CHEST

Medication Adherence in Patients With Severe Asthma Prescribed Oral Corticosteroids in the U-BIOPRED Cohort

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BACKGROUND: Although estimates of suboptimal adherence to oral corticosteroids in asthma range from 30% to 50%, no ideal method for measurement exists; the impact of poor adherence in severe asthma is likely to be particularly high.

RESEARCH QUESTIONS: What is the prevalence of suboptimal adherence detected by self-reporting and direct measures? Is suboptimal adherence associated with disease activity?

STUDY DESIGN AND METHODS: Data were included from individuals with severe asthma taking part in the U-BIOPRED (Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes) study and prescribed daily oral corticosteroids. Participants completed the Medication Adherence Report Scale, a five-item questionnaire used to grade adherence on a scale from 1 to 5, and provided a urine sample for analysis of prednisolone and metabolites by liquid chromatography-mass spectrometry.

RESULTS: Data from 166 participants were included in this study: mean (SD) age, 54.2 (± 11.9) years; FEV_1, 65.1% (± 20.5%) predicted; female, 58%; 37% completing the Medication Adherence Report Scale reported suboptimal adherence; and 43% with urinary corticosteroid data did not have detectable prednisolone or metabolites in their urine. Good adherence by both methods was detected in 49 of the 142 (35%) of participants in whom both methods were performed; adherence detection did not match between methods in 53%. Self-reported high adherers had better asthma control and quality of life, whereas directly measured high adherers had lower blood eosinophil levels.

INTERPRETATION: Low adherence is a common problem in severe asthma, whether measured directly or self-reported. We report poor agreement between the two methods, suggesting some disassociation between self-assessment of medication adherence and regular oral corticosteroid use, which suggests that each approach may provide complementary information in clinical practice.

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KEY WORDS: adherence; asthma; urinary corticosteroids

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ABBREVIATIONS: HADS = Hospital Anxiety and Depression Scale; ICS = inhaled corticosteroids; IQR = interquartile range; LC-HRMS = liquid chromatography coupled to high-resolution mass spectrometry; LoD = limit of detection; MARS = Medication Adherence Report Scale; OCS = oral corticosteroids; U-BIOPRED = Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes

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Take-home Points

Study Questions: What is the prevalence of suboptimal adherence in severe asthma detected using self-reporting and direct measures, and is suboptimal adherence associated with disease activity?

Results: Good adherence by both methods was detected in 35% of participants; self-reported high adherers had better asthma control and quality of life, whereas directly measured high adherers had lower blood eosinophil levels.

Interpretation: Poor adherence is common in severe asthma, and associated with worse outcomes.

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Severe asthma occurs when the disease is not controlled despite treatment with high-dose inhaled corticosteroids (ICS) plus second-line therapies, or when treatment with systemic corticosteroids is required to bring about control.[1] It occurs in up to 10% of the asthma population, but contributes disproportionately to the burden of disease in terms of morbidity, exacerbation rate, quality of life, and health-care costs.[2,3] The diagnosis of severe asthma is made on the presumption that the prescribed medication is taken, and decisions leading to treatment escalation are often made on the basis of presumed inadequate benefit; this despite evidence that suboptimal adherence is known to be common, although the estimated prevalence varies widely.[4] Low levels of adherence are associated with poor symptom control and lung function, increased exacerbation frequency, as well as high costs.[5-7]

Adherence is defined by the World Health Organization as "the extent to which a person's behaviour…corresponds with agreed recommendations from a health care provider."[8] Measuring adherence to medication in asthma is challenging. Prescription refill rates can be used to determine whether an appropriate number of inhalers has been collected, but do not indicate whether the medication has been taken, and are not available to treating physicians in many health-care systems.[9] Measures of self-reported adherence, through questionnaires such as the Medication Adherence Report Scale (MARS), rely on accurate patient recall and reporting.[10] Electronic inhaler monitoring devices are being developed and used in research[11] (and becoming available for clinical use in some health-care systems), but few record inhalation as well as actuation.[12] Direct measures of adherence, such as detection of drug in biological samples, are not widely available or validated,[13,14] although recently Mansur and colleagues[15] have shown the potential usefulness of serum prednisolone detection as a marker of adherence in severe asthma.

The Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes (U-BIOPRED) project, a collaboration between public and private sectors, aims to identify new phenotypes and targets in patients with severe asthma who are often prescribed systemic corticosteroids.[16] During the baseline visit, we collected urine samples for measurement of corticosteroids and metabolites, and also asked participants to fill out the MARS adherence questionnaire. In the present study we aimed to investigate the following: (1) the prevalence of poor

adherence in adult participants prescribed daily oral corticosteroids by each of these methods; (2) the performance of the MARS questionnaire in predicting adherence relative to urinary corticosteroid detection; and (3) the clinical characteristics of adherent and nonadherent participants identified by each method.

Study Design and Methods

Study Design and Participants

This study used cross-sectional data from the U-BIOPRED cohort.[16] We included adults with severe asthma participating in the baseline visit of the study, who were currently prescribed daily oral corticosteroids. Severe asthma was defined in patients with uncontrolled symptoms and/or frequent exacerbations despite high-intensity asthma treatment (fluticasone $\geq$ 1,000 μg/d or equivalent).[17] The inclusion criteria stated that adherence should be assessed before inclusion in the study, but there was no explicit requirement to exclude patients who were poorly adherent. Patients were not asked to withhold prednisolone and were not told that it specifically would be measured. As it is usual practice to instruct patients to take prednisolone in the morning, we would expect samples to have been taken within 8 to 10 h of dosing.

The Asthma Control Questionnaire, Asthma Quality of Life Questionnaire, and Hospital Anxiety and Depression Scale (HADS) were administered, and participants underwent spirometric measurements and fractional exhaled nitric oxide testing at 50 mL/s. Sputum was induced with hypertonic saline inhaled via ultrasonic nebulizer and analyzed by a standard protocol to measure the differential cell count.[18] Venous blood samples were analyzed to determine the differential WBC count.

Adherence Measurements

In the MARS questionnaire, five items assess how participants use their medicines, which includes unintentional and intentional behaviors: (1) "I forget to take them"; (2) "I alter the dose"; (3) "I stop taking them for a while"; (4) "I decide to miss out a dose"; and (5) "I take less than instructed." Each item was answered using a five-point response scale, ranging from very often (1 point) to never (5 points). The sum was calculated for each participant, ranging from 5 to 25. If the total MARS score was less than 23, the participant was considered nonadherent.[19] It is important to note that MARS is nonspecific for particular medications.

Urine samples were collected the same day the MARS questionnaire was completed, and analyzed for prednisolone, prednisone, and their metabolites, and for cortisol, by liquid chromatography-mass spectrometry.

Chromatographic Analysis

Samples were prepared and corticosteroid levels were determined on a robotic liquid-handling platform (Microlab STAR; Hamilton). Corticosteroids were analyzed from a sample preparation, using a 1-mL aliquot of urine fortified with internal standards, and subsequently hydrolyzed with β-glucuronidase (*Escherichia coli*). Purification was performed by mixed-mode solid-phase extraction in a 96-well plate format. Analysis of the extract was performed by reversed-phase liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) (Q-Exactive; Thermo Scientific Inc). Acquisition of raw LC-HRMS data was performed in full scan mode at a resolution of 35,000 with polarity switching.[20] The limit of detection (LoD) for all these compounds (prednisolone, prednisone, methylprednisolone, 16α-OH-prednisolone, 20β-dihydro-prednisolone, and cortisol) was 1 ng/mL. At this LoD prednisolone and its major metabolites would be detectable for more than 24 h after a 10-mg oral dose.[21]

Statistical Analysis

The data sets for this analysis were downloaded from tranSMART, an open-source knowledge management platform,[22] in November 2018. The prevalence of nonadherence by each method (MARS and urinary detection) was assessed using the cutoffs specified, that is, classed as "self-reported nonadherent" if MARS $<$ 23, and "objective nonadherent" if no exogenous steroids or metabolites were detected, and reported with 95% CIs (normal approximation method). Differences in clinical variables between adherent and nonadherent groups (including Asthma Control Questionnaire, FEV_1, HADS, fractional exhaled nitric oxide, and blood biomarkers) were investigated using parametric *t* tests if normally distributed, Mann-Whitney *U* tests if nonparametric, or χ^2 tests if categorical. To assess the agreement between the MARS questionnaire and urinary corticosteroid detection, the Cohen κ test was used, and the performance characteristics of MARS in predictive adherence by urinary steroid detection were reported (sensitivity, specificity, positive and negative predictive values) with 95% CI. Correlation between oral prednisolone dose and urinary levels was investigated, using the Spearman rank correlation coefficient. All statistical analyses were performed with SPSS for the Mac, version 22 (IBM). A *P* value less than .05 was considered significant. The performance characteristics of MARS (cutoff less than 23 of 25, indicating nonadherence) in predicting undetected urinary corticosteroids were calculated.

Results

Participant Characteristics

A total of 166 participants currently prescribed daily oral corticosteroids were included in this cohort study (Fig 1). The median (interquartile range [IQR]) daily dose of oral corticosteroids was 10.0 (7.5-20.0) mg. Demographic details are shown in Table 1. In summary, this cohort contained a majority of female patients, with clinically significant airflow obstruction (mean FEV_1/FVC ratio, 61%), a high BMI, and a heterogeneous smoking history.

Self-Reported Adherence Measured by MARS Questionnaire

Complete MARS data were available from 147 participants, of whom 54 (37%) were classed as having poor self-reported adherence (median score, 20; IQR, 19-22), giving an estimated prevalence of 37% (95% CI, 30%-44%). The prescribed dose of prednisolone was not different between individuals who were classed as having good or poor adherence (Table 2). Likewise, no differences were observed in

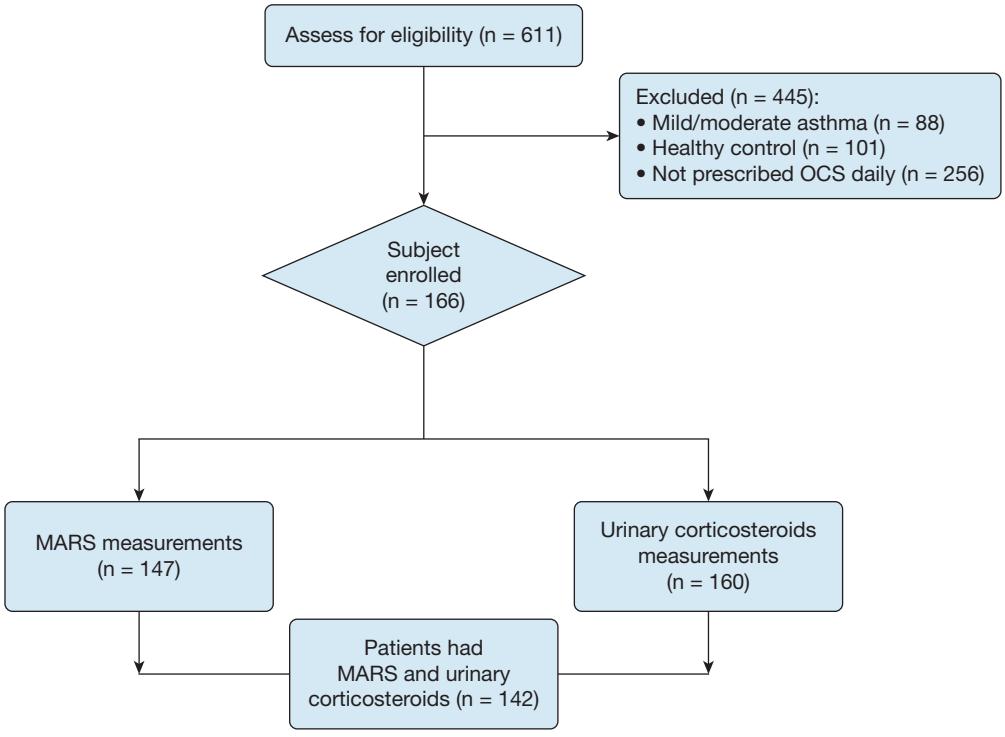

Figure 1 – *Study CONSORT diagram. CONSORT = Consolidated Standards of Reporting Trials; MARS = Medication Adherence Report Scale; OCS = oral corticosteroids.*

the urinary prednisolone level between groups, nor in the frequency of absence of detectable urinary cortisol. The poorly adherent group had statistically and clinically significant worse asthma control and quality of life than the group with good adherence. Although there were no differences in lung function or inflammatory biomarkers between groups, there were high levels of airflow obstruction and inflammatory biomarkers across both adherence categories.

TABLE 1] Participant Characteristics

Characteristic	Participants Using Oral Corticosteroids
Subjects, No.	166
Daily prednisolone dose, mg	10.0 (7.5-20.0)
Patients, female	96 (58)
Age, y	54.2 ± 11.9
BMI, kg/m^2	30.1 ± 6.5
FEV_1 % pred (pre-BD)	65.1 ± 20.5
FVC % pred (pre-BD)	86.5 ± 18.9
FEV_1/FVC % (pre-BD)	61.3 ± 13.1
Exacerbations over the previous year	3 (2-5)
Smoking status	105 (63): nonsmokers 54 (32): ex-smokers 7 (4): current smokers
Smoking history, pack-years	12.7 (4.8-22.5)
Intubation ever	15 (9)
ICU admission over the previous year	8 (5)

Data are expressed as mean ± SD, median (interquartile range), or No. (%). BD = bronchodilator.

Objective Adherence Measured by Urinary Corticosteroid Detection

Urinary corticosteroids and metabolite data were available for 160 participants, of whom 69 did not have detectable levels in their urine, despite the prescribed daily dose of prednisolone or prednisone being similar to those with detectable levels (Table 2). The estimated prevalence of nonadherence by urinary steroid detection was 43% (95% CI, 36%-50%). Other prednisolone metabolites (methylprednisolone, 16α-OH-prednisolone, and 20β-dihydro-prednisolone) were detected in 11 of the 91 who had corticosteroids detected. Almost all (89%) of the patients with detectable urinary corticosteroid metabolites had undetectable urinary cortisol, compared with about one-half (51%) of those with undetectable metabolites (χ^2, $P \leq .05$). There were no differences in asthma control, quality of life, exacerbation frequency, or in any of the

TABLE 2] Characteristics of Adherent and Nonadherent Participants Assessed Using Medication Adherence Rating Scale or Objective Urinary Corticosteroid Metabolites

Characteristic	MARS (n = 147)			Urinary Metabolites (n = 160)		
	Adherent	Nonadherent	Significance (*P* Value)	Adherent	Nonadherent	Significance (*P* Value)
Demographics						
Subjects, No.	93 (63%)	54 (37%)	…	91 (57%)	69 (43%)	…
Daily prednisolone dose, mg	10.0 (7.5-15) (n = 82)	10.0 (8.7-20) (n = 45)	.846	10.0 (7.5-18.7) (n = 81)	10.0 (7.5-20) (n = 59)	.940
Females, No. (%)	53 (57%)	31 (57%)	.938	49 (54%)	44 (63%)	.208
Age, y	55.1 ± 11.9	51.8 ± 11.9	.198	54.0 ± 12.7	54.8 ± 11.0	.667
BMI, kg/m^2	30.5 ± 7.1	29.4 ± 5.7	.336	30.0 ± 6.5	29.9 ± 6.7	.965
Asthma control, quality of life, and exacerbations						
ACQ-average	2.6 ± 1.4 (n = 89)	3.1 ± 1.2 (n = 51)	.015	2.7 ± 1.3 (n = 81)	2.9 ± 1.4 (n = 59)	.291
AQLQ	4.7 ± 1.2 (n = 89)	4.2 ± 1.3 (n = 53)	.020	4.7 ± 1.2 (n = 82)	4.4 ± 1.2 (n = 60)	.193
Exacerbations over the previous year, No.	3.0 (2.0-4.0) (n = 80)	3.0 (2.0-6.0) (n = 42)	.085	3.0 (2.0-5.0) (n = 74)	3.0 (1.7-4.2) (n = 62)	.449
Hospital Anxiety and Depression Score						
Total	12.4 ± 8.8 (n = 93)	14.0 ± 8.3 (n = 52)	.306	12.9 ± 9.2 (n = 89)	12.0 ± 7.5 (n = 67)	.529
Anxiety	6.9 ± 4.9 (n = 93)	7.8 ± 4.7 (n = 52)	.302	7.2 ± 5.1 (n = 89)	6.6 ± 4.2 (n = 67)	.454
Depression	5.5 ± 4.4 (n = 93)	6.2 ± 4.4 (n = 52)	.383	5.7 ± 4.6 (n = 89)	5.4 ± 4.1 (n = 67)	.702
Lung function						
FEV_1 % pred	66.0 ± 21.4 (n = 92)	62.0 ± 20.1 (n = 53)	.264	66.6 ± 21.4 (n = 89)	62.7 ± 19 (n = 68)	.239
FVC % pred	87.9 ± 20 (n = 92)	83.5 ± 18.7 (n = 53)	.195	87.7 ± 18.8 (n = 89)	85.3 ± 19.8 (n = 68)	.454
FEV_1/FVC	60.6 ± 12.9	61.1 ± 13.9	.819	62.0 ± 13.7	59.8 ± 11.9	.328
Biomarkers						
FENO	33 (22.0-53.0) (n = 83)	28 (15.7-72.5) (n = 51)	.924	33 (18.6-53.0) (n = 80)	29 (19.5-77.0) (n = 65)	.177
Sputum eosinophils, %	3.5 (1.0-18.9) (n = 40)	5.0 (0.2-19.7) (n = 24)	.720	5.2 (0.8-15.9) (n = 42)	5.0 (1.9-31.5) (n = 32)	.261
Sputum neutrophils, %	66.5 (44.1-86.7) (n = 40)	63.9 (30.3-93.6) (n = 24)	.650	69.5 (47.9-86.3) (n = 44)	44.6 (27.2-71.8) (n = 33)	.011
Blood eosinophils, × 10^3/μL	0.19 (0.1.0-0.4) (n = 93)	0.17 (0.1.0-0.4) (n = 51)	.649	0.1 (0.04-0.3) (n = 90)	0.30 (0.1-0.5) (n = 66)	.001
Blood neutrophils, × 10^3/μL	7.1 (4.9-8.7) (n = 93)	6.60 (4.0-8.4) (n = 51)	.539	7.4 (5.6-9.2) (n = 90)	5.30 (3.8-7.4) (n = 66)	.001

(Continued)

TABLE 2] *(Continued)*

Characteristic	MARS (n = 147)			Urinary Metabolites (n = 160)		
	Adherent	Nonadherent	Significance (*P* Value)	Adherent	Nonadherent	Significance (*P* Value)
Urinary prednisolone, ng/mL	1,579.7 (866.6-4,458.9) (n = 43)	1,561.1 (587.6-2,834.9) (n = 30)	.466	1,577.1 (690.7-3,064.7) (n = 79)	NA	NA
Detectable urinary cortisol, No. (%)	26 (28%)	13 (24%)	.617	10 (11%)	34 (49%)	< .001

Data are expressed as mean ± SD, median (interquartile range), or No. (%); between-group comparisons were made using parametric *t* tests if normally distributed, Mann-Whitney *U* tests if nonparametric, or χ^2 tests if categorical. ACQ = Asthma Control Questionnaire; AQLQ = Asthma Quality of Life Questionnaire; FENO = fractional exhaled nitric oxide; MARS = Medication Adherence Rating Scale; NA = not applicable.

HADS domains, between individuals with detectable urinary corticosteroid levels and the individuals with undetectable levels. Lung function parameters were similar between groups. There were differences in inflammatory biomarkers between groups, with sputum neutrophils (percentages) and blood neutrophils (counts) significantly higher, and blood eosinophils (counts) significantly lower in patients with detectable urinary corticosteroid metabolites. Of note, even in those with detectable urinary corticosteroid metabolites, the median (IQR) sputum eosinophils were still well above the normal range at 5.2% (0.8%-15.9%).

A daily prednisolone dose of at least 10 mg was prescribed in 100 participants, of whom 40% (n = 40) had undetectable corticosteroids in urine, compared with 43% (n = 19) of the 44 patients prescribed less than 10 mg (χ^2, $P = .744$). Moreover, no correlation was observed between the daily dose of prednisolone and the quantity of prednisolone in urine (Spearman $r = 0.095$, $P = .264$).

There was no difference in adherence measured by either MARS (Mann-Whitney *U*, $P = .582$) or steroid levels ($P = .723$) between nonsmokers and ex/current smokers.

Agreement Between Methods for Classifying Adherence

One hundred and forty-two participants had urinary corticosteroid metabolites analyzed and completed the MARS questionnaire (Table 3). The sensitivity and specificity of MARS to predict urinary corticosteroid detection were 59% (95% CI, 49%-66%) and 31% (95% CI, 20%-44%), respectively. The associated positive and negative predictive values were 54% (95% CI, 43%-64%) and 34% (95% CI, 22%-49%), respectively. There was poor agreement between the methods for determining medication adherence (κ test, –0.106; 95% CI, –0.266 to 0.054; $P = .268$).

Discussion

Poor adherence to oral corticosteroids is a major contributory factor to poor symptom control and hospitalizations[5,23]; poor adherence to ICS has been linked to death from asthma.[24] Despite recommendations that medication adherence should be routinely checked in primary care,[25] the optimal method to assess adherence is

TABLE 3] Agreement Between MARS and Urinary Corticosteroid Detection for Classifying Adherence

MARS	Urinary Prednisolone Metabolites		Total
	Detectable	Undetectable	
Good adherence (≥ 23)	49 (35%)	41 (28%)	90 (63%)
Poor adherence (< 23)	34 (23%)	18 (13%)	58 (37%)
Total	83 (58%)	59 (42%)	142

MARS = Medication Adherence Report Scale (rating score).

not clear. This is the first study to objectively determine adherence by direct measurement of urinary corticosteroid metabolites, and to compare this with self-reported adherence using the MARS questionnaire, in individuals with severe asthma prescribed daily oral corticosteroids. Our data suggest that MARS overestimates adherence to oral corticosteroids, considering urine corticosteroid metabolites as the "gold standard" comparator. We identified poor adherence in approximately 40% of individuals, using each method. Interestingly, however, the methods showed poor agreement, and the low adherers, identified via each method, were different in about one-half of all cases. Patients self-assessed as having poor adherence had worse asthma control and quality of life compared with self-reported good adherers, whereas objectively determined poor adherers do not appear to have more severe/uncontrolled disease. Importantly, patients with good adherence, assessed via either method, still displayed significant disease burden and raised inflammatory biomarkers, consistent with severe refractory asthma. Although the optimal method to assess medication adherence remains open to debate, we found that medication adherence remains suboptimal in a large number of patients with severe asthma, which should be considered by prescribers and discussed with patients during asthma reviews, particularly before the initiation of novel and expensive therapies such as biological therapies or bronchial thermoplasty.[13,26]

Identification of suboptimal medication adherence occurred despite application of the U-BIOPRED definition of severe asthma, recommending the exclusion of other, recognizable reasons for having "difficult" asthma such as clinical evidence of poor adherence.[17] Using the self-reported MARS questionnaire to determine adherence, 37% of the population had poor medication adherence. Previously, poor self-reported medication adherence, using the MARS questionnaire, had been observed in 69% of inner city adults with asthma[27] and 27% of children with persistent asthma.[28] Given the plethora of factors that may affect medication adherence (patient characteristics such as age, sex, socioeconomic level and ethnicity, social support, patient knowledge, psychological state, and patient's willingness to participate in self-management[29]), the divergence in adherence in our cohort of patients with severe asthma is no great surprise.

Adherence rates were similar when assessed by the self-reported MARS questionnaire and by urinary prednisolone detection. Importantly, however, the "poor adherers" were different in about one-half of cases. Our results highlight that the sensitivity and specificity for good adherence on the MARS questionnaire to identify individuals with detectable urinary prednisolone metabolites were 58% and 32%, respectively. These results indicate that relying solely on self-reported adherence would not be a useful assessment method in clinical practice. Although this is the first study to use the detection of urinary prednisolone metabolites to objectively assess medication adherence, our results are in line with adherence levels determined by blood plasma prednisolone detection in severe asthma.[13] It has been shown that challenging patients who claim to be adherent to medication, with objective evidence of poor adherence, in the form of blood prednisolone results or prescription refill rates, can facilitate frank and honest discussions on medication adherence.[13] More recently, Mansur and colleagues[15] have tested a sensitive liquid chromatography-tandem mass spectrometry-based assay for serum prednisolone, reporting detection for at least 3.5 h after witnessed dosing of 0.5 mg/kg in all 27 patients undergoing the test. The assay was also used for "spot testing" in 67 outpatients prescribed a median daily prednisolone dose of 10 mg (IQR, 15) and reported remarkably similar adherence levels to ours, with drug detected in approximately 58% of patients. We envisage a similar usefulness of urinary corticosteroid detection, which has the additional advantage of being less invasive than blood sampling and potentially offers a larger postdosing window for detection.[21]

Prednisolone metabolites are excreted mostly in the urine, and the peak concentration usually occurs after 4 to 8 h,[30] whereas the peak concentration for plasma prednisolone occurs much earlier (1.5-2 h) and becomes undetectable after 8 to 10 h.[31] In light of the results of the study by Mansur and colleagues,[15] it would have been of significant interest had we measured concomitant serum prednisolone in the patients, to determine whether the tests identify the same patients or whether they are complementary; we would propose this be the subject of further study. It seems likely that self-reported adherence contributes further supporting information; possible explanations for those reporting poor adherence but with detectable corticosteroid levels include sporadic poor adherence to systemic corticosteroid therapy, or good adherence to these drugs but poor adherence to others, such as inhaled medication.

Blood cortisol levels have also been used as surrogates for prednisolone adherence,[32,33] with adherence

considered satisfactory where there is detectable prednisolone and suppressed cortisol. It is more difficult, however, to interpret the situations in which only one of these tests is "positive." A detectable prednisolone level with normal cortisol may reflect intermittent prednisolone use, but there are no published data, to our knowledge, that support this interpretation; indeed, short-term use (up to 1 month) did not suppress 8 A.M. cortisol below 200 nM in approximately 75% of patients prescribed high-dose daily prednisolone (more than 25 mg/d), although no assessment of adherence was made in this study.[34] In contrast, suppressed cortisol without concomitant prednisolone detection could be found when prednisolone is present but below the LoD (due to dose and/or time since dosing), or when prednisolone is absent but persistent cortisol suppression is due to previous long-term prednisolone (and/or high-dose ICS) use, or primary hypoadrenalism.

Comparing the clinical characteristics between good adherers and poor adherers provides some interesting insights. First, self-reported poor adherers had worse asthma control and quality of life compared with self-reported good adherers. Although it is perhaps unsurprising that poor adherence would be associated with reduced asthma control and quality of life, these differences were observed despite the absence of differences in urinary corticosteroid levels, lung function, or inflammatory biomarkers. Possible explanations could be that patients with poor disease control and quality of life may be more self-analytical, or that they would be more likely to notice (and therefore report) when they had missed a dose of medication.

Somewhat surprisingly, there were no differences in markers of asthma control, quality of life, or severity of disease between those with and without detectable urinary corticosteroids. It may be that patients "self-regulate" their daily dose of corticosteroids to maintain relative disease stability. However, the patients with poor adherence measured in this way still had frequent exacerbations and poor control, and may represent a group in whom targeting of adherence as a "treatable trait" could potentially have an impact on these important outcomes. The relatively high blood eosinophil counts in these patients do suggest that regular corticosteroid therapy might be clinically effective.[35,36] In contrast, the finding of persistently raised median sputum eosinophils even in those with detectable corticosteroid levels suggests that some of these patients may represent a truly corticosteroid-insensitive phenotype,[37] and we propose that the concomitant measurement of corticosteroids in biofluids should be advocated in studies investigating this phenotype in future.

Many techniques are available to assess adherence to asthma medication; however, there is currently no gold standard.[38] This study benefits from using two such methods, but each technique has its own limitations. The 10-item MARS questionnaire is a validated tool to assess medication adherence with good test-retest reliability in asthma,[27] although the concordance of the five-item version used here with alternative objective measures has had mixed results when assessing inhaled corticosteroids in childhood asthma.[28] It is possible that using the 10-item MARS, or indeed other adherence questionnaires such as the eight-item Morisky Medication Adherence Scale,[39] would have given different results, although none are able to overcome the obvious shortcomings inherent in self-reporting. In the current study, we administered the MARS questionnaire to determine adherence to asthma medication in general, rather than to oral corticosteroids specifically. It has been shown that adherence may vary between types of asthma treatment, and therefore a patient's response to the MARS questionnaire may not reflect their oral corticosteroid adherence per se.

Mass spectrometry is highly sensitive for urinary prednisolone and its metabolites, with detection possible up to 24 h after a 10-mg dose, and 72 h after a 40-mg dose.[21] The median daily dose prescribed in our study was 10 mg, and so it is possible that we recorded false-negative results for some of those taking a lower dose. However, we believe that this is not likely to have been a common issue for two reasons: first, the patients were not asked to omit their oral corticosteroids (OCS) on the day of the study visit, and usual practice is to take it in the morning, with the study visit likely occurring within 8 to 10 h maximum; second, a similar proportion of those prescribed less than 10 mg had undetectable urinary levels (44%) as in those receiving 10 mg or more (41%). The significance of the differential detection of the unchanged drug and it metabolites is not known; the washout profile is specific to each, and it could be speculated that looking at their relative concentrations could give

more information on elapsed time since dosing. We did not record the specific formulation of oral corticosteroids taken; it is known that enteric coating slows the absorption of prednisolone,[38] and could therefore adversely have affected the sensitivity of the assay in this regard. A patient with occasional or sporadic medication use may therefore be categorized as having good adherence if they took their medication only on the days preceding the urine sample. Objective measures could have been further enhanced by the inclusion of direct measurement of inhaled corticosteroid metabolites in both blood and urine,[14,40] and the addition of inhaler monitoring using "smart inhalers." Indeed, a direct measure of ICS adherence would have allowed us to better understand any potential confounding effect that this may have had on our results (either through concordant or discordant relative ICS/OCS adherence), and whether the MARS data reflected behaviors related to inhaled or oral medication, or both.

Interpretation

The poor concordance that we identified between self-reported and objective adherence methods questions the validity of relying solely on self-reported adherence in clinical practice, although such questionnaires may provide insights into reasons for nonadherence, and therefore be useful in targeting interventions. The patients with asthma we identified with markedly raised inflammatory biomarkers despite good adherence to medication may represent patients with truly refractory disease. We suggest that objective measures of adherence (direct measurement in biofluids for OCS and smart inhaler use for inhaled therapies) should be used in clinical practice, to initiate discussions on medication adherence and to identify "steroid-unresponsive" patients for research and for novel biological treatments.

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References

1. British Thoracic Society/Scottish Intercollegiate Guidelines Network. BTS/SIGN British guideline on the management of asthma, 2019. https://www.brit-thoracic.org.uk/quality-improvement/guidelines/asthma/. Accessed February 25, 2021.
2. Chung KF, Wenzel SE, Brozek JL, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J*. 2014;43(2):343-373.
3. Moore WC, Bleecker ER, Curran-Everett D, et al. Characterization of the severe asthma phenotype by the National Heart, Lung, and Blood Institute's Severe Asthma Research Program. *J Allergy Clin Immunol*. 2007;119(2):405-413.
4. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention. Updated 2019. https://ginasthma.org/wp-content/uploads/2019/06/GINA-2019-main-report-June-2019-wms.pdf. Accessed February 26, 2021.
5. Krishnan JA, Riekert KA, McCoy JV, et al. Corticosteroid use after hospital discharge among high-risk adults with asthma. *Am J Respir Crit Care Med*. 2004;170(12):1281-1285.
6. Kandane-Rathnayake RK, Matheson MC, Simpson JA, et al. Adherence to asthma management guidelines by middle-aged adults with current asthma. *Thorax*. 2009;64(12):1025-1031.
7. O'Neill C, Gamble J, Lindsay JT, Heaney LG. The impact of nonadherence to inhaled long-acting β_2-adrenoceptor agonist/corticosteroid combination therapy on healthcare costs in difficult-to-control asthma. *Pharmaceut Med*. 2011;25:379-385.
8. World Health Organization. Adherence to Long-Term Therapies: Evidence for Action. 2003. https://www.who.int/chp/knowledge/publications/adherence_full_report.pdf. Accessed February 26, 2021.
9. Vollmer WM, Xu M, Feldstein A, Smith D, Waterbury A, Rand C. Comparison of pharmacy-based measures of medication adherence. *BMC Health Serv Res*. 2012;12:155.
10. Horne R, Weinman J. Self-regulation and self-management in asthma: exploring the role of illness perceptions and treatment beliefs in explaining non-adherence to preventer medication. *Psychol Health*. 2002;17(1):17-32.
11. Morton RW, Elphick HE, Rigby AS, et al. STAAR: a randomised controlled trial of electronic adherence monitoring with reminder alarms and feedback to improve clinical outcomes for children with asthma. *Thorax*. 2017;72(4):347-354.
12. McNicholl DM, Stevenson M, McGarvey LP, Heaney LG. The utility of fractional exhaled nitric oxide suppression in the identification of nonadherence in difficult asthma. *Am J Respir Crit Care Med*. 2012;186(11):1102-1108.
13. Gamble J, Stevenson M, McClean E, Heaney LG. The prevalence of nonadherence in difficult asthma. *Am J Respir Crit Care Med*. 2009;180(9):817-822.
14. George KE, Ryan DM, Keevil B, Niven R, Fowler SJ. A pilot study to investigate the use of serum inhaled corticosteroid concentration as a potential marker of treatment adherence in severe asthma. *J Allergy Clin Immunol*. 2017;139(3):1037-1039.e1.
15. Mansur AH, Hassan M, Duffy J, Webster C. Development and clinical application of a prednisolone/cortisol assay to determine adherence to maintenance oral prednisolone in severe asthma. *Chest*. 2020;158(3):901-912.
16. Shaw DE, Sousa AR, Fowler SJ, et al; U-BIOPRED Study Group. Clinical and inflammatory characteristics of the European U-BIOPRED adult severe asthma cohort. *Eur Respir J*. 2015;46(5):1308-1321.
17. Bel EH, Sousa A, Fleming L, et al. Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes (U-BIOPRED) Consortium, Consensus Generation. Diagnosis and definition of severe refractory asthma: an international consensus statement from the Innovative Medicine Initiative (IMI). *Thorax*. 2011;66(10):910-917.
18. Pavord I, Pizzichini M, Pizzichini E, Hargreave F. The use of induced sputum to investigate airway inflammation. *Thorax*. 1997;52(6):498-501.
19. Koster ES, Philbert D, Winters NA, Bouvy ML. Adolescents' inhaled corticosteroid adherence: the importance of treatment perceptions and medication knowledge. *J Asthma*. 2015;52(4):431-436.
20. Mullen JE, Thörngren JO, Schulze JJ, et al. Urinary steroid profile in females: the impact of menstrual cycle and emergency contraceptives. *Drug Test Anal*. 2017;9(7):1034-1042.
21. Ahi S, Beotra A, Dubey S, Upadhyay A, Jain S. Simultaneous identification of prednisolone and its ten metabolites in human urine by high performance liquid chromatography-tandem mass spectrometry. *Drug Test Anal*. 2012;4(6):460-467.
22. Athey BD, Braxenthaler M, Haas M, Guo Y. tranSMART: an open source and community-driven informatics and data sharing platform for clinical and translational research. *AMIA Jt Summits Transl Sci Proc*. 2013;2013:6-8.
23. Miller TP, Greenberger PA, Patterson R. The diagnosis of potentially fatal asthma in hospitalized adults: patient characteristics and increased severity of asthma. *Chest*. 1992;102(2):515-518.
24. Royal College of Physicians, Levy M, Andrews R, Buckingham R, et al. Why asthma still kills: the National Review of Asthma Deaths (NRAD) confidential

enquiry report. May 2014. https://www.rcplondon.ac.uk/file/868/download. Accessed February 26, 2021.

25. Heaney LG, Horne R. Non-adherence in difficult asthma: time to take it seriously. *Thorax*. 2012;67(3):268-270.
26. Lee J, Tay TR, Radhakrishna N, et al. Nonadherence in the era of severe asthma biologics and thermoplasty. *Eur Respir J*. 2018;51(4):1701836.
27. Cohen JL, Mann DM, Wisnivesky JP, et al. Assessing the validity of self-reported medication adherence among inner-city asthmatic adults: the Medication Adherence Report Scale for Asthma. *Ann Allergy Asthma Immunol*. 2009;103(4):325-331.
28. Garcia-Marcos PW, Brand PL, Kaptein AA, Klok T. Is the MARS questionnaire a reliable measure of medication adherence in childhood asthma? *J Asthma*. 2016;53(10):1085-1089.
29. Van Ganse E, Mörk A-C, Osman LM, et al. Factors affecting adherence to asthma treatment: patient and physician perspectives. *Prim Care Respir J*. 2003;12(2):46-51.
30. Garg V, Jusko WJ. Simultaneous analysis of prednisone, prednisolone and their major hydroxylated metabolites in urine by high-performance liquid chromatography. *J Chromatogr*. 1991;567(1):39-47.
31. Gambertoglio JG, Amend WJ Jr, Benet LZ. Pharmacokinetics and bioavailability of prednisone and prednisolone in healthy volunteers and patients: a review. *J Pharmacokinet Biopharm*. 1980;8(1):1-52.
32. Wilson AM, McFarlane LC, Lipworth BJ. Systemic bioactivity profiles of oral prednisolone and nebulized budesonide in adult asthmatics. *Chest*. 1998;114(4):1022-1027.
33. Wilson A, Lipworth B. Short-term dose-response relationships for the relative systemic effects of oral prednisolone and inhaled fluticasone in asthmatic adults. *Br J Clin Pharmacol*. 1999;48(4):579-585.
34. Henzen C, Suter A, Lerch E, Urbinelli R, Schorno XH, Briner VA. Suppression and recovery of adrenal response after short-term, high-dose glucocorticoid treatment. *Lancet*. 2000;355(9203):542-545.
35. Lassalle P, Sergant M, Delneste Y, et al. Levels of soluble IL-2 receptor in plasma from asthmatics: correlations with blood eosinophilia, lung function, and corticosteroid therapy. *Clin Exp Immunol*. 1992;87(2):266-271.
36. Sakae TM, Maurici R, Trevisol DJ, Pizzichini MMM, Pizzichini E. Effects of prednisone on eosinophilic bronchitis in asthma: a systematic review and meta-analysis. *J Bras Pneumol*. 2014;40(5):552-563.
37. Lehmann A, Aslani P, Ahmed R, et al. Assessing medication adherence: options to consider. *Int J Clin Pharm*. 2014;36(1):55-69.
38. Lee D, Taylor G, Walker J, James V. The effect of food and tablet formulation on plasma prednisolone levels following administration of enteric-coated tablets. *Br J Clin Pharmacol*. 1979;7(5):523-528.
39. Janežič A, Locatelli I, Kos M. Criterion validity of 8-item Morisky Medication Adherence Scale in patients with asthma. *PLoS One*. 2017;12(11):e0187835.
40. Hagan JB, Netzel BC, Matthews MR, Korpi-Steiner NL, Singh RJ. Urinary fluticasone propionate-17beta-carboxylic acid to assess asthma therapy adherence. *Allergy Asthma Proc*. 2012;33(4):e35-e39.