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ORIGINAL ARTICLE - PITUITARIES

Ki-67 labeling index and expression of p53 are non-predictive for invasiveness and tumor size in functional and nonfunctional pituitary adenomas

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Abstract

Background It is still controversial whether an increased proliferation index is correlated with the tumor invasiveness of pituitary adenomas. A homogeneous large monocentric series of pituitary adenomas was retrospectively analyzed. The correlation between the proliferation indices (Ki-67 and p53 expression levels) and invasiveness and size of pituitary adenomas was investigated in primary operated and recurrent adenomas.

Method Four hundred thirty-nine patients after resection of pituitary adenomas were retrospectively included (43 recurrent tumors, 196 null cell adenomas, 86 somatotroph adenomas, 55 corticotroph adenomas, 55 prolactinomas, 4 thyreotroph adenomas). The maximum tumor diameter and tumor invasiveness in Knosp grading were assessed and Ki-67 and p53 immunostaining was performed. The role of invasiveness was evaluated using a cumulative odds ordinal logistic regression. For calculating the effect of tumor size, a one-way analysis of variance (ANOVA) was conducted.

Results Overall and in the subgroups, no significant correlation between proliferation indices and mean tumor diameter was found. No significant predictive expression value of Ki-67 and p53 on tumor invasiveness and in recurrent tumors could be demonstrated. There was a tendency that Ki-67 LI and p53 LI are higher in recurrent corticotroph adenomas and lactotroph adenomas but values did not reach the significant level.

Conclusion Invasive character of pituitary adenomas is neither correlated with increased Ki-67 LI nor with increased p53 expression. Proliferation parameters are independent from adenoma size at initial presentation. The partly elevated expression of Ki-67 in recurrent tumors underlines the clinical importance of the marker.

Keywords Pituitary adenomas · p53 · Ki-67 · Invasiveness

Background

In the 2004 WHO classification of the endocrine organs, pituitary adenomas were divided into typical adenomas, atypical adenomas and pituitary carcinomas. The subgroup atypical adenoma was defined by morphological features suggestive of an aggressive clinical behavior such as invasive growth, an elevated mitotic index, Ki-67 labeling index (LI) > 3%, and an excessive nuclear p53 immunostaining [7]. This definition led to a wide discussion of the actual clinical implications and to a relatively variable report of the incidence of atypical adenomas [4, 20, 24, 32, 33].

Recently, the 2017 WHO classification was published [15]. The term atypical adenoma has been removed due to the low

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predictive value for aggressive behavior. p53 immunoreactivity is no more considered in the classification of pituitary adenomas because of controversial usefulness as a proliferative parameter in these neoplasms. Together with increased growth rate, radiological defined invasiveness, and mitotic rate, Ki-67 LI remains as a predictor for more aggressive clinical behavior of pituitary adenomas. Nevertheless, due to methodological differences, no clear cut-off value has been assigned [3, 14, 29]. As in the 2004 classification, tumor size is not included in the new classification. It is recommended to use invasiveness—defined by the intraoperative impression or imaging based—as an important prognostic feature in identifying individual cases for consideration of clinically aggressive adenomas. The available evidence shows a strong correlation of the radiologically defined invasiveness and the surgical outcome in terms of recurrence and persistence in functioning adenomas [2, 3, 14, 29]. Especially cavernous sinus invasiveness, as indicated by a high Knosp grade [13], impedes a total resection and is a highly significant factor for residual tumor and recurrence of the adenoma [3, 14]. It is still controversial whether an increased proliferation index is correlated with the tumor invasiveness—which is a major criterion for operability—and long-term remission after surgery [6]. While some publications showed a correlation of invasiveness and proliferation [31, 32], others found no evidence of a relationship [9, 27, 29].

To shed light into this unsettled issue, we retrospectively analyzed a homogeneous large series of pituitary adenomas. In this monocentric case study, we investigated the correlation between the proliferation indices (Ki-67 and p53 expression levels) and invasiveness and size of pituitary adenomas in primary operated and recurrent adenomas.

Method

Four hundred thirty-nine patients who underwent transsphenoidal or transcranial resection of pituitary adenomas from October 2004 to July 2012 were retrospectively included (mean 50.5 ± 17.1 years, 165 male, 41 recurrent tumors, 2 recurrent tumor with changing immunophenotype, 185 null cell adenomas, 86 somatotroph adenomas, 55 corticotroph adenomas, 55 prolactinomas, 11 silent-producing adenomas, 4 thyreotroph adenomas). One hundred eighty-six tumors were positive for alpha-SU. Of the clinically silent pituitary adenomas, immunohistochemical expression of ACTH ($n = 8$) and GH ($n = 3$) was observed. The diagnosis was determined by histology and additional immunostainings. All tumors were immunostained for the full spectrum of pituitary hormones (alpha-SU, GH, PRL, ACTH, LH, FSH, TSH).

Preoperative T1-weighted, contrast-enhanced magnetic resonance (MR) images (1-3 mm slice thickness, in coronal or axial orientation) were retrospectively evaluated independently by two raters (R.M. and J.H.) to assess the maximum tumor diameter in mm.

Tumor invasiveness was estimated in the established Knosp grading 0–4 [13]. Grade 0: normal condition of the cavernous sinus space. Adenoma not passing the tangent of the medial aspects of the supra- and intra-cavernous internal carotid artery (ICA). Grade 1: tumor extension not passing a line between the cross-sectional centers of the ICAs. Grade 2: tumor extending beyond the intercarotid line, not extending beyond or tangent to the lateral aspects of the intra- and supra-cavernous ICA. Grade 3: tumor extending lateral to the lateral tangent of the intra- and supra-cavernous ICA. Grade 4: total encasement of the intra-cavernous carotid artery.

In each case, slides from paraffin-embedded tumor samples were routinely immunolabeled using antibodies against Ki-67 (DakoCytomation, Glostrup, Denmark, clone MIB-1, dilution 1:200) and p53 (DakoCytomation, clone DO-7, dilution 1:2000) using an automated immunohistochemistry slide staining system (BenchMark®, Ventana Medical Systems, Tucson, Az, USA). The automated standard protocol is based on an indirect biotin-avidin system that uses a universal biotinylated immunoglobulin secondary antibody and diaminobenzidine substrate. The sections were counterstained with hematoxylin. Negative controls consisted of sections incubated in the absence of the primary antibody. For both antibodies, the labeling index (LI) was estimated by an experienced neuropathologist as an overall percentage of the immunostained tumor cells and recorded in the pathological report.

Statistics and data evaluation

Statistical analysis was performed in SPSS (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.).

To evaluate the role of invasiveness as given in the Knosp grading and separately the recurrence of the tumors, a cumulative odds ordinal logistic regression with proportional odds was chosen. The assumption of proportional odds was assessed by a full likelihood ratio test comparing the fit of the proportional odds model to a model with varying location parameters. A deviance goodness-of-fit test was used to indicate that the model fits to the observed data.

For calculating the effect of tumor size, a one-way analysis of variance (ANOVA) was conducted on the parameters. Data is presented as mean ± standard deviation. Multicollinearity was assessed by Pearson's correlation. Homogeneity was assessed by Box's test of equality of covariance matrices. In all items, significance level was set to $p \leq 0.05$.

Results

The descriptive statistics are illustrated in Table 1. Exemplary adenomas showing a high or low expression of Ki-67 or p53

Table 1 Descriptive statistics and group characteristic, (means ± standard deviation). The parameters of the primary operated, the recurrent cases, and the whole group are shown for the different groups of pituitary adenomas

Diagnosis		Knosp grade	Diameter (mm)	p53 (%)	Ki-67 (%)	α-Subunit (%)	Age (years)
Somatotroph adenoma	Primary	1.8 ± 1.3	20 ± 9	4.0 ± 3.6	2.3 ± 1.6	31 ± 38	46.9 ± 16.7
	Recurrent	1.8 ± 1.3	18 ± 6	4.9 ± 6.5	4.1 ± 6.1	18 ± 36	45.5 ± 10.3
	Total	1.8 ± 1.3	20 ± 9	4.1 ± 4.0	2.5 ± 2.5	27 ± 38	46.7 ± 16.1
Corticotroph adenoma	Primary	0.7 ± 0.9	16 ± 6	1.2 ± 1.7	1.9 ± 1.6	11 ± 27	43.5 ± 15.2
	Recurrent	1.1 ± 1.7	17 ± 9	6.4 ± 10.5	3.6 ± 2.1	5 ± 11	52.9 ± 20.4
	Total	0.8 ± 1.1	16 ± 6	1.9 ± 4.2	2.1 ± 1.8	7 ± 23	44.7 ± 16.1
Null cell adenoma	Primary	1.51 ± 1.1	24 ± 8	2.3 ± 4.3	2.3 ± 5.2	18 ± 27	59.1 ± 12.8
	Recurrent	1.8 ± 1.0	25 ± 5	2.6 ± 2.3	2.6 ± 2.5	17 ± 34	62.5 ± 9.6
	Total	1.5 ± 1.1	24 ± 7	2.3 ± 4.3	2.3 ± 5.2	17 ± 28	59.3 ± 12.8
Lactotroph adenoma	Primary	0.8 ± 1.0	17 ± 8	4.5 ± 4.0	2.9 ± 1.6	8 ± 16	29.8 ± 11.1
	Recurrent	1.7 ± 0.6	26 ± 10	8.7 ± 7.1	4 ± 2.7	4 ± 8	52 ± 20.9
	Total	0.8 ± 1.0	17 ± 9	4.7 ± 4.1	3.0 ± 1.6	7 ± 12	31 ± 12.6
Thyreotroph adenoma	Primary	1.5 ± 0.6	22 ± 9	5.5 ± 3.3	3.3 ± 0.5	76 ± 26	55.1 ± 9.9
	Total	1.5 ± 0.6	22 ± 9	5.5 ± 3.3	3.3 ± 0.5	76 ± 26	55.1 ± 9.9
Silent-producing adenoma	Primary	1.8 ± 1.7	25 ± 13	3.3 ± 3.6	1.9 ± 1.0	7 ± 13	62.4 ± 14.0
	Total	1.8 ± 1.7	25 ± 13	3.3 ± 3.6	1.9 ± 1.0	7 ± 13	62.4 ± 14.0
Switching immunophenotype	Recurrent	2.5 ± 1.5	27 ± 7	16 ± 14	3.5 ± 2.5	0 ± 0	73.9 ± 3.5
	Total	2.5 ± 1.5	27 ± 7	16 ± 14	3.5 ± 2.5	0 ± 0	73.9 ± 3.5
Total	Primary	1.4 ± 1.2	21 ± 9	2.9 ± 4.0	2.3 ± 3.9	20 ± 32	49.8 ± 17.3
	Recurrent	1.7 ± 1.2	22 ± 7	4.1 ± 5.7	3.2 ± 3.5	12 ± 30	56.6 ± 14.2
	Total	1.4 ± 1.2	21 ± 8	3 ± 4.3	2.4 ± 3.9	36 ± 35	50.5 ± 17.1

are illustrated in Fig. 1. Overall and in the subgroups, no significant correlation between proliferation indices and mean tumor diameter was found. There was no significant predictive expression value of Ki-67 and p53 on tumor invasiveness ($p > 0.05$) and no statistical overall significance in recurrent tumors. In the subgroup analysis, Ki-67 expression was significantly increased only in recurrent somatotroph adenomas ($p = 0.04$).

Invasiveness

The final statistic model significantly predicted the dependent variable over and above the intercept-only model, $\chi^2(9) = 55$, $p < 0.001$. The deviance goodness-of-fit test indicated that the model was a good fit to the observed data, $\chi^2(1100) = 1571$, $p = 0.5$. The assumption of proportional odds was met, as assessed by a full likelihood ratio test comparing the fit of the proportional odds model to a model with varying location parameters, $\chi^2(21) = 1$, $p = 0.9$.

Thyreotroph adenomas were excluded from the model due to the small number of cases ($n = 4$). The invasiveness according to the Knosp grading (Fig. 2) was neither correlated to the expression of Ki-67 (odds ratio 0.96, $p > 0.05$) nor to the expression of p53 (odds ratio 1.0, $p > 0.05$).

Only the odds ratios of being in a lower category of the Knosp grading for corticotroph adenomas (0.12, $p = 0.03$) and lactotroph adenomas (0.14, $p = 0.04$) showed a statistically significant effect (Table 2).

Tumor size

There was multicollinearity, as assessed by Pearson correlation ($r = 0.7$, $p < 0.002$) and homogeneity of variance-covariances matrices, as assessed by Box's test of equality of covariance matrices ($p < 0.003$). The descriptive statistics of the mean tumor size in the subgroups is shown in Table 1. No significant correlation of p53 expression and Ki-67 LI was found to the tumor diameter (Fig. 3).

A one-way ANOVA was conducted to determine if the maximum tumor diameter had an influence on the main factors. There was no significant effect regarding Ki-67 LI ($F = 1.56$, $p > 0.05$) and p53 expression ($F = 0.4$, $p > 0.05$).

The maximum tumor size was significantly different between the subgroups of pituitary adenomas ($F = 7.6$, $p < 0.001$, Fig. 3, Table 3). Tukey post hoc analysis revealed that the diameter difference between the subgroups somatotroph and corticotroph adenomas (4.4, 95% CI (1.4 to 7.4)) was statistically significant ($p = 0.001$), as well as between somatotroph and null cell adenomas (− 4.7, 95% CI (− 7.1 to − 2.4), $p < 0.001$) but no other group differences were statistically significant.

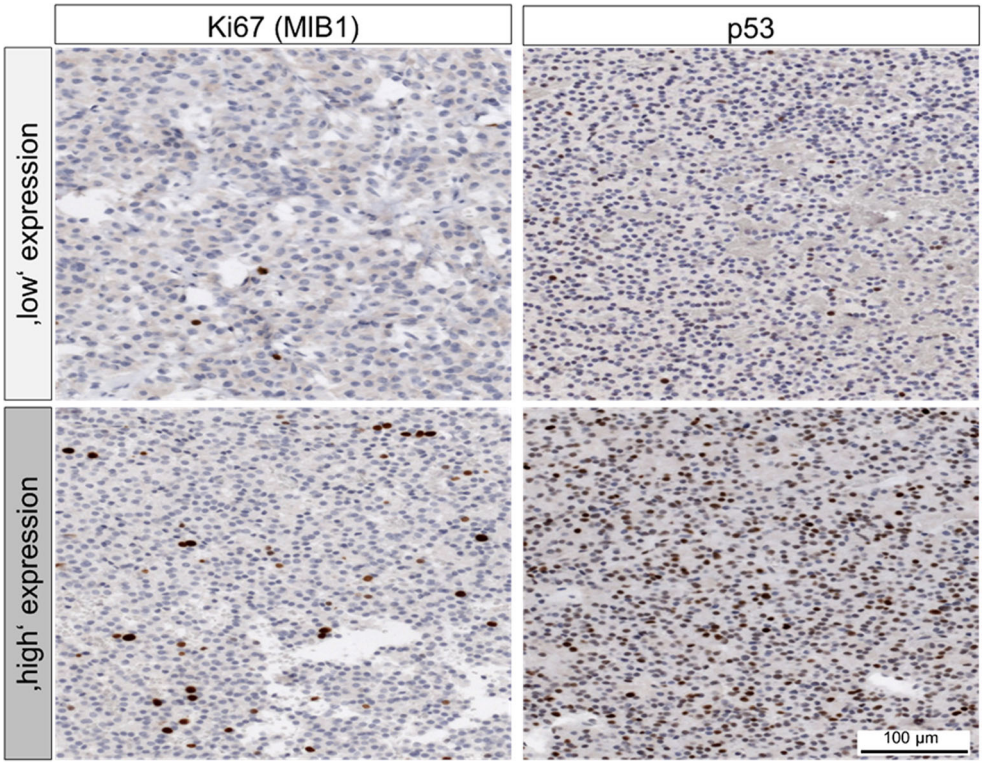

Fig. 1 Low and high immunohistochemical expression levels of Ki-67 and p53

Alpha-SU expression had statistically no effect on tumor size (Pearson correlation, $r = -0.09$, $p > 0.05$). The silent-producing adenomas did not show a significant group difference in the maximum tumor diameter compared with the overall collective and within the groups. There was a tendency for silent secreting corticotroph adenomas to have a larger diameter but missing significance due to a large scattering in the clinical manifest group (mean 19 mm ± 4 mm).

Tumor recurrence

A cumulative odds ordinal logistic regression with proportional odds was run to determine differences in primary operated and recurrent tumors. The deviance goodness-of-fit test indicated that the model was a good fit to the observed data, $\chi^2(160) = 152$, $p = 0.3$. The assumption of proportional odds was met, as assessed by a full likelihood ratio test comparing

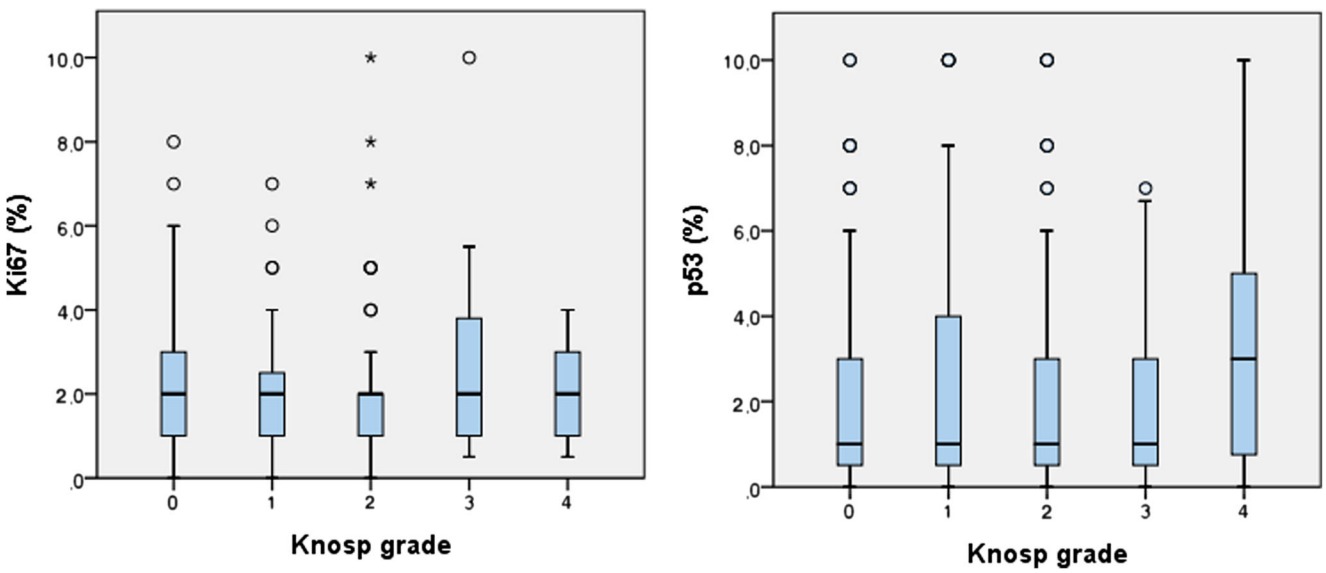

Fig. 2 Invasiveness and expression of Ki-67 LI and p53 LI

Table 2 Results of the ordinal logistic regression to determine factors for invasiveness. Given are the parameter estimates and odds ratio of Knosp grades with significance and confidence interval of the odds ratio. Operated corticotroph and lactotroph adenomas were significantly less invasive

	Estimate	Odds ratio	Std. Error	Wald	df	Sig.	95% confidence interval	
							Lower bound	Upper bound
Ki-67	− 0.03	1.0	0.03	1.4	1	0.2	0.9	1.0
p53	0.03	1.0	0.02	1.5	1	0.8	1.0	1.1
Age	0.01	1.0	0.01	0.0	1	0.4	1.0	1.0
Somatotroph adenoma	0.11	1.1	0.93	0.0	1	0.9	0.2	6.8
Corticotroph adenoma	− 1.7	0.2	0.97	3.2	1	*0.03	0.0	0.8
Null cell adenoma	− 0.03	1.0	0.92	0.03	1	0.9	0.1	5.8
Lactotroph adenoma	− 1.5	0.2	0.97	2.5	1	*0.04	0.0	1.4
Silent adenoma	− 0.05	1.0	1.1	0.02	1	0.9	0.1	9.4

the fit of the proportional odds model with a model with varying location parameters, $\chi^2(180) = 7$, $p = 0.2$.

There was a tendency that Ki-67 LI and p53 LI are higher in recurrent corticotroph adenomas ($p > 0.05$) and lactotroph adenomas ($p > 0.05$) but did not reach statistical significance (Fig. 4).

Taking into account all subgroups, there was no statistical significant effect of the recurrence to the expression value of Ki-67 (odds ratio 1.3, $p > 0.05$) and p53 (odds ratio 1.7, $p > 0.05$). Numbers of pituitary adenomas with observed changing of hormonal expression after recurrence were too low to observe statistical effects.

Discussion

Several studies evaluated the correlation between Ki-67, tumor size, invasiveness, and recurrence rate. Wide evidence exists that an expression of Ki-67 LI is associated with a shortened disease-free survival time and early recurrence of the adenomas even after gross surgical resection or following radiotherapy. Some studies found an increased incidence of tumor recurrence if a high level of Ki-67 was found at the first operation [8, 18, 21, 22]. Interestingly, due to methodological differences, a cut-off level for Ki-67 LI of 1.5% and 2% for a higher recurrence rate was used [1, 18, 29]. This differs considerably from the cut-off level of 3% suggested in the 2004 WHO classification [23]. Recent studies with larger case numbers highlighted the positive correlation of Ki-67 and recurrence rate [3, 14, 29].

A relevance of p53 as a predictor of progression and recurrence is controversial. While some authors reported on a positive correlation between p53 expression and mitotic rate and recurrence [14, 26], others could not confirm this finding [3, 8]. Due to the questionable usefulness of p53, it is omitted in the 2017 WHO classification of pituitary adenomas. Because of the clear correlation with a more aggressive tumor behavior, the evaluation of Ki-67 is maintained but no cut-off is defined anymore due to various levels reported [15].

Our results only partly deflect these circumstances. A prognostic role of the factors was not evaluated. In the subgroup of recurrent tumors, the estimated proliferative markers (both p53 and Ki-67 LI) were higher than in the primarily operated

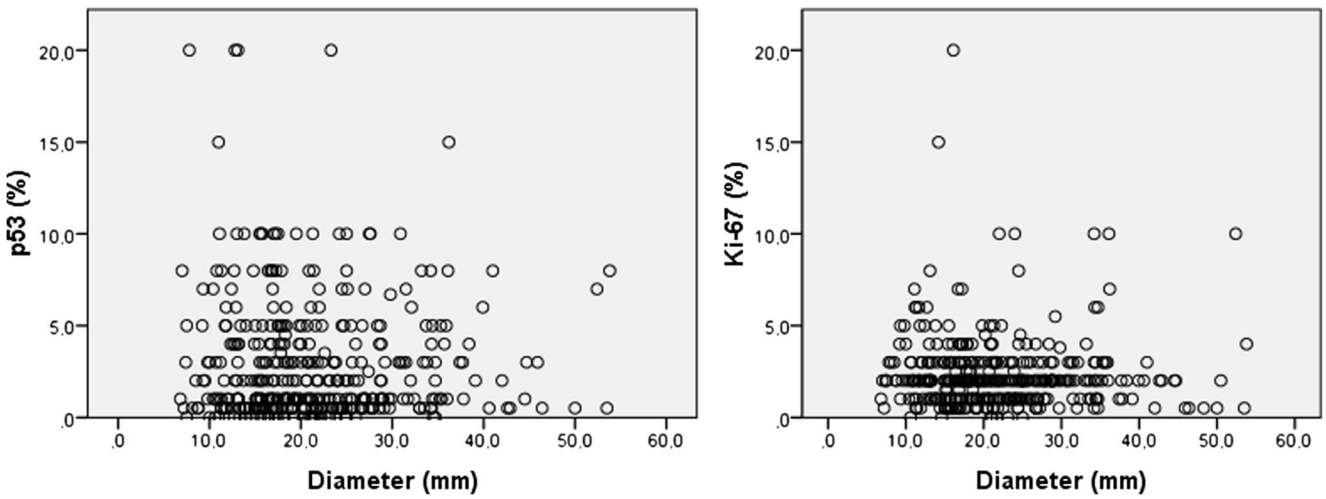

Fig. 3 Tumor diameter according to p53 expression and Ki-67 LI

Table 3 Results of the one-way ANOVA with dependent variable tumor diameter to determine if the maximum tumor diameter had influence on the main factors. There was no significant influence on Ki-67 and p53 expression. Only between the subgroups there was a significant group difference of the maximum tumor diameter

Source	Type III sum of squares	df	Mean square	*F*	Sig.
Corrected Model	4941	14	353	5.75	< 0.001
Intercept	9629	1	9629	156.86	< 0.001
Ki-67 expression	96	1	96	1.56	0.2
p53 expression	23	1	23	0.37	0.5
Age	41	1	41	0.67	0.4
Gender	115	1	115	1.88	0.2
Diagnosis	2328	5	466	7.59	< 0.001

tumors, but not statistically significant in the overall analysis. The elevated odds ratio suggested higher LIs in the recurrent tumors, but missing significance. Only looking at the subgroups on the diagnosis level, recurrent pituitary tumors in acromegaly showed a significantly higher expression of Ki-67.

In terms of tumor invasiveness, earlier studies with small case numbers found a significantly higher Ki-67 index in invasive adenomas [10, 12]. The finding was not confirmed by other studies and it was concluded that the invasive behavior is a feature independent of proliferative activity [9]. Recently, an association of cavernous sinus invasion and a high expression of KI-67 have been reported. In the same study, an increased tumor size causing hydrocephalus was associated with higher Ki-67 indices [2] which is contrary to other studies which could not correlate proliferative activity to tumor size [17, 26, 27]. Even in giant adenomas mitotic rates and labeling for p53 and Ki-67 were found to be only minimally increased [16].

Invasiveness and proliferation (as expressed by an increased Ki-67 LI) are often intermingled in recent studies and not regarded separately. In this context, the definition of aggressive tumors has emerged. Aggressive adenomas have been proposed to be invasive and proliferative. Combined invasive and proliferative tumors have been shown to have an increased probability of persistence or progression following surgery [29].

The results of our study at a large cohort do not indicate a correlation of an invasive growth with elevated LIs for Ki-67 or p53. In terms of tumor size, no statistical significant effect was found. There was no statistically significant correlation of invasiveness in Knosp grading with the expression of Ki-67 or p53. This issue has recently been raised and it has been proposed that the proliferative potential and the invasive character should be provided separately [25].

Silent adenomas often present as macroadenomas and frequently show a cavernous sinus infiltration or an osseous infiltration. Most common are silent corticotroph adenomas, which can account for up to 20% [5, 11, 19]. The silent corticotroph adenoma is recognized as “high-risk pituitary adenomas” in the new WHO classification. The diagnosis should gain priority due to the intrinsic aggression of these tumors. In our data, silent corticotroph adenomas are frequently represented and tend to be more extensive in diameter. However, we could not detect a greater invasiveness due to a scattering present in both groups.

As a risk for more aggressive tumors, the production of alpha-subunit is discussed. The role of expression is

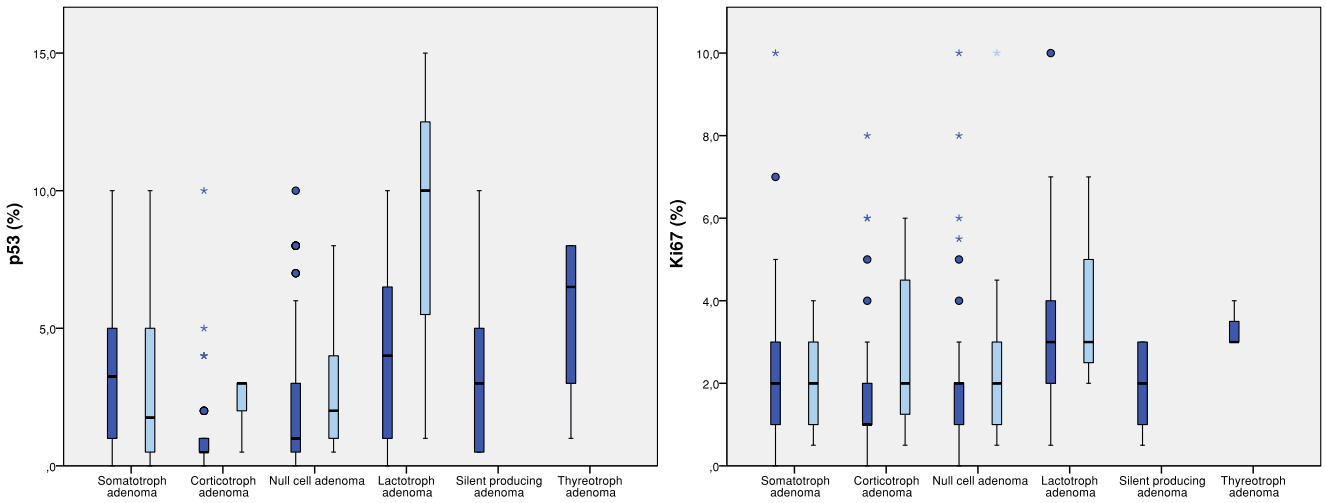

Fig. 4 p53 expression and Ki-67 LI according to diagnosis and to primary operated (blue) and recurrent (light blue) tumors

associated in some studies with either a greater suprasellar extension without increased invasiveness [28] or greater tumor extension [30]. In our large data collection, we could not detect any influence on tumor size or invasiveness.

Conclusion

We conclude that the invasive character of pituitary adenomas is neither correlated with increased Ki-67 LI nor with increased p53 expression. Similarly, proliferation parameters are independent from adenoma size at initial presentation. The partly elevated expression of Ki-67 in recurrent tumors underlines the clinical importance of the marker. These findings suggest that invasive behavior is a feature independent from proliferation.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (University Hospital Tübingen, Germany) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

For this type of study, formal consent is not required.

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