III° right nerve palsy and decreased vigilance. NMR imaging showed an impressive regrowth of the tumour with hydrocephaly, and the patient died 10 days after re-operation because of neurological complications. No autopsy could be performed. Histologically, the tumour showed an even more aggressive phenotype, with a mean LI exceeding 50% (Fig. 6b). The diagnosis of probable pituitary carcinoma was finally retained on the basis of the rapidly fatal clinical evolution and uncommon proliferative activity.

Discussion

Data presented herein confirm that, in agreement with previous large series (Kitz et al. 1991, Thapar et al. 1996a, Masstronardi et al. 1999, Schreiber et al. 1999) most pituitary tumours have a low LI, with mean values usually less than 3–4% and sporadic cases with higher proliferation rates. Careful analysis of this series and multivariate evaluation of

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**Table 5** Influence of pre-operative pharmacological treatment on GH and/or PRL-secreting adenomas and distribution of functional subgroups according to immunohistochemistry for pituitary hormones. The number of cases is indicated within brackets for each subgroup. (+) in GH-secreting adenomas, 'others' refer to treatment with DA drugs only or in association with OCT. For immunohistochemical classification, GH/glyP is for GH/glycoproteins and multiH for multimodal adenomas respectively.

<table>
<thead>
<tr>
<th></th>
<th>Pre-operative plasma hormone values</th>
<th>Immunohistochemistry (+)</th>
<th>LI (mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(means ± S.E.M.) (ng/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GH</td>
<td>IGF-I</td>
<td>PRL</td>
</tr>
<tr>
<td>GH-secreting adenomas</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated (25)</td>
<td>32.3±6.7</td>
<td>700.7±61.1</td>
<td>22.5±3.8</td>
</tr>
<tr>
<td>Treated (22)</td>
<td>10.6±2.2</td>
<td>443.5±36.0</td>
<td>12.8±3.2</td>
</tr>
<tr>
<td>OCT only (16)</td>
<td>6.9±1.5</td>
<td>418.9±47.1</td>
<td>10.9±3.3</td>
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<tr>
<td>Others (+) (6)</td>
<td>19.4±4.5</td>
<td>543.3±61.7</td>
<td>12.4±9.8</td>
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<tr>
<td>PRL-secreting adenomas</td>
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<tr>
<td>Untreated (6)</td>
<td>–</td>
<td>–</td>
<td>1665.5±972.3</td>
</tr>
<tr>
<td>Treated (9)</td>
<td>–</td>
<td>–</td>
<td>205.2±92.0</td>
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All mean values were compared by Mann–Whitney and the corresponding significant P values indicated by *P < 0.05, **P < 0.01 and °°°P < 0.001 vs untreated cases. LI variability among subgroups was evaluated by F-test and the corresponding significant P values are indicated by *P < 0.05 and **P < 0.005.
According to most (Landolt 1987, Pegolo et al. 1995, Thapar et al. 1996a, Mastronardi et al. 1999) but not all (Zhao et al. 1999) authors, CS tumours have a higher proliferative potential than CNS tumours. In this study, the difference between CS and CNS tumours appeared in untreated cases, with significantly greater LI variations and a trend towards higher LI values in the former subgroup. In contrast, because of the possible anti-proliferative effects of pre-operative pharmacological treatment, LI values in treated CS were very similar to those observed in CNS. In fact, in addition to the well-known anti-proliferative effects of dopaminergic drugs on prolactinomas, possible in vivo anti-proliferative effects of OCT on somatotroph adenomas could be pointed out by this study and by a very recent specific report (Losa et al. 2001). These data clearly indicate that the LI should not be interpreted only according to the functional status of the tumour, but also to its pre-operative management. Interestingly, in agreement with previous findings (Pegolo et al. 1995), nuclear atypias were also more frequent in CS than in CNS adenomas, although there was no clear relationship between mitosis and nuclear pleomorphism. Noteworthy, in CS adenomas, mitosis, but not atypias, were also found to significantly decrease with pre-operative medical treatment.

Another point of this study was the inverse relationship observed between the LI and the age of the patients, which was generally not reported previously (Buchfelder et al. 1996, Blevins et al. 1998, Losa et al. 1998). In this study, this age-related LI distribution was limited to CS tumours whereas, considering that CS and CNS adenomas were almost equally represented, the older age of patients with CNS adenomas was likely to explain the age-related pattern observed on the whole series. This finding may be of clinical importance since the age-related pattern observed in CS adenomas is in keeping with their higher aggressivity in young patients (Melmed 1990, Abe et al. 1999), and similar features have been recently proposed for CNS adenomas (Losa et al. 2000b). However, data from multivariate analysis indicate age as a secondary determinant factor when compared with pre-operative treatment or macroscopic features of the tumour itself.

Most (Landolt et al. 1987, Knosp et al. 1991, Thapar et al. 1996a, Mastronardi et al. 1999, Zhao et al. 1999) but not all (Gandour-Edwards et al. 1995, Kawamoto et al. 1995, Pegolo et al. 1995, Yonezawa et al. 1997, Losa et al. 2000b) studies have reported a higher proliferation rate in invasive than non-invasive tumours. Discrepancies may in part reflect differences in the definition of invasiveness, since about 40% of pituitary adenomas show gross invasion of the surrounding structures, contrasting with 70–95% showing histological evidence for dural infiltration, depending on tumour volume and functional characteristics (Scheithauer et al. 1986, Selman et al. 1986). Histologically proved dural invasion has been associated with higher LI values (Knosp et al. 1989, Kitz et al. 1991). In our series, the overall percentage of invasion was about 65%, which, with a few exceptions (Pegolo et al. 1995), was higher than commonly reported. This might be explained by the definition retained for the study — localised macroscopic dural infiltration was considered significant — and by the high percentage of macroadenomas referred to our Neurosurgery Units (Fraioli et al. 1995). Invasiveness was confirmed by multivariate analysis to be associated with a higher LI in the whole series and in...
CS adenomas. Supporting some (Knosp et al. 1991, Mastronardi et al. 1999) but not all (Kawamoto et al. 1995) previous data, invasion of the cavernous sinus, but not of the sphenoid sinus (Gandour-Edwards et al. 1995), was associated with a higher LI in most cases, although significance was not reached for CNS tumours, which only displayed a higher variability in LI values. This finding supports the hypothesis that peculiar biological mechanisms may lead to invasion of the different perisellar tissues by pituitary tumours (Jaffrain-Rea et al. 1998, Kawamoto et al. 1996a,b).

Most studies agree on the lack of correlation between tumour volume and proliferative activity in pituitary adenomas (Pegolo et al. 1995, Yonezawa et al. 1997). However, surgical indications for microadenomas are almost limited to CS tumours, so that ignoring the functional status of the adenomas may introduce a significant bias and mask volume-related variations. In the present study, only LI variability was found to increase with tumour volume on the whole series. However, mean LI values and its variability were markedly higher in macro- than in micro-CS adenomas, a finding which was further confirmed by multifactorial analysis. In addition, mean LI values and variability increased with SSE in this group. Higher LI values have also been recently reported in macro- vs micro-ACTH-secreting adenomas (Losa et al. 2000a) and in macro- vs micro-PRL-secreting adenomas (Turner et al. 2000). The high LI values observed herein in untreated prolactinomas, which were all macroadenomas, contrasting with the low values reported in poorly evolutive microprolactinomas (Nishioka et al. 2001) further reinforce this point. In this series, this finding could be extended to GH-secreting adenomas (data not shown). This is in keeping with the slowly growing potential of most CS microadenomas in clinical practice, while further supporting the frequent aggressivity of CS macroadenomas. In contrast, no significant LI variations was found in CNS adenomas according to their volume. This finding is in agreement with a recent report (Losa et al. 2000b) and further supports the slowly growing potential displayed by most CNS adenomas.

Despite some exceptions (Katoh et al. 1995), LI is currently viewed as a reliable marker of aggressivity for endocrine neoplasms (Vargas et al. 1997a,b, Clarke et al. 1998). Some authors have suggested that regrowing pituitary tumours have a higher LI than non-regrowing tumours (Shibuya et al. 1992, Ekramullah et al. 1996, Abe et al. 1997, Mizoue et al. 1997). Again, we recommend that the possible prognostic value of LI should be regarded differently in CS and in CNS tumours. In fact, although high LI values are more commonly seen in untreated CS tumours, most of them are GH and/or PRL-secreting tumours which will be responsive to post-operative pharmacological treatment, so that the LI is expected to be mainly useful for the management of CNS or CS resistant to pharmacological therapy. Our data support recent evidence that primary and recurrent CNS tumours do not significantly differ (Yonezawa et al. 1997), and that LI alone seems to poorly predict the risk of post-operative regrowth in this group (Losa et al. 2000b). This may be explained by factors other than cell proliferation being involved in the kinetics of tumour growth (i.e. tumour vascularisation and apoptotic index) and by the quality of surgical resection as a key prognostic factor. Nonetheless, since a correlation may exist between in vitro (Atkin et al. 1997) or in vivo (Hsu et al. 1993, Ekramullah et al. 1996) tumour doubling time, we believe that LI values should be kept in mind for an adequate follow-up of post-operative residual tumours.

Special attention should be paid to sporadic cases presenting with unusually high LIs. Despite the fact that data obtained on some endocrine neoplasms suggest that LI thresholds can be proposed which can help disclosing special aggressivity or malignancy (Vargas et al. 1997a,b, Clarke et al. 1998), evidence for metastasis is currently recognised as the sole criteria for the definition of pituitary carcinomas (Pernicone et al. 1997). According to this definition, these tumours are extremely rare. It should be remembered that pituitary carcinomas are believed to arise from benign proliferations secondary to de novo molecular events (Karga et al. 1992, Thapar et al. 1996b), an evolution which may take more than 10–15 years. Thus, despite high LI values reported in most documented pituitary carcinomas (Thapar et al. 1996a), ‘paradoxically’ low values can be observed at first surgery (Pernicone et al. 1997). We should also point out that very high LI values in other tumours are typically associated with malignancy, regardless of the presence of metastasis (Katoh et al. 1995, Garcia et al. 1997). Thus, as illustrated in the brief case report presented herein, current criteria may sometimes be too restrictive for clinical purposes, and very high LI values may help identify a subset of pituitary tumours with a highly aggressive, locally ‘malignant’ or life-threatening behaviour prevailing on their ability to metastasise. As a general rule, accepting the 90th percentile as a reasonable threshold to disclose ‘unusual LI values’, most pituitary adenomas with LI > 3% should be regarded with special attention. On the basis of the present study, we suggest to adapt this cut-off to the functional characteristics of the tumour, i.e. 2.5% for CNS adenomas or treated CS, and 6.0% for untreated CS. This may help the pathologist to disclose highly aggressive tumours and the physician to plan a more adequate short-term follow-up and, if necessary, a more aggressive therapeutic schedule (Knosp et al. 1995, Kaltas et al. 1998).

In conclusion, we believe that immunostaining for the MIB-1 LI should be part of the routine examination of operated pituitary tumours, provided that both pathologists and physicians keep aware of the functional status of the tumour and the presence of any pre-operative pharmacological treatment. In fact, despite its value for clinical decision making being restricted to a subset of tumours, it may represent a precious tool for the diagnosis and management of tumours.
displaying an unusually aggressive phenotype and/or pharmacological resistance to conventional medical therapy. On the other hand, pitfalls in its interpretation should be kept in mind by researchers interested in pituitary tumour pathogenesis and in disclosing factors associated with an increased kinetic activity.

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References


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