Crosstalk Between Tumor Blood Vessels Heterogeneity and Hormonal Profile of Pituitary Adenomas: Evidence and Controversies

BOGDAN BĂLINIŞTEANU[1], ANCA MARIA CIMPEAN[1], EUGEN MELNIC[2], MIHAIL COCULESCU[3], RALUCA AMALIA CEAUŞU[1] and MARIUS RAICA[1]

[1]*Department of Microscopic Morphology/Histology, Angiogenesis Research Center "Victor Babes", University of Medicine and Pharmacy, Timisoara, Romania;*
[2]*Department of Pathology, "Nicolae Testemiţanu" University of Medicine and Pharmacy, Chisinau, Moldova;*
[3]*Department of Endocrinology, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania*

Abstract. *Aim: Pituitary adenomas are intracranial tumors with controversial histopathology and heterogeneous clinical behaviour. Angiogenesis and tumor blood vessels' role in pathogenesis, remain one of the great pituitary tumor mysteries. No connection between tumor vessel heterogeneity, hormonal profile and biological behaviour has been reported. We aimed to study pituitary adenomas blood vessels concerning their immature, intermediate or mature phenotype and microvessel density, correlated with immunohistochemical hormonal profile and hormone values in serum and cerebrospinal fluid. Materials and Methods: We classified pituitary adenomas according to hormone profile and we applied a double immunostaining highlighting both endothelial and perivascular cells for a more accurate assessment of blood vessel types. Results: Overall microvessel density was found to be highest in growth hormone-secreting adenomas (48.51±12.15) and lowest in prolactinomas (29.15±18.78). When we differentially counted tumor blood vessels we observed a predominance of immature and intermediate blood vessels compared to mature ones. A significant correlation was found between immature tumor blood vessels and tissue prolactin expression, as assessed by immunhistochemistry (p=0.044). A partial correlation was found between serum (p=0.036) and cerebrospinal prolactin values (p=0.006) with immature and intermediate blood vessels. Also, a partial correlation has been reported only between mature blood vessels and cerebrospinal fluid prolactin values (p=0.008). No correlation was obtained for other types of pituitary adenomas. Conclusion: Our results suggest a strong involvement of prolactin with a dual role in pituitary adenomas vasculature remodelling by acting both on endothelial and perivascular cells, a finding that could partially explain discrepancies between clinical diagnosis and hormonal profile.*

Correspondence to: Professor Anca Maria Cimpean, MD, Ph.D., Department of Histology, Angiogenesis Research Center, "Victor Babeş" University of Medicine and Pharmacy, Timişoara 300041, Piata Eftimie Murgu nr.2, Timisoara, Romania. Tel: +40 720060955, e-mail: ancacimpean1972@yahoo.com

Key Words: Pituitary adenomas, tumor blood vessels, prolactinomas, angiogenesis.

Pituitary tumors are the most frequent intracranial tumors (10-15%) after meningiomas and gliomas (1). The biology of pituitary adenomas is complex and they produce a variety of clinical disorders based on hormone profile of proliferating cells. Some of the pituitary tumors are hormone-inactive adenomas with no clinical significance during the lifetime of the patient.

In the past, pituitary tumors were classified as acidophilic, basophilic and chromophobic cell adenomas based on cell cytoplasm microscopic appearance. Modern pathological classification is based mainly on immunohistochemistry and electron microscopy (2, 3). Today, classification of pituitary adenomas reflects specific clinical features and genetic changes that predict targeted treatments, as well as prognostic information for patients with pituitary adenomas (4). Despite the updated classification of pituitary adenomas, several steps of pituitary tumorigenesis remain unclear. Among them, angiogenesis and the role of tumor blood vessels in adenoma progression and invasion is far from being fully elucidated.

Unlike many other solid tumors, where angiogenesis seems to drive the malignancy, pituitary adenoma tumorigenesis often fails to correlate with microvessel density or other angiogenic markers (5).

Both benign and aggressive pituitary adenomas have a lower value of blood vessel density compared to normal hypophysis (6). Except prolactinomas, where the lower microvessel density may be partially explained by the

presence of a 16-kDa fragment of prolactin with inhibitory effects on tumor angiogenic growth factors and endothelial cells (7), none of other pituitary adenomas have any scientific data to support the decrease of blood vessel density. Hence, angiogenesis, in pituitary adenomas without describing its angiogenic mechanism and factors involved in this process, seems to be questionable. However, recent data reported a smaller size of capillaries in pituitary adenomas compared with normal hypophysis and a significant correlation between this vessel type and nestin expression (8). Previous articles published on pituitary adenomas blood vessel assessment were focused on their morphologic and ultrastructural changes (9, 10) and few of them on immunohistochemical or molecular profile (11). Heterogeneity of pituitary adenoma blood vessels was first suggested in prolactinomas by Schechter *et al*. in 1988 (12) based on an electron microscopic study of endothelial and perivascular cells. He observed the presence of abnormal capillaries surrounded by several layers of perivascular cells in prolactinomas compared to normal pituitary fenestrated capillaries. None of other types of pituitary adenomas were characterized concerning blood vessels morphology and immunophenotype. Moreover, tumor blood vessel heterogeneity impact on pituitary adenomas clinical behaviour has not been previously reported.

Based on previous evidence and controversies in the field, we aim to study tumor blood vessel heterogeneity in pituitary adenomas and to answer if their heterogeneity is correlated with tumor type, serum and cerebrospinal fluid hormonal levels.

Materials and Methods

We included in our study 39 specimens obtained from patients previously diagnosed with pituitary adenomas in the Neuroendocrine and Pituitary Disease Department of C.I. Parhon Institute of Endocrinology, Bucharest, Romania and five normal pituitary glands harvested during autopsy. Tumors were removed by the transsphenoidal approach. Each diagnosis was certified by clinical, biological and imaging data. We were also able to detect serum and cerebrospinal fluid (CSF) hormone values in 14 cases. Biopsies were fixed in 10% buffered formalin for 48 h and paraffin embedded. Three micrometer-thick serial sections were performed from each paraffin block. Histopathology was assessed on hematoxylin and eosin stained slides. Based on morphologic evaluation, additional sections from each case were selected to perform hormone profile of pituitary adenomas and to highlight tumor blood vessels endothelial and perivascular cells. For this we used specific antibodies against each of the pituitary hormones as Growth Hormone (GH), prolactin (PRL), Adrenocorticotroph Hormone (ACTH), Thyroid Stimulating Hormone (TSH), Follicle Stimulating Hormone(FSH) and Luteinizing Hormone (LH). (Dako, Carpinteria,CA, USA). Visualization of the immune reactions was done by means of streptavidin- biotin-peroxidase technique with use of 3, 3'-diaminobenzidine as chromogen. Endothelial cells were labelled with anti CD34 antibodies (clone QBEnd10, Dako) and perivascular cells with smooth muscle actin antibodies (clone 1A4, Dako). The Envision Doublestain G system kit (Dako), used as visualisation system, highlighted CD34 positive endothelial cells in brown and Smooth Muscle Actin (SMA) positive perivascular cells in red. The counterstain step was performed by applying modified Lillie Hematoxylin. The entire immuno-histochemical procedure was performed with a DakoCytomation Autostainer (Dako). Microscopic evaluation was performed by three independent observers using a Nikon Eclipse E600 Microscope (Nikon Corporation, Tokio, Japan). Images were captured and processed with the Lucia G software system (link or supplier). The presence of more than 10% of hormone immuno-positive cells was considered as a secretory tumor. The immunostaining results for each patient were graded as being 0 (negative), 1+ (10-30% of cells), 2+ (30-50% of cells) or 3+ (over 50% of cells). The tumors with high co-expression GH/PRL immunoreactivity (>50% of cells) were considered mammo-somatotrophic adenomas.

Tumor blood vessels were quantified for CD34/SMA expression according to Gee *et al*. (13). Based on double immunostaining with the endothelial marker CD34 and the perivascular cell marker-smooth muscle actin (SMA), three morphological types of blood vessels were identified: immature, intermediate and mature. The immature blood vessels showed no obvious lumen and were positive only for CD34; the intermediate blood vessels were positive for CD34, had a well defined perfused or non perfused lumen, thin walls and were occasionally surrounded by perivascular cells (positive for SMA); the mature vessels showed a well structured wall with CD34 positive cells, doubled by SMA positive perivascular cells. Blood vessels were counted in the tumor area using the hot spot method, according to the procedure published by Weidner N *et al*. (14).

Image acquisition was done with the Nikon Eclipse E 600 microscope equipped with a Nikon Photo Camera following by picture processing with the Lucia G Software Analysis System. Statistical analysis was performed by using the SPSS software version 17 package (IBM Corporation, Chicago, USA). Statistical methods included correlation tests as Pearson, Kendall and Spearman tests. Correlations were considered as significant for a $p<0,05$. A total correlation was obtained if all three mentioned correlations showed significance and a partial correlation was reported for cases when two out of all three correlation tests showed significance.

The local research ethics committee approved the study protocol and informed consent was obtained from all subjects according to the World Medical Association Declaration of Helsinki.

Results

Based on the immunohistochemical profile, we classified 39 pituitary adenomas included in the present study as follows: 7 GH-secreting adenomas, 7 PRL-secreting adenomas, 7 mixed GH-cells/PRL-cells adenomas, 3 LH-secreting adenomas, 11 null-cell adenomas (non-secreting) and 4 plurihormonal adenomas. Detailed immunohistochemical profile of each case is shown in Table I.

As a particular clinical aspect, for mixed GH-cells/PRL-cells adenomas, the patients were admitted to the hospital mainly because of acromegaly clinical signs. The same situation was reported for plurihormonal adenomas. For null-cell adenomas, headache and/or imagistic detection of tumor mass by radiologic methods were the most frequent reasons for admittance.

By applying double stain for CD34/SMA on normal hypophysis and pituitary adenoma tissues, we observed a significant decrease of microvessel density in pituitary adenomas compared to normal tissues (Figure 1A). Also, blood vessels morphology and perivascular cells coverage were different between normal and pathological conditions.

Inside normal pituitary parenchyma, we detected capillaries with a well-defined lumen highlighted by CD34-positive endothelial cells without SMA positive pericyte coverage, organized as a rich vascular network (Figure 1A, yellow arrow). Mature blood vessels of normal pituitary with positive reaction for both CD34 and SMA were grouped at the periphery of normal tissue. Microvessel density was 75vessels/field (×200) for CD34+/SMA– fenestrated capillaries inside the normal pituitary parenchyma. At the periphery of the pituitary parenchyma predominated the mature CD34+/SMA+ blood vessels (27 vessels/field; x200) with arteriole, venule and capillary like morphology (13 vessels/field; ×200). Overall vessel density (immature, intermediate and mature) dramatically decreased in pituitary adenomas; with this phenomenon being independent by their hormonal status (Figure 1A, red arrow).

Overall tumor microvessel density (MVD) was not correlated with pituitary adenoma tumor types and no significant correlation was detected by statistical analysis.

Despite the lack of statistical significant correlations between histopathology and overall MVD, we observed that the hormonal subtype with the highest MVD was the GH-secreting adenoma (mean=48.51; SD=±12.15), whereas prolactin cell adenomas had the lowest (29.15±18.78). Other pituitary adenomas types had the following MVD values: 35.40±11.58 for LH-secreting adenomas, 30.82±5.94 for mixed GH-cell/PRL-cell adenomas, 36.30±21.11 for null cell adenomas and 36.62±15.49 for plurihormonal adenomas. Values of differentially microvessel density for immature, intermediate and mature tumor blood vessels specific for each type of pituitary adenoma are summarized in Table II.

Morphology of tumor blood vessels was particular for each type of pituitary adenomas. Null-cell adenomas (non secreting adenomas) had a capillary vascular network composed of vessels with a large and perfused lumen, mainly grouped in the peritumoral area (Figure 1B). No perivascular SMA positive cells have been detected by immunohistochemistry. Isolated CD34 positive endothelial cells characterized the intra-tumor area of plurihormonal adenomas. The capillary walls were discontinuous and intravascular pillars can be observed (Figure 1C). Some of these structures have intra-cytoplasmic vacuoles suggesting their tendency to aquisite lumen (Figure 1D). The peritumor area contained scattered mature blood vessels composed by both CD34-positive endothelial cells surrounded by SMA-positive perivascular cells (Figure 1D, insert). GH-secreting adenomas presented CD34-positive endothelial cells arranged in cords between tumor cells. Some of these cord-like structures aquisite small lumen and scattered perivascular cells have been observed attaching to them (Figure 1E). Mature blood vessels (CD34-positive/ SMA-positive) were present in GH-secreting adenomas but, most of them, were collapsed (Figure 1E). Blood supply of PRL secreting adenomas was composed of a low number of tumor blood vessels with small lumen and lacking SMA positive perivascular cells. Most of these blood

Table I. *Pituitary adenoma classification according to immuno-histochemical expression of pituitary hormones for each case.*

Case	STH	PRL	ACTH	TSH	FSH	LH	IHC-based diagnosis
1	0	0	0	0	0	0	NULL
2	3	1	0	0	0	0	MIXT
3	0	0	0	0	0	3	LH
4	0	0	0	0	0	0	NULL
5	2	0	0	0	0	0	GH
6	0	3	0	0	0	0	PRL
7	0	3	0	0	0	0	PRL
8	2	0	0	0	0	0	GH
9	2	2	0	2	0	0	PLURI
10	1	3	0	0	0	0	MIXT
11	0	0	0	0	0	0	NULL
12	3	1	1	0	0	0	PLURI
13	0	0	0	0	0	1	LH
14	0	3	0	0	0	0	PRL
15	3	3	0	0	0	0	MIXT
16	0	3	0	0	0	0	PRL
17	1	0	3	0	0	0	PLURI
18	3	1	0	0	0	0	MIXT
19	0	0	0	0	0	1	LH
20	1	0	0	0	0	0	GH
21	3	2	0	0	0	0	MIXT
22	0	3	0	0	0	0	PRL
23	0	0	0	0	0	0	NULL
24	1	3	0	0	0	2	PLURI
25	0	0	0	0	0	0	NULL
26	0	0	0	0	0	0	NULL
27	1	0	0	0	0	0	GH
28	0	0	0	0	0	0	NULL
29	0	3	0	0	0	0	PRL
30	0	0	0	0	0	0	NULL
31	0	0	0	0	0	0	NULL
32	3	1	0	0	0	0	MIXT
33	3	0	0	0	0	0	GH
34	0	3	0	0	0	0	PRL
35	3	0	0	0	0	0	GH
36	0	0	0	0	0	0	NULL
37	0	0	0	0	0	0	NULL
38	3	2	0	0	0	0	MIXT
39	3	0	0	0	0	0	GH

NULL, -No positive reaction for any hormones; MIXT, combined GH/PRL expression; GH, STH secreting adenoma; PRL, prolactinoma; PLURI, 2 (other than GH/PRL) or three hormones shared positive reaction; LH, luteinizing hormone secreting adenoma.

Table II. *Tumor microvessel density (MVD; mean±SD) values for each morphological type of blood vessels.*

Hormonal Subtype of adenoma	Immature vessels	Intermediate vessels	Mature vessels
GH-secreting adenomas	27.87±4.81	18.50±12.79	2.14±2.30
Prolactin-secreting adenomas	13.14±7.43	14.12±12.43	1.65±2.15
LH-secreting adenomas	20.53±13.53	12.53±10.95	2.33±2.08
Mixed GH-cell/PRL-cell adenomas	14.27±4.96	14.27±5.20	2.28±2.56
Null-cell adenomas	19.33±13.84	15.70±9.65	1.36±2.01
Plurihormonal adenomas	21.15±12.63	14.07±5.68	1.40±1.14

vessels were not perfused. Intermediate blood vessels constantly showed intravascular pillars suggesting an intense intussusceptive angiogenesis (Figure 1F). Mixed GH/PRL secreting adenoma blood vessels were similar with those from prolactinomas rather than those of GH secreting adenomas.

Fourteen out of 39 cases of pituitary adenomas included in the present study had serum and CSF hormone profiles, as highlighted in Table III.

For these cases we correlated tumor blood vessel types with hormone values. A significant correlation was found between immature tumor blood vessels and tisular expression of immunohistochemically-detected prolactin in tumor tissue (p=0.044) (Figure 2). When we tried to separately correlate immature and intermediate tumor blood vessels with serum and CSF levels of prolactin, no significant correlation was obtained. Instead, the sum of two types showed a partial correlation with serum level (p=0.036) and also with CSF level of prolactin (p=0.006). Also, mature blood vessel value has been partially correlated with CSF level of prolactin (p=0.008) (Figure 3) but not with its serum level (p=0.689). Except serum and CSF values of prolactin, no other hormones showed a significant correlation with tumor blood vessel types.

Discussion

Pituitary adenomas represent one of the most mysterious groups of disease. Their clinical behaviour does not always superpose with their immunohistochemical hormonal profile. Also, pituitary adenoma pathogenesis and mechanisms involved in tumor progression and invasion are not fully elucidated.

Angiogenesis remains a controversial issue in pituitary adenoma pathogenesis. Abnormal vascular pattern in pituitary adenomas was early reported by angiographic studies (15, 16). These preliminary observations were then supported by ultrastructural changes in the capillary bed of pituitary adenomas (9). Ultrastructural and immunohistochemical comparative evaluation of pituitary adenomas and normal pituitary blood vessels number showed a decrease of vessel density in pituitary adenomas compared with normal hypophysis (17). The same finding was observed in our study. Many controversies still exist concerning different MVD values depending on pituitary adenoma hormonal profile. If most published studies agreed that the lowest microvessel density assessed with the CD34 and/or CD31 endothelial markers is specific for prolactinomas (18, 19), the MVD for other pituitary adenoma types remain still questionable (5, 20) and depends on various factors. Our overall MVD values showed that the highest MVD was found in GH secreting adenomas, while mixt GH/PRL and PRL secreting adenomas exhibited the lowest MVD. Our results concerning overall MVD in different types of pituitary adenomas partially corroborate with findings reported by Pawlikowski (21) who also reported the lowest MVD for mixed GH/PRL and PRL secreting adenomas; however, the highest MVD was reported for FSH expressing adenomas. This apparent controversy could derive from the use of different anti-endothelial antibodies to assess tumor blood vessels in both studies. Pawlikowski *et al.* used the Factor VIII-related antigen compared with our method, which used anti CD34 antibodies to highlight endothelial cells. The Factor VIII-related antigen is an endothelial antigen expressed later in the endothelial cell development (after fully development of Weibel Palade bodies) compared with CD34 known to be expressed from early stages of endothelial development. The lowest MVD obtained by Pawlikowski for mixed GH/PRL and PRL secreting adenomas by using theFactor VIII-related antigen should be an evidence which support the presence of immature and intermediate blood vessel types from mixed GH/PRL and PRL secreting adenomas.

None of the studies concerning pituitary adenoma angiogenesis has assessed tumor blood vessels according to their maturation degree. Together with overall MVD, by using CD34/SMA immunohistochemical double-stain procedure, we differentially quantified, tumor blood vessel types according to their degree of maturation, as assessed by presence or absence of perivascular smooth muscle cells. Scattered $CD34^+/SMA^+$ mature tumor blood vessels were observed inside the tumor tissue from all types of pituitary adenomas included in the present study. In contrast, immature and intermediate blood vessels are frequently observed in all types of pituitary adenomas. For such types of blood vessels,

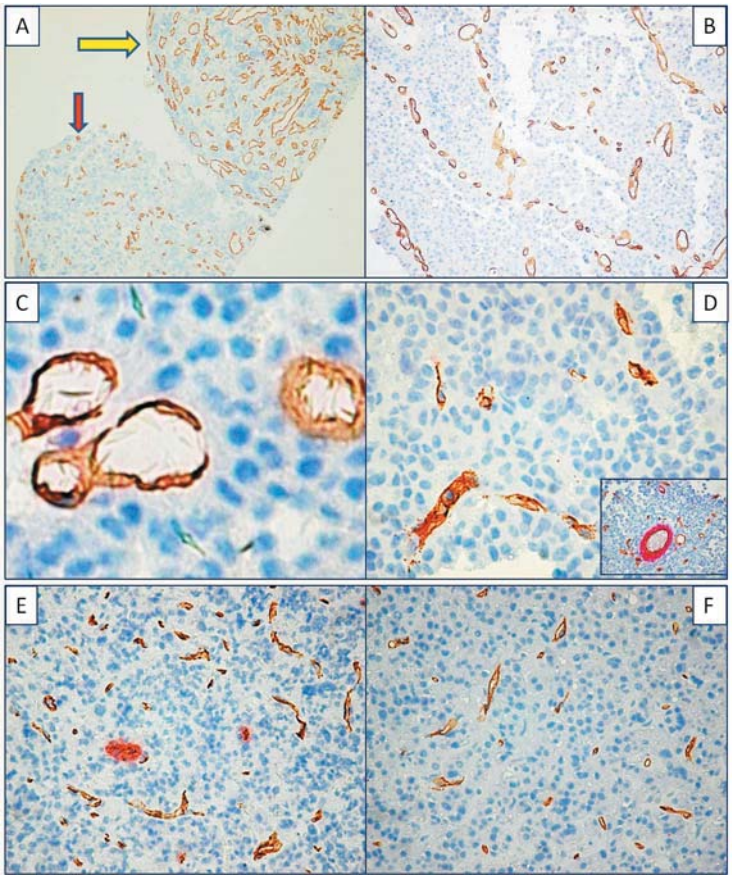

Figure 1. *A lower MVD was observed in pituitary adenoma (A, red arrow) compared to normal hypophysis (A, yellow arrow). Tumor blood vessels in null-cell adenomas grouped at the periphery of tumor areas (B) with discontinuous vascular wall lacking SMA-positive perivascular cells (C). Plurihormonal adenomas (D) with isolated CD34$^+$/SMA$^-$ endothelial cells, and mature CD34$^+$/SMA$^+$ blood vessels grouped in peritumor area only (D, inset). For GH secreting adenomas, intratumor CD34$^+$/SMA$^+$ tumor blood vessels with a well-defined lumen were mixed with collapsed CD34$^+$/SMA$^+$ mature blood vessels and cord-like structures (E). Intratumor blood vessels from prolactinomas had a particular morphology and were characterized by a small lumen and the absence of SMA$^+$ perivascular cells. CD34$^+$ intravascular pillars were frequently seen in prolactinomas. Note CD34$^+$ intravascular pillars (yellow arrows) frequently seen in prolactinomas blood vessels suggesting an active intussusceptive process (F).*

microvascular density showed a high variability dependent on the pituitary adenoma hormonal profile. No data about correlations between vessel types and hormone profiles have been found in the literature. Differences between vascular network morphology and immunohistochemical profile of tumor blood vessels from different types of pituitary adenomas included in the present study suggested that the development of tumor blood vessels in pituitary adenomas could be influenced by a different tumor microenvironment dependent on the hormonal profile of such tumors. These findings are sustained by a recently published study of Di Ieva and coworkers (22). Based on their own observations about heterogeneity of pituitary adenoma microvasculature, they proposed a more complex approach of tumor blood vessel assessment in pituitary adenomas by using a panel of methods including fractal analysis, new and more specific immunological techniques or combinations of different types of immunostaining procedures. They concluded that such an approach of pituitary adenoma angioarchitecture will have an important impact on the study of angiogenesis with relative scientific, medical and surgical implications.

Among pituitary hormones, prolactin has been identified as having a dual and still controversial role in angiogenesis. Blood vessels are well-known targets of both the full length prolactin which exerts an intense angiogenic effect on endothelial cells (23, 24) and also of the 16kDa prolactin fragment which inhibits angiogenesis and lymphangiogenesis (25, 26). Recently, Nguyen *et al*. (27) demonstrated that anti-angiogenic16kDa prolactin, reduced pericyte coverage and disruption of the Platelet Derived Growth Factor B and its corresponding receptor (PDGF-B/PDGFR-B), Angiopoietins and Tie2 and Delta/Notch pathways, leading to a dysfunctional tumor vasculature in a

IICPRL

```
- - -  P A R T I A L   C O R R E L A T I O N   C O E F F I C I E N T S  - - -
Controlling for..    Overall MVD
             IIMVD       C-PRL
IIMVD        1.0000      -.7669
             (    0)     (    9)
             P= .        P= .006
C-PRL         -.7669      1.0000
             (    9)     (    0)
             P= .006     P= .
(Coefficient / (D.F.) / 2-tailed Significance)
```

IISPRL

```
- - -  P A R T I A L   C O R R E L A T I O N   C O E F F I C I E N T S  - - -
Controlling for..    Overall MVD
             IIMVD       S-PRL
IIMVD       1.0000      -.6348
             (    0)     (    9)
             P= .        P= .036
S-PRL       -.6348      1.0000
             (    9)     (    0)
             P= .036     P= .
(Coefficient / (D.F.) / 2-tailed Significance)
```

MATCPRL

```
- - -  P A R T I A L   C O R R E L A T I O N   C O E F F I C I E N T S  - - -
Controlling for..    OVERALL MVD
          MATVESSELS    C-PRL
MATVESSELS   1.0000       .7487
             (    0)     (    9)
             P= .        P= .008
C-PRL          .7487      1.0000
             (    9)     (    0)
             P= .008     P= .
(Coefficient / (D.F.) / 2-tailed Significance)
```

Figure 2. *Partial correlation between cerebrospinal value of prolactin (IICPRL) and serum level of prolactin (IISPRL) and sum of intermediate and immature tumor blood vessels of prolactinomas. Mature blood vessels had partial correlation with CSF value of prolactin, only (MATCPRL).*

		IMMVES	PRL-IHC
IMMVES	Pearson Correlation	1.000	-.324
	Sig. (2-tailed)	.	.044
	N	39	39
PRL-IHC	Pearson Correlation	-.324	1.000
	Sig. (2-tailed)	.044	.
	N	39	39

Correlations between immature tumor blood vessels and +3 score expression of PRL in pituitary adenomas

* Correlation is significant at the 0.05 level (2-tailed).

Figure 3. *Significant and direct correlation between immature tumor blood vessels and +3 score of PRL immunohistochemical expression in prolactinomas.*

murine B16-F10 tumor model. Based on these findings concerning prolactin we can argue, on one hand, for the lowest MVD found in prolactinomas and GH/PRL mixed adenomas and, on the other hand, the presence of immature and intermediate blood vessels lacking smooth muscle actin coverage. Also, the significant correlation found exclusively for prolactinomas in our study between immunohistochemical tissue expression of PRL, immature blood vessels types and the low MVD could emerge from the inhibitory activity of the 16 kDa fragment of prolactin which exerts a stronger anti angiogenic effect impairing blood vessel development and maturation followed by disturbance of prolactin release into bloodstream. The predominance of CD34+/SMA- immature blood vessels in prolactinomas sustains the hypothesis that immature endothelial cells are the main targets of prolactin's inhibitory effect in pituitary tumors, probably by its action on endothelial lumen acquisition process. Lack of endothelial cell lumen acquisition has, as a main consequence, the inability to organize a functional vascular network. This malfunction may explain the high content of prolactin in prolactinomas and GH/PRL mixed adenomas cells scored as +3 by immunohistochemistry as demonstrated in our study.

Because we are not able to find significant -complete or partial- correlations between blood vessel types and other pituitary hormones we speculate that prolactin has an indirect, but important impact on clinical and biological behaviour of prolactinomas and mixed GH/PRL-secreting adenomas by modulating the blood vessel maturation degree.

Mature blood vessels were partially correlated with CSF but not with serum prolactin levels in the present study. Normal pituitary gland has a rich capillary network composed of fenestrated capillaries with few or no perivascular cells. This particular structure of the capillaries in normal condition facilitates a quick and direct release of the pituitary hormones into the bloodstream. Defective maturation of tumor blood vessels by acquisition and presence of several layers of perivascular cells reduces the diffusion of prolactin through capillary walls, impairs prolactin release into bloodstream and facilitates its diffusion directly into CSF. Several years ago, Schechter *et al.* (12) published a paper describing prolactinoma "abnormal arteries" as vessels with fenestrated endothelium surrounded by a variable number of smooth muscle cells, well formed vessels with multiple layers of smooth muscle cells and normal fenestrated capillaries similar with those of anterior normal pituitary. The authors called this phenomenon arteriogenesis. We consider that the description of these "abnormal arteries" is similar with the mature blood vessels of our study, having a defective endothelial layer, which characterizes tumor blood vessel endothelium surrounded by smooth muscle cells found either isolated in the pericapillary connective tissue space or in small cords in some distance from the vessel lumen. Based on Schechter's previous ultrastructural description of blood vessels from prolactinomas

that have been correlated with a partially significant correlation between immature and intermediate tumor blood vessels (with both serum and CSF prolactin values) and also mature blood vessels (with CSF prolactin value exclusively), we may launch the hypothesis that development of tumor blood vessels in prolactinomas is a dynamic process closely related with tumor progression. This suggests that in the first stage of prolactinoma development predominate immature and intermediate blood vessels, while in the later stages tumor blood vessels mature. By this behaviour, tumor blood vessels can modulate serum and CSF values of prolactin with a direct impact on clinical and biological diagnostic parameters.

It is certified that prolactin, by acting through its correspondent receptor, has autocrine and/or paracrine effects on various malignant cells in human tumors or experimental models (28, 29) and is able to induce angiogenic and tumorigenic factors (30-33). But, as a paradox, prolactin effects on tumor cells and blood vessels from prolactinomas and other pituitary adenomas has not been extensively studied.

Conclusion

To the best of our knowledge, this is the first study reporting a correlation between both serum and cerebrospinal fluid hormone levels and tumor blood vessels heterogeneity for different types of pituitary adenomas in humans. Pituitary adenoma blood vessels have particular features depending on their hormonal profile. Development of pituitary adenoma tumor blood vessels is a dynamic process closely related with their special and heterogeneous microenvironment, their progression and clinical behaviour. Except for prolactin effects on endothelial and perivascular cells, no other pituitary hormones have been certified to be directly involved in tumor or non-tumor angiogenesis. Assessment of pituitary adenoma blood vessels by their maturation degree related to hormonal profile specific for each type of pituitary adenomas, might partially explain clinical and biological discrepancies still persisting in pituitary tumors.

Acknowledgements

The Authors we are grateful to Diana Tătucu for her excellent support of specimens' processing and to medical team from C.I. Parhon Insitute of Endocrinology, Bucharest, Romania for collecting clinical and biological data.

Conflicts of Interest

None to declare.

References

1 Saeger W, Ludecke DK, Buchfelder M, Fahlbusch R, Quabbe HJ and Petersenn S: Pathohistological classification of pituitary tumors: 10 years of experience with the German Pituitary Tumor Registry. Eur J Endocrinol *156*: 203-216, 2007.

Table III. *Serum (S) and cerebrospinal (C) hormone values for 14 out of 39 patients together with microvessel density, clinical diagnosis and immunohistochemical profile.*

Case	Immature (CD34+)	Intermediate (CD34+/Act–)	Mature (CD34+/Act+)	Overall MVD	Clinical diagnosis	IHC profile	SGH	CGH	SPRL	CPRL	SLH	CLH	SFSH	CFSH	STSH	CTSH
1	27	40	0	67	ACM	PRL	39.84	1.67	0.000	0.000	0.000	0.000	4.897	1.398	1.55	0.000
2	44	20	0	64	NFA	NUL	0.56	0.00	16.130	4.600	0.000	3.680	1.870	4.730	0.32	0.000
3	32	16	0	48	NFA	PLURI	3.35	0.28	5.300	1.840	0.200	0.310	0.270	0.160	0.00	0.020
4	5	9	0	14	PRL	PRL	2.29	0.02	0.320	0.030	0.520	1.240	0.650	0.770	0.00	0.000
5	14	18	0	32	PRL	PRL	0.43	0.09	13.190	2.980	0.510	2.220	1.720	4.930	0.47	0.150
6	11	14	0	25	NFA	NUL	0.00	0.00	8.030	1.410	1.780	2.930	15.220	4.350	6.10	0.510
7	32	14	0	46	NFA	STH	0.00	0.00	19.920	11.410	0.940	2.150	2.460	1.510	0.62	2.210
8	20.6	18	0	38.6	ACM	PRL	95.54	1.94	35.610	2.720	3.450	1.620	2.730	0.480	1.90	0.130
9	12.3	5	0	17.3	NFA	NUL	0.00	0.00	1.380	0.190	0.860	0.980	0.820	0.000	0.00	0.000
10	12.6	14	5	31.6	ACM	PRL	41.15	14.92	39.100	26.460	0.153	1.415	1.000	5.280	9.69	0.760
11	6.3	11.6	2	19.9	NFA	NUL	0.00	0.00	3.790	1.620	4.520	8.380	5.370	7.780	0.97	1.550
12	5.6	17.3	3	25.9	NFA	LH	0.00	0.00	21.240	3.390	0.000	0.000	7.310	1.570	3.99	0.440
13	8.3	6	2.6	16.9	NFA	PLURI	0.00	0.00	0.000	0.000	2.470	1.250	1.750	0.620	0.00	0.000
14	8.6	15.6	4	28.2	NFA	NUL	0.00	0.00	14.690	7.530	6.190	3.310	12.730	4.000	5.97	5.800

ACM, Acromegaly; NFA, non-functional adenoma; PRL, prolactinoma.

2 Aron DC, Howlett TA: Pituitary incidentalomas: Endocrinology and Metabolism Clinics of North America *29*: 205, 2000.

3 De Lellis RA, Lloyd RV, Heitz PU: Pathology and genetics of tumours of endocrine organs. World Health Organization classification of tumours IARC Press, Lyon, 2004.

4 Mete O and Asa SL: Clinicopathological correlations in pituitary adenomas. Brain Pathol *22*: 443-453, 2012.

5 Niveiro M, Aranda, FI, Peiró G, Alenda C and Picó A: Immunohistochemical analysis of tumor angiogenic factors in human pituitary adenomas. Hum Pathol 36: 1090-1095, 2005.

6 Cohen AB, Lessell, S: Angiogenesis and pituitary tumors. Semin. Ophthalmol *24*: 185-189, 2009.

7 Ferrara N, Clapp C and Weiner R: The 16K fragment of prolactin specifically inhibits basal or fibroblast growth factor stimulated growth of capillary endothelial cells. Endocrinology *129*: 896-900, 1991.

8 Perez-Millan MI, Berner SI, Luque GM, De Bonis C, Sevlever G, Becu-Villalobos D and Cristina C: Enhanced nestin expression and small blood vessels in human pituitary adenomas. Pituitary *16*: 303-310, 2013.

9 Schechter J: Ultrastructural changes in the capillary bed of human pituitary tumors. Am J Pathol *67*: 109-126, 1972.

10 Erroi A, Bassetti M, Spada A and Giannattasio G: Microvasculature of human micro- and macroprolactinomas. A morphological study. Neuroendocrinology *43*: 159-165, 1986

11 Sav A, Rotondo F, Syro LV, Scheithauer BW and Kovacs K: Biomarkers of pituitary neoplasms. Anticancer Res *32*: 4639-4654, 2012.

12 Schechter J, Goldsmith P, Wilson C and Weiner R: Morphological evidence for the presence of arteries in human prolactinomas. J Clin Endocrinol Metab *67*: 713-719, 1988.

13 Gee MS, Procopio WN, Makonnen S, Feldman MD, Yeilding NM and Lee WM: Tumor vessel development and maturation impose limits on the effectiveness of anti-vascular therapy. Am J Pathol *162*: 183-193, 2003.

14 Weidner N, Folkman J, Pozza F, Bevilacqua P, Allred EN, Moore DH, Meli S and Gasparini G: Tumor angiogenesis: a new significant and independent prognostic indicator in early-stage breast carcinoma. J Natl Cancer Inst *84*: 1875-1887, 1992.

15 Berkmen YM: Angiographically demonstrated abnormal vascular pattern in a pituitary adenoma. Clin Radiol *18*: 197-199, 1967.

16 Cophignon J, Djindjian R, Caron JP, Houdart R: Angiographic study of vascularization of hypophyseal tumors. Neurochirurgie *15*: 439-450, 1969.

17 Viacava P, Gasperi M, Acerbi G, Manetti L, Cecconi E, Bonadio AG, Naccarato AG, Acerbi F, Parenti G, Lupi I, Genovesi M and Martino E: Microvascular density and vascular endothelial growth factor expression in normal pituitary tissue and pituitary adenomas. J Endocrinol Invest *26*: 23-28, 2003.

18 Turner HE, Nagy Z, Gatter KC, Esiri MM, Harris AL and Wass JA: Angiogenesis in pituitary adenomas - relationship to endocrine function, treatment and outcome. J Endocrinol *165*: 475-481, 2000.

19 Turner HE, Nagy Z, Gatter KC, Esiri MM, Harris AL and Wass JA: Angiogenesis in pituitary adenomas and the normal pituitary gland. J Clin Endocrinol Metab *85*: 1159-1162, 2000.

20 Vidal S, Horvath E, Kovacs K, Lloyd RV and Scheithauer BW: Microvascular structural entropy: a novel approach to assess angiogenesis in pituitary tumors. Endocr Pathol *14*: 239-247, 2003

21 Pawlikowski M, Pisarek H and Jaranowska M: Immunocyto-chemical Investigations on the Vascularization of Pituitary Adenomas. Endocr Pathol. Autumn *8*: 189-193, 1997.

22 Di Ieva A, Weckman A, Di Michele J, Rotondo F, Grizzi F, Kovacs K, Cusimano MD: Microvascular morphometrics of the hypophysis and pituitary tumors: From bench to operating theatre. Microvasc Res *89*: 7-14, 2013.

23 Reuwer AQ, Nowak-Sliwinska P, Mans LA, van der Loos CM, von der Thüsen JH, Twickler MT, Spek CA, Goffin V, Griffioen AW and Borensztajn KS: Functional consequences of prolactin signalling in endothelial cells: a potential link with angiogenesis in pathophysiology? J Cell Mol Med *16*: 2035-2048, 2012.

24 Clapp C, López-Gómez FJ, Nava G, Corbacho A, Torner L, Macotela Y, Dueñas Z, Ochoa A, Noris G, Acosta E, Garay E and Martínez de la Escalera G: Expression of prolactin mRNA and of prolactin-like proteins in endothelial cells: evidence for autocrine effects. J Endocrinol *158*: 137-144, 1998.

25 Lkhider M, Seddiki T and Ollivier-Bousquet M: Prolactin and its cleaved 16 kDa fragment. Med Sci (Paris) *26*: 1049-1055, 2010.

26 Kinet V, Castermans K, Herkenne S, Maillard C, Blacher S, Lion M, Noël A, Martial JA and Struman I: The angiostatic protein 16K human prolactin significantly prevents tumor-induced lymphangiogenesis by affecting lymphatic endothelial cells. Endocrinology *152*: 4062-4071, 2011.

27 Nguyen NQ, Castermans K, Berndt S, Herkenne S, Tabruyn SP, Blacher S, Lion M, Noel A, Martial JA and Struman I: The antiangiogenic 16K prolactin impairs functional tumor neovascularization by inhibiting vessel maturation. PLoS One, *6*: 11, 2011.

28 González-Lucano LR, Muñoz-Valle JF, Ascencio-Cedillo R, Domínguez-Rosales JA, López-Rincón G, Del Toro-Arreola S, Bueno-Topete M, Daneri-Navarro A, Estrada-Chávez C, Pereira-Suárez AL: Increased expression of the prolactin receptor is associated with malignant laryngeal tumors. Exp Ther Med *3*: 603-607, 2012.

29 Hammer A, Rider L, Oladimeji P, Cook L, Li Q, Mattingly RR and Diakonova M: Tyrosyl Phosphorylated PAK1 Regulates Breast Cancer Cell Motility in Response to Prolactin through Filamin A. Mol Endocrinol *27*: 455-465, 2013.

30 Dagvadorj A, Collins S, Jomain JB, Abdulghani J, Karras J, Zellweger T, Li H, Nurmi M, Alanen K, Mirtti T, Visakorpi T, Bubendorf L, Goffin V and Nevalainen MT: Autocrine prolactin promotes prostate cancer cell growth via Janus kinase-2-signal transducer and activator of transcription-5a/b signaling pathway. Endocrinology *148*: 3089-3101, 2007.

31 Banerjee SK, Zoubine MN, Tran TM, Weston AP and Campbell DR: Overexpression of vascular endothelial growth factor164 and its co-receptor neuropilin-1 in estrogen-induced rat pituitary tumors and GH3 rat pituitary tumor cells. Int J Oncol *16*: 253-260, 2000.

32 Too CK, Knee R, Pinette AL, Li AW and Murphy PR: Prolactin induces expression of FGF-2 and a novel FGF-responsive NonO/p54nrb-related mRNA in rat lymphoma cells. Mol Cell Endocrinol *137*: 187-195, 1998.

33 Harvey PW: Hypothesis: prolactin is tumorigenic to human breast: dispelling the myth that prolactin-induced mammary tumors are rodent-specific. J Appl Toxicol *32*: 1-9, 2012.

Received June 6, 2014
Revised July 15, 2014
Accepted July 16, 2014