

# Proliferating cell nuclear antigen (PCNA) expression in pituitary adenomas: relationship to the endocrine phenotype of adenoma

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**Abstract:** The expression of proliferating cell nuclear antigen (PCNA) correlates to cell proliferation and for this reason it is commonly considered as one of proliferation markers. Since proliferation rate is an important factor determining the tumor aggressiveness, the evaluation of PCNA index (the percentage of PCNA-immunopositive nuclei in the investigated tumor sample) is suggested as useful in predicting pituitary adenoma outcome. Seventy three unselected, surgically removed pituitary adenomas were immunostained with antibodies against the pituitary hormones or their subunits and against the proliferating cell nuclear antigen (PCNA). The highest PCNA index was found in ACTH-immunopositive tumors without the manifestation of the Cushing's disease ("silent" corticotropinomas). This value was significantly different in comparison to other adenoma subtypes including corticotropinomas manifesting themselves by Cushing's disease. The lowest PCNA index was noticed in monohormonal GH-secreting tumors. The adenomas which express more than one hormone (plurihormonal adenomas) seem to have a higher PCNA indices than monohormonal ones; the difference was significant in the case of mono- and plurihormonal prolactinomas. The recurrent tumors presented a higher mean PCNA index as compared to the primary tumors, although the difference was significant only in the case of prolactinomas. These findings suggest that the proliferative potential of pituitary adenomas is related to the tumor recurrence and hormone expression. ([www.cm-uj.krakow.pl/FHC](http://www.cm-uj.krakow.pl/FHC))

**Key words:** Pituitary adenomas - Immunohistochemistry - PCNA

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## Introduction

Proliferating cell nuclear antigen (PCNA) is a nuclear auxiliary protein of deoxyribonucleic acid polymerase delta. Its expression correlates to cell proliferation and for this reason it is commonly considered as one of proliferation markers. Since proliferation rate is an important factor determining the tumor aggressiveness, the evaluation of PCNA index (the percentage of PCNA-immunopositive nuclei in the investigated tumor sample) is suggested as useful in predicting the pituitary adenoma outcome. Hsu *et al.* [6] showed that PCNA index was higher in pituitary adenomas that would recur, than in nonrecurrent tumors. This observation was confirmed in further studies [2, 16]. PCNA

indices were also found to be higher in invasive vs non-invasive pituitary adenomas [1, 14, 19]. PCNA expression in pituitary adenomas correlates also with another proliferation marker, nucleolar organizing regions (NOR) [10]. However, other authors did not find the correlation between PCNA expression and pituitary tumor recurrence and/or invasiveness [5, 7, 9, 17]. The data concerning the proliferative activity of different types of pituitary adenomas are not univocal. Otsuka *et al.* [15] did not show differences in PCNA expression between GH-producing, PRL-producing, FSH-producing and nonfunctioning adenomas. On the other hand, the higher proliferation rate was reported in the adrenocorticotrophic tumors in comparison to other types of pituitary adenomas [12]. Our earlier studies showed that adenomas expressing gonadotropins or free alpha subunit (gonadotropinomas/alphomas) and alpha-subunit co-expressing tumors had higher PCNA indices vs alpha-SU-immunonegative

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adenomas [10]. The aim of the present study was to answer the following questions: (1) Does PCNA expression vary between non-recurrent and recurrent adenomas? (2) Does PCNA expression depend on the endocrine phenotype of pituitary adenoma?

## Materials and methods

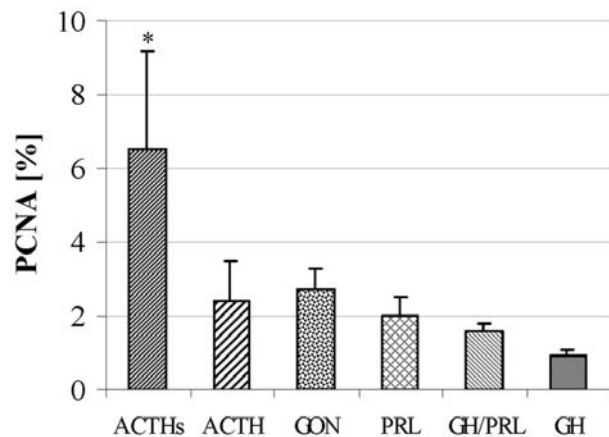
Seventy three unselected, surgically removed pituitary adenomas were studied. The tumors were fixed in Bouin-Hollande fixative and embedded in paraffin wax. Five  $\mu\text{m}$  sections were immunostained with antisera against the pituitary hormones and alpha-subunit (alpha-SU). The following antisera were used: polyclonal anti-human PRL (Dako, Denmark, prediluted by the manufacturer), polyclonal anti-human GH (Immunon, USA, prediluted by the manufacturer), monoclonal anti-human LH (Dako, prediluted by the manufacturer), monoclonal anti-human FSH (Dako, working dilution 1:50), monoclonal anti-human TSH (Immunotech, France, working dilution 1:100), monoclonal anti-alpha SU (Immunotech, working dilution 1:100), polyclonal anti-ACTH (Sigma, USA, DAKO or Immunon, working dilution 1:100). The sections were incubated with the primary antibodies for 24 hours at 4°C. The primary antibodies were then detected using the appropriate biotinylated secondary antibodies. The immunoreaction was visualized by the streptavidin-peroxidase complex (StreptABC/HRP, Dako) and 3,3'-diaminobenzidine. PCNA expression was assessed using the anti-PCNA monoclonal antibody (DAKO, prediluted by the manufacturer) and EnVision System AP (Dako). The number of PCNA-positive nuclei was estimated in 1000 randomly scored cells of each tumor and expressed in percent as PCNA index.

The numerical data were analyzed statistically by means of ANOVA and Mann-Whitney tests.

## Results

Twenty four adenomas were found to express FSH and/or LH or free alpha SU and were diagnosed as gonadotropinomas. Fourteen tumors were diagnosed as prolactinomas, 11 expressed both GH and PRL (somatotroprolactinomas), 5 expressed solely GH (somatotropinomas). Fourteen tumors expressed ACTH. One of them was an adenoma removed from a patient suffering from Cushing's disease submitted prior to bilateral adrenalectomy (Nelson's syndrome), 9 were removed from patients with Cushing's disease and the other 4 were found in patients without any symptoms of hypercortisolism (silent corticotropinomas). The remaining 4 adenomas were immunonegative for all the investigated pituitary peptides (null cell adenomas).

The relations between PCNA index and the expression of pituitary hormones in the tumor are shown in Figure 1. The highest PCNA index was found in ACTH-immunopositive tumors without the manifestation of the Cushing's disease (silent corticotropinomas -  $6.47 \pm 2.74\%$ ; Figs. 2 and 3). This value is significantly different in comparison to other adenoma subtypes including corticotropinomas manifesting themselves by Cushing's disease ( $2.4 \pm 1.1\%$ ). The lowest PCNA index was noticed in monohormonal GH-secreting tumors ( $0.94 \pm 0.22\%$ ). It seems that the adenomas immunone-

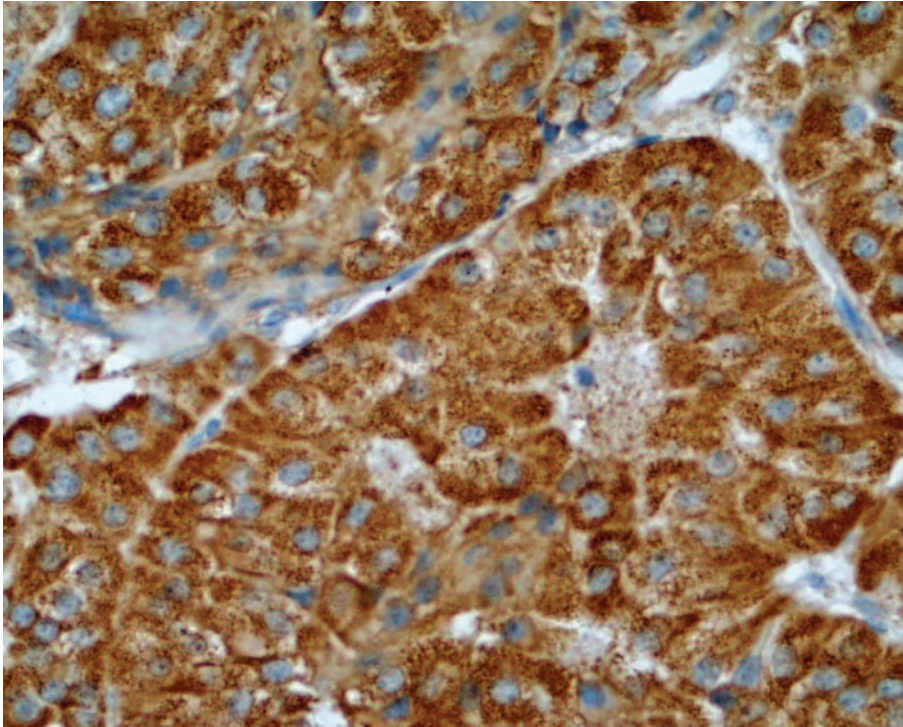


**Fig. 1.** PCNA indices (means  $\pm$  SEM) of pituitary adenomas in relation to endocrine phenotype. Asterisk indicates the statistically significant difference ( $p < 0.05$ ) vs the remaining types of adenomas.

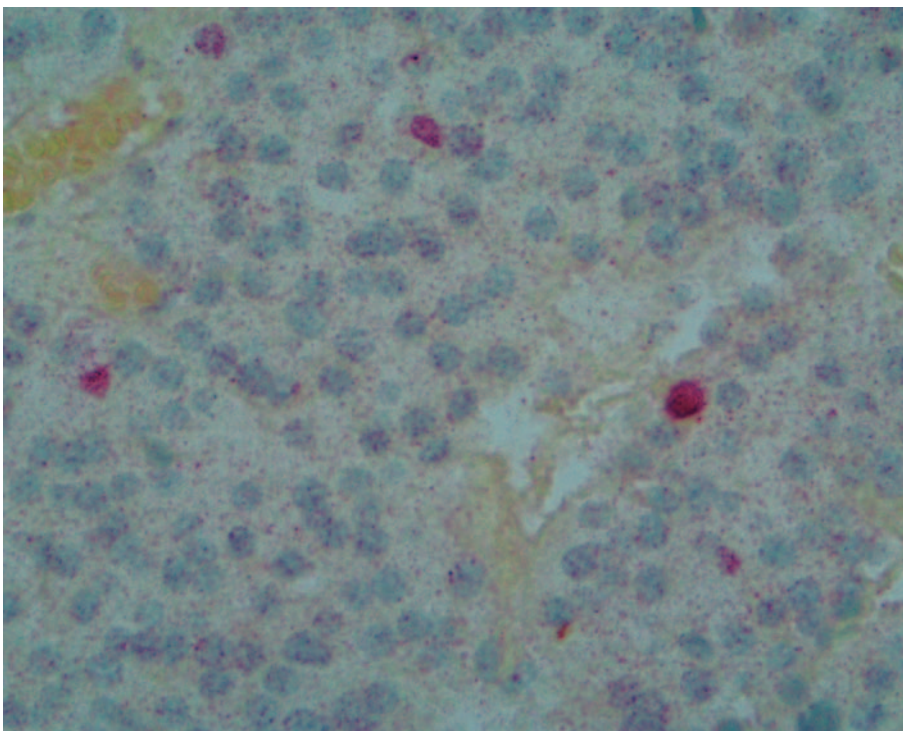
gative for all the investigated pituitary hormones (null cell adenomas) have in the majority low PCNA expression. This study included 4 such adenomas. All but one had low PCNA index ( $< 1\%$ ) and the remaining unexpectedly had it very high ( $13.7\%$ ). Because of this heterogeneity we did not calculate the mean value for this adenoma subtype and we did not consider it in statistical evaluation. The adenomas which expressed more than one hormone (plurihormonal adenomas) seemed to have higher PCNA indices than monohormonal ones; the difference was significant in the case of mono- and plurihormonal prolactinomas ( $1.3 \pm 0.5\%$  vs  $3.1 \pm 0.7\%$ , respectively). Moreover, in prolactinomas and gonadotropinomas, a tendency towards higher PCNA indices in men than in women was observed (Fig. 4). However, the difference was not statistically significant. The recurrent tumors presented a higher mean PCNA index ( $3.2 \pm 0.6\%$ ) as compared to the primary tumors ( $2.5 \pm 0.4\%$ ), but the difference was again statistically insignificant, except recurrent vs primary prolactinomas ( $3.15 \pm 1.15\%$  vs  $1.47 \pm 0.38\%$ ,  $p < 0.05$ , see Fig. 5).

## Discussion

The data presented above indicate that the proliferative potential of pituitary adenomas, estimated as PCNA expression, is, at least in part, related to the endocrine phenotype of adenoma. The most interesting observation concerns the high PCNA expression in ACTH-immunopositive tumors without clinically and/or biochemically manifested hypercortisolism ("silent" corticotropinomas). It is well known that ACTH-secreting adenomas become very aggressive in patients suffering from Cushing's disease after bilateral adrenalectomy (so-called Nelson's syndrome [8, 11, 13]). It is assumed that the fast growth of these tumors depends on the lack of feedback inhibition by cortisol.



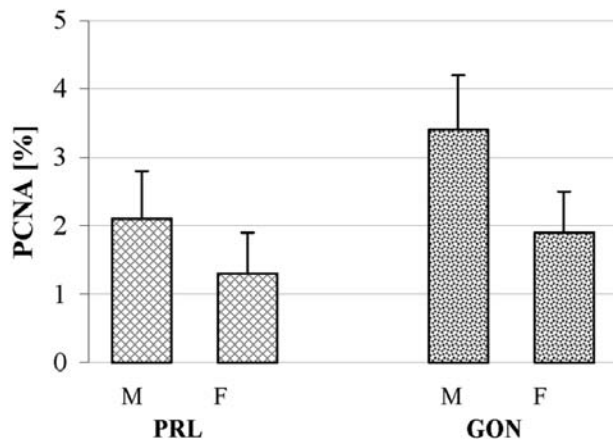
**Fig. 2.** ACTH immunostaining in "clinically non-functioning" adenoma from 62-year old man.  $\times 400$ .



**Fig. 3.** PCNA-immunopositive nuclei (deep purple) in the same tumor as in Figure 1.  $\times 400$ .

Nowadays the Nelson's syndrome becomes rare because the bilateral adrenalectomy is not recommended for the treatment of Cushing's disease. In our material we have only one case of ACTH-immunopositive tumor from patient with Nelson's syndrome. This tumor presented a high PCNA index comparable with those found in "silent" corticotropinomas (6.1%). It seems to us that in the case of "silent" corticotropinomas the

situation may be close to that observed in Nelson's syndrome. "Silent" corticotropinomas express ACTH or ACTH-like peptide(s) but do not secrete biologically active ACTH. In the majority of cases included in this study, not only was hypercortisolism absent but the function of the adrenal cortex was impaired. It means that the growth of tumoral corticotrophs was not restrained by the endogenous cortisol like in Nelson's syndrome.

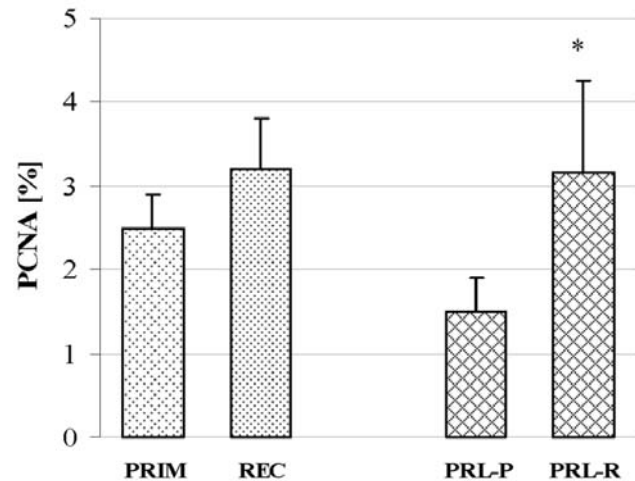


**Fig. 4.** PCNA indices (means±SEM) in prolactinomas (PRL) and gonadotropinomas (GON) in relation to patients' gender. F - women, M - men.

The relatively high PCNA indices were also observed in gonadotropinomas. This observation is in agreement with our earlier findings [10,16]. These tumors are assumed to grow slowly but they are diagnosed usually in the late stage as the giant macroadenomas. They exhibit also a high recurrence rate after surgical treatment.

Another observation worth to be underlined is higher PCNA expression in plurihormonal vs monohormonal prolactinomas. The plurihormonal prolactinomas in our material co-expressed, in addition to PRL, the following pituitary hormones: free alpha-SU (2 cases), ACTH (2 cases), GH (2 cases), FSH (1 case) and TSH (1 case). A slight difference in PCNA indices between mixed GH/PRL and "pure" GH-secreting adenomas in acromegalic patients in favor of the former should also be noticed. It is worth to recall that Desai *et al.* [4] have found that co-expression of glycoprotein hormones or free alpha-subunit by ACTH-secreting adenomas is associated with their enhanced aggressiveness. In our material, the recurrence rate of plurihormonal adenomas is twice as high as in the monohormonal ones, when we exclude the gonadotropin-expressing tumors which have very high recurrence rate (unpublished observations). The question whether plurihormonality of pituitary adenomas can be a predictive factor of aggressiveness needs further studies.

Last but not least, the patient's gender can also influence the proliferation potential and aggressiveness of the pituitary adenoma. Such an observation concerning prolactinomas was reported by French authors [3,18]. Our findings confirm it and suggest that the same may also concern another phenotype of adenomas, namely gonadotropinomas. The relationship between the recurrence and proliferative potential of pituitary adenomas is a matter of controversy (see papers cited in Introduc-



**Fig. 5.** PCNA indices (means±SEM) in all primary (PRIM) and recurrent (REC) tumors and primary (PRL-P) and recurrent prolactinomas (PRL-R). Asterisk indicates the statistical significance ( $p < 0.05$ ).

tion). Nevertheless, the data presented in the present study taken together with earlier observations from our and some other laboratories [1, 2, 6, 14, 16, 19] strongly suggest such a relationship.

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