Diagnosis of a malignant adrenal mass:

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Diagnosis of a malignant adrenal mass: the role of urinary steroid metabolite profiling.

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Abstract:

Purpose of review – Adrenal masses are highly prevalent, found in 5% of the population. Differentiation of benign adrenocortical adenoma from adrenocortical carcinoma is currently hampered by the poor specificity and limited evidence base of imaging tests. This review summarizes the results of studies published to date on urine steroid metabolite profiling for distinguishing benign from malignant adrenal masses.

Recent findings: Three studies have described cohorts of at least 100 patients with adrenal tumors showing significant differences between urinary steroid metabolite excretions according to the nature of the underlying lesion, suggesting significant value of steroid metabolite profiling as a highly accurate diagnostic test.

Summary: Steroid profiling is emerging as a powerful novel diagnostic tool with a significant potential for improving the management for patients with adrenal tumors. While the current studies used gas chromatography-mass spectrometry for proof-of-concept, widespread use of the method in routine clinical care will depend on transferring the approach to high-throughput tandem mass spectrometry platforms. The use of computational data analysis in conjunction with urine steroid metabolite profiling, i.e. steroid metabolomics, adds accuracy and precision.

Key words: steroid profiling, adrenal tumor, adrenocortical carcinoma, diagnostic test, mass spectrometry, steroid metabolomics

Introduction

Adrenal masses are common, with a reported overall prevalence of at least 5% in the population [1, 2]. Prevalence increases with age, ranging between <0.5% in children and around 10% in 70-year-old patients [3, 4]. Most patients are diagnosed incidentally on cross-sectional abdominal imaging performed for indications other than suspected adrenal disease. In the United States alone, the number of computed tomography (CT) imaging performed quadrupled from 21 million per year in 1995 to more than 80 million per year in 2014 [5]. Increase in widespread use coupled with enhancement in the quality of cross-sectional imaging explains the growing numbers of patients newly diagnosed with an adrenal tumor requiring further diagnostic assessment.

Management of patients with a newly detected adrenal mass is dictated by 1) whether there is evidence of associated adrenal hormone excess and 2) whether the adrenal mass is malignant or benign. However, due to the limited accuracy of currently applied diagnostic tools, reaching a confident conclusion in regards to these two key questions is challenging for many patients with adrenal tumors. Patients frequently require additional immediate and/or longitudinal follow-up tests and visits, and invasive procedures to ascertain the precise diagnosis, such as adrenal biopsy or unilateral adrenalectomy, add to the burden of disease associated with adrenal incidentalomas.

In this review, we will discuss the evidence on currently available tests to diagnose adrenal malignancy and review the data on urinary steroid profiling as a potential non-invasive diagnostic test to detect adrenocortical carcinoma (ACC) in patients with adrenal masses.

Diagnosis of a malignant adrenal mass

Most adrenal tumors are discovered incidentally on cross-sectional imaging study performed for a reason other than suspected adrenal disease, e.g. unspecific abdominal pain. Imaging characteristics can be helpful in clarifying the nature of the adrenal mass. Pheochromocytomas can be taken out of the equation by highly sensitive and specific biochemical screening with measurement of plasma metanephrines. Doing this first, helps avoid difficulties in interpreting imaging findings as there is a significant overlap between pheochromocytomas and adrenocortical carcinomas.

Following biochemical exclusion of a catecholamine-producing pheochromocytoma, imaging is the next step to detect or exclude adrenocortical malignancy. While a large tumor diameter has limited sensitivity and specificity for detecting an adrenocortical carcinoma [6, 7], additional features such as irregular borders, inhomogeneity of the mass and signs of local invasion are pointing to underlying adrenocortical malignancy. Tumor density on unenhanced, non-contrast computed tomography (CT) scans is helpful if a homogeneous density value of less than 10 Hounsfield Units (HU) is measured in the tumor area, which invariably indicates a fat-containing benign adrenal tumor. Conversely, while HU values >10 are suspicious of malignancy, the majority of such masses eventually turn out to be benign. Similarly, on magnetic resonance imaging (MRI) scans, the loss of signal intensity in the so-called chemical shift analysis also correlates with the amount of fat contained in the adrenal tumor, with a large drop in signal intensity between in-phase and out-of-phase T1 sequences suggestive of a benign adrenal tumor. Fluorodeoxyglucose positron emission tomography (FDG-PET) has been proposed as a better test to diagnose a malignant adrenal mass. However, surprisingly little evidence exists on the diagnostic accuracy of the imaging characteristics of the commonly employed imaging studies, mainly due to the fact that few studies provide an optimal reference standard for comparison [8]. While evidence for the accurate diagnostic performance of non-contrast CT HU

cutoff of 10 is more robust, confidence intervals of estimates for other imaging characteristics are wide, based on the very small sample size of studied cohorts (**Table 1**), and therefore their use, though regularly employed, is not based on high quality evidence[8].

Because of the limited specificity of imaging tests in making the diagnosis of adrenocortical malignancy in the setting of a high prevalence of benign adrenal tumors, the current diagnostic approach, which almost exclusively employs imaging tests, has a significant impact on the economic burden of disease. Many patients undergo either immediate additional imaging tests to better characterize the adrenal mass or are advised to return for imaging studies to assess tumor growth after an interval of 3-12 months [9]. This exposes patients to radiation and increases health care costs, without evidence that performing multiple imaging modalities improves the diagnostic performance with regard to differential diagnosis between benign and malignant adrenal masses. Some guidelines recommend annual imaging follow for 5 years after the initial detection of an adrenal mass [10, 11]. However, following this strategy in patients with a conclusively benign presentation on initial imaging, e.g. HU<10 on unenhanced CT, may result in significant radiation exposure and health care costs for no good reason. Therefore, the recently published joint clinical guidelines of the European Society for Endocrinology (ESE) and the European Network for the Study of Adrenal Tumors (ENSAT) have recommended that such patients should not undergo repeat imaging[9].

In a proportion of patients, image-guided adrenal biopsy or adrenalectomy are performed due to concerns about the potentially malignant nature of the incidentally detected adrenal mass. Adrenal biopsy is an expensive procedure with a reported rate of non-diagnostic biopsies of 8.7% and a complication rate of 2.5% [12]. In addition, adrenal biopsy has poor accuracy in making the diagnosis of ACC (**Table 1**), due to inherent difficulties for pathologists to making an accurate diagnosis based on very limited amounts of tissue, and therefore is not recommended for patients with suspected ACC [9, 13]. In principle, adrenal biopsy is mainly considered in

patients with extra-adrenal malignancy and only, as stated by the ESE-ENSAT guidelines, if “all of the following criteria are fulfilled: (i) the lesion is hormonally inactive (in particular, a pheochromocytoma has been excluded), (ii) the lesion has not been conclusively characterized as benign by imaging and (iii) management would be altered by knowledge of the histology” [9].

As adrenalectomy series suggest, one third to half of all patients undergo adrenalectomy unnecessarily as pathology is consistent with a benign non-functioning adrenal tumor [7, 9]. This is likely due to underlying concern for malignancy, possibly related to larger tumor diameters. Indeed, despite the poor accuracy of tumor size in predicting malignancy, several published guidelines recommend adrenalectomy at various cutoffs of tumor size of 4 to 6 cm [10, 11, 14]. Recent guidelines on management of adrenal tumors acknowledge the lack of evidence on the natural history of large apparently benign adrenal tumors to suggest a certain adrenal tumor cutoff for adrenalectomy but allow for a highly individualized consideration of surgery in patients with tumors >4 cm [9]. Given the shortcomings of the currently employed imaging tests, a non-invasive, accurate and inexpensive test to distinguish ACC from other adrenal tumors is urgently needed, especially in patients with large adrenal tumors and in patients with adrenal masses with indeterminate imaging characteristics.

Steroid metabolite profiling

Serum and urinary steroid analysis traditionally play an important role in the diagnosis of adrenal hormone excess and disorders of steroidogenesis. Around 15% of adrenal tumors present with either clinically overt adrenal cortex hormonal excess including overt Cushing syndrome, primary hyperaldosteronism and, uncommonly, hyperandrogenism [7, 15]. In addition, up to 30-50% of patients with adrenal tumors present with evidence of mild autonomous cortisol excess, previously termed subclinical Cushing syndrome; the diagnosis of these patients is

challenging and management is usually delayed until cortisol-induced comorbidities occur [16]. While serum steroid measurements are susceptible to differences occurring due to the diurnal variation in adrenal steroid secretion, 24-hour urine steroid excretion represents a more accurate estimate of net total adrenal hormone production [17, 18] and thus also holds diagnostic potential for the assessment of adrenal hormonal excess.

Quantitatively, the majority of steroids excreted in the urine of adult individuals are androgens and glucocorticoids with a significantly lower output for mineralocorticoids and steroid precursors [18-20]. Adrenal steroid production and metabolism are affected by gender, age and body mass index [18, 21, 22]. Glucocorticoid metabolites are higher in men than in women and in obese versus lean persons [21, 22]. Looking at ratios of steroids that are substrates and products, respectively, of a distinct steroidogenic enzyme activity allows for representative assessment of elements of the steroidogenic pathway, which is particularly informative in the diagnosis of inborn steroidogenic disorders including congenital adrenal hyperplasia. However, by reflecting steroidogenic output of the adrenal glands, urinary steroid profiling inherently has great potential in diagnosing adrenal hormone excess.

Steroid metabolite profiling in diagnosis of ACC

Most ACCs are large tumors at the time of initial diagnosis, and a significant number is found to produce adrenal hormones in excess, though this is not always clinically apparent. In general, the concurrent excess production of multiple steroids, in particular when including androgens, is considered as indicative of adrenocortical malignancy. The majority of ACCs are classified as non-functioning based on conventional serum steroid analysis, which mostly assesses end products of steroidogenesis. However, adrenocortical carcinoma cells can be considered to represent a more immature, dedifferentiated cell type as is typical for cancer cells. Hence, it is

likely that the steroidogenic pattern produced by adrenal cancer cells is characterized by large amounts of steroid precursors rather than end products of mature and complete steroidogenesis. Consistent with this assumption, a significant number of patients with ACC show increased production of the glucocorticoid precursor 17-hydroxyprogesterone (17OHP), up to half of ACC patients reported by one series [18]. However, serum 17OHP only provides limited sensitivity for detecting enhanced steroid precursor production in adrenocortical carcinoma patients.

Decades ago, small case series demonstrated differences in urine steroid metabolite excretion in some patients with ACC [23, 24]. However, the first large study systematically investigating steroid metabolite excretion in 147 patients with confirmed adrenocortical tumors, 45 patients with ACC and 102 patients with benign adrenocortical carcinoma (ACA), was published in 2011 by Arlt et al [18]. In this proof-of-concept study the authors described steroid profiling by gas chromatography-mass spectrometry (GC-MS) combined with computational data analysis utilizing a machine learning-based approach [18]. The employed GC-MS steroid profiling included measurement of 32 adrenal steroids including metabolites of glucocorticoid, mineralocorticoid and androgen precursors, providing comprehensive coverage of adrenal steroid output (**Figure 1**). This study revealed a distinct steroid pattern, a “malignant steroid fingerprint”, that indicated the presence of an adrenocortical carcinoma with 90% sensitivity and specificity. Three steroid metabolites were most informative in distinguishing ACC from ACA: the glucocorticoid precursor metabolite tetrahydro-11-deoxycortisol (THS) derived from 11-deoxycortisol and the adrenal androgen precursor metabolites pregnenediol (5-PD) and pregnenetriol (5-PT), derived from pregnenolone and 17-hydroxypregnenolone, respectively. In addition, six further steroids were identified on the machine learning algorithm, based on their relevance towards discriminating ACC from ACA: the progesterone metabolite pregnanediol (PD), the 17OHP metabolite pregnanetriol (PT), the 11-deoxycorticosterone metabolite tetrahydro-11-deoxycorticosterone

(THDOC), the corticosterone metabolite 5α-tetrahydrocortisol (5α-THA), the cortisol metabolite 5α-tetrahydrocortisol (5α-THF), and the androgen metabolite etiocholanolone (Etio). Receiver operating characteristic (ROC) curve analysis demonstrated the diagnostic information of all 32 steroids resulted in 90% sensitivity and specificity, while the 9 most informative and the top 3 biomarker steroids provided only slightly lower diagnostic accuracies of 87.7% and 87.2%, respectively. Ten of the 45 ACC patients in this study had metastatic disease at the time of 24-h urine collection; however, their steroid profile did not differ from ACC patients who harbored the primary tumor only. Steroid output did not correlate with tumor size.

In 2015, Kerkhofs et al presented their data on an independent cohort of patients with 27 ACCs and 125 ACAs [19]. They performed GC-MS analysis of 22 steroid metabolites but did not employ computational analysis to refine the diagnostic performance of the test. They identified 15 individual steroid markers with a sensitivity of 90% or above in detecting ACC, with the 11-deoxycortisol metabolite THS again representing the most informative markers. Other informative markers identified by that study included PD, PT, Etio, the major androgen metabolite androsterone (An) and the major glucocorticoid metabolites tetrahydrocortisol (THF) and tetrahydrocortisone (THE). However, specificities varied widely (2-83% for individual steroids). They defined a cut-off of 2.35 µmol/24h for THS excretion as 100% sensitive and 99% specific for the detection of ACC. However, their ACC cohort was considerably smaller and 67% of their ACC patients showed evidence of cortisol excess while this found in only 18% of their ACA patients. (**Table 2**). This selection bias might explain why they found the glucocorticoid metabolites THE and THF to be apparently distinguishing between ACC and ACA.

A recent study by Velikanova et al. [20], carrying out GC-MS analysis of 32 steroid metabolites in 24-h urine samples from 31 patients with ACC and 96 patients with ACA authors confirmed the findings reported by Arlt et al. They demonstrated that patients with ACC have significantly higher THS, PD, 5PT, and 5PD than patients with ACA. However, increased urinary excretion of

THS was demonstrated only in 74% of their patients with ACC and the authors suggested consideration of additional steroid parameters and ratios [20]. Similar to Kerkhofs et al. they did not employ unbiased computational data analysis of the steroid excretion data.

All three studies identified THS as the most informative steroid marker indicative of ACC. THS is the metabolite of 11-deoxycortisol, which is converted to cortisol by the adrenal-specific steroidogenic enzyme CYP11B1. Measurement of 11-deoxycortisol is not routinely used clinically, though recently serum results were reported in a cohort of benign adrenal tumors [25]. Abundance of THS in patients with ACC suggests a relative deficiency of CYP11B1, and may be due to de-differentiation and mutational changes occurring in ACC. Similarly, the accumulation of the androgen precursor steroids 5PT and 5PD indicate a relative inefficiency of CYP17A1 (17,20 lyase) activity in converting 17-hydroxypregnenolone to the major adrenal androgen DHEA. Based on the available studies, it is evident that the excretion of steroid precursor metabolites is significantly higher in ACCs than in ACAs and we propose that this is due to the relative steroidogenic immaturity of the adrenocortical carcinoma cells. Steroid precursor metabolite excretion was equally observed both in patients with clinically evident adrenal hormone excess as well as in patients with clinically “endocrine inactive” ACC. Interestingly, steroid profiling revealed higher glucocorticoid metabolite excretion in ACA versus healthy volunteers, including those classified as non-functioning [17, 18, 20]. At this time, studies systematically exploring potential diagnostic implications of urine steroid profiling in hormonal excess are currently lacking.

CHALLENGES and FUTURE DIRECTIONS

Steroid profiling has particular promise as a valuable tool for differential diagnosis in patients with adrenal tumors presenting with indeterminate imaging characteristics. These patients proceed to have additional studies, most commonly repeat CT imaging which add to the lifetime exposure to radiation, but also expose affected patients to cost, inconvenience, and anxiety of waiting another 6-12 months. Steroid profiling is an attractive noninvasive alternative which could help rule out ACC much earlier in the diagnostic pathway. Earlier diagnosis would allow for adrenalectomy without delay in patients with suspected ACC and avoid additional procedures and tests in patients in whom ACC is conclusively excluded.

While the above initial results suggest impressive differences between 24-h urine steroid metabolite excretion in patients with ACC when compared to ACAs, at this time there are some barriers which need to be overcome before widespread use of steroid profiling as a non-invasive diagnostic tool in patients with adrenal tumors. All initial results are based on retrospective studies with still relatively small numbers of patients totaling <500 patients. Prior to implementation in routine clinical practice, urinary steroid metabolome profiling as a diagnostic tool will need to be prospectively validated in a much larger cohort of prospectively enrolled patients and compared to a reference standard comprising results of histopathology and clinical and imaging follow-up investigations. Recently, a large prospective international multi-center test validation study carried out with support of the European Network for Study of Adrenal Tumors (ENSAT) has completed after successfully recruiting more than 2000 patients with newly diagnosed adrenal mass. Results from this study, EURINE-ACT (Evaluation of URINE steroid metabolomics in the differential diagnosis of AdrenoCortical Tumors), will conclusively determine the diagnostic accuracy and performance of the steroid profiling in a non-selected cohort of patients in a “real-life” setting.

Even after appropriate validation is completed, there are certain challenges associated with universal implementation of steroid profiling into the clinical laboratory. All available current evidence on steroid profiling relies on measurements performed by GC-MS. However, while GC-MS allows the concurrent profiling of the entire steroid metabolome, the method requires special expertise and is only offered by a small number of institutions and laboratories. The method is relatively laborious and hence not cheap. However, these obstacles could be overcome by transfer of the steroid profiling method to the high-throughput liquid chromatography-tandem mass spectrometry (LC-MS/MS) platform and these approaches have now been developed and are currently being investigated for their diagnostic performance. For widespread use of this diagnostic test it will also be critical to provide a straightforward diagnostic algorithm and this is likely to be achieved by the machine learning-based algorithm described by Arlt et al [18].

Conclusion

In conclusion, mass spectrometry-based 24-h urine steroid metabolome profiling is a highly promising non-invasive diagnostic tool in patients with adrenal tumors, with a diagnostic sensitivity and specificity that exceeds that of diagnostic imaging. While proof-of-concept and subsequent studies were carried out in retrospective cohorts, the outcome of a large prospective test validation study will be available soon. Results will determine whether steroid profiling can become part of widely implemented routine care in the diagnostic evaluation of patients with adrenal masses.

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