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Cushing Syndrome due to black adrenal adenoma: difficulties in the differential diagnosis of a patient with an adrenal adenoma

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Abstract

Signs and symptoms of endocrine dysfunction related to adrenal nodules usually are linked to their specific hormonal hypersecretion. Still, these diagnoses are not always a straightforward process and there have been cases in which the clinical expression has been discordant with the tumour histologic features.

A 59-year-old woman was observed at the endocrinology outpatient department due to uncontrolled diabetes mellitus. Furthermore, the patient referred paroxysmal high blood pressure and weight loss. Her medical history was remarkable by uncontrolled hypertension, chronic hepatitis C, past pelvic actinomycosis and an adrenal nodule diagnosed due to an unrelated purpose. Endocrine evaluation showed serum morning cortisol, urinary cortisol and metanephrines within the reference range, but supressed adrenocorticotropic hormone (ACTH), loss of cortisol circadian rhythm and a non-suppression in the dexamethasone test, suggesting the diagnosis of Cushing's syndrome. The imaging procedures were inconclusive. The CT scan revealed a lesion of 20 mm in the left adrenal with a spontaneous density of 33 HU, well-defined contours, a relative wash-out of 61%, and 120HU on portal venous phase. The MRI showed a hyperintense nodule in T1-weighted images, without a signal dropout in out-of-phase T1 imaging. There was no evidence of tumour invasion of adjacent tissues, ganglion or distant metastasis. MIBG scintigraphy did not show abnormal findings.

The patient underwent unilateral left adrenalectomy. Histopathologic examination showed an adenoma with foci of oncocytic cells and neuromelanin pigments. The surgical procedure resulted in Cushing's syndrome remission, and an improvement in blood pressure and glucose control.

This medical report illustrates the difficulties in the differential diagnosis of an adrenal adenoma. The patient presented some clinical features suggestive of pheochromocytoma, while the typical clinical findings of a Cushing syndrome were absent. Nevertheless, she presented a history of diabetes mellitus, hypertension, chronic hepatitis C and actinomycosis, for which the Cushing Syndrome might have contributed. Additionally, the atypical imaging findings made the differential diagnosis more complicated, when it was in fact a black adrenal adenoma with peculiar histologic characteristics.

Keywords: adrenal gland; Cushing's syndrome; adrenal adenoma; pheochromocytoma; neuromelanin; black adenoma.

Resumo

Uma das disfunções da glândula supra-renal é a síndrome de Cushing, podendo ser causada por um tumor benigno ou maligno. Contudo, alguns dos diagnósticos clínicos podem ser autênticos quebra-cabeças.

Uma mulher de 59 anos foi observada na Consulta Externa de Endocrinologia por diabetes melitus descompensada. Adicionalmente, a doente referia episódios paroxísticos de pressão arterial elevada e emagrecimento. Nos seus antecedentes pessoais, destacavam-se os diagnósticos um nódulo da supra-renal, hipertensão arterial mal controlada, hepatite C crónica e actinomicose pélvica.

A avaliação endócrina subsequente revelou valores séricos de metanefrinas, de cortisol sérico e urinário dentro dos parâmetros de referência. Contudo, o valor sérico da ACTH encontrava-se suprimido, registou-se perda do ritmo circadiano do cortisol e o teste de supressão pela dexametasona revelou uma não-supressão do cortisol, sugerindo o diagnóstico de síndrome de Cushing.

Os exames imagiológicos revelaram-se inconclusivos relativamente à natureza da lesão: a TC mostrou uma lesão de 20 mm na supra-renal esquerda com densidade espontânea de 33HU, com contornos bem-definidos, um *washout* relativo de 61% e densidade de 120HU na fase portal venosa. A ressonância magnética nuclear revelou um nodulo hiperintenso em T1, sem sinal após contraste, sem evidencia de invasão tumoral de tecidos adjacentes, nem adenopatias ou metástases à distância. Foi também realizada uma cintigrafia com MIBG que não revelou alterações.

A doente foi submetida a uma adrenalectomia esquerda. A análise histopatológica revelou um adenoma com focos de células oncocíticas e pigmentos de neuromelanina. O procedimento cirúrgico resultou numa remissão da síndrome de Cushing e redução da pressão arterial e dos níveis glicémicos.

Este caso medico ilustra as dificuldades no diagnóstico diferencial de um adenoma da supra-renal. A doente apresentava alguns sintomas sugestivos de feocromocitoma e não

apresentava os sintomas e sinais típicos de uma Síndrome de Cushing. Contudo, apresentava diabetes melitus, hipertensão, hepatite C crónica e história prévia de actinomicose pélvica para os quais a Síndrome de Cushing poderá ter contribuído. Os exames imagiológicos também não foram esclarecedores, quando de facto se tratava de um adenoma preto da supra-renal com uma histologia muito peculiar.

Palavras-chave: glândula supra-renal; Síndrome de Cushing; adenoma suprarrenal; feocromocitoma; neuromelanina; adenoma preto.

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Introduction

An adrenal incidentaloma is the designation of a clinically silent adrenal mass, discovered accidentally during imaging procedures [1]. Due to the greater availability of imaging procedures and exams, adrenal incidentalomas are increasing in frequency in the clinical practice [2]. They can be subdivided according to their aetiology: an adenoma is the most common type of adrenal tumour and it is usually non-functioning. However, 5-47% may secrete cortisol and 1.6-3.3% secrete mineralocorticoids [3]. More rare diagnosis can also be made, including pheochromocytoma, adrenocortical carcinoma and metastatic cancer [3].

The management of a patient with an adrenal adenoma includes clinical and laboratorial search for symptoms and signs of adrenal hormone excess and the assessment of the risk of malignancy through imaging procedures [4].

As previously reported, adrenal adenomas can be responsible for symptoms and signs related to hormonal excess, e.g., pheochromocytoma, Conn's or Cushing's syndrome. The symptoms depend mostly on the main compound secreted by the lesion [5]. Cushing syndrome is the most common presentation in functioning adenomas, being ACTH-independent, and consisting of a constellation of symptoms and signs secondary to prolonged exposure to elevated cortisol levels. Cortisol secretion from adrenal adenomas are responsible for 15-20% of all Cushing syndromes. These functional adenomas are most common in the fourth and fifth decades and females are more likely to be affected [6]. Nevertheless, more rarely, pheochromocytomas are also capable of secreting various hormones, including ACTH, and Cushing's syndrome has been reported in the literature secondary to this type of tumour.

The laboratorial assessment includes a screening for Cushing syndrome with an overnight (1 mg) dexamethasone suppression test, screening for pheochromocytoma with fractionated plasma metanephrines and screening for hyperaldosteronism measuring the ratio of plasma aldosterone to plasma renin if the patient has concomitant hypertension or unexplained hypokaliema. If there is a possibility of adrenocortical carcinoma, sex-hormones and steroids precursors should be measured as well. In parallel, a non-contrast CT should be done initially. If the results are uncertain, washout CT, chemical shift MRI and FDG-PET should be considered [4].

If an adrenal mass is responsible for hormonal excess or has radiological findings suspicious of malignancy, adrenalectomy is the standard treatment [4].

After surgery, the histopathological examination of the mass is one of foremost importance for the diagnosis, discerning malignity. The Weiss scoring system is the most common evaluating approach. However, this system has its limitations and may not be applicable so easily in tumours with uncommon histopathology, such as a tumour with oncocytic characteristics [7].

Therefore, some adrenal tumours can represent a diagnostic challenge. Herein, we report an extremely rare case of adrenal tumour with atypical clinical and imaging findings.

Clinical Case

A 66-years-old caucasian woman was observed at the endocrinology outpatient department due to uncontrolled Diabetes Mellitus. The patient had the diagnosis of Diabetes Mellitus from the age of 53, with diabetic retinopathy treated with photocoagulation. Her medical records were also noteworthy of a chronic hepatitis C diagnosed at the age of 30 treated with interferon without cure. She also had a past pelvic actinomycosis infection with resection of two thirds of the rectum, colostomy and salpingo-oophorectomy at the age of 50, and a history of a fibromyoma treated by hysterectomy at 53.

In terms of symptomatology, the patient referred paroxysmal hypertension episodes (approximately one per week), followed by a "heat feeling in the mouth" and tremors. Additionally, the patient referred to a feeling of sadness and crying spells.. The patient also referred asthenia and an unexplained and poorly specified weight loss during the last years before her appointment in the outpatient clinic. She also had a record of a 15 mm adrenal mass discovered at the age of 55 in a context that the patient does not know of, nor the reason for this imaging procedure. The clinical examination revealed a patient with 49 kg, 1.62m and a BMI of 18.7 Kg/m^2. The blood pressure laying down indicated 167/85mmHg and a heart rate of 100 bpm, measured in the left arm. In an orthostatic position, after 1-minute standing, the values were similar. She presented general muscle weakness. Physical examination was otherwise unremarkable, namely there was no evidence of tremor, cardiac arrhythmia, fever, diaphoresis, virilization, striae, ecchymoses, buffalo-hump fat distribution, gynecomastia, any palpable mass in the abdomen or adenomegaly.

She was medicated daily with 100 mg of sitagliptin per day, 825 mg of metformin three times per day, 14 international units (IU) of detemir insulin at breakfast and 7 IU at supper, 10 mg of sinvastatin per day, 10 mg of perindopril per day, 2.5 mg of indapamide per day, 5 mg of amlodipine and 5 mg of bisoprolol per day. Even with this medication, hemoglobin A1c (HbA1c) was around 10-12% and blood pressure values around 160/80mmHg.

Her laboratorial work-up, performed under nifedipine and insulin therapy (see table 1) showed a supressed ACTH, 24 h urinary cortisol levels slightly elevated, normal morning serum cortisol values with abolishment of its circadian rhythm, and no suppression after

the administration of 1 mg de dexamethasone (23 mcg/dL). Catecholamine 24h urine testing was completed showing normal values except for a raised value of epinephrine. The clonidine suppression test was then made, which showed normal levels of catecholamines, both baseline and after clonidine administration.

The EKG and chest x-ray were normal. The 24-hours ambulatory blood pressure monitoring (under nifedipine 30mg per day) revealed mild systolic diurnal and nocturnal hypertension, with non-dipper pattern, without spikes evocative of pheochromocytoma (table 2).

The computed tomography showed a thickening of the left adrenal gland, with a nodule of 20 mm with an attenuation of 33 HU and well-defined contours. It had a relative washout of 61% and a gain of absolute contrast in the portal phase of 120HU. The MRI showed a 20-mm nodule in the left adrenal gland, with a discrete hyperintensity regarding the adjacent adrenal parenchyma in T1-weighted images, without a signal dropout in out-of-phase T1 imaging. The imaging procedures did not reveal any organ invasion or metastasis. MIBG scintigraphy did not show abnormal findings.

With the clinical features, biochemical evidence of hypercortisolism and normal catecholamines, she was diagnosed to have a functioning left adrenal tumour with Cushing syndrome.

The patient was submitted to laparoscopic left adrenalectomy. In the surgery, the mass was confined to the left adrenal gland. Preoperative medical management for pheochromocytoma was made with 0.9 % sodium chloride solution, doxazosin 12mg per day and propranolol 40 mg 8/8h. No intraoperative hemodynamic fluctuations were noted during anaesthesia and surgery, and the postoperative period was uneventful, medicated with nifedipine 60 mg per day.

Histopathological examination revealed an adrenal gland with 3.2x1.7x1.7 cm, with a well-demarcated and capsulated nodule in the cranial pole of the gland, with 1.9x1.5 cm of dimension, of brown colour with yellow spots. Adrenal tissue was identified grossly around it. Light microscopic examination of H&E-stained sections revealed an adrenal cortical adenoma with oncocytic cell foci and extended areas where cells showed an abundant brown pigment in the cytoplasm, compatible with neuromelanin – black adenoma.

After the surgery, she did oral cortisol replacing treatment (hydrocortisone), in progressively lower doses, during the next 8 months. Six months after surgery she presented a morning cortisol level of 1µg/dL. Between 8 months after the surgery and 1 year and two months, adrenal function was apparently restored, as evidenced by a morning serum cortisol level of 15µg/dL one year after surgery and 22 µg/dL one year and to months after surgery (and she also presented a normal suppression by 1mg dexamethasone). Even though adrenal function could have been apparently restored, she complained of lack of strength when glucocorticoid replacement was withdrawn, and therefore, she returned to take hydrocortisone 10 mg in alternate days, until 18 months after surgery.

One month after surgery, in the endocrinology follow-up appointment, the patient revealed not only an improvement of the hypertension control, having a mean blood pressure value of 115/65, heart rate of 80 bpm, having reduced her antihypertensive medication (eliminating amlodipine, maintaining only telmisartan 80mg/hydrochlorothiazide 25mg). Control of diabetes mellitus was also significantly improved, she had progressively lower glycemia values comparatively to the values before surgery (hemoglobin A1c of 7,4%, and random plasma glucose between 120-160 mg/dL), taking a unique dosage of 24 IU of glargine insulin at breakfast one month after the surgery and complete elimination of insulin after two months. Approximately 4 months after the surgery, the patient had gained 2kg of body weight and 9 kg after four years.

The patient was also followed up by a gastroenterologist in the outpatient department due to chronic hepatitis C. The patient was treated with interferon for several years without response, and the infection was only eradicated in 2016 after taking sofosbuvir and ledipasvir, showing a sustained virologic response.

Discussion

In this study we report a left laparoscopic adrenalectomy performed for a tumour with some findings suggestive of pheochromocytoma and laboratory findings of subclinical Cushing syndrome which, however, had histopathologic features of an adrenal black adenoma.

Hereby, we describe a patient with an adrenal nodule, detected in an imaging procedure performed for un unrelated purpose [3]. Due to the increasingly availability of imaging for diagnostic or treatment aims, incidentalomas are becoming more and more frequent, however, the majority of the studies are retrospective resulting from autopsy or radiology, having referral bias, unsatisfactory clinical information, and multiple patient selection criteria [2]. Cortical adenomas are the most common entities in this diagnosis (up to 80% all masses) [8, 9]. Even though the majority of cortical tumours are non-functioning, 5-47% secrete cortisol and 1.6-3.3% secrete mineralocorticoids. [3]. Other causes include cortical carcinomas accounting with 1.4% of the cases of adrenal masses or medullar tumours such as pheochromocytoma accounting for 1.8-4.3% [10, 11]. The reported frequencies differ, depending on the study and its criteria. There is, however, clear evidence that the vast majority of adrenal incidentalomas are benign adrenocortical adenomas. [4]

So, in the initial work-up of an incidentaloma, it is relevant and likely decisive to distinguish between a benign and malignant lesion, its origin - derived from the adrenal cortex, the medulla or metastatic - as well as differentiate if the mass is functioning or non-functioning. [4] Malignant lesions may need expeditious surgical intervention and/or some other therapies, therefore, any delay should be avoided.

Concerning their origin, if the mass is medullar, it should be differentiated in pheochromocytoma (benign or malignant), ganglioneuroma, neuroblastoma, or ganglioneuroblastoma. [11] If cortical, these can arise from the three zones (zona glomerulosa, zona fasciculata and zona reticularis). If the lesion is functional, it can result in a hormonal hypersecretion (mineralocorticoids, glucorticoids, sexual steroids and catecholamines) with its associated respective clinical syndromes (Conn syndrome, Cushing syndrome, adrenal hyperandrogenism, pheochromocytoma). [12]

According to the recommended guidelines from the European Society of Endocrinology, there should be an assessment of hormone excess and of the risk of malignancy through clinical history, physical examination, measurement of hormone levels by biochemical testing and imaging procedures [4]. For pheochromocytoma and paraganglioma, genetic testing can also be necessary, in particular patients with suspected germline mutations [13].

Thus, clinical history and physical examination should be guided towards the symptoms and signs related to hormonal secretion and malignant disease [4]. In this case, the patient presented symptoms compatible with pheochromocytoma, such as headache, diaphoresis, palpitation, tachycardia, anxiety episodes and weight loss, with onset 5 years before the incidentaloma diagnosis. However, she also demonstrated clinical comorbidities which could be associated with Cushing syndrome, such as medical history depression, hepatitis C, actinomycosis, Diabetes Mellitus, insulin resistance and hypertension are suggestive of both clinical situations. Although the presence of paroxysmal episodes are suggestive of pheochromocytoma, those episodes were not confirmed by the ambulatory blood pressure monitoring, showing nevertheless, high blood pressure . [14]

Secondly, after the initial clinical history and physical examination, hormonal assessment is critical in the work-up for an adrenal lesion. Concerning the differential diagnosis, for the initial testing of our patient must include the screening of Cushing syndrome, with tests that measure excessive glucocorticoid levels; and concerning pheochromocytoma, measuring plasma free metanephrines or urinary fractionated metanephrines. Based on such reasoning, levels our patient of 24h-urinary cortisol level was slightly increased. Although the morning cortisol level was within normal range, ACTH was supressed. Concerning catecholamines, urinary 24h samples showed slightly raised epinephrine. The other values such as urinary metanephrines and normetanephrines were normal.

The patient underwent a 1 mg overnight dexamethasone suppression test, which has a 90-96% sensitivity [15]. The latter has a 30% false-positive rate, more often in patients with obesity, chronic illness, psychiatric disorders, and alcohol abuse [16]. Therefore, this test cannot give a diagnosis of Cushing syndrome with certainty, and supplementary tests are thus necessary, such as measurement late-night salivary cortisol test (sensitivity of 90-96%, specificity > 90%) or 24-hour urine free cortisol (sensitivity of 90-98%, specificity 90%) [15, 17-19]. It should be noted that there is a substantial heterogeneity in the

specificity and sensibility between the studies according to differences in diagnostic protocols and definitions of outcome. Some authors advocated that they should be used in concert to complement each other and two of them should be done simultaneously, even though the European guidelines advise for the 1mg overnight dexamethasone suppression test as the first test.

In order to rule out false-positive and negative responses, some authors suggest the measurement of both cortisol and dexamethasone to ensure satisfactory plasma dexamethasone concentrations [>5.6 nmol/liter (0.22 μg/dl)].[20] Additionally, several studies have used a cortisol cut-off value between 50 and 138 nmol/L (1.8–5.0 μg/dL) in the 1-mg DST to insure the diagnosis of 'autonomous cortisol secretion', expanding the sensitivity to 100%. If the values are <1.8, the diagnosis of this syndrome is unlikely. The patient had a supressed ACTH, even if the baseline morning cortisol level was within normal range. Moreover, the normal circadian rhythm of cortisol was lost, and the cortisol levels were not supressed in the dexamethasone test. Besides, the 24h urinary cortisol was high, corroborating the Cushing syndrome by hormonal functioning adrenal mass. [21, 22]. The patient did not present the typical classic signs of this syndrome, as mentioned previously, but the reported prevalence of subclinical Cushing's syndrome among patients with adrenal incidentaloma is not rare, ranging from 5% to 20% [23-26].

Low age-and-gender–adjusted DHEAS levels are found in Cushing's syndrome due to adrenal adenoma, even though that it not fully understood, whereas DHEAS levels are a marker of adrenal androgen excess and may signal an adrenal carcinoma. In this patient, DHEAS levels were low, therefore, by itself, not suggesting a malignant lesion. Part of the explanation comes from the fact that many of the steroid biosynthesis enzymes are defective in adrenocortical carcinomas, having therefore an inefficient steroid production, increasing plasma level patterns of steroid precursors. However, the sensitivity and specificity of this parameter are low (51% and 65%, respectively [27].The studies relating to DHEAS and adrenal carcinomas are still very uneven and heterogeneous, limiting its practical applicability and therefore, a normal value will not exclude a carcinoma and further tests will be necessary [28, 29].

The presence of hypertension in Cushing syndrome is about 80% [30]. The cortisol related contributing factors to hypertension seem to result from the interaction between numerous pathophysiological mechanisms modifying plasma volume, cardiac output and

peripheral vascular resistance and pressor response to angiotensin II, all increased in this syndrome. Besides this, studies have shown that cortisol in excess can bind to mineralocorticoid receptors, thus mimic an excess of aldosterone. There is also a decrease of nitric oxide, enhanced vasoconstriction and reduced vasodilatation by increased levels of endotelin-1 [31, 32]. Concerning glucose metabolism, cortisol in excess seems to contribute as well to inhibit insulin secretion and sensitivity, glucose uptake and glycogen synthesis, therefore resulting in insulin resistance and Diabetes Mellitus. [33-37]

Concerning primary aldosteronism, aldosterone/renin ratio as well as measurement of plasma potassium, should be made in patients with high blood pressure or unexplained hypokalemia [38]. Both were normal in the patient.

Urinary fractionated metanephrines were measured and were within the reference range. This analysis has a sensitivity of 85-100%. So, even though the patient had hypertension, fractionated metanephrines were within the normal reference range, against the possible diagnosis of a pheochromocytoma.

Concerning imaging procedures, although most adrenal incidentalomas are benign adenomas, the morphological characteristics by itself cannot consistently differentiate between an adenoma and a malignant lesion. [39, 40] Following the ESE guidelines, it is recommend that all patients with adrenal incidentalomas should undergo a non-contrast CT to ascertain that the mass is homogeneous, lipid-rich and consequently benign. [4]. The attenuation value in unenhanced CT, with a cut-off of <10 HU, expressed in Hounsfield Units (HU), can be used to differentiate between an adenoma and other abnormalities[41] calculating the likelihood of being an adenoma. This method has more than 97% of sensitivity [42]. Nevertheless, we cannot forget the fact that 30% of all adenomas are lipid-poor and can be indistinguishable of a carcinoma in an unenhanced CT. [43] If that is the case, the percentage of change in the washout of the contrast material on the dynamic and delayed contrast-enhanced CT can be a good method to distinguish. [44, 45] These suggestions are based on the remark that adrenal adenomas display faster and superior washout of the contrast material than adrenal nonadenomas. >40% relative washout is highly suggestive of adrenal adenoma [46]. Typically, adenomas lose their contrast more quickly than nonadenomas (assessment 15 minutes after the administration of the contrast), such as malignant lesions that have abnormal vasculature, and therefore, a higher microvascular density, show slower blood flow and

abnormal endothelial permeability. Consequently, the contrast material is accumulated within the tissue a longer time. In our patient's case, the CT scan showed a lesion of 20 mm, spontaneous density of 33 HU, well-defined contours and a relative wash-out of 61%. Pheochromocytomas, for example, may exhibit a high percentage washout on delayed images, but they typically enhance to a greater degree than adenomas do on portal phase images.[47] Therefore, the CT scan is not clear in the definition of a benign or malignant mass, having still the possibility of being a pheochromocytoma.

The MRI imaging may be important to make a diagnosis in some cases, particularly when the CT scan results are ambiguous. The most characteristic feature of an adrenal adenoma is the presence of intracellular lipid, in which chemical shift imaging is the most reliable technique for its diagnosis [48]. Most adrenal adenomas demonstrate a loss of signal intensity on out-of-phase images. The loss of the signal intensity on opposed-phase images relative to the spleen is indicative of the presence of intracytoplasmic lipids and therefore is diagnostic of adenoma [49]. Concerning pheochromocytoma, T2-weighted sequences are more specific than CT and infrequently lose signal in out-of-phase [49]. The patient did not present this loss of signal in the left adrenal gland and presented hyperintensity in T1, and so, it is not in complete favour of an adenoma nor pheochromocytoma.

If, in one hand, CT and MRI are made to provide anatomical details of the adrenal mass, other imaging procedures can be made to target elements of adrenal function, enlightening metabolic pathways. For example, PET/CT may be a good method to evaluate adrenal masses, using radiopharmaceuticals targeted to various features of adrenocortical and medulla function. They have the potential to distinguish benign from malignant adrenal lesions, characterize the lesions, stage them in case of carcinoma and identify neuroendocrine tumours of chromaffin origin. PET/CT uses ^{18}F-FDG [50] and ^{67}Ga for malignant tumours [51]. Hydroxyephedrine-PET [52] may be useful to detect chromaffin tissue lesions. Besides this, scintigraphies may also be made that include ^{131}I-6-iodomethylnorcholesterol for cortical adenomas [53] and ^{123}I-metaiodobenzylguanidine (MIBG) [53], for medullary chromaffin tissue lesions, including pheochromocytomas and ganglioneuromas [54]. ^{131}I-6-iodomethylnorcholesterol (NP-59), is a radiotracer that is selectively taken up in the adrenal cortex, accumulating bilaterally in the normal adrenal glands and unilaterally in case of a hormonally active adenoma or carcinoma [55].

Regarding our patient, after uncertain CT and MRI scans, a MIBG scintigraphy was made that did not report abnormal hyperfixation zones. This exam was made to rule out pheochromocytoma [56]. In summary, the imaging procedures did not conclude any undoubtful diagnosis. Many patients still go to recurrent imaging scans and probably still do adrenalectomies that are not evidence-based necessary. However, in our clinical case, since the laboratorial work showed hormonal hyperfunction of the adrenal, adrenalectomy was evidence-based regardless of the imaging procedures.

Although the probability was low (normal laboratory and MIBG scintigraphy), since the diagnosis of pheochromocytoma could not be completely ruled-out (suspect CT and MRI), left adrenalectomy was performed with pre-operative preparation for this condition.

Several studies have reported that adrenalectomy is more beneficial than conservative management in patients presenting subclinical Cushing syndrome in an incidentaloma context, allowing remission of its co-morbidities [57]. That is, indeed, what happened to our patient, improving most of the laboratory hormonal alterations, improving arterial blood pressure values and glycemic control, reducing antihypertensive, oral antihyperglycemic agents and insulin need, improving body mass index and quality of life.

Anatomopathological examination revealed that the lesion was an adrenal adenoma with pigment compatible with neuromelanin and foci of oncocytic cells. Several factors are considered for determining the malignancy nature of adrenal cortical tumours, such as tumour size, presence of necrosis, nodularity, and Weiss criterion (considered the standard). 9 parameters are evaluated: high nuclear grade, mitotic rate >5 per 50 HPF, atypical mitotic figures, eosinophilic tumour cells (≥75% of tumour), diffuse architecture (≥33% of tumour), necrosis, venous invasion (smooth muscle in wall), sinusoidal invasion (no smooth muscle in wall) and capsular invasion [58, 59]. The presence of 3 or more of the nine criteria is suggestive of an adrenal cortical carcinoma, while two or less would be more evocative of an adenoma. So, according to these criteria, the mass was considered an adenoma.

Since the lesion had a black colouring due to what was considered as neuromelanin pigment, it was designated as “black adenoma”. The term “pigment” refers to the different

colouring matter in the cell or tissue depositions and frequently are granules or cytoplasmic inclusions. The precise nature of the pigment is not always identifiable without complete histochemical characterization or electron microscopy and their identification not only helps in its correct diagnosis, but also provides important prognostic information. The main sources of tumour pigmentation are melanin or lipofuscin nodules, primary or secondary melanoma, haemangioma or adrenal myelolipoma. Lipofuscin, the main pigment found in cortical adrenal adenomas, is brown and develops inside of granules of senescent epithelial cells or in cells in degenerative altered cells (known as brown atrophy). The function of neuromelanin has yet to be sorted out. This substance is perceived as a waste product of catecholamine metabolism (data *in vitro* indicate that neuromelanin pigment is formed from the excess cytosolic catecholamine not accumulated into synaptic vesicles), and is indistinguishable from lipofuscin in its ultrastructure [60]. While the presence of lipofuscin in adrenal tumours is very common, interestingly, neuromelanin is not. There is a study that reaffirms that the presence of neuromelanin implies necessarily that catechol precursors were present in the pigmented cells, so they could be oxidized and polymerized to produce this pigment [61]. So, it is important to distinguish neuromelanin and lipofuscin pigments from melanin, since the latter is frequently present in primary and secondary malignancies, and the first ones are more often present in benign lesions [62]. There are some studies concluding that, in very rare cases, adrenal adenomas could be a source of catecholamines, eventually explaining some symptomatology of the patient, which was suggestive of pheochromocytoma, whose clinical presentation could be attributable to the presence of epinephrine noneccentric granules [63]. Alternatively, some studies have raised the possibility of a mass effect of the cortical tumour resulting in adrenal medullary hyperplasia [64, 65], the adrenal tumour itself can trigger the adrenal medulla to secrete catecholamines even without hyperplasia [66]. Adenomas are usually negative for S-100 and HMB45 markers, positive low-molecular-weight keratins, limited and small-sized, measuring less than 5 cm [67]. Carcinomas are usually bigger in size and melanomas are positive Melan-A, HMB45 and S-100 [68]. Therefore, immunohistochemical analysis and electron microscopy can elucidate the true biphasic nature of these tumours.

Besides the neuromelanin pigment described, oncocytic foci was also mentioned in the histopathological examination. Oncocytic cells are large epithelial cells with a deeply eosinophilic, granular cytoplasm. Oncocytic tumours, also designated as oncocytomas,

can also be found as adrenal incidentalomas. 75% are benign and 31% are associated with Cushing syndrome, virilizing syndrome or clinical features of pheochromocytoma [70]. Histopathologically, these tumours present a granular eosinophilic cytoplasm and numerous mitochondria. The salivary gland, kidney, thyroid, parathyroid and hypophysis are the main organs in which oncocytomas develop, but cases have been reported in the adrenal glands [71]. Usually, they have a considerable tumour size (8,5 cm in length), round, well- circumscribed, encapsulated, solid with possible areas of necrosis and/or haemorrhage that correspond to brown areas [72]. No decisive features can discriminate benign from malignant adrenal oncocytic neoplasm on imaging scans, lacking clarity [73]. In fact, this agrees with the findings in the present case. However, absence of mitosis, tumour necrosis or vascular invasion helps to exclude the possibility of malignancy features [74]. This association, that is to say, neuromelanin pigments co-existing with oncocytic foci of cells in the adrenal gland, is extremely rare and we found no case reported in literature [69].

In the presence of a suspicious nodule, only microscopic criteria are able to determine a precise histology description and clinical behaviour, so adrenalectomy is the standard treatment, being laparoscopy the most diffuse approach [75].

In conclusion, this communication describes an extremely rare case of adrenal black adenoma with a clinical presentation mimicking pheochromocytoma and associated with a subclinical Cushing syndrome suggested by increased values cortisol, findings which posed some diagnostic dilemma pre-operatively. Therefore, we must reaffirm the relevance of the biochemical assessment in any patient with an adrenal mass, aside from any clinical symptoms and signs. It also shows the need for the development of new methods of study concerning the adrenal gland, since in this patient, CT and MRI were not able to reflect the intrinsic nature of the black adenoma.

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Tables

Laboratory values	**Before surgery (09/2011)**	**One month after the surgery (04/2012)**	**Five months after the surgery (08/2012)**	**12 months after the surgery (03/2013)**	**2 years after the surgery (2017)**
Sodium (133–145mmol/l)	143	N	141	142	141
Potassium (3.3–5.1mmol/l)	4,5	N	4,1	4,4	4,8
Fasting glycemia (mg/dL)	**335**	106	147	123	130
HbA1c (%)	**9,7**	**7,4**		**6,9**	**7**
ACTH (6–50pg/ml)	**<5**	24	9	81	55
Cortisol (6.7–22.6µg/dl)	19	5,1	1,1	15	21,5
Cortisol after low-dose DST test (normal suppression <1.8µg/dl)	23				
Renin in orthostatic position (4.4 -46.1 µUI/mL)	6,50			13	
Aldosterone (orthostasis: 97 - 626; decubitus: 42 - 202 pg/mL)	310,2		65,0	165,3	
DHEA-S (94.0–246µg/dl)	< 15	<15	<15	<15	9
24-h urinary free cortisol (55.5-286 µg/24h)	**456**	76	206		68
Urinary creatinine (600-1800 mg/24h).	**456,6**	**405,0**		556,7	610
Urinary free cortisol µg/g-creatinine	**1000**	187			111
Urinary aldosterone (2.8 - 30 µg/24hrs)	6.9	7.3	16.7		
24-h urinary norepinephrine (15-80 µg/24h)	29				
24-h urinary epinephrine (0-20 µg/24h)	**33**				
Normetanephrine-sulfate (44-540 µg/24h)	149,3				
Metanephrine-sulfate (26-230 µg/24h)	110,9				
Vanillylmandelic acid (1.8-6.7 mg/ 24h)	**8,1**				
5-Hydroxyindoleacetic Acid (0.7-8.2 mg/24h)	**11,0**				

Table 1: Pre-operative and postoperative biochemical evaluation in the presented case. Surgery day was 16/03/2012.

	average sYSTOLIC bp (MMHG)	% of high SYSTOLIC bP	AVERAGE DIASTOLIC BP (MMHG)	% OF HIGH DIASTOLIC BP
Daytime	150	100%	78	11%
Nightime	146	100%	75	86%
Total	149		78	

Table 2: 24-hours ambulatory blood pressure monitoring in mmHg (under nifedipine).