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Title: Medication adherence in patients with severe asthma prescribed oral corticosteroids in the U-BIOPRED cohort

Short title: Measuring adherence in severe asthma

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Preliminary results from this study have been presented in abstract form at the European Respiratory Society: Alahmadi F, Simpson A, Gomez C, Wheelock C, Shaw D, Fleming L, et al. Measures of adherence in patients with severe asthma prescribed systemic steroids in the UBIOPRED cohort. European Respiratory Journal. 2018;52(suppl 62):PA3992.

ABSTRACT

Background: Whilst estimates of sub-optimal 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: 1. What is the prevalence of suboptimal adherence detected using self-reporting and direct measures? 2. 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 study prescribed daily oral corticosteroids. Participants completed the MARS, 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, 58% female. 37% completing the MARS reported sub-optimal 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 35% participants who had both 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 eosinophils.

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.

KEYWORDS: Asthma, Adherence, Urinary corticosteroids

ABBREVIATIONS:

ACQ: Asthma Control Questionnaire

AQLQ: Asthma Quality of Life Questionnaire

FEV_1: forced expiratory volume in first second

FVC: forced vital capacity

FeNO: fractional exhaled Nitric Oxide

HADS: Hospital Anxiety and Depression Scale

ICS: inhaled corticosteroids

LC-HMRC: liquid chromatography coupled to high-resolution mass spectrometry

LoD: limit of detection

MARS: Medication Adherence Report Scale

U-BIOPRED: Unbiased BIOmarkers for the Prediction of REspiratory Disease outcomes

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 eosinophils.

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

Severe asthma is defined where the disease is not controlled despite treatment with high dose inhaled corticosteroids (ICS) plus second line therapies, or where treatment with systemic corticosteroids is required to bring about control [1]. It comprises 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 healthcare costs [2,3]. The diagnosis of severe asthma assumes that the prescribed medication is taken, and decisions leading to treatment escalation are often made on the basis of presumed inadequate benefit. This is despite the 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, lung function, and increased exacerbation frequency, as well as high costs [5-7].

Adherence is defined by the World Health Organization as "'the degree to which the person's behaviour corresponds with the 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 have been collected, but do not indicate whether the medication has been taken, and are not available to treating clinicians in many healthcare systems [9]. 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 healthcare 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 have shown potential utility of serum prednisolone detection as a marker of adherence in severe asthma [15].

The Unbiased BIOmarkers for the Prediction of Respiratory Disease Outcomes (U-BIOPRED) project is a collaboration between public and private sectors, which 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 the MARS adherence questionnaire. In the present study we aimed to investigate: 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 by urinary corticosteroids detection; and 3. the clinical characteristics of adherent and non-adherent participants identified by each method.

STUDY DESIGN AND METHODS

Study Design and Participants

This study used the 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 where patients had uncontrolled symptoms and/or frequent exacerbations despite high intensity asthma treatment (at least 1000 mcg/day fluticasone or equivalent) [17]. The inclusion criteria stated that adherence should be assessed prior to 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 prescribe prednisolone to be taken in the morning, then we would expect samples to have been taken within 8-10 hours of dosing.

Asthma Control Questionnaire (ACQ), Asthma Quality of Life Questionnaire (AQLQ) and Hospital Anxiety and Depression Scale (HADS) were administered, and participants underwent measurement of spirometry and fractional exhaled nitric oxide (FeNO) test at 50ml/sec. Sputum was induced using hypertonic saline inhaled via ultrasonic nebulizer and analysed by a standard protocol to measure the differential cell count [18]. Venous blood samples were analysed for differential white cell count.

Adherence Measurements

In the MARS questionnaire, five items assess how participants use their medicines, which includes unintentional and intentional behaviours: 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-graded response scale, ranging from very often (1) to never (5). The sum was calculated for each participant ranging from 5 to 25. If the total score of MARS was below 23, the participant was considered non-adherent [19]. It is important to note that MARS is non-specific to particular medications.

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

Chromatographic analysis:

The sample preparation for determination of corticosteroids was performed on a robotic liquid-handling platform (Microlab STAR, Hamilton Robotics, Bonaduz, Switzerland). The corticosteroids were analysed from a simple preparation using a 1mL aliquot of urine fortified with internal standards and subsequently hydrolyzed using β-glucuronidase (*E. coli*). Purification was performed using mixed mode solid phase extraction (SPE) in 96 well plate format. The analysis of the extract was performed with reversed phase liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS, Thermo Q-Exactive, Thermo Fisher Scientific Inc, Waltham, USA). Acquisition of raw LC-HRMS data was performed in full scan mode at a resolution of 35000 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 over 24h after a 10mg oral dose [21].

Statistical analysis:

The datasets for this analysis were downloaded from tranSMART, an open-source knowledge management platform [22] on November 2018. The prevalence of non-adherence by each method (MARS and urinary detection) was assessed using the cut-offs specified, i.e. classed as "self-reported non-adherent" if MARS < 23, and "objective non-adherent" if no exogenous steroids or metabolites were detected, and reported with 95% confidence intervals (CI, normal approximation method). Differences in clinical variables between adherent and non-adherent groups [including ACQ, forced expiratory volume in 1 second (FEV1), HADS, FeNO, and blood biomarkers] were investigated using parametric t-tests if normally distributed or Mann-Whitney U tests if nonparametric, or Chi-square tests if categorical. For assessing the agreement between MARS questionnaire and urinary corticosteroids detection, Cohen Kappa test was used, and the performance characteristics of MARS in predictive adherence by urinary steroid detection reported (sensitivity, specificity, positive and negative predictive values) with 95% CI. Correlation between oral prednisolone dose and urinary levels was investigated using Spearman's rank correlation coefficient. All statistical analysis was performed using SPSS for MAC version 22 (SPSS, Chicago, IL). A *p*-value of less than 0.05 was considered significant. The performance characteristics of the MARS (cut-off less than 23 out of 25 indicating non-adherence) 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 (figure 1). The median (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 females, with clinically significant airflow obstruction (mean forced expiratory 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 which 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 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. Whilst 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.

Objective adherence measured by urinary corticosteroid detection

Urinary corticosteroids and metabolite data were available for 160 participants, of which 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 non-adherence 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 have corticosteroids detected. Almost all (89%) of patients with detectable urinary corticosteroid metabolites had undetectable urinary cortisol, compared to around half (51%) of those with undetectable metabolites (chi squared $p \leq 0.05$). There were no differences in asthma control, quality of life, exacerbation frequency, or in any of the HADS domains, between individuals with detectable urinary corticosteroids levels and the individuals with undetectable levels. Lung function parameters were similar between groups. There were differences in inflammatory biomarkers between groups, with sputum (percentages) and blood (counts) neutrophils significantly higher, and blood eosinophils (counts) significantly lower in patients with detectable urinary

corticosteroids 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 41 % (n=40) had undetectable corticosteroids in urine, compared to 44% (n=19) of the 44 patients prescribed less than 10mg (chi squared p = 0.744). Moreover, no correlation was observed between daily dose of prednisolone and the quantity of prednisolone in urine (Spearman's r= 0.095, p = 0.264).

There was no difference in adherence measured by either MARS (Mann-Whitney U p=0.582) or steroid levels (p=0.723) between non-smokers and ex/current smokers.

Agreement between methods for classifying adherence

One-hundred and forty-two participants had urinary corticosteroid metabolites analysed and completed the MARS questionnaire (Table 3). The sensitivity and specificity of MARS (95% CI) to predict urinary corticosteroids detection were 59 (49-66) % and 31 (20-44) % respectively. The associated positive and negative predictive values were 54 (43-64) % and 34 (22-49) % respectively. There was poor agreement between the methods for determining medication adherence (kappa test = -0.106, 95% CI -0.266-0.054, p = 0.268).

DISCUSSION

Poor adherence to oral corticosteroids is a major contributory factor to poor symptom control and hospitalisations [23,24]; poor adherence to ICS has been linked to death from asthma [25]. Despite recommendations that medication adherence should be routinely checked in primary care [26], the optimal method to assess adherence is not clear. This is the first study to objectively determine adherence by direct measurement of urinary corticosteroid metabolites, and to compare this to

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 around half of all cases. Patient self-assessed as having poor-adherence had worse asthma control and quality of life compared with self-reported good-adherers, whilst 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. Whilst the optimal method to assess medication adherence remains open to debate, we identified that medication adherence remains sub-optimal in a large number of severe asthma patients, which should be considered by prescribers and discussed with patients during asthma reviews, particularly prior to the initiation of novel and expensive therapies such as biological therapies or bronchial thermoplasty [13,27].

The identification of sub-optimal medication adherence occurred despite the application of the U-BIOPRED definition of severe asthma, recommending the exclusion of other, recognisable 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 has been observed in 69% of inner city asthmatic adults [28] and 27% of children with persistent asthma [29]. Given the plethora of factors that may affect medication adherence (patient characteristics such as age, gender, socio-economic level and ethnicity, social support, patient knowledge, psychological state and patient's willingness to participate in self-management [30]), the divergence in adherence in our cohort of severe asthma patients is of no great surprise.

Adherence rates were similar when assessed using the self-reported MARS questionnaire and using urinary prednisolone detection. Importantly, however, the 'poor-adherers' were different in around half of cases. Our results highlight 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. Whilst this is the first study to utilise 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 have tested a sensitive liquid-chromatography tandem mass spectrometry based assay for serum prednisolone, reporting detection for at least 3.5 hrs following witnessed dosing of 0.5mg/kg in all 27 patients undergoing the test [15]. The assay was also used for "spot testing" in 67 outpatients prescribed median (IQR) 10 (15) mg daily prednisolone and reported remarkably similar adherence levels to us, with drug detected in approximately 58% of patients. We envisage a similar utility of urinary corticosteroid detection, which has the additional advantage of being less invasive than blood-sampling and potentially offer a larger post-dosing window for detection [21].

Prednisolone metabolites are mostly excreted in the urine, and the peak concentration usually occurs after 4-8 hours [31], whilst the peak concentration for plasma prednisolone occurs much earlier (1.5-2 hours) and becomes undetectable after 8-10 hours [32]. In light of the results of the Mansur study [15], it would have been of significant interest had we measured concomitant serum prednisolone in our 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 includes sporadic poor adherence

to systemic corticosteroids 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 [33,34], with adherence considered satisfactory where there is detectable prednisolone and suppressed cortisol. It is more difficult however to interpret the situations where only one of these tests is "positive". A detectable prednisolone level with normal cortisol may reflect intermittent prednisolone use, but there are no data published to our knowledge that support this interpretation; indeed short term (up to a month) use did not suppress 8am cortisol below 200nmol/L in approximately 75% of patients prescribed high dose daily prednisolone (over 25mg/day), although no assessment of adherence was made in this study [35]. On the other hand, suppressed cortisol without concomitant prednisolone detection could be found where prednisolone is present but below LoD (due to dose and/or time since dosing), or where prednisolone is absent but persistent cortisol suppression 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. Firstly, self-reported poor-adherers had worse asthma control and quality of life compared to 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 no difference in urinary corticosteroid levels, nor any difference in 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 between those with and without detectable urinary corticosteroids. It may be that patients "self-regulate" their daily dose of corticosteroids in order 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 [36,37]. On the other hand, the finding of persistently raised median sputum eosinophils even in those with detectable corticosteroids levels suggests that some of these patients may represent a truly corticosteroid-insensitive phenotype [38], 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 [39]. 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 [28], 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 [29]. It is possible that using the 10-item MARS, or indeed other adherence questionnaires such as the 8-item Morisky Medication Adherence Scale [40], 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, 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 hours after a 10 mg dose, and 72 hours after 40mg [21]. The median daily dose prescribed in our study was 10mg, and so it is possible that we recorded false-negative results for some of those taking a lower dose. However we feel that this is not likely to have been a common issue for two reasons: first our patients were not asked to omit their 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 8-10

hrs maximum; second a similar proportion of those prescribed less than 10mg had undetectable urinary levels (44%) as in those on 10mg 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 [39], 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 categorised as having good-adherence if they only took their medication on the days preceding the urine sample. Objective measures could have been further enhanced by the inclusion of direct measurement of inhaled corticosteroids metabolites in both blood and urine [14,41], 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 upon our results (either through concordant or discordant relative ICS/OCS adherence), and whether the MARS data reflected behaviours 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 non-adherence, and therefore be useful in targeting interventions. The asthmatics we identified with markedly raised inflammatory biomarkers despite good adherence to medication may represent patients with truly refractory disease. We suggest that objective (direct measurement in biofluids for OCS and smart inhaler use for inhaled therapies) measures of adherence should be utilized in clinical practice, to initiate discussions on medication adherence and identify 'steroid-unresponsive' patients for research and for novel biologic treatments.

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Table 1. Participant characteristics

	Participants using oral corticosteroids
Subjects, n	166
Daily prednisolone dose, mg	10.0 (7.5-20.0)
Females, n (%)	96 (58)
Age, years	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, n	3 (2-5)
Smoking status, n (%)	105 (63) non-smoker 54 (32) ex-smoker 7 (4) current smoker
Smoking history, pack years	12.7 (4.8-22.5)
Intubation ever, n (%)	15 (9)
ICU admission over the previous year, n (%)	8 (5)

Data are expressed as mean ± SD, median (interquartile range) or n/N (%). BMI: body mass index; FEV_1: forced expiratory volume in 1 second; BD: bronchodilator; FVC: forced vital capacity; ICU: intensive care unit.

Table 2. Characteristics of adherent and non-adherent participants assessed using the Medication Adherence Rating Scale (MARS) or objective urinary corticosteroids metabolites.

		MARS (n=147)			Urinary metabolites (n=160)		
		Adherent	Non-adherent	Significance	Adherent	Non-adherent	Significance
Demographics	Subjects, n	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)	P=0.846	10.0 (7.5-18.7) (n=81)	10.0 (7.5-20) (n=59)	P=0.940
	Females, n (%)	53 (57%)	31 (57%)	P=0.938	49 (54%)	44 (63%)	P=0.208
	Age, years	55.1 ±11.9	51.8±11.9	P=0.198	54.0± 12.7	54.8± 11.0	P=0.667
	BMI (kg/m2)	30.5 ±7.1	29.4 ± 5.7	P=0.336	30.0±6.5	29.9± 6.7	P=0.965
Asthma control, quality of life and exacerbations	ACQ-average	2.6±1.4 (n=89)	3.1±1.2 (n=51)	P=0.015	2.7±1.3 (n=81)	2.9±1.4 (n=59)	P=0.291
	AQLQ	4.7±1.2 (n=89)	4.2±1.3 (n=53)	P=0.020	4.7±1.2 (n=82)	4.4±1.2 (n=60)	P=0.193
	Exacerbations over the previous year, n	3.0 (2.0-4.0) (n=80)	3.0 (2.0-6.0) (n=42)	P=0.085	3.0 (2.0-5.0) (n=74)	3.0 (1.7-4.2) (n=62)	P=0.449
Hospital Anxiety and Depression Score	Total	12.4±8.8 (n=93)	14.0±8.3 (n=52)	P=0.306	12.9±9.2 (n=89)	12.0±7.5 (n=67)	P=0.529
	Anxiety	6.9±4.9 (n=93)	7.8± 4.7 (n=52)	P=0.302	7.2±5.1 (n=89)	6.6±4.2 (n=67)	P=0.454

	Depression	5.5±4.4 (n=93)	6.2±4.4 (n=52)	P=0.383	5.7±4.6 (n=89)	5.4±4.1 (n=67)	P=0.702
Lung function	FEV_1 %pred.	66.0±21.4 (n=92)	62.0±20.1 (n=53)	P=0.264	66.6±21.4 (n=89)	62.7±19 (n=68)	P=0.239
	FVC %pred.	87.9±20 (n=92)	83.5±18.7 (n=53)	P=0.195	87.7±18.8 (n=89)	85.3±19.8 (n=68)	P=0.454
	FEV_1/FVC	60.6±12.9	61.1±13.9	P=0.819	62.0±13.7	59.8±11.9	P=0.328
Biomarkers	FeNO	33 (22.0-53.0) (n=83)	28 (15.7-72.5) (n=51)	P=0.924	33 (18.6-53.0) (n=80)	29 (19.5-77.0) (n=65)	P=0.177
	Sputum eosinophils, %	3.5 (1.0-18.9) (n=40)	5.0 (0.2-19.7) (n=24)	P=0.720	5.2 (0.8-15.9) (n=42)	5.0 (1.9-31.5) (n=32)	P=0.261
	Sputum neutrophils, %	66.5 (44.1-86.7) (n=40)	63.9 (30.3-93.6) (n=24)	P=0.650	69.5 (47.9-86.3) (n=44)	44.6 (27.2-71.8) (n=33)	P=0.011
	Blood eosinophils (X 10^3/ul)	0.19 (0.1.0-0.4) (n=93)	0.17 (0.1.0-0.4) (n=51)	P=0.649	0.1 (0.04-0.3) (n=90)	0.30 (0.1-0.5) (n=66)	P=0.001
	Blood neutrophils (X 10^3/ul)	7.1 (4.9-8.7) (n=93)	6.60 (4.0-8.4) (n=51)	P=0.539	7.4 (5.6-9.2) (n=90)	5.30 (3.8-7.4) (n=66)	P=0.001
	Urinary prednisolone (ng/mL)	1579.7 (866.6-4458.9) (n=43)	1561.1 (587.6-2834.9) (n=30)	P=0.466	1577.1 (690.7-3064.7) (n=79)	NA	NA
	Detectable urinary cortisol n (%)	26 (28%)	13 (24%)	p=0.617	10 (11%)	34 (49%)	p<0.001

Data are expressed as mean ±SD, median (interquartile range) or n (%); Between-group comaprisons were made using parametric t-tests if normally distributed, Mann-Whitney U tests if nonparametric, or Chi-square tests if categorical; BMI: body mass index; FEV_1: ACQ: Asthma Control Questionnaire; AQLQ: Asthma Quality of Life Questionnaire; FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity

Table 3. Agreement between MARS and urinary corticosteroids detection for classifying adherence

		Urinary prednisolone metabolites		Total
		Detectable	Undetectable	
MARS	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 rating score

Figure 1 legend: Study CONSORT diagram