Endocrine (2019) 65:683–691
https://doi.org/10.1007/s12020-019-02022-8

ORIGINAL ARTICLE

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H-score of 11β-hydroxylase and aldosterone synthase in the histopathological diagnosis of adrenocortical tumors

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Received: 5 March 2019 / Accepted: 12 July 2019 / Published online: 22 July 2019

Abstract

Purpose To assess the diagnostic performance of the H-score of 11β-hydroxylase (CYP11B1) and aldosterone synthase (CYP11B2) in the histopathological diagnosis of adrenocortical tumors (ACT).

Methods We retrospectively evaluated 199 cases of ACT, of which 85 were diagnosed as aldosterone-producing adenoma (APA), 66 as cortisol-producing adenoma (CPA), 9 as aldosterone–cortisol co-secreting adenoma, 30 as non-hyperfunctioning adenoma, and 9 as adrenocortical carcinoma (ACC). Immunohistochemical staining was performed using anti-CYP11B1 and anti-CYP11B2 monoclonal antibodies. The staining was quantified by the McCarty's H-score system. The diagnostic performance was assessed by the receiver operating characteristic curve (ROC).

Results The H-score of CYP11B1 is highest in the CPA group and lowest in the ACC group. The H-score of CYP11B2 in the APA group is significantly higher than other ACT groups. The area under ROC (AUC) of an increased H-score of CYP11B2 (>65) for the diagnosis of APA was 0.971 (95%CI 0.937–0.990). The AUC of an increased H-score of CYP11B1 (>204) for the diagnosis of CPA was 0.725 (95%CI 0.658–0.786). The AUC of a decreased H-score of CYP11B1 (<85) for the diagnosis of ACC was 0.960 (95%CI 0.923–0.983).

Conclusions H-score of CYP11B1 and CYP11B2 are reliable tools for the histopathological subtyping of functional benign ACT and may offer some value in the histopathological diagnosis of malignant ACT.

Keywords Adrenocortical Tumors · 11β-hydroxylase · Aldosterone synthase · H-score · Histopathological diagnosis

Introduction

Adrenocortical tumors (ACT) are classified into benign and malignant tumors. Adrenocortical adenoma is the most common cause of benign ACT with a prevalence of 1.4–8.9% in general population [1]. Varied in steroid secretion, adrenocortical adenoma is mainly classified into aldosterone-producing adenoma (APA), cortisol-producing adenoma (CPA), nonhyperfunctioning adenoma (NHFA), and rarely aldosterone–cortisol co-secreting adenoma (ACA). APA, CPA, and ACA commonly show resistant hypertension [2] and cardiovascular, cerebrovascular, and metabolic complications [3–5]. Adrenocortical carcinoma (ACC) is a rare adrenocortical malignancy with a prevalence of ~1–2 cases per million in the general population [6]. Despite its rarity, ACC has drawn much attention due to its aggressive behavior and poor prognosis [7]. The subtyping of ACT is relatively complicated and entails a comprehensive assessment of clinical/biochemical

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Supplementary information The online version of this article (https://doi.org/10.1007/s12020-019-02022-8) contains supplementary material, which is available to authorized users.

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characteristics, imaging, histopathological findings, and follow-up.

Histopathological examination is crucial for the differential diagnosis of ACT, and consists of morphological evaluation and immunohistochemical staining [8]. Morphological evaluation is the basis of histopathological diagnosis, and immunohistochemical staining with the appropriate biomarkers can significantly enhance the diagnostic accuracy [9].

11β-hydroxylase (CYP11B1) and aldosterone synthase (CYP11B2) are the key enzymes for the synthesis of cortisol and aldosterone, respectively [10–12]. The strong immunopositivity for CYP11B1 or CYP11B2 in ACT suggests CPA or APA, respectively [13–15]. Besides, it has been reported that the immunoreactivity of CYP11B1 in ACC is significantly lower than the normal adrenal cortex [16]. However, there have been reports of APA with absent CYP11B2 immunoreactivity, but positive for CYP11B1 staining [17, 18]. Furthermore, few studies have compared the immunoreactivity of CYP11B1 between ACC and NHFA. Given the fact that the patterns of CYP11B1 and CYP11B2 immunoreactivity were varied in some ACT and were less documented in others, their roles in the pathological subtyping of ACT remain to be investigated.

This study retrospectively evaluated 199 cases of common causes of ACT, and the immunoreactivity of CYP11B1 and CYP11B2 in all tumor specimens were examined and quantified by the McCarty's H-score system. We aimed to assess the diagnostic performance of the H-score of CYP11B1 and CYP11B2 immunoreactivity in the histopathological diagnosis of ACT.

Materials and methods

Study design and participants

This study was approved by the ethics committee of the first affiliated hospital of Chongqing medical university and a written informed consent was obtained from each participant. One hundred and ninety-nine patients with ACT who underwent unilateral adrenalectomy between November 2012 and December 2017 were retrospectively enrolled in this study, including 85 cases of APA, 66 cases of CPA, 9 cases of ACA, 30 cases of NHFA, and 9 cases of ACC (Supplemental Fig. 1).

The diagnosis of APA was based upon the Endocrine Society's clinical practice guideline [19] and the Primary Aldosteronism Surgical Outcome Study (PASO) consensus [20], which required all of the following criteria to be met: (1) the diagnosis of primary aldosteronism (PA) was established: positive for PA screening [aldosterone-rennin ratio (ARR) ≥20 $pg{\cdot}ml^{-1}/mIU{\cdot}l^{-1}$ (101 $pmol{\cdot}l^{-1}/mIU{\cdot}l^{-1}$)] and positive for at least one confirmatory test [fludrocortisone suppression test (FST), post-FST of plasma aldosterone concentration (PAC) >6 ng/dL; saline infusion test (SIT), post-SIT PAC >6 ng/dL; captopril challenge test (CCT), post-CCT PAC> 11 ng/dL]; (2) typical adenoma was detected on computed tomography (CT) scan (unilateral radiolucent nodule with a diameter at least 10 mm, with the remaining ipsilateral and contralateral adrenal glands appearing smooth and not enlarged) and/or lateralization was confirmed by adrenal vein sampling (AVS); (3) one single adenoma was confirmed at pathology; and (4) biochemical cure of hyperaldosteronism was achieved after unilateral adrenalectomy (PASO standard).

The diagnosis of CPA was based upon the Endocrine Society's clinical practice guideline [21], which encompassed the following requirements: (1) Cushing's syndrome was confirmed on the basis of at least two of the followings: (a) 24 h urinary free cortisol greater than the detection upper limit of normal values for at least two measurements; (b) 8 am plasma cortisol post 1 mg-overnight dexamethasone suppression test (DST) > 50 nmol/l; (c) cortisol post 2 mg low-dose DST > 50 nmol/l; (2) serum ACTH lower than 10 pg/ml or inhibiting rate after 8 mg DST less than 50%; (3) unilateral adrenal nodule was detected on CT scan or magnetic resonance imaging (MRI); and (4) an adenoma is detected at pathology.

Nine cases, meeting the diagnostic criteria for both PA and Cushing's syndrome, were classified as ACA and were either enrolled in the CPA group when evaluating the diagnostic performance of the H-score of CYP11B1 or enrolled in the APA group when evaluating the diagnostic performance of the H-score of CYP11B2.

The diagnosis of NHFA [22] required all of the following criteria: (1) normal biochemical test results and no catabolic signs; (2) unilateral adrenal nodule is detected on CT scan or MRI; and (3) an adenoma is detected at pathology. These NHFAs were surgically resected due to suspicious radiological features or surgical desire from patients. The diagnosis of ACC was based on morphological parameters assessed using the revised Weiss scoring system [23], an ACT with a Weiss score >3 was histologically confirmed as ACC (Supplemental Table 1).

All clinical diagnoses were confirmed according to these comprehensive criteria by two experienced endocrinologists who reviewed the medical records of all study participants and were used as reference standards.

Immunohistochemistry

All surgically removed adrenal specimens were subsequently fixed by 10% buffered formalin and embedded in paraffin. Immunohistochemical staining was performed using rat anti-CYP11B1 (Millipore, MABS502, 1: 500) and

Table 1 Clinical characteristics of subjects

	APA ($n = 85$)	CPA ($n = 66$)	ACA ($n = 9$)	NHFA ($n = 30$)	ACC ($n = 9$)
Gender (M/F)	29/56	7/59*	2/7	20/10*	1/8
Age (years)	45.15 ± 10.61	45.89 ± 12.97	52.56 ± 13.69*	55.20 ± 9.86*	54.22 ± 18.25
History of hypokalemia (n)	76	12*	8	2*	2*
Duration of HT (years)	4.00 (1.00, 9.00)	0.60 (0.00, 4.00)*	6.00 (2.50, 11.00)	0.60 (0.00, 7.75)*	0.00 (0.00, 0.65)*
BMI (kg/m^2)	23.66 ± 3.31	23.93 ± 3.37	23.11 ± 3.92	25.00 ± 2.60*	23.19 ± 2.08
WC (cm)	82.70 ± 9.54	85.03 ± 9.20	84.21 ± 12.42*	80.33 ± 4.51	–
SBP (mmHg)	159.36 ± 17.98	145.46 ± 19.34*	166.94 ± 10.40	135.73 ± 21.65*	153.22 ± 23.80
DBP (mmHg)	98.89 ± 13.39	93.45 ± 16.36*	95.17 ± 14.67*	81.58 ± 12.08*	85.56 ± 11.99*
FPG (mmol/l)	5.24 ± 0.67	5.56 ± 1.69	6.06 ± 1.11	5.56 ± 1.03	5.34 ± 1.32
TC (mmol/l)	4.13 ± 0.89	5.44 ± 1.17*	4.10 ± 0.48	4.81 ± 1.09*	4.62 ± 0.74
TG (mmol/l)	1.14 (0.80, 1.60)	1.36 (0.92, 1.85)	0.89 (0.61, 1.41)	1.88 (1.26, 3.36)*	–
LDL-c (mmol/l)	2.54 ± 0.69	3.43 ± 1.00*	2.48 ± 0.56	2.93 ± 1.14	2.32 ± 1.17
HDL-c (mmol/l)	1.28 ± 0.37	1.64 ± 0.48*	1.34 ± 0.50	1.37 ± 0.57	1.39 ± 0.69
Serum K^+ (mmol/l)	2.93 ± 0.62	3.81 ± 0.47*	2.58 ± 0.63	4.02 ± 0.31*	3.49 ± 0.53*
Serum Na^+ (mmol/l)	143.29 ± 3.16	142.47 ± 2.87	144.56 ± 3.88	142.13 ± 2.49	137.67 ± 15.33
ARR ($pg{\cdot}ml^{-1}/mIU{\cdot}l^{-1}$)	301.64 (113.09, 718.57)	6.25 (2.81, 14.51)*	241.43 (87.67, 793.57)	7.83 (1.99, 22.70)*	3.50 (1.58, 7.01)*
Cortisol post-1 mg DST (nmol/l)	27.23 (20.17, 33.66)	467.31 (278.64, 679.50)*	78.32 (58.79, 116.70)*	27.87 (20.17, 37.10)	505.81 (400.62, 653.01)*
Total weiss sore	0.00 (0.00, 0.00)#	0.00 (0.00, 1.00)#	1.00 (0.00, 1.50)#	0.00 (0.00, 0.00)#	5.00 (3.50, 5.50)#

Data were expressed as mean ± SD or median (interquartile range) except for gender and history of hypokalemia

APA aldosterone-producing adenoma, *ACA* aldosterone–cortisol co-secreting adenoma, *CPA* cortisol-producing adenoma, *NHFA* nonhyperfunctioning adenoma, *ACC* adrenal cortical carcinoma, *HT* hypertension, *BMI* body mass index, *WC* waist circumference, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *FPG* fasting plasma glucose, *TC* total cholesterol, *TG* triglyceride, *LDL-c* low density lipoprotein cholesterol, *HDL-c* high density lipoprotein cholesterol, *Serum* K^+ concentration of serum potassium, *Serum* Na^+ concentration of serum sodium, *ARR* aldosterone to renin ratio, *DST* dexamethasone suppression test

*significantly different from APA group ($P < 0.05$)

#significantly different from ACC group ($P < 0.05$)

mouse anti-CYP11B2 (Millipore, MABS1251, 1: 200) monoclonal antibodies, which were developed by Gomez-Sanchez CE et al. [24] and commercialized. For immunohistochemical staining, sections of 6 μm thickness from paraffin-embedded tumor specimen were deparaffinized and rehydrated, and then incubated with citrate 0.1 mM (pH 6.0) for 30 min at 98 °C for antigen retrieval. Immediately after incubation of H_2O_2 to eliminate endogenous peroxidase, the slides were incubated overnight at 4 °C with the primary antibody. After rinsing in phosphate-buffered saline with tween, the goat anti-mouse (ZSGB-BIO, PV-9002), and goat anti-rat (ZSGB-BIO, PV-9004) peroxidase-labeled polymer were incubated as secondary antibody for 20 min. The proteins were then visualized with DAB and counterstained with hematoxylin. All stained sections were scanned and captured by Image Scope DM4000 (Leica) for analysis.

H-score analysis

For each section stained, ten high-power fields (400×) were randomly selected for analysis. Immunoreactivity of CYP11B1 and CYP11B2 in tumor specimen were quantified by the McCarty's H-score system, which incorporates both the intensity of the specific staining and the percentage of positive cells. The relative intensity of specific staining was defined as not present (0), weak but detectable above control (1+), distinct (2+) and very strong (3+) [25]. The final score was the sum of the relative intensity of specific staining multiplied by the percentage of positive cells. The H-score analysis was carried out independently by two experienced pathologists who were blinded to the final clinical diagnosis of all cases studied. A third pathologist would review the score when there was an inconsistency between the two pathologists.

Statistical analysis

For descriptive statistics, data distributions were analyzed by Kolmogorov–Smirnov test. Normally distributed variables were expressed as mean ± standard deviation (SD) and were analyzed by the student *t* test. Skewed distributed variables were expressed as median (quartile range) and analyzed after a natural logarithm transformation. H-scoring data were expressed as median (interquartile range) and analyzed by Kruskal–Wallis test. SPSS 21 was used for statistical analysis. MedCalc software 8.1.1.0 was used to assess the diagnostic accuracy of the H-score of CYP11B1 and CYP11B2 immunoreactivity for the histopathological diagnosis of ACT and the clinical comprehensive diagnosis

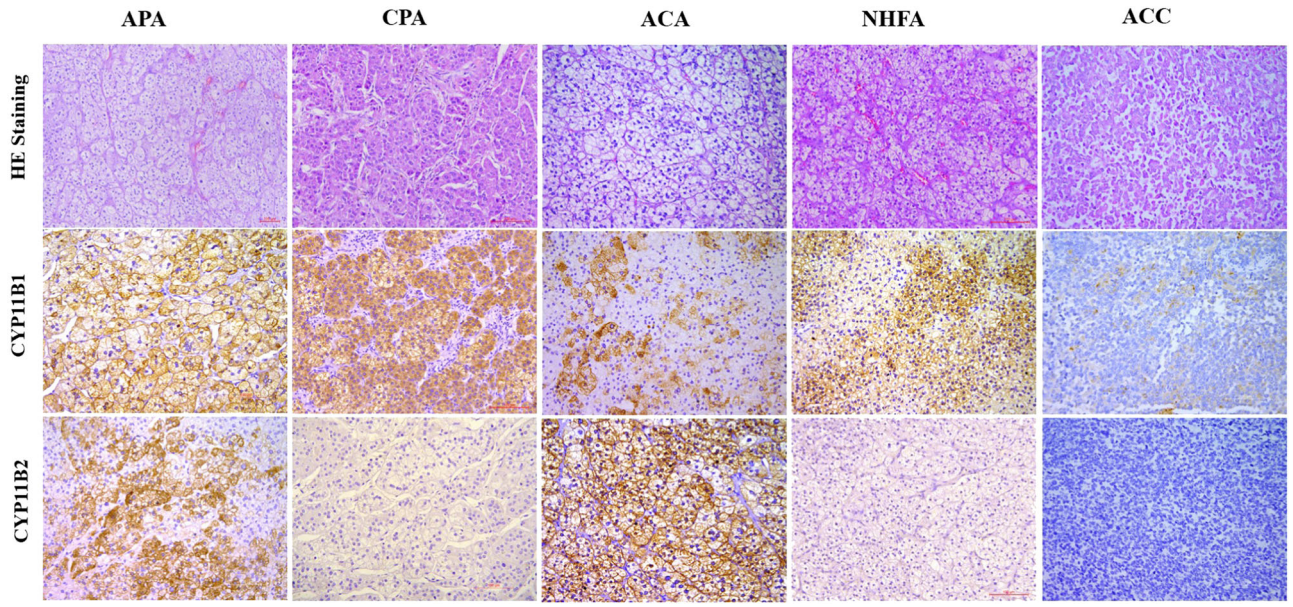

Fig. 1 Representative histopathological finding in ACT groups (original magnification ×200). Sections from formalin-fixed, paraffin-embedded tumor specimens in different ACT were stained by hematoxylin–eosin (H&E) staining (upper panel), CYP11B1 (middle panel), and CYP11B2 (lower panel). APA aldosterone-producing adenoma, ACA aldosterone–cortisol co-secreting adenoma, CPA cortisol-producing adenoma, NHFA nonhyperfunctioning adenoma, ACC adrenocortical carcinoma

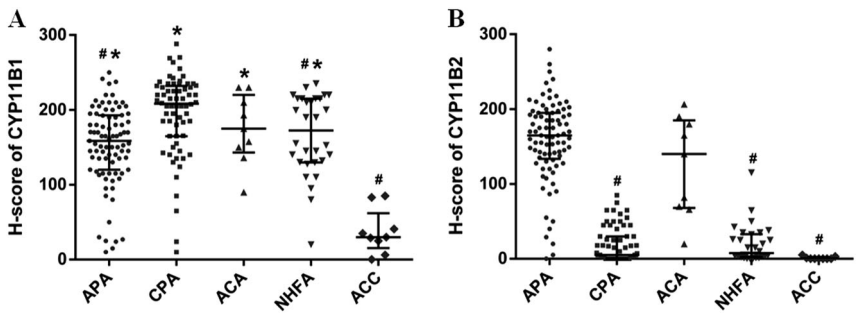

Fig. 2 H-score of CYP11B1 and CYP11B2 immunoreactivity in ACT groups. **a** H-score of CYP11B1 immunoreactivity in ACTs. Data were presented as median (interquartile range). The hash symbol indicates significantly different from CPA group ($p < 0.05$); an asterisk indicates significantly different from ACC group ($p < 0.05$); **b** H-score of CYP11B2 immunoreactivity in ACTs; data were presented as median (interquartile range). The hash symbol indicates significantly different from APA group ($p < 0.05$)

described above was used as the reference standard. Two-tailed test was used in all analyses and a P-value < 0.05 was considered statistically significant except for multiple comparisons.

Result

Clinical and biochemical parameters of subjects

We reviewed 111 patients with PA who underwent unilateral adrenalectomy. Among them, 105 achieved biochemical cure, including 11 cases of adrenocortical hyperplasia and 94 cases of APA. Of all APA patients enrolled, 9 out of 94 cases were also meeting the diagnostic criteria for Cushing's syndrome and were established as aldosterone–cortisol co-secreting adenoma (ACA). In nine cases ACC enrolled in present study, eight out of nine cases manifested as clinical overt hypercortisolism and one case has both normal ARR and 8 am plasma cortisol post 1-mg-overnight DST. Preoperative clinical and biochemical parameters in all ACT groups are summarized in Table 1. ARR and the incidence of hypokalemia were significantly higher in the APA group compared with other ACT groups with the exception of the ACA group (all P values < 0.05). Systolic blood pressure in the APA group was significantly higher than that in the CPA and NHFA groups (all P values < 0.05), while the serum potassium was significantly lower in

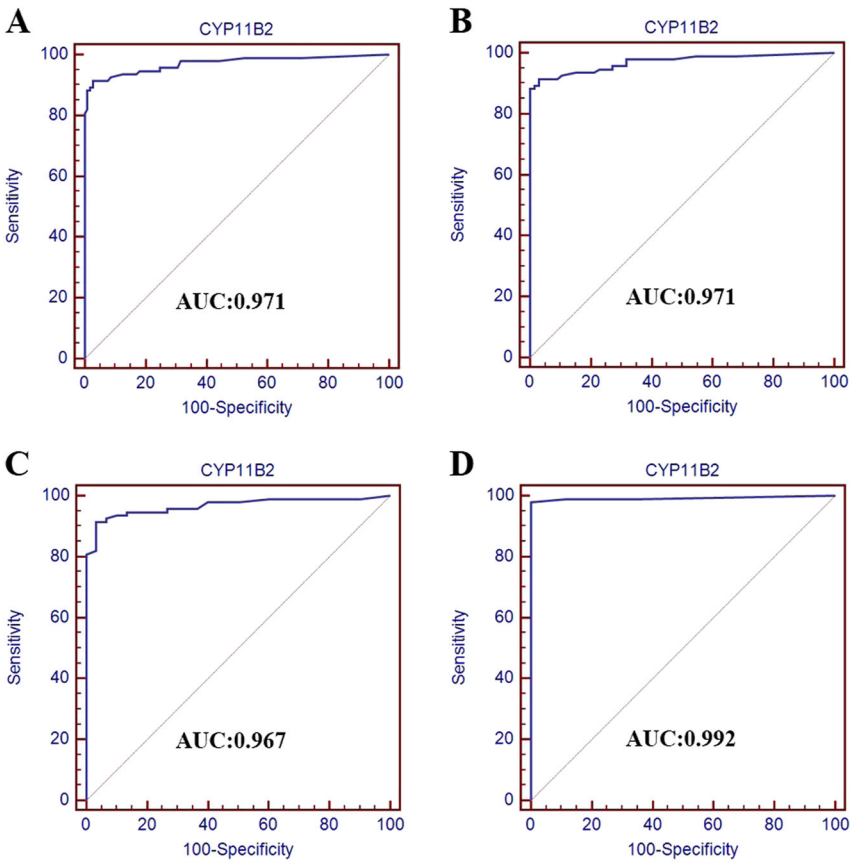

Fig. 3 ROC curve of the H-score of CYP11B2 immunoreactivity for the histopathological diagnosis of APA. ROC curve for differentiating APA from non-APA (**a**), CPA (**b**), NHFA (**c**), and ACC (**d**). Non-APA: all ACT enrolled in present study except APA

the APA group compared with other ACT groups except the ACA group (all P values < 0.05). Post-1 mg-DST plasma cortisol concentration was markedly elevated in the CPA, ACA, and ACC groups compared with the APA group (all P values < 0.05).

Immunohistochemical findings of studied cases

The specificity of antibodies against CYP11B1 and CYP11B2 were validated (Supplemental Fig. 2) and normal zona fasciculata and aldosterone-producing Cell Cluster were used as natural positive control and negative control [26]. Representative histopathological findings and immunohistochemical results of CYP11B1 and CYP11B2 in all groups were shown in Fig. 1. The immunoreactivity of CYP11B1 and CYP11B2 in the tumor sections of all cases examined were quantified by the McCarty's H-score system and the results are shown in Fig. 2 and Supplemental Tables 2 and 3. The H-score of CYP11B1 immunoreactivity in the CPA group was significantly higher than that in the APA, NHFA, and ACC groups (all P values < 0.05), but was not significantly different from the ACA group ($P = 0.176$). The H-score of CYP11B1 immunoreactivity in the ACC group was significantly lower than all other ACT groups (all P values < 0.05) (Fig. 2a). The H-score of CYP11B2 immunoreactivity in the APA group was highest among all groups, which was significantly higher than the CPA, NHFA, and ACC groups (all P values < 0.05) except the ACA group ($P = 0.161$) (Fig. 2b).

Accuracy of the H-score of CYP11B2 immunoreactivity in the histopathological diagnosis of ACT

When using an increased H-score of CYP11B2 immunoreactivity to differentiate APA from other ACT group, the receiver operating characteristic curve (ROC) analysis revealed that the AUC was 0.971 (95%CI 0.937–0.990) (Fig. 3a). Using the maximum Yoden index (YI) for cutoff selection, the optimal cutoff was set at an H-score of CYP11B2 immunoreactivity >65, which showed a sensitivity of 91.5% (95%CI 83.9–96.3%) and specificity of 97.1% (95%CI 91.9–99.4%). Subgroup analysis revealed that, when using an increased H-score of CYP11B2 immunoreactivity to differentiate APA from CPA, NHFA, and ACC, the AUC was 0.971, 0.967, and 0.992, respectively (Fig. 3b–d).

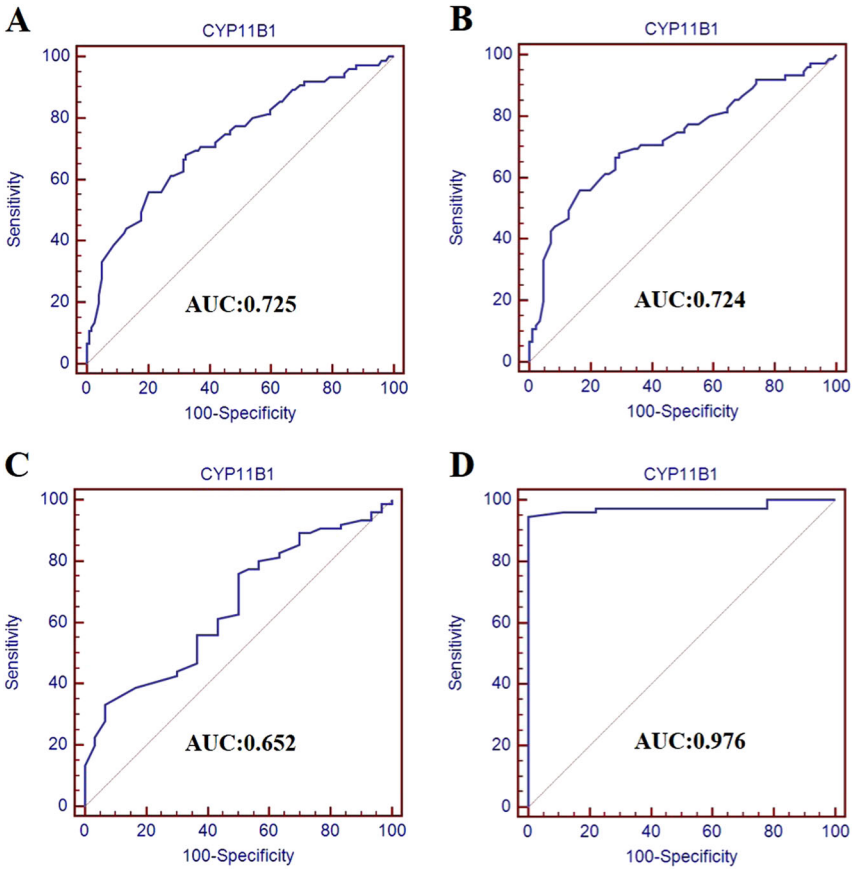

Fig. 4 ROC curve of the H-score of CYP11B1 immunoreactivity for the histopathological diagnosis of CPA. ROC curve for differentiating CPA from non-CPA (**a**), APA (**b**), NHFA (**c**), and ACC (**d**). Non-CPA: all ACT enrolled in present study except CPA

Accuracy of the H-score of CYP11B1 immunoreactivity in the histopathological diagnosis of ACT

When using an increased H-score of CYP11B1 immunoreactivity to differentiate CPA from other ACT, the ROC analysis revealed that the AUC was 0.725 (95%CI 0.658–0.786) (Fig. 4a). For a maximum YI, the optimal cutoff was set at an H-score of CYP11B1 immunoreactivity greater than 204, which showed a sensitivity of 56.0% (95% CI 44.1–67.5%) and a specificity of 79.8% (95CI 71.7–86.5%). Subgroup analysis revealed that, when using an increased H-score of CYP11B1 immunoreactivity to differentiate CPA from APA, NHFA, and ACC, the AUC was 0.724, 0.652, and 0.976, respectively (Fig. 4b–d).

When using a decreased H-score of CYP11B1 immunoreactivity to differentiate ACC from other ACT, the ROC analysis revealed that the AUC was 0.960 (95%CI 0.923–0.983) (Fig. 5a). Using the maximum YI for cutoff selection, the optimal cutoff was set at an H-score of CYP11B1 immunoreactivity lower than 85, which showed a sensitivity of 100.0% (95%CI 66.4–100.0%) and a specificity of 92.1% (95%CI 87.3–95.5%). Further subgroup analysis revealed that, when using a decreased H-score of CYP11B1 immunoreactivity to differentiate ACC from APA, CPA, and NHFA separately, the AUC was 0.944, 0.976, and 0.967, respectively (Fig. 5b–d).

Discussion

In the present study, we found that the H-scores of CYP11B1 and CYP11B2 are reliable tools for the histopathological diagnosis of ACT. An increased H-score of CYP11B2 (>65) could accurately distinguish APA from other ACT; an increased H-score of CYP11B1 (>204) offers some value for the histopathological diagnosis of CPA; and a decreased H-score of CYP11B1 (<85) could facilitate the differentiation of ACC from other ACT. To our knowledge, this is the first study to assess the diagnostic accuracy of the H-scores of CYP11B1 and CYP11B2 in the histopathological diagnosis of a wide range of ACT with a relatively large sample size.

CYP11B2 immunohistochemistry has been reported to be valuable in the histopathological diagnosis of APA [13, 14, 27, 28]. A study of 25 cases of APA demonstrated positive CYP11B2 staining in all cases [26]. In another study of 29 patients with unilateral PA, Nanba et al. divided them into APA, diffuse adrenocortical hyperplasia, unilateral multiple adrenocortical micronodules and multiple

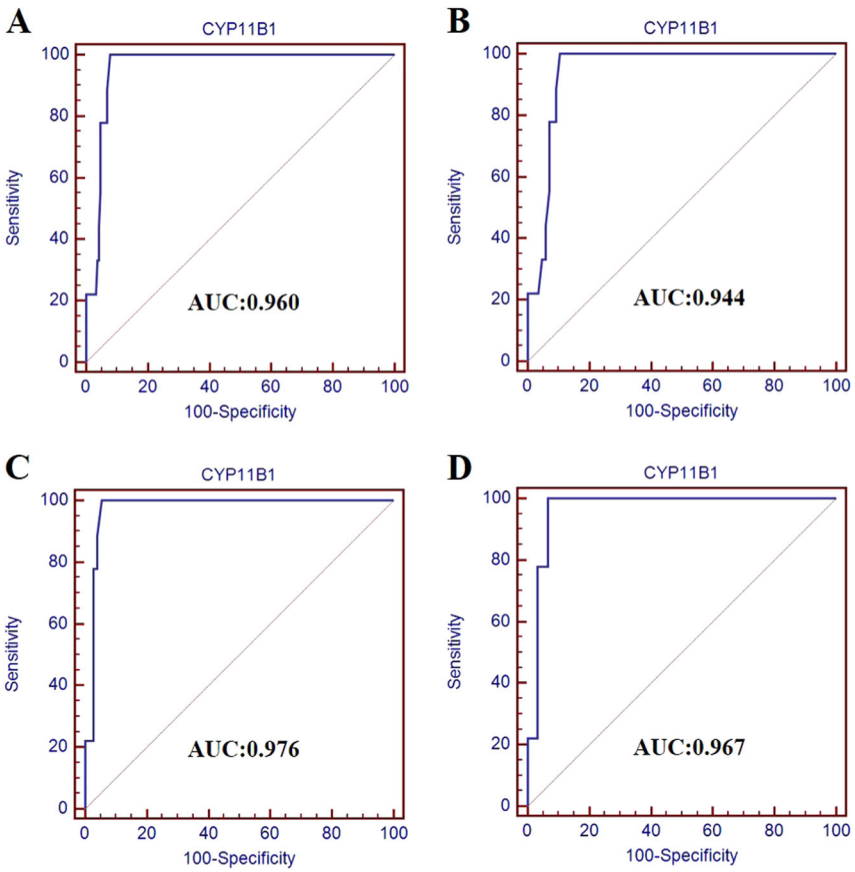

Fig. 5 ROC curve of the H-score of CYP11B1 immunoreactivity for the histopathological diagnosis of ACC. ROC curve for differentiating ACC from non-ACC (**a**), APA (**b**), CPA (**c**), and NHFA (**d**). Non-ACC all ACT enrolled in present study except ACC

aldosterone-producing cell clusters according to the CYP11B2 staining patterns of their adrenal specimens [29]. Positive correlations were found between the immunoreactivity of CYP11B2 and PAC, serum potassium concentration, tumor size, cellular composition, and mutation status in patients with APA [18, 29, 30]. These studies suggested that CYP11B2 is a reliable biomarker for the histopathological diagnosis of APA. Although there are limited reports of APA specimens with low or absent CYP11B2 immunoreactivity [17, 18], our study with a large number of APAs, revealed that the AUC of the H-score of CYP11B2 for the diagnosis of APA reached 0.971. The CYP11B2 H-score remained a robust test for subtyping of APA when compared with all other ACT, including CPA, NHFA, and ACC in subgroup analyses. The dominant nodule on imaging was not always the source of aldosterone excess in patients with PA. Due to the technically demanding nature of AVS, it was not offered to all patients. Those with typical characteristics of APA, including young age and hypokalemia, underwent unilateral adrenalectomy without the guide of AVS. For these patients, immunostaining of CYP11B2 is particularly relevant in verifying the clinical diagnosis and determining the site of aldosterone overproduction in adrenal specimen. Our study supports earlier studies that call for the use of CYP11B2 immunostaining in the histological diagnosis of APAs.

The diagnostic value of CYP11B1 immunohistochemistry in the histopathological diagnosis of CPA has not been fully investigated. Fumie Kubota et al. [15] and Koshiro Nishimoto et al. [26] performed CYP11B1 staining in a total of 84 samples of CPA which were all diffusely positive for CYP11B1. However, a study by Koshiro Nishimoto et al. [26] revealed that the majority of APA (24 out of 25 cases) were also immuno-positive for CYP11B1. Using the McCarty's H-score system to quantify CYP11B1 immunoreactivity in various ACT, we found that the H-score could facilitate the differentiation of CPA from other ACT, showing an intermediate level of AUC (0.725). Further validation is required to establish CYP11B1 as a reproducible histopathological biomarker of CPA.

We clearly demonstrated that a decreased H-score of CYP11B1 immunoreactivity was a distinct feature of ACCs in contrast to all other ACT. A previous study of nine cases of ACC showed that three cases had absent CYP11B1 immunoreactivity while the remaining six cases showed spotted CYP11B1 staining predominantly in small carcinoma cells with a compact/clear cytoplasm and minimum morphologic nuclear atypia [16]. In line with this study, we

also showed a decreased CYP11B1 immunoreactivity in the majority of our ACC tumor specimens. When quantified using the McCarty's H-score system, we found that the H-score of CYP11B1 immunoreactivity in ACC was significantly lower than other ACT groups, and further ROC analysis confirmed its role in the histopathological subtyping of ACC. While the diagnosis of typical ACC does not pose a challenge, adrenal cortical tumors with borderline/atypical features aresometimes proved hard to be clearly diagnosed [31]. The addition of H-score of CYP11B1 to the histopathological examination of adrenal tumor specimens may complement the Weiss score and other biomarkers as a novel diagnostic tool for the diagnosis of ACCs, which are variable in morphology and behavior.

Our study has several important strengths. Firstly, this study included five types ACT with a relatively large sample size, which virtually covered the entire spectrum of ACT. Secondly, the diagnostic tests for each condition were performed in a rigorous and standardized manner. A comprehensive diagnosis, which incorporated clinical and biochemical characteristics, imaging, histopathological finding, and follow-up after surgery, was used as the reference standard. Thirdly, pathologists were blinded to the clinical final diagnosis of all cases examined in H-score analysis.

The main limitation of our study is its single-centered nature with only Chinese participants included. Moreover, due to retrospective nature of present study, we failed correlated CYP11B1 immunohistochemistry with disease prognosis in ACC.

Conclusion

This study demonstrated that CYP11B1 and CYP11B2 are reliable immunohistochemical biomarkers for the histopathological subtyping of functional benign ACT and may offer some value in the histopathological diagnosis of malignant ACT. This conclusion should be validated in prospective study.

Acknowledgements The authors thank Laboratory of Endocrine and Laboratory of Lipid & Glucose Metabolism, the First Affiliated Hospital of Chongqing Medical University. The authors also thank Chuan Peng, Rufei Gao, and Xiaoqiu Xiao (from Laboratory of Lipid & Glucose Metabolism, the First Affiliated Hospital of Chongqing Medical University) for suggestions of study design and revision.

Funding This study was funded by National Key Clinical Specialties Construction Program of China to the Department of Endocrinology, the First Affiliated Hospital of Chongqing Medical University; and the National Natural Science Foundation of China (81670785) to Q.L.; and the National Natural Science Foundation of China (81800701) to Y.S.; and the Fundamental Science & Advanced Technology Research of Chongqing (Major Project, cstc2015jcyjBX0096) to Q.L.; and Chongqing Science and Technology Committee Innovation Project (Technology Development and Application of Precision Medicine, cstc2016shms-ztzx1003) to Q.L.; and the Joint Medical Research Project of Chongqing Science and Technology Commission and Chongqing Health and Family Planning Commission (Youth Project, 2018QNXM001) to J.H.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent A written informed consent was obtained from each participant enrolled.

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