IMMUNOHISTOCHEMISTRY WITH INHIBIN ALPHA, MELAN A AND MNF 116 IN ADRENAL TUMORS

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Abstract

Aim. The goal was to study immunostaining with Inhibin alpha, Melan-A and MNF 116 in tumors located in the adrenals (benign adrenocortical tumors and metastatic lesions in the adrenal gland) because sometimes pathology cannot distinguish between the two.

Patients and Methods. We included 35 patients with benign adrenal tumors and 15 patients with adrenal metastases from nonadrenal tumors submitted to laparoscopic (n=40) or classical (n=10) surgery. In our study we have explored immunostaining with inhibin α-subunit, melan-A, MNF 116 in adrenocortical tumors and metastatic lesions in the adrenal gland in order to make the distinction between primary adrenal cortical lesions and metastatic lesions.

Results. All nonsecreting adrenocortical adenomas were stained with inhibin α-subunit and melan-A, but did not stain with MNF 116. All adrenal metastases stained with MNF 116 but were negative for inhibin α-subunit and melan-A with the exception of the 2 melanomas, which stained for melan-A.

Conclusion. Inhibin α-subunit and melan-A were sensitive for benign adrenocortical tumors, while MNF 116 was sensitive for metastases from extraadrenal tumors.

Key words: adrenal adenomas, adrenal metastasis, adrenal tumors, immunohistochemistry, melan-A, inhibin α-subunit, MNF 116.

INTRODUCTION

The percentage of adrenal masses found incidentally increased with the development of imaging techniques. Although the imaging appearance, hormonal tests and histological appearance show the type of tumor in most of the cases, some of the adrenal tumors can be real challenges and immunohistochemistry helps us to make the distinction between them. Previous studies have shown inhibin α-subunit, melan-A and others (BCL-2, calretinin, vimentin, SF-1, D11) to be useful as markers of adrenal cortical differentiation (1-5) and MNF 116 as marker of epithelial tissue differentiation (6).

Inhibin is a dimeric glycoprotein that is structurally and functionally related to transforming growth factor-beta (TGF-β). Ovarian granulosa cells
and testicular Sertoli cells are the main sources of the circulating inhibin. Inhibin α-subunit gene expression (which is needed for inhibin synthesis) has also been detected in extragonadal organs such as: adrenal glands, placenta, pituitary gland, central nervous system, liver. Thus, extragonadal expression of the inhibin occurs mainly in steroid-producing organs and immunopexpression for inhibin alpha-subunit is useful for making distinction of adrenal cortical tumors (7-10). Inhibin immunoreactivity may also be seen in hepatocellular carcinoma (11), renal cell carcinoma (11), and pheochromocytoma (9).

Melan-A 103 is a monoclonal antibody directed against an antigen on melanoma cells recognized by T lymphocytes termed Melan-A or MART-1 (12). Although this antibody has been most commonly used as a marker for malignant melanoma, Busam and coworkers (13) have reported A103 immunoreactivity in adrenal cortical tumors and other steroid-producing cells (1, 11). Subsequent studies have supported the utility of A103 immunostaining in the diagnosis of adrenal cortical tumors (11, 14).

MNF 116 is an antibody which shows an especially broad pattern of reactivity with human epithelial tissue from simple glandular to stratified squamous epithelium and can be used in the detection and classification of normal and neoplastic cells of epithelial origin such as: epidermis, trachea, mammary gland (6, 15).

**Aim of study**

In this study we have examined the immunoreactivity of inhibin alpha-subunit and melan-A as markers of adrenal cortical differentiation and of MNF 116 as marker of human epithelial tissue differentiation, in tumors of adrenal cortical origin (nonfunctional cortical adenomas) and extraadrenal origin (adrenal metastasis from other cancers) in order to make the distinction between primary adrenal cortical lesions and metastatic lesions in the adrenal gland. This can be helpful when we cannot make this distinction morphologically, clinically or through hormonal or imagistic evaluation.

**MATERIAL AND METHODS**

The pathology files of the Clinical Emergency Hospital Bucharest were surveyed to retrieve samples of adrenal tumors and adrenal metastasis. Cases were selected in which the diagnosis was well established on the basis of the histological features of the tumor and the history provided on the pathology report. There were 50 adrenal tumors: 15 metastasis of extraadrenal tumors (lung and breast adenocarcinomas, melanomas) and 35 nonfunctional cortical adenomas diagnosed between 2005-2012 in “C.I.Parhon” National Institute of Endocrinology Bucharest. All patients had adrenal CT and hormonal evaluation: plasma and urinary cortisol, 1 mg DST (dexamethasone) over-night suppression test (cut off value of 1.5μg/dL), plasma and urinary MN (metanephrines) and NMN (normetanephrines) and aldosterone/renin ratio for patients with high blood pressure (HBP).
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Surgery has been performed for all these adrenal tumors in the same period in the Clinical Emergency Hospital Bucharest. We reviewed hematoxylin-eosin–stained slides and selected routinely processed, formalin-fixed, paraffin blocks containing well-preserved tumor. Immunostaining was performed (Bucharest, “Victor Babes” Institute) using three types of antibodies for each tumor sample: Alpha Inhibin - (Novocastra) clone AMY82, Melan-A/ MART1- (Dako) clone A103, MNF116(Dako). Staining of at least 10% of the tumor with moderate or greater intensity was required for a positive reaction. Statistical analysis was made using statistic functions from Microsoft Office Excel 2003 (AVERAGE, STDEV, TTEST with a p value <0.05 being considered with statistical significance).

RESULTS

We included 50 adrenal tumors: 35 nonfunctional unilateral adrenal adenomas and 15 adrenal metastases from other cancers. In the group of the 35 nonfunctional cortical adenomas there were 28 women and 7 men. Mean age of women was 48.07±12.08 years and mean age of men was 49.5± 9.19 years.

The smallest tumor size was 4.1/3.87 cm (left adrenal adenoma) and the biggest tumor size was 6.27/8.31 cm, (left adrenal adenoma). Tumor size was comparable for left and right adenomas (p=0.012). Plasma and urinary MN and NMN were normal. The secretory pattern of plasma and urinary cortisol was normal.

However, 5 patients had bigger values of plasma cortisol (29.7±6.64 μg/dL, N: 8-24 μg/dL) and urinary cortisol (380.8±47.5μg/24h, N: 39-348 μg/24h) but they were suppressed with 1 mg DST. Mean value of plasma cortisol after 1 mg DST was 0.9±0.2 μg/dL with a cut off value of 1.5μg/dL. Mean tumor size for these patients was 4.7/4.8 cm ± 0.42/1.1 cm and it was comparable with mean tumor size of all the other nonfunctional adrenal adenomas (p=0.017). Fourteen patients (40%) had

| Table 1. Median tumor size of nonfunctional adrenal adenomas (n=35) |
|------------------------|------------------------|
| **Left nonfunctional adenomas** | **Right nonfunctional adenomas** |
| Median tumor size ( cm ) | 4.3/4.9 +/- 1.85/1.48 | 4.8/5.1 +/- 1.24/1.47 |

| Table 2. Mean hormonal values for nonfunctional adrenal adenomas (n=35) |
|------------------------|------------------------|
| Mean value |
| Plasma MN ( N: 10-90 pg/mL) | 23 |
| Plasma NMN ( N: 15-180 ) | 54 |
| Urinary MN ( N: 50-350 μg/24h ) | 112 |
| Urinary NMN ( N: 100-600 μg/24h ) | 150 |
| Plasma Cortisol ( N: 8-24 μg/dL ) | 12 |
| Urinary Cortisol ( N: 39-348 μg/24h ) | 125 |
high blood pressure (HBP) and for them it was performed aldosterone/renin ratio which was normal: 11.63±4.37 with a ratio in excess of 30 being considered abnormal. Four patients had symptoms: 3 patients abdominal pain and 1 patient low back pain.

Laparoscopic approach was attempted in all patients and only in 3 cases the conversion to open surgery was needed because of the big tumor size.

All the paraffin blocks were analyzed by Immunohistochemistry using the 3 types of antibodies: Alpha Inhibin - (Novocastra) clone AMY82, Melan-A/ MART1 - (Dako) clone A103, MNF116 (Dako). Alpha Inhibin and Melan-A were positive in all nonfunctional adrenal adenomas and MNF 116 was negative in all the 35 nonfunctional adrenal adenomas.

We also included 15 paraffin blocks of adrenal metastases derived mainly from lung adenocarcinomas (12 cases), mammary adenocarcinoma (1 case), melanomas (2 cases). Mean age of patients with adrenal metastases was 59±9 years old. There were 9 men

**Table 3. Immunoreactivity of nonfunctional adrenal adenomas (arrows show positive cells)**

<table>
<thead>
<tr>
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<th>Alpha Inhibin</th>
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<td>Nonfunctional adrenal adenomas</td>
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![Figure 1a. CSR Adenoma HE10X](image1)

![Figure 1b. CSR Adenoma Melan A pos 20X](image2)

![Figure 1c. CSR Adenoma HE 10X](image3)

![Figure 1d. CSR Adenoma Inhibin pos 10X](image4)
Immunohistochemistry of adrenal tumors

(mean age was 62±12 years old) and 6 women (mean age was 56±8 years old). Mean size of adrenal metastases was: 4.2/3.8±1.23/0.87 cm (minimum size 2 cm and maximum size 7 cm). In 4 cases adrenal metastases were bilateral. Just in 3 cases adrenal metastases were isolated. In the other cases besides the involvement of adrenal gland there were metastases in the spleen, pancreas, kidney, lymph nodes. Adrenal metastases have been detected at the same time with the primary tumor only in 2 cases. In the rest of the cases metastases were found at an average interval of 6.7±3 months. Plasma Cortisol, 1mg DST, plasma and urinary MN and NMN were normal. None of the patients had adrenal insufficiency.

Ten patients had laparoscopic adrenalectomy and in 2 cases conversion to open surgery was necessary. In the other 5 cases open surgery was the procedure of choice from the beginning because of tumor invasion. All patients had specific chemotherapy for the primary tumor.

All the paraffin blocks were analyzed by Immunohistochemistry using the 3 types of antibodies as in the case of the preceding tumors: alpha Inhibin - (Novocastra) clone AMY82, Melan-A/ MART1 - (Dako) clone A103, MNF116 (Dako). MNF 116 was positive in all the 15 adrenal metastases from nonadrenal tumors. Alpha Inhibin was negative in all the 15 adrenal metastases and Melan-A was negative in adrenal metastases with the exception of the 2 melanomas.

**DISCUSSION**

We studied immunoreactivity with inhibin α-subunit and melan-A as markers of adrenocortical differentiation but a lot of other markers have been used to make this distinction in previous studies. For example, D11 is a monoclonal antibody which has been used to mark the nuclei of adrenal cortical cells and it can be helpful in distinction of adrenocortical

| Table 4. Mean hormonal values in patients with adrenal metastases (n=15) |
|-----------------------------|----------------|
| Mean value                  |                |
| Plasma MN ( N: 10-90 pg/mL ) | 34             |
| Plasma NMN ( N: 15-180 )    | 73             |
| Urinary MN ( N: 50-350 μg/24h ) | 100           |
| Urinary NMN ( N: 100-600 μg/24h ) | 132           |
| Plasma Cortisol ( N: 8-24 μg/dL ) | 14            |
| Urinary Cortisol ( N: 39-348 μg/24h ) | 202           |

| Table 5. Immunohistochemistry of adrenal metastases (n=15) |
|-----------------------------|----------------|
| Origin of adrenal metastasis | Alpha Inhibin | Melan A | MNF116 |
| Pulmonary metastases (n=12) | -              | -       | +      |
| Mammary metastases (n=1)    | -              | -       | +      |
| Melanoma metastases (n=2)   | -              | +       | +      |
tumors, but it may also show reactivity with hepatocellular carcinomas, lung carcinomas, and renal cell carcinoma (5). Another known marker for adrenocortical differentiation is SF-1. It has a high sensitivity and specificity to determine the adrenocortical origin of an adrenal mass and its expression is of stage-independent prognostic value in patients with ACC (adrenocortical carcinomas) (4).

Turányi et al. (16) studied the immunoreactivity of Delta-like protein (DLK) which is expressed in fetal and adult adrenal glands in tumors of adrenal and nonadrenal origin and he showed that DLK is a very sensitive marker for adrenal tumors of cortical and medullary origin but renal cell carcinomas, presenting the major differential diagnostic problem for cortical tumors, were all negative, as well as melanomas.

Ankur et al. (17) performed immunoreactivity for the adrenal cortical antigens SF-1, calretinin, inhibin, and melan-A and for the renal epithelial antigens hKIM-1, PAX-8 (18) and showed that the use of novel renal epithelial markers hKIM-1 (clone AKG7) and/or PAX-8 and the adrenocortical marker SF-1 in an immunohistochemical panel for distinguishing adrenal cortical lesions from metastatic CC-RCC improves diagnostic sensitivity and
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Specificity.

Although melan-A and inhibin are highly sensitive and specific in differentiating adrenal cortical tumors from medullary tumors (1), none are highly specific for adrenal cortical tumors. Melan-A and inhibin are both known to be positive in a number of nonadrenal tumors, such as melanomas and angiomyolipomas (melan-A) and ovarian epithelial and sex-cord stromal tumors (melan-A and inhibin) (8, 9, 14, 19). However, these markers (inhibin α-subunit and melan-A) are not useful in differentiating benign from malignant adrenal cortical tumors as long as adrenocortical carcinomas stained with these two markers (3, 7, 20, 21).

Older studies showed positive immunoreactivity for inhibin in normal adrenal cortex: McCluggage et al. (3), Spencer et al. (10), and in benign and malignant adrenocortical tumors (9). Nishi et al. (22) provided data to suggest that tumors associated with Cushing’s syndrome secreted higher levels of inhibin-like material than those causing other clinical syndromes linking inhibin to cortisol production. This was also reported by Pelkey et al. (9).

All literature to date shows that A103 immunoreactivity is very rare in extra-adrenal carcinomas (23). Busam and coworkers found no A103 immunostaining in 111 extra-adrenal carcinomas, including 14 renal cell carcinomas, 8 lung carcinomas, 12 ovarian carcinomas, and 5 hepatocellular carcinomas (12, 13). Renshaw and Granter (11) found no A103 immunoreactivity in 33 renal cell carcinomas and 25 hepatocellular carcinomas. It also should be noted that A103 immunostaining may be seen in angiomyolipomas, lymphangioleiomyomatosis, clear cell sugar tumors, and extra-adrenal steroid-producing tumors such as Leydig cell tumors and granulosa cell tumors (12, 13).

There are a variety of tumors metastases in the adrenal gland and the most frequent are breast, lung, kidney, stomach, pancreas, ovary, colon tumors (24). It is often difficult to appreciate if the tumor is primary adrenal or it is a metastasis of another neoplasm especially in situations in which the patient was not known with a malignancy. This distinction is very important because a diagnosis of adrenal metastasis indicates stage IV disease when examining patients with cancers and the presence of metastases almost always influences the choice of treatment (25). Immunohistochemistry helps us to make this distinction and MNF 116 as marker for epithelial tissue is an important tool in this distinction with the limitations imposed by the wide range of tumours which may be positive with this marker. We found no particular focused studies on immunohistochemistry with MNF 116 in adrenal tumors but in various tumors with epithelial origin which showed positivity for MNF 116: lung adenocarcinomas, mammary, liver cancer, melanomas, etc.

In conclusion, in our study all nonsecreting adrenocortical adenomas stained with inhibin alpha-subunit and melan-A, but did not stain with MNF 116. All adrenal metastases from extra adrenal tumors stained with MNF 116, but they were negative for inhibin alpha-
subunit and melan-A with the exception of the 2 melanomas which stained for melan-A. Thus we can say that MNF 116, inhibit alpha-subunit, melan-A were sensitive in differentiating benign adrenal tumors from adrenal metastases. Our study also confirms the value of inhibit alpha-subunit and melan-A in the diagnosis of adrenocortical tumors and the value of MNF 116 in the diagnosis of tumors of epithelial tissue.

**Conflict of interest**
We declare that there is no conflict of interest.

**Acknowledgement**
This paper is supported by the Sectorial Operational Programme Human Resources Development (SOP HRD) 2007-2013, financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/107/1.5/S/82839”.

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