

Urinary fluticasone propionate-17 β -carboxylic acid to assess asthma therapy adherence

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ABSTRACT

Although the National Asthma Education and Prevention Program Expert Panel Report 3 recommends referral to specialists to address adherence, guidelines do not provide a tool to determine nonadherence. This study was designed to prospectively evaluate the characteristics of urinary analysis of fluticasone propionate-17 β -carboxylic acid (FP17 β CA) as a test to verify if a specific patient has not taken fluticasone propionate (FP) within 16–24 hours. Urine of asthmatic subjects was prospectively analyzed 16–24 hours after witnessed administration of orally inhaled FP using liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis; limit of quantitation was 10.3 pg/mL. Results were compared with those from asthmatic subjects not receiving inhaled FP. Thirty asthmatic subjects receiving inhaled FP (2 oral inhalations of FP at 110 micrograms each or 1 oral inhalation twice daily of fluticasone and salmeterol in fixed combination at 250/50 micrograms for 1 week) were compared with 30 asthmatic subjects not receiving FP. FP17 β CA was detected in the urine of 30 of 30 asthmatic subjects receiving FP (median, interquartile range [IQR; 413.5, 212.8–1230.0] range 12.4–3290.0 pg/mL [corrected for urine creatinine: median, IQR {576.2, 188.1–1306.6} range 6.3–5425.9 ng/g Cr]) and was undetectable in 30 of 30 subjects not receiving inhaled FP. The sensitivity and specificity of LC-MS/MS to detect FP17 β CA in urine were 100% (95% exact binomial confidence interval, 88–100) and 100% (95% exact binomial confidence interval, 88–100), respectively. Analysis of FP17 β CA in urine provides a sensitive method that may be used to verify that a specific patient may not have administered FP within a 16- to 24-hour window before testing.

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Each year asthma accounts for 10.5 million office visits in the United States.¹ The Guidelines for the Diagnosis and Management of Asthma Expert Panel Report 3 recommend assessments of asthma control, trigger factors, and for patients with prescribed medications, assessment of adherence during physician visits. Unfortunately, patient nonadherence to prescribed medication is high and except for referral to a specialist, Expert Panel Report 3 guidelines do not provide either primary care or specialty health care providers tools to assess adherence in specific patients.² Underestimation of adherence issues may lead to poor clinical outcome, neglect of issues that may accompany poor adherence, and unnecessary escalation of prescription medications

that, estimated at \$5.9 billion yearly, are reported to be the single largest direct medical cost related to asthma in the United States.^{3–7} Data from the Medical Expenditure Panel Survey using medical expenditures, productivity, and chronic comorbidity reported that the total national medical expenditure attributable to adult asthma was \$18 billion/year.⁸ Medical decisions to “step up” asthma controller therapy are largely based on a health care providers synthesis of recent asthma control based on current and previous symptoms, understanding of recent environmental (including viral) exposures, spirometry measurements, and assessment of adherence perhaps using verbal questioning, interpretation of body language, or visual inspection of inhaler device counters if available. However, during patient and health care provider interactions, answers to simple questions such as “Did you take your inhaler last night?” may not be all that forthcoming or reliable. We recently developed a highly sensitive tool to measure urinary fluticasone propionate-17 β -carboxylic acid (FP17 β CA), a metabolite of fluticasone propionate (FP), by liquid chromatography tandem mass spectrometry (LC-MS/MS).⁹ This study sought to prospectively evaluate the characteristics of urinary analysis of FP17 β CA as a test to verify that a specific patient may not have administered FP within a 16- to 24-hour window before testing.

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METHODS

Controlled asthmatic patients, aged 12–80 years, were recruited from those who had undergone recent clinical evaluation by an asthma specialist and were receiving medium daily adult doses of FP. Subjects using FP (Flovent 110 [GlaxoSmithKline, London, UK], 2 puffs, or Advair [GlaxoSmithKline] 250/50, twice daily) and asthmatic controls with or without other inhaled corticosteroid (ICS) medications were studied. Inclusion criteria included a physician diagnosis of asthma. A significant smoking history defined as 10 pack-years of cigarettes over a lifetime, current tobacco smoking that included any tobacco use within the past year, or clinical suspicion of poor adherence was used as an exclusion criterion.

After enrollment, informed consent, and an educational session with an asthma research nurse providing instructions to document in a diary, twice-daily administration of FP for 7 days, the patients returned to the clinic and underwent a witnessed administration of inhaled FP in a controlled setting. An extender tube was provided and used by subjects receiving FP (Flovent). The gold standard for compliant inhaler use within 16–24 hours included a signed medication administration log reporting compliant use of 2 oral inhalations of FP, 110 μg each (Flovent), or 1 oral inhalation, twice-daily fluticasone/salmeterol at 250/50 μg (Advair) $\times$ 1 week for 1 week, in addition to the allergy research nurse's direct observation of the subject's last administration of inhaler. The patient withheld the next inhaler dose until returning to the clinic within 16–24 hours to submit a urine specimen. Urine samples from asthmatic subjects not taking FP were used as controls. FP nasal spray was allowed in study subjects in the FP inhaler group previously using the medication. However, control subjects using FP nasal spray were initially excluded from the study and provided an alternative nasal steroid of their choice for 2 weeks such that they could enroll later as an asthmatic control. After acetonitrile precipitation, urine samples were extracted with dichloromethane, washed, and dried. Reconstituted extract (60 μL) was subjected to reversed-phase chromatography and positive-ion mode LC-MS/MS analysis. Fluorometholone was used as the internal standard. Linear range of the FP17 β CA assay was 10.3–9510 pg/mL. Limit of quantitation was 10.3 pg/mL and recovery ranged from 85.8 to 111.9%. Interassay CVs were 7.4–12.0% for FP17 β CA concentrations of 11.1–5117 pg/mL. Measurement of FP17 β CA by LC-MS/MS was previously found to be an acceptable analytical performance for clinical use.⁹ Spirometric data were collected from recent testing where methods were performed in accordance with the American Thoracic Society criteria for acceptability and reproducibility. The study was approved by the

Mayo Clinic Institutional Review Board. The test referenced in this article is provided through Mayo Medical Laboratories, a Division of the Mayo Clinic Department of Laboratory Medicine and Pathology.

Statistical Analysis

Unless otherwise specified, data are summarized as the raw numerical values with percentages or median values and interquartile ranges (IQRs). A standard contingency table was used to compare the positive and negative results of steroid testing compared with witnessed administration of inhaled FP. Calculations were performed to yield the sensitivity and specificity of the test. A value of $p < 0.05$ was considered significant. Statistical analyses were performed using JMP Software release 8.0 (Statistical Discovery™ SAS Institute, Cary, NC).

RESULTS

All of the patients enrolled in this study had a physician's diagnosis of asthma with demographic data shown in Table 1. Sixty-one patients were enrolled in the study, but one patient was excluded from the study as an asthmatic control because the subject initially reported not taking FP but on later questioning admitted to using a sample inhaler provided elsewhere containing FP. Of the remaining 60 study subjects, 30 subjects submitted a urine specimen 16–24 hours after witnessed administration of their inhaler (Flovent 110, 2 puffs, or Advair 250/50, twice daily $\times$ 1 week) and were compared with urine samples from 30 asthmatic subjects not receiving FP as negative controls. FP17 β CA was detected in 30 of 30 asthmatic subjects receiving FP (median, IQR [413.5, 212.8–1230.0] range 12.4–3290.0 pg/mL [corrected for urine creatinine: median, IQR [576.2, 188.1–1306.6] range 6.3–5425.9 ng/g Cr]). FP17 β CA was undetectable in 30 of 30 subjects not receiving inhaled FP. The sensitivity and specificity of LC-MS/MS to detect FP17 β CA in urine were 100% (95% exact binomial confidence interval, 88–100) and 100% (95% exact binomial confidence interval, 88–100), respectively. It is to be noted that the excluded study subject was shown to have FP17 β CA in the urine after analysis at 419 pg/mL. The urine sample of this specific patient was reanalyzed and confirmed to have FP17 β CA in the urine (422 pg/mL).

DISCUSSION

We report a highly sensitive, novel, noninvasive method that may be used to verify that a specific patient may not have administered FP within a 16- to 24-hour window before testing. Gossec and colleagues reported adherence to medications in recent randomized controlled trials of six chronic diseases including asthma. From 24 asthma studies, only 11 (45.8%) de-

Table 1 Patient characteristics

	Asthma with FP	Asthma without FP	<i>p</i> Value
No. in study group	30	30	
Inhaled steroid treatment			
Total FP	30	0	
FP	14	0	
FP/salmeterol xinafoate	16	0	
Total not FP	0	30	
None	0	13	
Ciclesonide	0	1	
Mometasone furoate	0	2	
Budesonide	0	8	
Beclomethasone dipropionate	0	5	
Budesonide/formoterol	0	1	
Gender, <i>n</i> (% with/without FP)			
Male	9 (30)	12 (40)	
Female	21 (70)	18 (60)	
Age, median (IQR)	40 (31–53)	48 (38–56)	>0.05
Weight (kg), median (IQR)	81.5 (64.8–90.6)	77.7 (62.6–93.6)	>0.05
Height (cm), median (IQR)	167 (161.3–175.1)	164 (159.3–170.3)	>0.05
BMI, median (IQR)	28.1 (24.1–31.5)	28.3 (24.2–31.1)	>0.05
Pulmonary function			
FVC, median (IQR)	4.01 (3.50–4.56)	3.59 (2.95–4.88)	>0.05
FVC %, median (IQR)	101 (89–107)	100 (90–117)	>0.05
FEV ₁ , median (IQR)	3.06 (2.63–3.49)	2.77 (2.15–3.66)	>0.05
FEV ₁ %, median (IQR)	90 (83–101)	91 (74–101)	>0.05
FEV ₁ /FVC ratio, median (IQR)	75.9 (70.7–82.4)	74.4 (65.3–77.2)	>0.05
Urinary creatinine mg/dL, median (IQR)	91.5 (57.8–179.5)	97.5 (70.8–163.0)	>0.05

BMI = body mass index; FEV₁ = forced expiratory volume 1 s; FP = fluticasone propionate; FVC = forced vital capacity; IQR = interquartile range.

scribed assessment of adherence with none of the 24 documenting drug or metabolite assessment.¹⁰ Although this methodology only applies to subjects using FP, it is unique and the use of a comparative assessment tool particularly for ICS agents in the current literature appears to be absent. There has been much concern regarding the heterogeneity of asthma and variable effect of ICS on different asthma phenotypes.^{11,12} Documentation of medication use may reduce the variables that underlie suboptimal response to inhaled FP. In the present study, an evaluation of the clinical performance of a novel LC-MS/MS assay for detecting the metabolic product of FP in urine showed 100% clinical sensitivity in the ability to verify that a patient has not taken a moderate dose of inhaled FP within a recent 16- to 24-hour window.

Estimates of poor adherence range from 20 to 80% of all patients who have been prescribed ICS medications. Reports also suggest that 30% of asthma fatalities, substantial morbidities, and costs occur because of poor adherence.^{4,6,13–15} The projected cost of asthma hospitalization in 2010 was \$5.5 billion.³ Suboptimal adher-

ence is reported to account for the majority of asthma-related hospitalizations.¹⁶ In addition, physicians who do not adequately consider the potential effect of poor adherence may unnecessarily escalate medical therapy, which through 10.5 million office visits may already influence the \$5.9 billion in prescription medication costs that occur yearly in the United States. Self-reporting of adherence is insufficient to assess adherence.^{17,18} Some authors have recommended that objective tools should be used to confirm adherence before prescribing expensive novel therapies for the treatment of difficult asthma.¹⁹ Although there could be potential clinical limitations when using an assessment tool that is restricted to checking adherence in a 16- to 24-hour window after inhaler use, low compliance with prescribed medications including reports that more than one-half of asthmatic subjects fill no greater than a 1-month prescription each year,²⁰ suggest ample opportunities exist to use such a tool in the clinical setting. Verifying poor adherence has significant clinical ramifications. Appreciation of poor adherence issues may allow for recognition of other health issues such as

psychological disorders. Addressing such issues or implementing strategies to address suboptimal adherence may lead to improved outcomes at least for the short term.^{21–23} This modality may represent only one of multiple diagnostic and interventional tools including those capable of a longer window of assessment, which may be required to address adherence in the management of a complex and chronic disease such as asthma.²⁴

A recent case report and prospective study showed the feasibility of assessing for inflammation and current adherence by simultaneous analysis of sputum eosinophils and FP using induced sputum.^{25,26} Documenting persistent bronchial inflammation despite verified inhaled FP use may allow for a strategy to determine inadequacy of anti-inflammatory treatment and hence the need to escalate therapy in difficult to control asthmatic patients. Analysis of FP17 β CA may be performed on a urine sample obtained from a clinical or research setting perhaps in association with a monitor of asthma control such as a questionnaire or exhaled nitric oxide where sputum induction may not be readily available. This technique could potentially allow for retrospective case detection of nonadherent subjects in previously performed asthma clinical trials that used FP if stored urine specimens were available. A recent 2012 review of the Childhood Asthma Management Program clinical trial that began recruitment in 1993 confirmed the importance of independent monitoring in clinical research trials. In this trial, suboptimal adherence defined by a subject's use of <80% of prescribed medication was self-reported by only 6% of patients. In contrast, suboptimal adherence was documented by objective measurements in 75% of the same patients. The authors concluded that researchers should use objective measurements to identify subjects with poor adherence in clinical trials.²⁷

In summary, analysis of FP17 β CA in urine provides a highly sensitive, novel, noninvasive method that may be used to verify that a specific patient may not have administered FP within a 16- to 24-hour window before testing. We speculate that the potential usefulness of specific measurements of asthma control such as questionnaires, exhaled nitric oxide, or sputum eosinophilia may be augmented by concomitant assessments of anti-inflammatory treatment used at the time of testing. This study advances the concept that specific biomarkers may be used to identify individual subjects who, although proven to be adherent, may have persistent sputum eosinophilia or perhaps other surrogate markers of suboptimal asthma control that may benefit from novel biological therapies.

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