

## Clinical aggression of prolactinomas: correlations with invasion and recurrence

MIHAELA N. POPESCU<sup>1)</sup>, ELENA IONESCU<sup>2)</sup>, L. C. IOVĂNESCU<sup>2)</sup>, B. V. COTOI<sup>2)</sup>,  
 A. I. POPESCU<sup>3)</sup>, ANCA ELENA GĂNESCU<sup>4)</sup>, ADINA GLODEANU<sup>5)</sup>,  
 CRISTIANA GEORMĂNEANU<sup>2)</sup>, ANDREIANA MORARU<sup>6)</sup>, ANCA PĂTRAȘCU<sup>7)</sup>

<sup>1)</sup>Department of Endocrinology

<sup>2)</sup>Department of Nursing

<sup>3)</sup>Department of Internal Medicine

Faculty of Medicine,  
 University of Medicine and Pharmacy of Craiova

<sup>4)</sup>Faculty of Chemistry,  
 University of Craiova

<sup>5)</sup>Faculty of Dentistry,  
 University of Medicine and Pharmacy of Craiova

<sup>6)</sup>Floreasca Emergency Hospital, Bucharest

<sup>7)</sup>Department of Obstetrics and Gynecology,  
 Faculty of Medicine,  
 University of Medicine and Pharmacy of Craiova

### Abstract

Some hypophyseal adenomas are discrete, well-marked lesions, which can be curatively removed by surgical resection, while others seem aggressive, invasive, recidive-prone, most often progressing in spite of any pharmacological, surgical or radiotherapeutical interventions. There is also a great variability within the incidence of aggression and invasion among the various types of hypophyseal immunotypes. Despite their well-differentiated nature and their "benign" constitution, an important number of hypophyseal tumors will be surely invasive within the sclerous, osseous and neural structures. Because the aggressive behavior of invasive adenomas is generally resolved by surgical treatment, this aspect of their biology does not perfectly reflect within their histopathological aspect. In fact, the invasive tumors with local extreme aggression are most often deceiving due to their relatively harmless histopathological aspect. The usual morphological signs of tumor aggression, namely pleomorphism, nuclear atypia, hemorrhage, high cellular and mitotic activity, poorly correlate with the invasive potential of pituitary tumors, with their proliferation capacity, their tendency of post-surgical recurrence or with their global biological behavior.

**Keywords:** prolactinomas, immunohistochemistry, invasiveness.

### Introduction

Hypophyseal adenomas are known due to their considerable variations regarding the clinical aggression. While some adenomas are discrete, well-marked lesions that may be curatively removed by surgical resection, others seem aggressive, invasive, recidive-prone, most often progressing in spite of any pharmacological, surgical or radiotherapeutical interventions. There is also a great variability within the incidence of aggression and invasion among the various types of hypophyseal immunotypes. The global frequency of invasion mostly depends on the used criteria (imagistic, intraoperative, morphological). Several statistics are quoted: 10%, 35%, 85% invasion; there has been decided to consider tumors as "invasive" based on the imagistic examination and the intraoperative observations [1, 2].

The histological evaluation of the aggressive behavior in the hypophyseal adenomas is a difficult one, because the characteristic features that may be emphasized in other tumors, like the nuclear and cellular pleomorphism, nuclear

atypias, mitoses frequency, hemorrhages and necroses, poorly correlate with the invasive potential, with their proliferation capacity, recidive tendency or the global biological behavior, and therefore they cannot be taken into consideration as determinant indicators of aggression. Due to this poor correlation, the prognosis of the hypophyseal tumor clinical behavior proved to be one of the most ambiguous aspects of their biology. Hence, there have been experienced several strategies in the question of proliferation potential of these tumors, some of them concerning only the research activity so far [3].

Prolactin (PRL) is the most frequently excess secreted hormone by a hypophyseal adenoma, and it is also increased in the patients with hypothalamus affection or shank compression [4]. That is why the PRL determination is essential in the evaluation of a patient suspect of hypophyseal affection and, thus, it should be determined in those suffering from galactorrhea, gonadic dysfunction, hypogonadotropic hypogonadism or any cause of sella turcica growth [5–8].

Despite their well-differentiated nature and their “benign” constitution, an important number of hypophyseal tumors will be surely invasive within the sclerous, osseous and neural structures. The invasion degree is most often a variable one, from focalities, tumor small parts invading the adjacent areas, to massive and destructive infiltrations of the structures around the sella turcica. Even though the latter are not easy to distinguish, the “invasion” involves the destructive infiltration, while the “expansion” involves a directional growth of the tumor, accompanied by compression. Generally speaking, invasive tumors present both characteristics. Lateral expansion and invasion of the cavernous sinus is the most frequent pattern of the massive local invasion [2].

Because the aggressive behavior of invasive adenomas is generally resolved by surgical treatment, this aspect of their biology does not perfectly reflect in their histopathological aspect [9]. In fact, the invasive tumors with local extreme aggression are most often deceiving due to their relatively harmless histopathological aspect.

A number of prognostic factors with unfavorable effects on a broad spectrum of human malignant tumors might be implicated in a sustained aggressiveness of the tumor [2].

For over two decades, the Ki67 antibody is used as a proliferation marker, in spite of the limited knowledge regarding the proteins it identifies. Ki67 represents a major basic protein with high DNA affinity, having a role in maintaining its structural order during meiosis. Thus, the presence of Ki67 is necessary in cell proliferation. A high correlation was evidenced between the increased Ki67 activity and other markers of aggressivity, mitotic activity, histological grading [1, 10–12].

PCNA (proliferating cell nuclear antigen), the nuclear antigen of cell proliferation, is a non-histonic nuclear protein, having a 36 kDa molecular weight and acting as a cofactor for DNA-polymerase. PCNA has various expressions during the cellular cycle; its synthesis rate correlates directly with the cellular proliferation degree.

P53: The necessity of additional information to those offered by the histopathological examination led to the investigation of specific genomic mutations, whose presence in the tumor tissue may serve as markers of the aggressive clinical phenotype. While mutations of the p53 oncosuppressor gene represent the most frequent genomic alterations in the human neoplasms, they have been considered markers of this disease [14].

## Objective

In this study, we proposed to evaluate the clinical invasion of prolactinomas by using immunohistochemical techniques.

## Materials and Methods

We performed a retrospective study including 20 patients suffering from hyperprolactinemia. The patients were hormonally, imagistically and immunohistochemically evaluated.

The magnetic resonance imaging (MRI) protocols consisted of T1 sections in the coronal and sagittal plans, before and after gadolinium intake, with a slice thickness up to 2 mm. The criosectioned or paraffin-embedded

specimens were immunohistochemically processed by the Avidin–Biotin–Peroxidase complex method in the Laboratory of Histopathology, “Victor Babeș” National Institute for Research and Development in Pathology and Biomedical Sciences, Bucharest, Romania. Polyclonal anti-human pituitary hormone was used as primary antibody. The histological check-up was performed through Hematoxylin–Eosin staining. The sections containing more than 5% immunoreactive cells were considered positive. The Avidin–Biotin method is based upon the high affinity of a glycoprotein in the egg white, Avidin, to bind the biotin fixed on the Fc fragment of an antibody. Each Avidin molecule can bind up to four molecules of peroxidase-bound Biotin. The secondary antibody directed against the primary antibody (mono- or polyclonal) is biotinylated. This will subsequently bind the Avidin–Biotin-peroxidase complex.

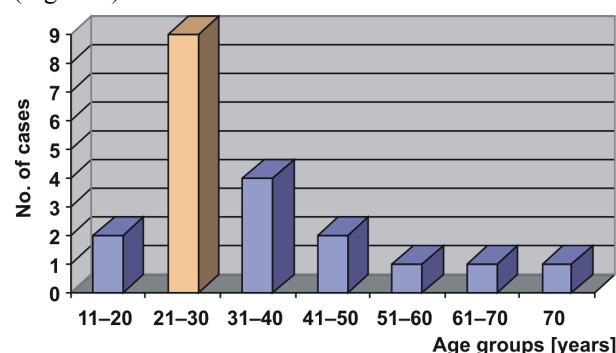
The hypophysis fragments were fixed by immersion in 10% buffered formalin solution, ethanol dehydrated and paraffin-embedded. There were performed cross-sections of 5 µm thickness, stained with Hematoxylin–Eosin (HE), respectively, following the classical methodology, and examined under the optical microscope afterwards.

The predominantly diffuse or submembrane cytoplasmic layout of PRL is quoted by some authors as being specific for the plurihormonal adenomas.

## Results

In performing the present study, we started from the investigation of a group of 20 patients (15 with macroprolactinomas and five with microprolactinomas) and five with growth hormone (GH) and PRL secretion, who accused menstrual cycle disorders in various stages (amenorrhea, bradymenorrhea with oligo- or hypomenorrhea), infertility, premenstrual syndrome, but also galactorrhea present in 19 patients.

The age-group distribution of PRL secretant adenomas disclosed a greater incidence in the young patients (21–30-year-old – nine cases, 31–40-year-old – four cases) (Figure 1).



**Figure 1** – Age-group distribution of patients with PRL secretant adenomas.

Because the time for tumoral mass duplication is generally comprised between 100 and 700 days, the onset event arises earlier. Therefore, the age-group distribution of patients with hypophyseal tumors shows that the tumors having a rapid growth rate, like the PRL-secretant tumors, present a higher incidence in the young patients. In the second and fourth age decade, there predominate the prolactinomas.

The determination of a complete diagnosis is based on the general clinical examination and on the endocrinological one. The errors regarding the diagnosing of hypophyseal adenomas are extremely rare, especially in the first stages, due to an incompletely established clinical picture.

The hypophyseal clinical examination pointed out cephalalgia as the first symptom, met in 12 patients. Galactorrhea was met in 19 patients. Dizziness was noted in seven patients, while eyesight blurring in three patients.

The thyroid examination indicated the signs and symptoms specific to hypothyroidism (rugged teguments, bradycardia, bradypsychia) found in eight patients.

The clinical ovary examination determined the presence of menstrual cycle disorders in all the 20 patients, in various stages: amenorrhea, bradymenorrhea with oligo- or hypomenorrhea. Moreover, it also pointed out the presence of infertility (normal anovulatory cycles) in 15 patients and of the premenstrual syndrome in 12 patients.

By analyzing the determined symptoms in the studied patients, there were established two syndromes: the endocrine syndrome (prolactin excess – PRL), and the tumoral syndrome (result of the adenoma compression upon other anatomical structures around the hypophysis – macroadenomas).

The first stage of the algorithm for establishing the hypophyseal adenoma diagnosis was represented by the hypophyseal hormone determination: PRL.

The PRL determinations presented a large value range (Table 1). The confirmation regarding the fact that hypophyseal adenomas were prolactinomas were performed according to a single criterion, namely that PRL must exceed 100 ng/mL, although the specialty literature includes a multitude of different opinions regarding the validity of this minimum prolactin value as a single criterion in diagnosing a prolactinoma, when the hypophyseal adenoma had already been imagistically detected.

**Table 1 – Statistical indicators for PRL in prolactinomas**

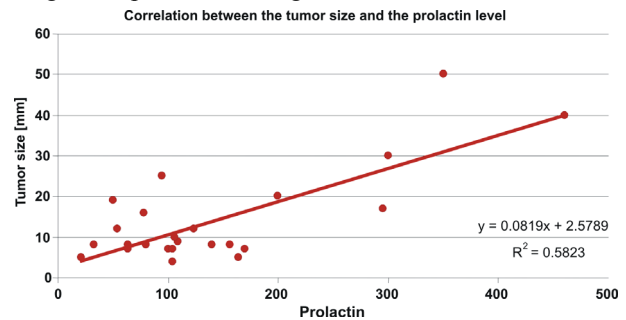
Indicator	PRL [ng/mL]
No. of patients	25
Mean	141.28
Standard deviation	106.25
Dispersion	11289.54
Standard error	21.25
Mode	104
Minimum	45
Quartile Q1	64
Median	106
Quartile Q3	170
Maximum	460

There may be noticed a mean of 141.28 ng/mL, with a minimum of 45 and a maximum of 460 ng/mL.

From the performed statistical estimations, we determined a good, statistically significant correlation between the tumor size and the prolactin value.

The Pearson correlation coefficient is  $r=0.763$ , namely 76.3%, with a statistically high significant value. The dependence equation between the tumor size and the prolactin is  $y=0.0819x + 2.5789$ , where  $y$  represents the tumor size in [mm] and  $x$  represents the prolactin level. The determination coefficient between the tumor size

and the prolactin level is  $R^2=0.5823$ . This means that approximately 58.2% of the tumor variation is explained by the variation of the prolactin level. The correlation diagram is presented in Figure 2.



**Figure 2 – Prolactin value–tumor size correlations.**

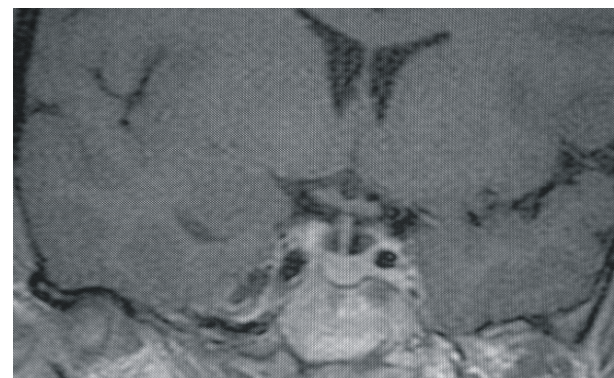
The hypothalamo–hypophyseal pathology usually requires the imagistic support for establishing the diagnosis and for monitoring the efficiency of the performed treatment.

Usually, the radiological exploration is used, starting with common techniques and ending with the most performant ones.

Classical radiology allows the diagnosis of hypophyseal tumoral processes when the latter present large sizes and when calcifications are present within the proliferative process, but it also has the disadvantage of creating confusion among the numerous anatomical varieties, as well as of the method limitations.

The evaluation of hypophyseal adenomas and their extension degree was performed through the MRI method, by using the sagittal and coronary plane reconstruction techniques with standard examination protocols. Thus, there was determined the tumor size, the suprasellar, parasellar, frontal and retrosellar extensions and there were obtained information about the tumor structure. The hypophyseal adenoma was identified as a hypodense mass, which appears as hyperdense after the injection of the contrast substance (Figure 3). The convex surface of the gland was found at 60% of the microadenoma.

A macroadenoma is presented in Figure 3 (MRI hyposignal aspect in T1).



**Figure 3 – MRI aspect of a pituitary macroadenoma.**

Of the macroprolactinoma patients, 10 patients accepted hypophysectomy, being hospitalized and operated in the Clinic of Neurosurgery, “Bagdasar–Arseni” Emergency Clinical Hospital, Bucharest, Romania. The surgical parts were prepared for examination by classical histological techniques, and subsequently by immunohistochemistry.

The used material, representing fragments of tumoral hypophysis, was intraoperatively sampled and placed in specific buffering necessary for the subsequent processing in order to examine the structural aspects of the immunohistochemical examination (which was performed in the Laboratory of Histopathology, "Victor Babeș" National Institute for Research and Development in Pathology and Biomedical Sciences, Bucharest).

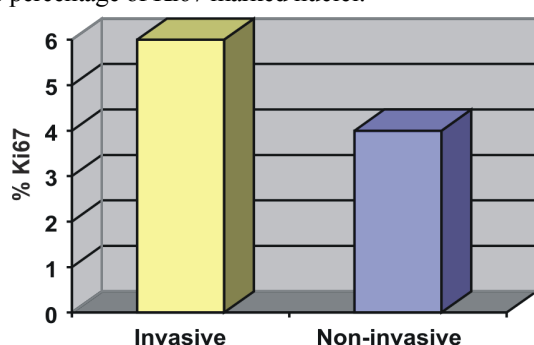
The PRL reaction was present in a tumor with randomly placed cells, of variable intensity and focal representation, presenting a diminished intensity and a peri- or para-nuclear or submembrane intracytoplasmatic layout.

Given the particular aspects and, sometimes, inconsistent behavior of the hypophyseal tumors, we performed the analysis of some markers known as unfavorable prognosis factors for a large number of human neoplasms, which may be involved in the assertion of the aggressive potential of the tumor as important prognosis factors: Ki67, p53, PCNA.

We intended to obtain certain correlations between the histopathological aspect of hypophyseal adenomas and the tumor proliferation factors, thus marking the invasive character and the recurrence potential.

The above-mentioned immunohistochemical markers were investigated in the 10 operated patients.

The histopathological examination pointed out the morphological signs of aggression: cellular and nuclear pleomorphism in various stages (moderate or severe) with nuclear atypias, binucleate and trinucleate cells, hemorrhagic phenomena, hypercellularity, mitotic activity (rarely) in the majority of invasive adenomas. These phenomena were also determined in the non-invasive adenomas, but less frequently. Even though these criteria did not suffice for a clearly enough differentiation among adenomas, still, the adenomas where there was determined an accentuated nuclear pleomorphism and mitoses could be considered as atypical (after WHO classification level 3) (Figure 4). The majority of those adenomas presented a high-level of Ki67 (MIB1 monoclonal antibody). In the end, there was determined the Ki67 marker, expressed as the percentage of Ki67 marked nuclei.



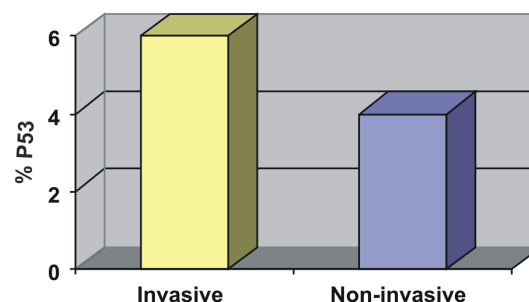
**Figure 4 – Ki67 percentage expression in operated adenomas.**

The PCNA (PC10 monoclonal antibody) ranged between 0 and 20%, with  $14.33 \pm 1.11$  in the recurrent adenomas and  $7.69 \pm 1.46$  in the non-recurrent adenomas.

A nuclear positivation for p53 was identified in six tumors, all among the invasive adenomas (Figure 5).

Among the invasive adenomas, p53 was identified in a single case of very low positivation of one, two nuclei that practically could be considered as indicating a certainly positive reaction. In all reactions, the positivation for p53 was determined only in the nuclei of tumoral

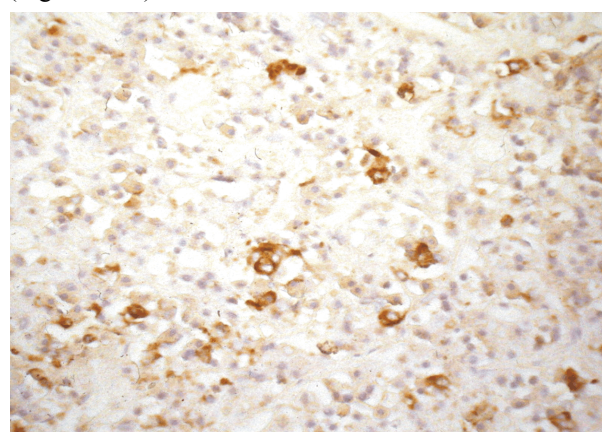
cells. The non-tumoral interstitial adenohypophyseal cells and those of the vascular endothelium were regularly immunonegative. Immunopositivation for p53 was limited to the nuclei. The cytoplasmatic positivation was not established in any of the cases. We determined considerable variations of the intra- and inter-tumoral immunoreaction intensity, considered as the result of regional differences in tissue fixation.



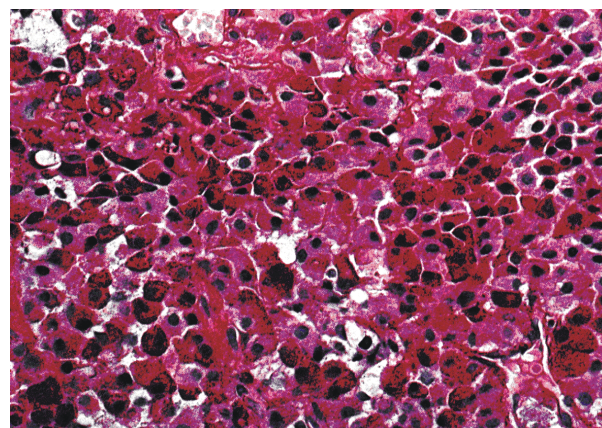
**Figure 5 – P53 percentage expression in the operated adenomas.**

The p53 expression and the tumoral behavior were closely correlated, the presence of the oncosuppressor protein being determined only in the invasive adenomas (50% of the invasive adenomas).

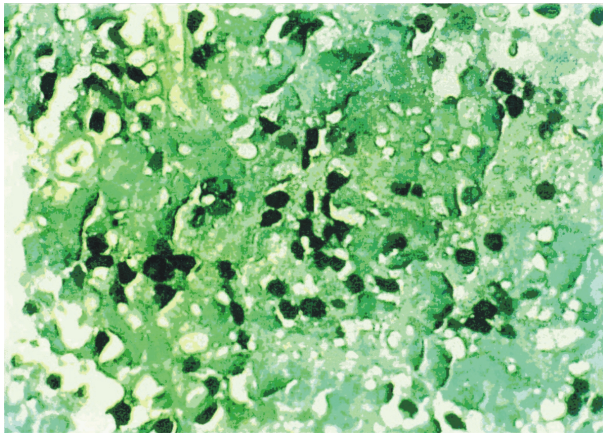
The following figures show the most representative histological aspects found in the studied patients (Figures 6–8).



**Figure 6 – Prolactinoma. Intracytoplasmatic staining in the tumoral cells. Immunohistochemical aspect, antiprolactin monoclonal antibody,  $\times 200$ .**



**Figure 7 – Pituitary adenoma. The adenoma cells are medium sized with a smooth granular eosinophilic cytoplasm, with round nuclei, small nucleoli and the presence of Crooke bodies. HE staining,  $\times 400$ .**



**Figure 8 – The PCNA expression in a pituitary recurrent adenoma. Immunohistochemical staining,  $\times 400$ .**

## Discussion

Pituitary adenomas are benign tumors with major consequences on the human organism. The hormonal-active tumors manifest with well-known syndromes including amenorrhea, galactorrhea (PRL-secreting adenoma – prolactinoma) [7, 8].

Prolactinomas were more frequent in younger subjects – nine (45%) cases and four (20%) cases between 31 and 40-year-old. This confirms the findings that in younger patients these tumors become clinically manifest at puberty [15, 16].

Considering the symptoms by the onset of the disease, headache was registered more frequently in the patients with macroadenomas. The headache had an intense and persistent character.

Hypopituitarism was frequent in macroadenomas and was absent in microadenomas. Despite the fact that in our study the macroadenomas prevail (15 cases out of 20), the hypopituitarism was present only in a minority of cases (25%), as other studies have reported, too [17].

The secondary amenorrhea or oligomenorrhea were present in 49.3% of the studied women, in concordance with the literature [17].

## Imaging

The gland's dimension and morphology are influenced by the age and the gender of the normal subjects. Although a convex surface of the gland was considered abnormal or at least unusual in the beginning, later studies have evidenced convex glands in more than 53% of normal subjects. In our study, this aspect was recorded in 47% of the microadenomas.

## Pathology

The usual morphological signs of tumor aggression, namely pleomorphism, nuclear atypia, hemorrhage, high cellular and mitotic activity, poorly correlate with the invasive potential of pituitary tumors, with their proliferation capacity, their tendency of post-surgical recurrence or with their global biological behavior [10].

The targeted and efficient therapeutic strategy in the clinical practice is based on the precise and complete histopathological diagnosis. Using the conventional optical

microscopy techniques in the histopathological examination, the diagnostic concordance may not be greater as 75%. Thus, it was necessary the introduction of new techniques, such as immunohistochemistry, in order to identify the cellular or tissue constituents, by the antigen-antibody reaction. This qualitative method, beside the conventional pathology, has become the most valuable in the complete structural analysis.

The frequent reports of invasive pituitary tumors having various sizes, reflects the difference between the criteria used in their definition. The invasivity is still insufficient understood on biological bases. The morphological markers that correlate with aggressivity in the cellular system such as nuclear polymorphism, cytological atypia, great cellularity, necrosis and mitotic activity are limited to the pituitary adenomas, especially those related to invasive tendency, growth rate, recurrence potential or their general biology. The mitotic aspects are rarely observed in the pituitary adenomas. The mitotic cells are often enough in the invasive adenomas, but rare enough in the non-invasive tumors to be used as a routine test. Thus, immunohistochemistry is essential in the classification of pituitary adenomas, especially in the identification of the aggressive variants [10, 18].

Recently, because of the understanding of the phenomena of the cellular cycle, the variations of specific nuclear antigens were identified, allowing a immunohistochemical estimation of the tumor growth fraction. Ki67 is one of the nuclear proteins that, despite its physiological function uncertainty, provided useful prognostic information pertaining to cell proliferation in pituitary adenomas [11].

Our study has proven, as well as other studies [3, 10, 12], the high risk of tumor recurrence.

The PCNA level is very low in the beginning of the G1 phase of the cellular cycle, then it grows rapidly toward the middle of the cycle, then remains constant during the S phase; then, it starts to rise again in G2 and M. Thus, PCNA is associated with mitotic activity and a higher degree of development. It also plays a crucial role in initiating the cell proliferation [13]. The p53 immunoreactivity is an acknowledged marker of the aggressive behavior of the human tumors and is present especially in the high-grade or invasive/metastatic neoplasms. The p53 gene mutations occur in the tumors with a strong capability of local aggressiveness [10]. Thus, a new strategy is required for identifying the differences in the pituitary tumors' behavior focused on searching the specific genomic alterations that could serve as markers of tumor aggressivity).

## Conclusions

The clinical manifestations of hypophyseal tumors have the mechanism of high or defficitary tumoral secretion and the compression exerted by the tumor upon the adjoining structures. The clinical signs and symptoms with the highest diagnosis specificity were: galactorrhea, menstrual cycle disorders, eyesight-blurring, cephalalgia. From the performed statistical estimations, we determined a good statistically significant correlation between the tumor size and the prolactin value. The invasion estimate may be also performed on immunohistochemical criteria, as, for example, the percentage of immunoreactivity for

Ki67, PCNA and p53. In the present study, we used these markers to show a high risk for tumoral recurrence. The diagnosis algorithm in hypophyseal adenomas is significantly improved through the association of some modern paraclinical investigations.

## References

- [1] Mastronardi L, Guiducci A, Spera C, Puzzilli F, Liberati F, Maira G, *Ki-67 labelling index and invasiveness among anterior pituitary adenomas: analysis of 103 cases using the MIB-1 monoclonal antibody*, J Clin Pathol, 1999, 52(2):107–111.
- [2] Kumar V, Abbas AK, Fausto N, Mitchell RN (eds), *Robbins and Cotran basic pathology*, 8<sup>th</sup> edition, Elsevier, Philadelphia, USA, 2007, 860–882.
- [3] Kovacs K, Horvath E, *The differential diagnosis of lesions involving the sella turcica*, Endocr Pathol, 2001, 12(4):389–395.
- [4] Wang AT, Mullan RJ, Lane MA, Hazem A, Prasad C, Gathaiya NW, Fernández-Balsells MM, Bagatto A, Coto-Yglesias F, Carey J, Elraiyah TA, Erwin PJ, Gandhi GY, Montori VM, Murad MH, *Treatment of hyperprolactinemia: a systematic review and meta-analysis*, Syst Rev, 2012, 1:33.
- [5] Peña KS, Rosenfeld JA, *Evaluation and treatment of galactorrhea*, Am Fam Physician, 2001, 63(9):1763–1770.
- [6] Serri O, Chik CL, Ur E, Ezzat S, *Diagnosis and management of hyperprolactinemia*, Can Med Assoc J, 2003, 169(6):575–581.
- [7] Casanueva FF, Molitch ME, Schlechte JA, Abs R, Bonert V, Bronstein MD, Brue T, Cappabianca P, Colao A, Fahlbusch R, Fideleff H, Hadani M, Kelly P, Kleinberg D, Laws E, Marek J, Scanlon M, Sobrinho LG, Wass JA, Giustina A, *Guidelines of the Pituitary Society for the diagnosis and management of prolactinomas*, Clin Endocrinol (Oxf), 2006, 65(2):265–273.
- [8] Melmed S, Casanueva FF, Hoffman AR, Kleinberg DL, Montori VM, Schlechte JA, Wass JA; Endocrine Society, *Diagnosis and treatment of hyperprolactinemia: an Endocrine Society clinical practice guideline*, J Clin Endocrinol Metab, 2011, 96(2):273–288.
- [9] Babey M, Sahli R, Vajtai I, Andres RH, Seiler RW, *Pituitary surgery for small prolactinomas as an alternative to treatment with dopamine agonists*, Pituitary, 2011, 14(3):222–230.
- [10] Thapar K, Scheithauer BW, Kovacs K, Pernicone PJ, Laws ER Jr, *p53 expression in pituitary adenomas and carcinomas: correlation with invasiveness and tumor growth fractions*, Neurosurgery, 1996, 38(4):765–770; discussion 770–771.
- [11] Gözü H, Bilgiç B, Hazneci J, Sargın H, Erkal F, Sargın M, Sönmez B, Orbay E, Şeker M, Bozbuğa M, Bayındır Ç, *Is Ki-67 index a useful labeling marker for invasion of pituitary adenomas?* Turk J Endocrinol Metab, 2005, 9(4):107–113.
- [12] Ogawa Y, Ikeda H, Tominaga T, *Clinicopathological study of prognostic factors in patients with pituitary adenomas and Ki-67 labeling index of more than 3%*, J Endocrinol Invest, 2009, 32(7):581–584.
- [13] Pawlikowski M, Gruszka A, Kurnatowska I, Winczyk K, Kunert-Radek J, Radek A, *Proliferating cell nuclear antigen (PCNA) expression in pituitary adenomas: relationship to the endocrine phenotype of adenoma*, Folia Histochem Cytobiol, 2006, 44(1):37–41.
- [14] Yu R, Heaney AP, Lu W, Chen J, Melmed S, *Pituitary tumor transforming gene causes aneuploidy and p53-dependent and p53-independent apoptosis*, J Biol Chem, 2000, 275(47):36502–36505.
- [15] Kane LA, Leinung MC, Scheithauer BW, Bergstralh EJ, Laws ER Jr, Groover RV, Kovacs K, Horvath E, Zimmerman D, *Pituitary adenomas in childhood and adolescence*, J Clin Endocrinol Metab, 1994, 79(4):1135–1140.
- [16] Catli G, Abaci A, Bober E, Büyükgebiz A, *Clinical and diagnostic characteristics of hyperprolactinemia in childhood and adolescence*, J Pediatr Endocrinol Metab, 2013, 26(1–2):1–11.
- [17] Cannavò S, Venturino M, Curtò L, De Menis E, D'Arrigo C, Tita P, Billeci D, Trimarchi F, *Clinical presentation and outcome of pituitary adenomas in teenagers*, Clin Endocrinol (Oxf), 2003, 58(4):519–527.
- [18] Tănase C, Codreanu E, Ardeleanu C, Terzea D, Ciubotaru V, OGREZEAN I, CRUCERU ML, POPESCU M, *Significance of some proliferating cell markers in predicting pituitary adenomas behavior*, 10<sup>th</sup> Meeting of the European Neuroendocrine Association (ENEA), Munich, 12.–14. September, 2002, 92.

## Corresponding author

Mihaela N. Popescu, Lecturer, MD, PhD, Department of Endocrinology, Faculty of Medicine, University of Medicine and Pharmacy of Craiova, 2 Petru Rareș Street, 200349 Craiova, Romania; Phone +40723–572 738, e-mail: mihaelavoica123@yahoo.co.uk, elena.ionesc@yahoo.com

Received: April 10, 2013

Accepted: December 19, 2013