Biomedical Laboratory Sciences

Mónica Silva

Clinical Case

Histotechnology

BLS ACADEMY
BIOMEDICAL LABORATORY SCIENCE

BioTriCK

Polytechnic of Coimbra

Introduction

PATIENT

Diagnosed with hypercortisolism in 2019

Diagnosed with independent Cushing's Syndrome ACTH

Nodule in the right adrenal gland with 35 millimeters, producing cortisol

Underwent surgery by laparoscopy for removal of the right adrenal gland

Cushing's Syndrome

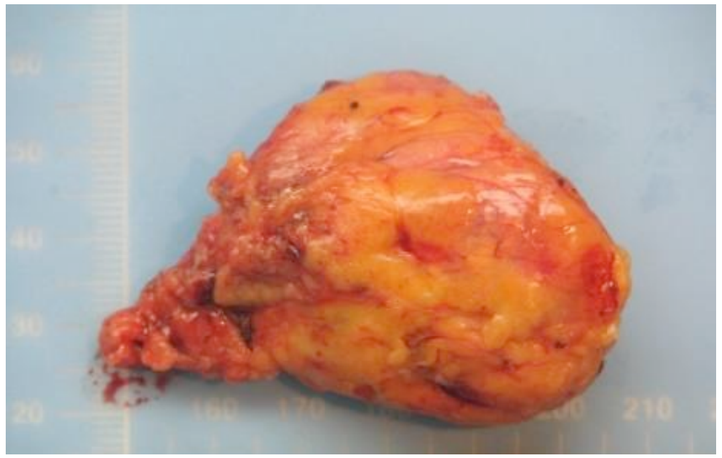
Endocrine disorders that generate an exaggerated secretion of cortisol

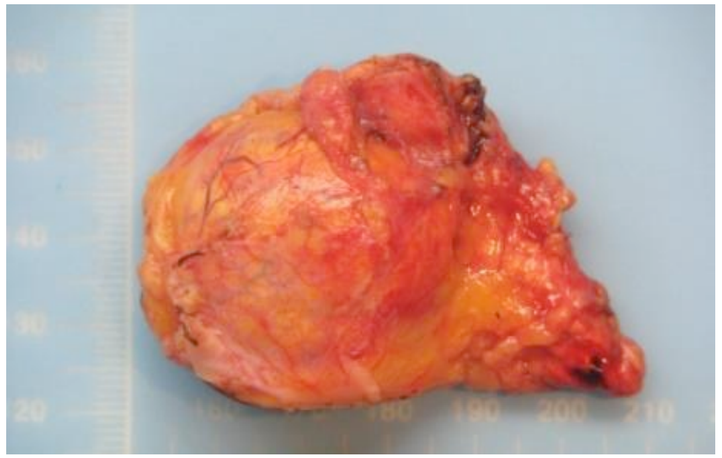
Independent ACTH originates from endogenous causes such as adrenal tumors or nodular hyperplasias

Methods and results

MACROSCOPY

- Right adrenalectomy piece with 19.5 grams
- Dimensions: 6 x 4,6 x 2,7 cm
- Preserved adrenal gland, yellowish, with congestive foci, no particularities

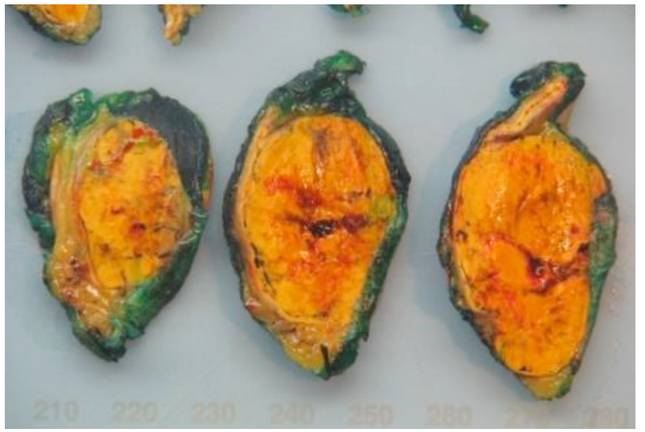




Methods and results

MACROSCOPY

In the performance of sections, a nodular formation with 2.8 cm was observed, consisting of Orange tissue, with congestive foci, well delimited and coinciding with the surgical margin.



Subsequently, fragments representative of the areas of interest for the diagnosis were collected and placed in histological cassettes.

Methods and results

Histological Processing

- Conducted in a closed system, overnight

3 stages

Dehydration → removal of water from tissue using alcohol in increasing concentration

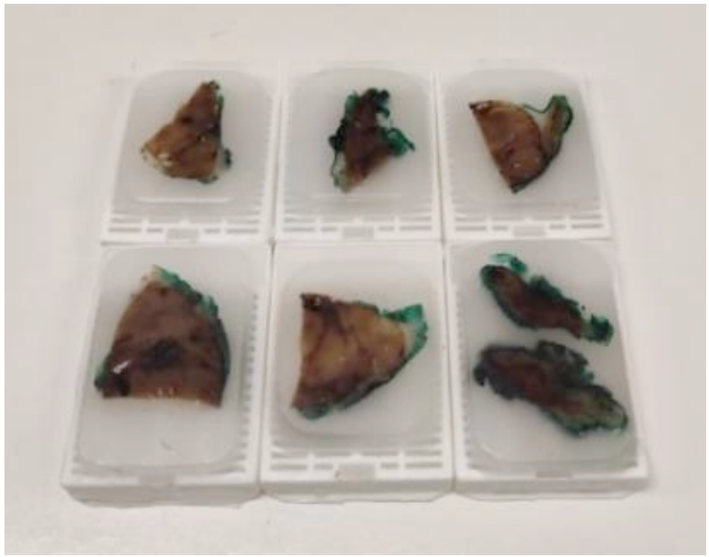
Diaphanization → xylol is used to remove the dehydration medium and subsequent impregnation in paraffin

Impregnation → used paraffin, which replaces the diaphanizing agent of tissues and gives them support and rigidity

Methods and results

Inclusion

It allows to obtain a block that is easy to cut and that allows to obtain fine and quality histological cuts, without distortion or fragmentation of the tissue structures paraffin and suitable molds are used.



Methods and results

Microtomy

Realized in semi-automatic Rotary Microtome type Minot

- Block roughing - thickness of 25 µm
- Histological sections - thickness of 3 µm

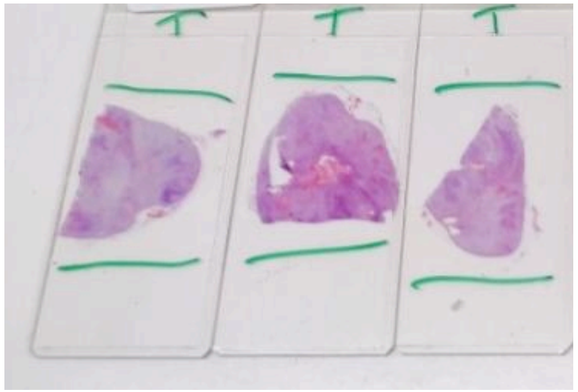
The obtained sections are placed in a histological tub so that their extension occurs and are subsequently placed on slides.

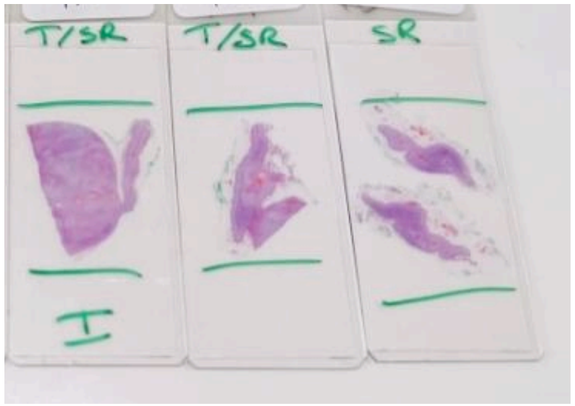
Methods and results

Coloring

Hematoxylin - eosin technique performed in an automatic device

1. Greenhouse-complete adhesion of the cut to the blade
2. Dewaxing-used xylol
3. Hydration-alcohol used in decreasing concentration
4. Hematoxylin
5. Bluing
6. Eosin
7. Dehydration - using alcohol in increasing concentration
8. Diaphanization-using xylol
9. Assembly





Methods and results

Immunohistochemistry

Search for markers:

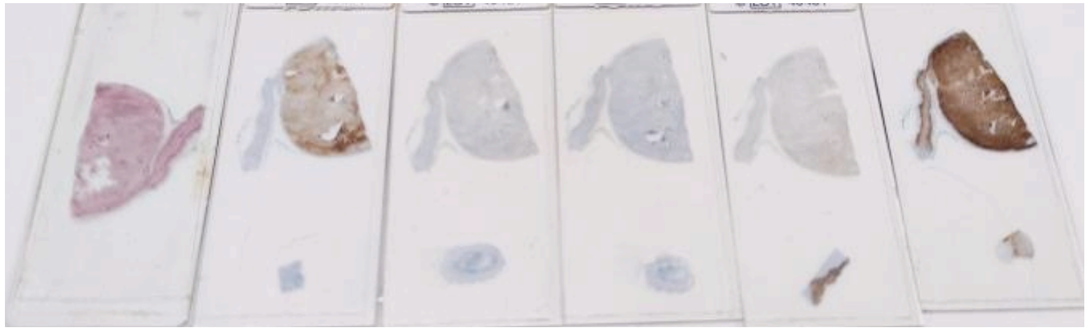
Melan A - melanocyte marker, very associated with melanoma, but which allows distinguishing adrenal cortical tumors from renal cell carcinoma, positively marking the first case.

Alpha-inhibin - a protein that inhibits or activates the secretion of FSH and that marks positively for adrenocortical tumors.

Chromogranin A – a neuroendocrine marker that negatively marks adrenocortical tumors.

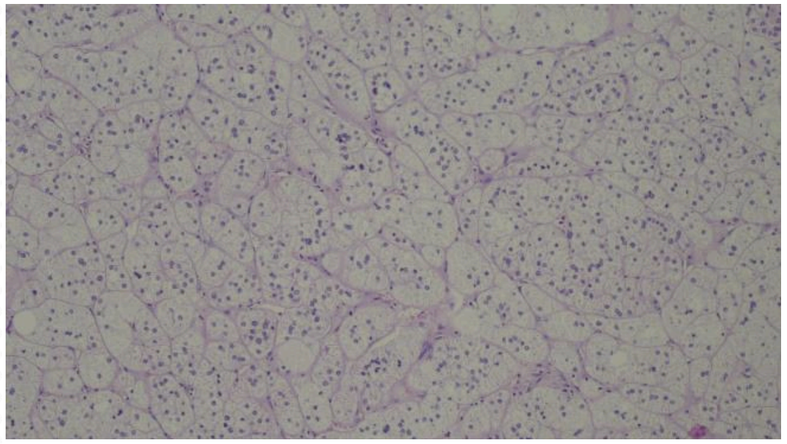
Ki-67 - cell proliferation marker.

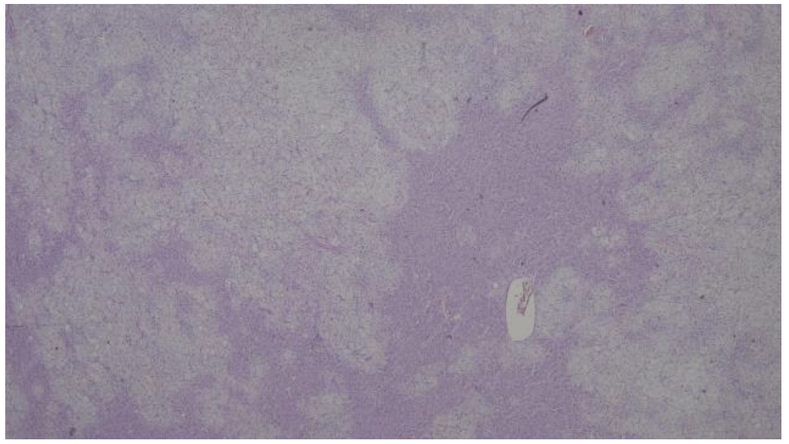
p53 - tumor suppressor gene

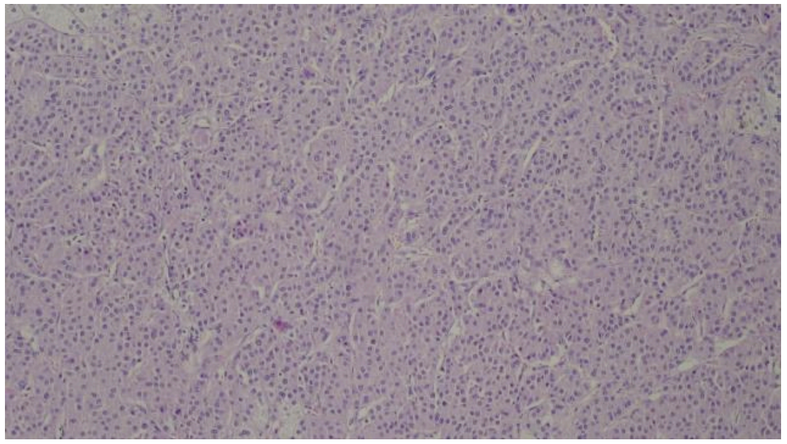


Discussion

At the microscopic level, a nodular lesion with more or less defined limits was described, with a pseudo-capsule that separates it from the normal parenchyma. Tumor cells have small and uniform nuclei, and mitotic activity is absent, as well as venous invasion and necrosis.

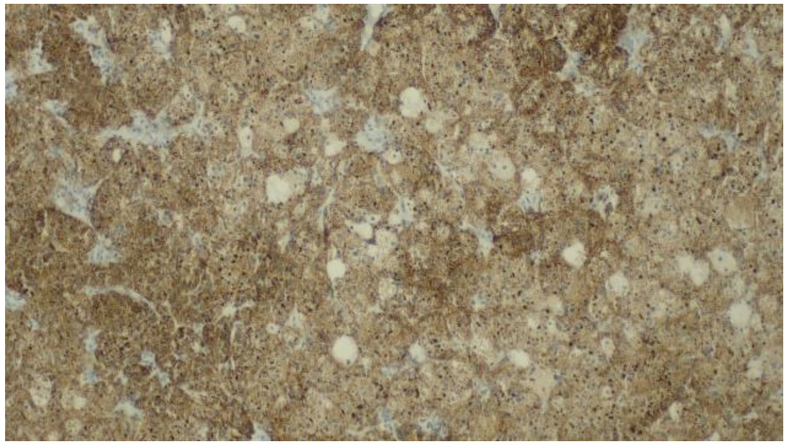






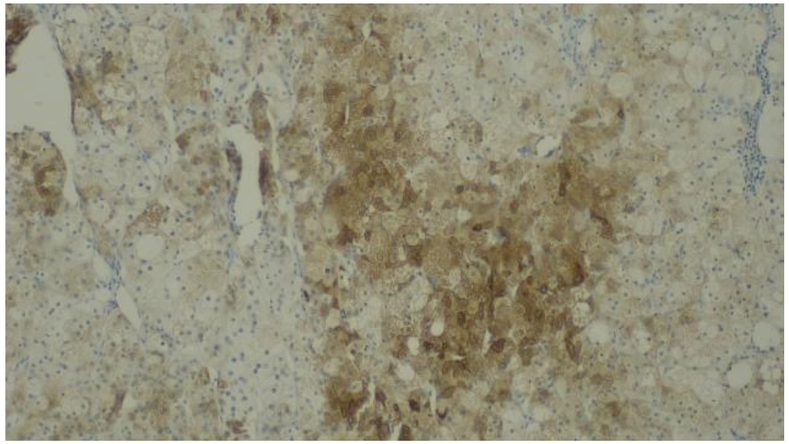
Discussion

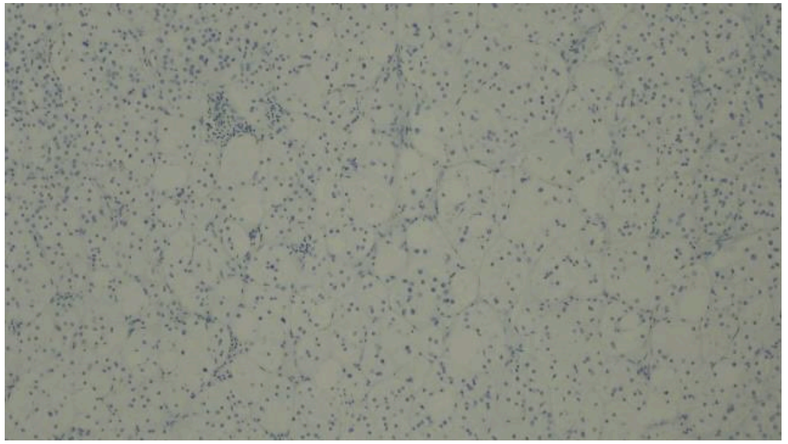
In slides subjected to immunohistochemical techniques it is possible to identify that the tumor positively expresses melanin and Alpha-inhibin, and that there is no labeling for Chromogranin A, Ki-67 is less than 1% and p53 is not mutated.



Melan A

Alpha-inhibin





Chromogranin A

Discussion

To help in the diagnosis, a set of criteria are analyzed that helps differentiate adenomas from carcinomas → **Weiss criteria**

- Presence of necrosis
- Presence of atypical mitoses
- Capsule invasion
- Presence of clarified tumor cells

Tumors that meet less than 3 criteria - adenomas with no risk of metastatic spread

Tumors that fit more than 3 criteria - adrenal adenocarcinomas

In this case, none of the criteria were met → **ADENOMA**

Conclusion

Hematoxylin – Eosin Staining → cells without characteristics of malignancy, without mitosis, without necrosis

Immunohistochemistry → Melan A and Alpha-inhibin positive and Chromogranin a negative

Weiss criteria → no criteria verified

Adenoma of the cortex of the right adrenal gland

Bibliography

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Mónica Silva

monicaars01@gmail.com

Polytechnic of Coimbra

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