Case Report

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Synchronous aldosterone- and cortisol-producing adrenocortical adenomas diagnosed using CYP11B immunohistochemistry

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Abstract

Immunohistochemistry with antibodies targeting enzymes responsible for the final conversion steps of cortisol (CYP11B1) and aldosterone (CYP11B2) is gaining ground as an adjunct tool in the postoperative evaluation of adrenocortical nodules. The method allows the pathologist to visualize hormone production for each lesion, thereby permitting a more exact assessment regarding the distinction between adrenocortical adenomas and adrenocortical hyperplasia, with implications for patient follow-up. We describe how immunohistochemistry facilitated the histopathological diagnosis of twin adenoma (one cortisol- and one aldosterone-producing) from suspected hyperplasia in a patient with hypertension, mild autonomous cortisol secretion and concurrent adrenocorticotropic hormone–producing adrenomedullary hyperplasia. As the nodules were similar in size and displayed rather analogous histology, CYP11B1 and B2 immunohistochemistry was needed to exclude adrenocortical hyperplasia, allowing us to discharge the patient from further surveillance. We conclude that the application of functional immunohistochemistry has direct clinical consequences and advocates the prompt introduction of these markers in clinical routine.

Keywords

Adrenocortical adenoma, adrenocortical hyperplasia, hyperaldosteronism, Cushing syndrome, CYP11B1, CYP11B2

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Introduction

The underlying cause of primary hyperaldosteronism as well as subsets of cases with hypercortisolism is a hyperfunctioning adrenal mass, either an adrenocortical adenoma (ACA) or adrenocortical hyperplasia (ACH). While the treatment often is multimodal, surgery remains the curative option for patients with ACA, while ACH patients display an increased risk of subsequent hormonal hypersecretion from the contralateral adrenal.[1,2] The distinction between ACA and ACH therefore confers a more correct follow-up of these patients. The introduction of monoclonal antibodies targeting the CYP11B1 and CYP11B2 enzymes responsible for the conversion to cortisol and aldosterone, respectively, allows correlation between individual nodules and their functional capacity.[3,4] Without these antibodies, the pathologist must rely on the quantity of the lesions (single nodules imply ACA, while multiple expansions suggest ACH), in addition to the histological appearance (lipid-rich cell dominance suggests cortisol-producing lesion, while lipid-poor cell dominance implies aldosterone production). Numerous

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reports have validated the clinical value of utilizing CYP11B1 and CYP11B2 antibodies, perhaps best exemplified in patients with hyperaldosteronism—as subsets of patients diagnosed with ACH have been re-assigned as aldosterone-producing ACAs.[3]

We present an exceptionally rare manifestation of a patient displaying twin ACAs (one cortisol- and one aldosterone-producing) as well as adrenomedullary hyperplasia with ACTH production and show how the use of CYP11B1 and CYP11B2 antibodies aided in the final diagnosis in a case otherwise highly suspicious for ACH.

Case report

The patient is a 72-year-old male of Swedish ethnicity with a medical history including excessive consumption of alcohol, sleep apnea, glaucoma and cardiac insufficiency, lacking family history indicative of adrenal tumors. In 2012, the patient developed septicemia following a core-needle biopsy of the prostate, and an abdominal computed tomography (CT) scan was performed revealing two distinct nodules measuring 27 mm × 22 mm, Hounsfield unit (HU) 9 and 18 mm × 12 mm, HU 1, respectively, in the left adrenal gland. Physical examination revealed hypertension, abdominal obesity and relatively slim extremities. Hormonal analysis demonstrated a pathological overnight dexamethasone suppression test (s-cortisol 361 nmol/L after suppression), which was repeated with similar result (s-cortisol 388 nmol/L after suppression). A 24-h cortisol-ACTH curve (six separate samples) showed a stiff cortisol curve (lowest s-cortisol value 339 nmol/L at 06:00 h, highest s-cortisol value 408 nmol/L at 12:00 h) and s-ACTH suppressed (<1.1 pmol/L) at all times. Plasma renin (4.9 ng/L, reference: 3.0–16 ng/L), aldosterone (273 pmol/L, reference: 80–440 pmol/L) and methoxycathecolamines were within normal ranges. The patient also displayed hypokalemia intermittently, with lowest values of 3.0 mmol/L. However, the biochemistry did not prove a diagnosis of primary aldosteronism, and adrenal vein sampling was not considered.

To summarize, these hormonal investigations indicated mild autonomous cortisol secretion (MACE), previously defined as subclinical Cushing syndrome. Treatment with anti-hypertensive agents (losartan, metoprolol, amlodipine) had limited effect. After delay caused by loss of follow-up, the patient was offered an adrenalectomy in 2016—but declined. In a follow-up CT scan performed approximately 4 years after diagnosis, the smaller tumor had increased from 18 mm × 12 mm to 26 mm × 20 mm, while the size of the larger tumor was relatively unchanged. At this time, the patient reported a subjective weakening of muscle strength.

The patient underwent left adrenalectomy in 2018. The removed adrenal weighted 52 g, and the pathology grossing identified two adrenocortical lesions; one larger measuring 27 mm × 20 mm × 14 mm displaying a yellow and solid cut surface, as well as a second nodule measuring 22 mm × 17 mm × 17 mm with a yellow-orange appearance (Figure 1(a)). In addition, the adrenal medulla was macroscopically observed with an increased width, suggesting adrenomedullary hyperplasia (AMH). Microscopically, the larger lesion was demarcated and encapsulated by a thin capsule and composed of cells reminiscent of zona fasciculata with abundant, finely vacuolated cytoplasm and small nuclei arranged in nests and cords (Figure 1(b)). In addition, a smaller cell population (5%) was seen exhibiting an eosinophilic phenotype. The Weiss score was 0. All immunohistochemical analyses were performed using a clinically accredited platform and validated antibodies (Ventana Medical Systems, Oro Valley, AZ, USA) in a routine pathology laboratory setting. The tumor cells were positive for Melan A, Inhibin alpha and calretinin—proving an adrenal origin. The Ki-67 index was 1%. Surprisingly, no CYP11B1 immunoreactivity was noted, while a strong and diffuse CYP11B2 staining was seen in most tumor cells—verifying the lesion as aldosterone-producing (Figure 1(c) and (d)). The separate 22-mm large lesion was similar in appearance, but with a larger proportion of eosinophilic cells (20%) (Figure 1(e)). In addition, myelolipomatous changes of unknown significance were observed. The Weiss score was 0 and the Ki-67 index was 1.5%. Melan A, Inhibin alpha, calretinin and CYP11B1 stained positive, while CYP11B2 immunoreactivity was absent (Figure 1(f) and (g)). AMH was confirmed microscopically and investigated for ACTH production given the twin adrenocortical nodules and the coupling between AMH and intra-adrenal ACTH production.[5] The ACTH antibody is a rabbit polyclonal antibody stained in a "ready-to-use" setting with an antigen retrieval step using Ventana Ultra CC1 buffer as recommended by the manufacturer. The antibody is used in our clinical routine practice and displayed expected staining outcomes of positive and negative controls (data not shown). Subsets of adrenomedullary cells were positive for ACTH (Figure 1(h)). The final histopathological diagnosis was consistent with twin ACAs, one aldosterone- and one cortisol-producing. The concurrent ACTH-producing AMH was not believed to play a causal role in the development of these nodules given the different hormonal profiles of the lesions. The patient's hypertension was reversed postoperatively and he is normotensive 6 months after surgery. Also, the patient developed transient hypocortisolemia postoperatively and required cortisone (first intravenous hydrocortisone treatment, followed by per oral administration in successive reduction), which was terminated 7 months postoperatively following ACTH stimulation tests displaying normal adrenal function. His intermittently observed hypokalemia also resolved postoperatively.

Ethical approval was acquired by the local ethical review board. Informed consent was obtained.

Discussion

The advent of functional immunohistochemistry has revolutionized the histopathological assessment of multiple adrenocortical lesions in patients with hyperaldosteronism and

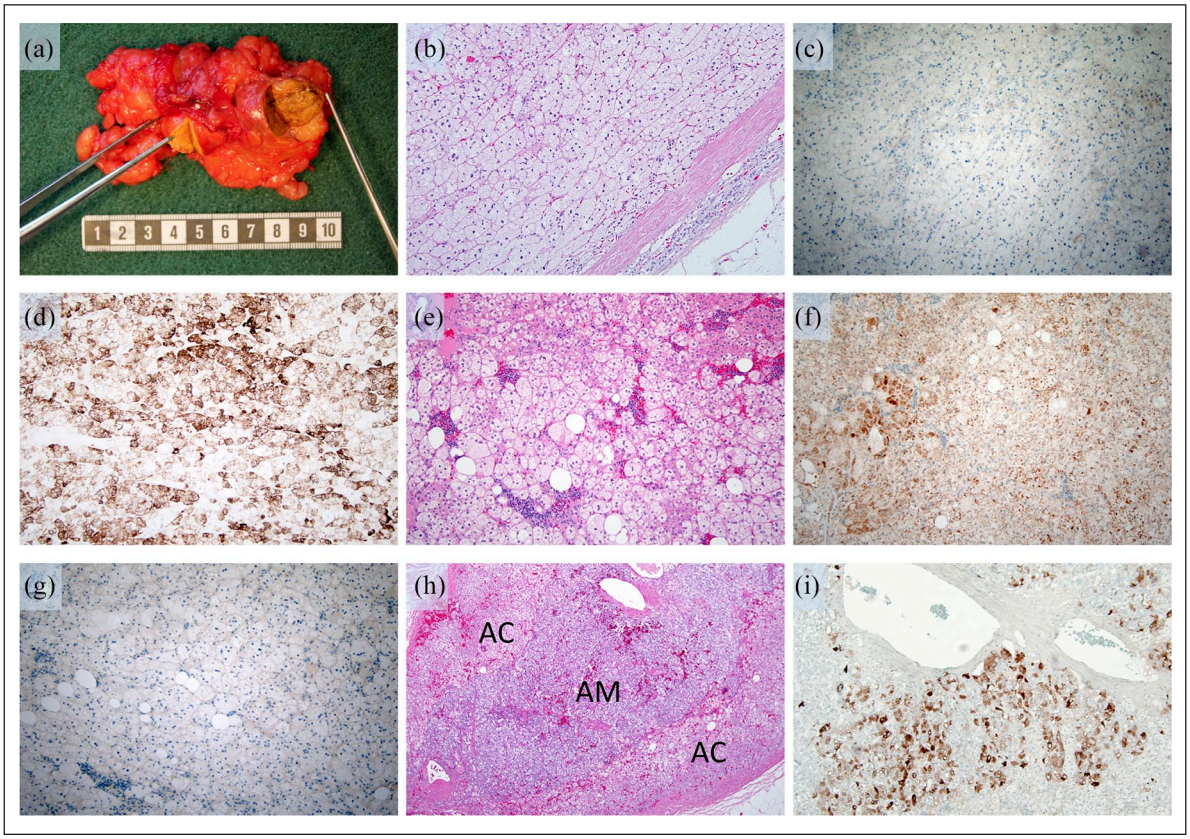

Figure 1. Macro- and microscopic findings of the excised adrenal. All microscopic images are magnified ×100 unless otherwise stated. (a) The resected adrenal specimen with postoperative incisions into the twin ACAs aldosterone-producing to the left, cortisol-producing to the right). Note the canary-yellow cut surface of the left nodule and the yellow-orange color tone of the right lesion. (b) Routine hematoxylin and eosin (H&E) staining of the aldosterone-producing ACA displaying predominance of lipid-rich cells reminiscent of the zona fasciculata arranged in nests. (c)–(d) Same case stained negative for CYP11B1 and intensely positive for CYP11B2, respectively. (e) Routine H&E staining of the cortisol-producing ACA. Note the increased proportion of eosinophilic cells as well as the myolipomatous changes. (f)–(g) Same case stained positive for CYP11B1 and negative for CYP11B2, respectively. (h) Adjacent area of the adrenal tail with prominent adrenal medulla magnified ×40, indicating adrenal medullary hyperplasia (AMH). The adrenal medulla clearly exceeds one third of the gland's thickness. (i) ACTH immunostaining of the AMH component visualizes ACTH production in subsets of the chromaffin cells.

hypercortisolism, and numerous studies report the clinical benefits of employing the CYP11B1/2 markers.[3,4,6] The advantage compared to routine histology alone is perhaps best exemplified by patients with hyperaldosteronism—as subsets of patients diagnosed with ACH have been re-assigned as aldosterone-producing ACAs after the application of CYP11B1/2 immunohistochemistry. This patient category exhibited a high cure rate following surgery, further motivating the re-classification from a clinical standpoint.[3]

From a diagnostic perspective, there are numerous instances arguing for an ACH in our case: the multinodularity, the comparable histological appearance as well as the intra-adrenal ACTH production from the AMH component. Indeed, without CYP11B1 and CYP11B2 immunohistochemistry, there is a likelihood of this case being signed out as an ACH—in which case the patient would have been followed clinically for contralateral recurrence. Instead, the markedly different hormonal profiles of each adrenocortical lesion allowed us to pinpoint the occurrence of double ACAs. A similar case was described in 1997 when numerous analyses were needed for this distinction, also demonstrating the use of CYP11B1/2 immunohistochemistry as an overall diagnostic improvement for these lesions.[7] Moreover, previous studies have pinpointed continuous zona glomerulosa (ZG) hyperplasia in patients with aldosterone-producing adenomas—a phenomenon which was not seen in our case.[8] As both adrenocortical nodules were macroscopically distinct and encapsulated, there is little risk of confusion between the occurrence of double adenomas and widespread ZG hyperplasia in this study.

The patient exhibited normalized blood pressure postoperatively and required hydrocortisone supplementation therapy—suggesting that the MACE phenotype was associated with the CYP11B1-positive ACA. The occurrence of a separate adenoma with CYP11B2 immunoreactivity could in theory also suggest a concomitant hyperaldosteronism

profile, but the only preoperative aldosterone value recorded for our patient (measured 6 years prior to the actual surgery) was normal, although he exhibited intermittent hypokalemia that resolved following the adrenalectomy. As no further preoperative investigations along this line were made, we cannot, with certainty, rule out whether the patient suffered from simultaneous primary hyperaldosteronism or not.

To summarize, we present a rare case of twin ACAs and exemplify how the application of functional immunohistochemistry in clinical routine was crucial in establishing the final diagnosis, with implications for future patient follow-up. The current case thus demonstrates the benefit of utilizing CYP11B1/2 antibodies as a complement to routine histopathology, and we strongly recommend tertiary units to implement these markers in clinical practice.

Declaration of conflicting interests

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