

Original Article

Serum Inhaled Corticosteroid Detection for Monitoring Adherence in Severe Asthma

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What is already known about this topic? Adherence to inhaled corticosteroids in asthma is poor, and yet few reliable methods exist to assess adherence in clinic.

What does the article add to our knowledge? Blood detection of budesonide, beclomethasone dipropionate, and fluticasone propionate may represent a new method for monitoring adherence in severe asthma, and (for fluticasone) relate to important markers of disease severity.

How does this study impact current management guidelines? Blood detection of inhaled corticosteroids merits further testing as a potential measure of adherence in severe asthma.

BACKGROUND: Daily inhaled corticosteroids (ICSs) are fundamental to asthma management, but adherence is low.

OBJECTIVES: To investigate (1) whether LC-MS/MS could be used to detect ICSs in serum and (2) whether serum levels related to markers of disease severity.

METHODS: We collected blood samples over an 8-hour period from patients with severe asthma prescribed at least 1000 µg daily of beclomethasone dipropionate equivalent. Following baseline sampling, patients were observed taking their usual morning dose. Subsequent blood samples were obtained 1, 2, 4, and 8 hours postinhalation and analyzed by LC-MS/MS. Correlations between serum ICS levels and severity markers were investigated.

RESULTS: A total of 60 patients were recruited (41 females; 39 prescribed maintenance prednisolone; mean age, 49 ± 12 years; FEV₁, 63 ± 20 %predicted). Eight hours postinhalation, all patients using budesonide (n = 10) and beclomethasone dipropionate (15), and all but 1 using fluticasone propionate

(28), had detectable serum drug levels. Fluticasone furoate was detected in 2 patients (of 4), ciclesonide in none (of 7). Low adherence by repeat prescription records (<80%) was identified in 43%. Blood ICS levels correlated negatively with exacerbation rate, and (for fluticasone propionate only) positively with FEV₁ %predicted.

CONCLUSIONS: Commonly used ICSs can be reliably detected in the blood at least 8 hours after dosing, and could therefore be used as a measure of adherence in severe asthma. Higher exacerbation rates and poorer lung function (for fluticasone propionate) were associated with lower blood levels. © 2021 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2021;■:■-■)

Key words: Asthma; Adherence; Inhaled corticosteroids; Disease severity

INTRODUCTION

Inhaled corticosteroids (ICSs) are the cornerstone of treatment in asthma.¹ Poor adherence to ICSs is recognized in 30% to 70% of people with asthma.^{2,3} This is associated with an increased number of exacerbations and hospital admissions, increased morbidity, and reduced lung function.^{4,5} It is therefore important to ensure adherence before patients step up their asthma management, particularly before considering treatment with mAbs.⁶

Although a significant concern in the management of asthma, measuring the level of adherence is challenging,⁷ with no method considered as a criterion standard. Subjective measurements such as self-reporting show that patients tend to overestimate their adherence.⁸ Medicines possession ratio, calculated using prescription refill rate over a specific period, represents an objective method of adherence and is calculated as a percentage of total amount prescribed,^{1,9} with the patient usually considered adherent if the number of collected doses is more than 80%.^{10,11} The most obvious drawback of this method is that even with

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Abbreviations used

AUC- Area under the curve
BDP- Beclomethasone dipropionate
BMI- Body mass index
BUD- Budesonide
CIC- Ciclesonide
DPI- Dry powder inhaler
FF- Fluticasone furoate
FP- Fluticasone propionate
iAUC- Incremental area under the curve
ICS- Inhaled corticosteroid
INCA- Inhaler Compliance Assessment Device
LOD- Limit of detection
PK- Pharmacokinetics

100% prescription collection, there is no way to confirm whether the patient has taken the treatment. Recently, electronic monitoring devices have been developed, including where the patient is provided with feedback (eg, *via* a mobile application).¹² Such systems have been shown to improve adherence and reduce the exacerbation rate in people with poorly controlled asthma but have significant cost implications.¹³

Direct biological measurements of adherence (ie, drug assay) could give complementary information through measurement of drug exposure,¹⁴ although disease-specific data on pharmacokinetics (PK) are required to guide interpretation of results. High-performance LC-MS has been widely used as a method for sensitive and specific drug measurement in clinical laboratories, and has significant advantages over immunoassays.¹⁵ LC-MS has shown high accuracy in detecting the level of urinary fluticasone propionate (FP) in mild to moderate asthma.¹⁶

In this study, we assessed the level of adherence by taking serum blood samples over an 8-hour time period in patients with severe asthma treated with high-dose ICSs by LC-MS/MS. The secondary aim was to identify any correlation between serum ICS concentration and markers of asthma severity. Preliminary results of this study have been reported in abstract form.¹⁷

METHODS

Participants

Consecutive patients under the care of the regional severe asthma service at Wythenshawe Hospital, Manchester University NHS Foundation Trust attending as a day-case or elective admission (eg, for assessment, treatment, and/or investigations) were considered for recruitment. Inclusion criteria were asthma diagnosis confirmed by severe asthma multidisciplinary team and treated with high-dose ICS (1000 µg beclomethasone dipropionate [BDP]/d equivalent minimum) at a stable dose (ie, participants were not using their combination inhalers as both reliever and maintenance therapy) for at least 2 months. Exclusion criteria were patients unwilling or unable to participate in the study. Ethical and R&D approvals were obtained (refs: M2014-23; IRAS107832; R&D study ID: 2005TM013), and patients provided written informed consent. Demographic and clinical data including smoking history, medications, and exacerbation rate were collected from the patient and their clinical notes.

Study design and measurements

The study was conducted during a single visit over approximately 8.5 hours. At baseline (typically between 8 and 9 am), spirometry

and fractional exhaled nitric oxide were recorded, and a venous blood sample collected. Prescription collection records for all participants were collected from their general practitioner. Patients were then observed using their usual morning dose of ICS, and inhaler technique assessed by the researcher using a specific inhaler checklist.¹⁸ Where inhaler technique was deemed inadequate, instruction was given and the participant was asked to take a further inhalation with good technique before further study procedures. Repeat venous blood samples were collected at 1, 2, 4, and 8 hours postinhalation. Blood samples were processed and stored for later analysis for quantification of BDP, budesonide (BUD), ciclesonide (CIC), FP, and fluticasone furoate (FF) by LC-MS/MS. Further details of measurements and analyses are given in this article's Online Repository at www.jaci-inpractice.org.

Sample size calculation and statistical methods

The primary outcome of the study was to estimate the proportion of patients in whom ICSs could be detected (ie, above the limit of detection [LOD]) at 8 hours. Based on our previous study,¹⁹ expecting detectable ICS in 90% (95% CI ± 10%) at 8 hours, we would need a sample size of 62.

Data analyses were performed using SPSS software version 22 (IBM corporation, Chicago, Ill) and Prism version 7 for graphical figures (Graph Pad Software Inc, La Jolla, Calif). Data are expressed as median (Q1; Q3); intergroup comparisons (by ICS type) were made using Kruskal-Wallis tests. Serum concentration for FP, BUD, BDP, CIC, and FF were plotted against time. PK properties (area under the curve [AUC]₀₋₈), maximum concentration, and time to maximum concentration were calculated. The trapezoid test determined the AUC. Correlation (*r*) between continuous data was performed using Spearman test. Partial coefficients were calculated for the relationship between PK (total AUC₀₋₈, incremental AUC₁₋₈, maximum concentration, time to maximum concentration) and clinical parameters (age, sex, body mass index [BMI], exacerbation rate, and FEV₁ %predicted) after adjusting for any potential confounding factors. The significant level (*P*) was set to less than .05 (2-sided).

Using repeated measurements for each patient, mixed linear models were applied to examine the actual changes from baseline serum concentration over time adjusted by baseline measurements for the 3 main ICS inhaler types (FP, BUD, and BDP). All the data models were calculated using STATA V.13, and data are presented as means (95% CI).

RESULTS

Demographic characteristics

We recruited 60 patients with severe asthma (mean age, 49 ± 12 years; 69% females, and 65% patients prescribed maintenance [daily] oral corticosteroids). Poor adherence based on general practitioner prescription refill rates (<80% within 12 months) was identified in 47% (*n* = 28) of the participants. Inhaled FP was most commonly used (43%), whereas 23%, 15%, 11%, and 8% were using BDP, BUD, CIC, and FF, respectively. Full demographic characteristics are presented in Table 1.

ICS detection in blood

At 8 hours, blood ICSs were detected in 96% of participants taking FP, 100% BUD, 100% BDP, 75% FF, and 0% CIC. Because the individual PK are ICS-specific, we present each separately.

TABLE I. Clinical characteristics of study participants

Characteristic	All participants	ICS type					P value (all)	P value* (FP-BUD-BDP)
		FP	BUD	BDP	CIC	FF		
Subjects, n† (%)	60	28 (43)	10 (15)	15 (23)	7 (11)	5 (8)	NA	NA
Sex: female, n (%)	41 (69)	19 (68)	8 (80)	9 (60)	7 (100)	3 (60)	.338	.576
Age (y)	52 (45-58)	50 (36-58)	53 (46-58)	51 (48-58)	53 (45-60)	54 (37-62)	.893	.719
BMI (kg/m ²)	30 (27-36)	33 (28-39)	28 (27-31)	27 (25-29)	29 (27-34)	34 (26-39)	.027	.006
Exacerbations, n in last year	2 (1-6)	3 (1-10)	3 (2-6)	1 (0-2)	1 (0-2)	1 (1-4)	.010	.011
Hospital admissions, n in last year	1 (1-4)	2 (1-4)	1 (1-4)	1 (1-3)	1 (1-4)	1 (1-4)	.456	.347
Daily prednisolone use (mg/d), dose (Q1-Q3) (n)	13 (8-20) (n = 39)	7.5 (0-15) (n = 18)	10 (5-20) (n = 8)	5 (0-15) (n = 9)	10 (5-15) (n = 6)	20 (0-55) (n = 2)	.740	.437
BDP equivalent daily ICS dose (μg), median (IQR)	2000 (1700-2000)	2000 (2000-4000)	2000	1600 (800-1600)	4000	2000	<.001	<.001
Spacer use, n	12	5	0	6	1	0	NA	NA
Poor inhaler technique, n	5	3	2	0	1	0	NA	NA
GP prescription refill %	80 (58-100)	77 (58-89)	80 (54-80)	75 (66-100)	92 (58-100)	92 (66-100)	.686	.750
FEV ₁ , mean ± SD % predicted	60 (50-72)	64 (56-71)	51 (45-83)	51 (40-84)	58 (43-60)	65 (59-79)	.275	.343
FVC, mean ± SD % predicted	72 (59-90)	71 (60-85)	84 (68-107)	66 (53-96)	71 (54-100)	74 (58-84)	.764	.400
FENO (ppb), median (IQR) (n)	24 (11-50) (n = 43)	28 (8-41) (n = 18)	20 (6-45) (n = 4)	45 (14-76) (n = 14)	33 (13-43) (n = 7)	15 (8-28) (n = 5)	.515	.392

FENO, Fractional exhaled nitric oxide; FVC, forced vital capacity; GP, general practitioner; IQR, interquartile range.

Data presented median (Q1-Q3).

*P value for comparison of FP, BUD, and BDP inhalers only.

†Five of 60 patients were taking 2 types of ICSs.

Fluticasone propionate. Blood samples were collected from 27 of 28 individuals prescribed FP (1 provided insufficient samples for analysis; data not shown); 74% ($n = 20$) were taking 1000 μg and 26% ($n = 7$) 2000 μg daily as either Seretide or Flixotide. Eighteen patients were on metered-dose inhaler, 5 with a spacer, and 9 on dry powder inhaler (DPI). At baseline, typically 10 to 12 hours after the previous dose and before witnessed ICS administration, we were able to detect serum FP in 21 participants (78%). Eight hours after witnessed ICS, FP was detectable in all but 1 (96%). Most of the participants (23 of 27) had peak serum concentration 1 to 2 hours after FP administration (see Figure E1, A, in this article's Online Repository at www.jaci-inpractice.org). Inhaled FP detection in serum of individuals over 8 hours is listed in Table E1 in this article's Online Repository at www.jaci-inpractice.org. We examined the level of serum concentration changes from baseline by linear mixed model (Figure 1). The mean change at each time point after FP was above the LOD; however, the lower 95% CIs at 4 and 8 hours post-ICS were below the LOD. Poor inhaler technique was identified in 3 participants, 2 of whom had low serum concentration of FP compared with the other participants (see Figure E2 in this article's Online Repository at www.jaci-inpractice.org). Four patients had less than 50% inhaler pick up, and a further 10 had 50% to 79% pick up; 3 of these 14 (21%) had undetectable baseline FP, *versus* 3 of the 13 (23%) with good ($\geq 80\%$ pick up) adherence.

Budesonide. Nine individuals used DPI Symbicort (400/12) and 1 DPI DuoResp Spiromax (320/9). Seven (70%) had detectable BUD at baseline (premorning ICS dosing). Serum BUD was detected in all patients 8 hours post-ICS dosing. The maximum concentration of serum BUD occurred 1 hour after inhalation (Figure E1, B). From the mixed linear model, the lower 95% confidence limit was above the LOD at all time points (Figure 1). Detection rates at each time point are presented in Table E2 in this article's Online Repository at www.jaci-inpractice.org. Two participants had poor inhaler technique with BUD (Figure E2). A good ($\geq 80\%$) prescription refill rate was observed in 6 participants, 1 of whom had undetectable baseline level. Two of the 4 subjects with low adherence ($\leq 80\%$) had undetectable level at baseline.

Beclomethasone dipropionate. Most participants in the BDP group (11 of 15) used metered-dose inhaler Fostair, 3 DPI Fostair NEXThaler, and 1 Clenil. Serum ICS was not detected in 1 patient at baseline. Serum BDP was detectable in all participants 8 hours after using the inhaler. The highest concentration among most of the participants took place after 1 hour of using the medication (Figure E1, C); serum detection of individuals over the 8-hour period is presented in Table E3 in this article's Online Repository at www.jaci-inpractice.org. Results of mixed linear modeling indicated that the lower 95% CI was above the LOD (Figure 1). All participants taking BDP had acceptable inhaler technique. Satisfactory prescription refill rate ($\geq 80\%$) was recorded in 46% ($n = 7$). Undetectable baseline levels were noted in 1 patient (considered as adherent based on a prescription refill).

Ciclesonide. Seven patients used metered-dose inhaler Alvesco at a dose of 80 or 160 μg . Three patients used it along with another ICS (FP, BUD, or BDP). We were not able to detect serum CIC at 8 hours post-ICS in any participant; 1

patient had a detectable level at baseline (see Table E4 in this article's Online Repository at www.jaci-inpractice.org). Good inhaler technique was observed in all the patients using CIC. Four of the 7 patients were collecting 50% to 79% of their ICS prescriptions, and the rest collecting more than 80%.

Fluticasone furoate. Blood samples were collected from 4 of 5 individuals prescribed FF (1 provided insufficient samples for analysis; data not shown), prescribed in all as 1 puff daily of DPI Relvar (184/22). FF was not detected at baseline in any samples, and in 2 patients after 8 hours (see Table E5 in this article's Online Repository at www.jaci-inpractice.org). All 4 patients had good inhaler technique; 2 collected less than 80% of prescriptions.

Because of low numbers and low levels of detection, CIC and FF were not included in further analyses.

PK and adherence

We compared PK (maximum concentration, AUC, baseline concentration) between participants with a suboptimal rate of prescription refill and participants filling above 80% of prescription refill in the past 6 or 12 months. There were no differences between these 2 groups in any PK variable for each individual inhaler (see Table E6 in this article's Online Repository at www.jaci-inpractice.org).

Serum PK and markers of asthma severity

We investigated relationships between serum PK (baseline concentration, AUC for FP, BUD, and BDP both together [$n = 52$] and individually) and markers of asthma severity. Exacerbation rate, ICS dose, and BMI were associated with PK parameters in the bivariate correlation analyses, and so these variables were also included in the partial correlation analyses to explore whether any were independently associated with the PK parameters of interest.

Baseline concentration. Higher ICS baseline concentration correlated with lower exacerbation frequency, with lower BMI and ICS dose (Table II). The significant correlation with exacerbation frequency held after adjusting for BMI and ICS dose. For FP, there was a positive correlation ($r = 0.384$; $P = .048$) between serum concentration at baseline and FEV₁ % predicted.

Area under the curve. The AUC for BDP and BUD was considerably higher than for FP, and because of the strong association between the AUC and baseline levels for each inhaler, we used incremental AUC (iAUC). This was calculated by subtracting the area under the baseline level. Higher iAUC correlated with lower BMI (see Figure E3 in this article's Online Repository at www.jaci-inpractice.org), exacerbation rate (Figure 2), and ICS dose (Table III). The FEV₁ % predicted was associated with iAUC concentration for FP ($r = 0.401$; $P = .038$) (Figure 3). No significant correlations of iAUC for BUD and BDP against asthma markers were identified as presented in Table III.

DISCUSSION

Although indirect methods, such as prescription refill or self-report diaries, have commonly been used to identify non-adherence to ICSs in asthma, the only direct and therefore reliable way of determining ICS exposure is through detection in biological samples. In this observational study, we have examined

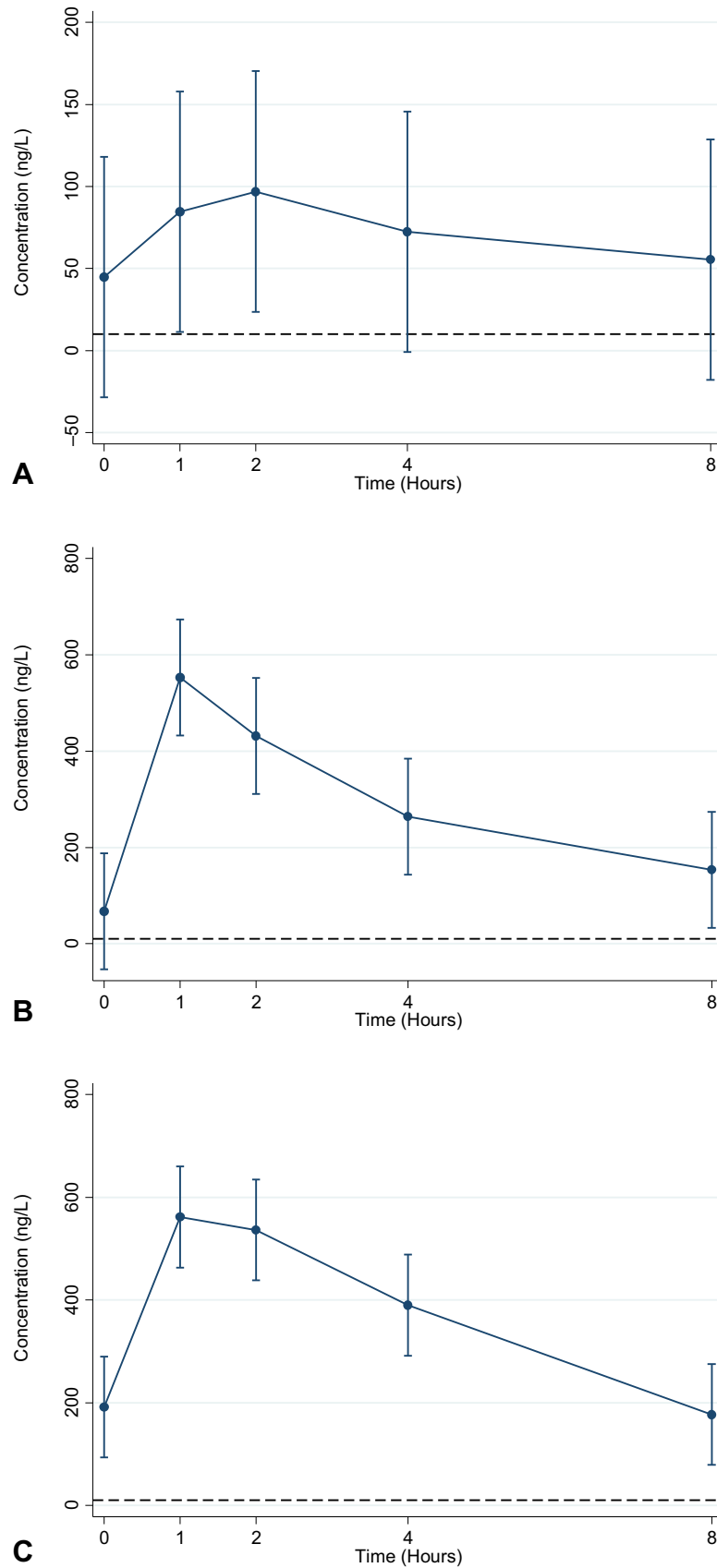


FIGURE 1. Linear mixed model predictions for mean (95% CIs) serum concentration of (A) FP, (B) BUD, and (C) BDP. Dashed line indicates LOD.

TABLE II. Correlation coefficients (*P* value) of C_{base} of participants using FP, BUD, and BDP inhalers and markers of asthma

	All		FP	BUD	BDP
	Unadjusted	Adjusted*			
Age	0.188 (<i>P</i> = .181)		0.436 (<i>P</i> = .023)	0.405 (<i>P</i> = .246)	−0.400 (<i>P</i> = .139)
BMI	−0.379 (<i>P</i> = .006)	Exacerbation −0.090 (<i>P</i> = .532) ICS dose −0.185 (<i>P</i> = .198)	−0.378 (<i>P</i> = .052)	−0.635 (<i>P</i> = .066)	0.261 (<i>P</i> = .348)
Exacerbation	−0.298 (<i>P</i> = .032)	BMI −0.308 (<i>P</i> = .029) ICS dose −0.323 (<i>P</i> = .021)	−0.249 (<i>P</i> = .210)	−0.006 (<i>P</i> = .986)	0.220 (<i>P</i> = .431)
FEV ₁	0.000 (<i>P</i> = .999)		0.384 (<i>P</i> = .048)	−0.305 (<i>P</i> = .391)	0.123 (<i>P</i> = .704)
Prednisolone dose	−0.069 (<i>P</i> = .625)		0.006 (<i>P</i> = .974)	−0.408 (<i>P</i> = .242)	0.411 (<i>P</i> = .128)
Total daily ICS dose†	−0.397 (<i>P</i> = .004)	BMI −0.312 (<i>P</i> = .027) Exacerbation −0.269 (<i>P</i> = .056)	−0.093 (<i>P</i> = .645)	NA	0.164 (<i>P</i> = .560)

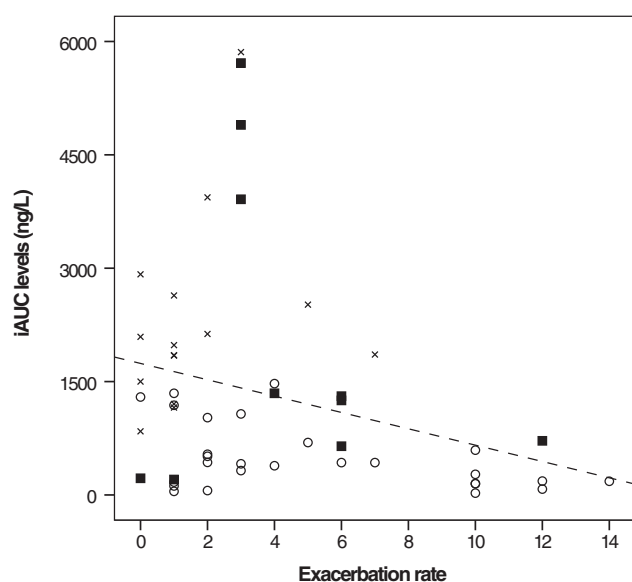
C_{base} , Baseline concentration; NA, all patients having the same dose.

Example to guide interpretation: Higher BMI correlated with lower C_{base} , but this did not remain significant after adjusting for exacerbation rate or daily ICS dose; however, higher exacerbation rate correlated with lower C_{base} , and this remained significant after adjusting for BMI and ICS dose.

Bold indicates significant (*P* < .05) correlations.

*Partial correlation controlled by variables as shown.

†Dose converted to BDP equivalent.

**FIGURE 2.** Scatter plot showing serum iAUC for FP (open circle), BUD (solid square), or BDP (x) against exacerbation rate. The dashed line represents the line of correlation ($r = -0.297$; $P = .032$).

the feasibility of using LC-MS/MS to detect ICS in serum after direct observation of drug administration. Up to 8 hours after inhalation, we were able to detect ICS in the serum of all patients using BUD and BDP, and all but 1 using FP. In contrast, we could not reliably detect CIC or FF in samples, although the number of participants taking these ICSs was low. The lower 95% CIs for predicated levels of BUD and BDP were above the LOD at 8 hours. The implication is that the test will have clinical utility in a typical daytime outpatient clinic. A positive result would be interpreted as confirming short-term adherence; a negative result would mean (for patients prescribed FP, BUD, or BDP) that there was a lower than 1 in 20 chance that they had taken their ICS that morning. This information could then be used to guide a personalized discussion and education about inhaler technique and adherence.

Reliable detection of ICS in the serum may have significant advantages over other methods of detecting adherence. Electronic monitoring devices such as the Inhaler Compliance Assessment Device (INCA) are gaining popularity in assessing adherence and currently considered the most accurate and reliable method.²⁰ The INCA, unlike most other devices, is designed to detect inhalation as well as actuation, allowing an estimation to be made of whether each dose has been taken. However, the INCA relies on an acoustic sensor and automated detection software and could therefore be prone to false positives or negatives, where either an environmental sound has been picked up or an inhalation sound missed. Furthermore, the INCA device is available only for the DPI Diskus,²¹ and there are many different inhaler devices on the market for ICSs that cannot currently be monitored.²²

The most commonly used indirect assessments of adherence are prescription refill counting and self-assessment. The former is useful only if the source of all prescriptions is traceable, and even then will be prone to overestimate adherence because no measure is made of actual dosing.¹⁴ Self-assessment is also known to overestimate adherence, although the responses to some questionnaires are able to give insights into possible reasons behind nonadherence in an individual and could therefore potentially guide intervention.²³⁻²⁶

Unsurprisingly, there was marked variability in serum PK between ICS types and between patients, although the mean serum concentrations of FP, BUD, and BDP were similar to those previously reported.²⁷⁻³⁰ The main sources of variation in PK relevant to this study are likely to be deposition (lung *vs* oropharyngeal), oral bioavailability (the fraction of a drug that is not completely metabolized on first pass through the hepatic circulation), lipophilicity (and hence lung residency time), and volume of distribution.³¹ The higher detection rates for BUD and BDP that we have reported are likely a consequence of the interaction between a number of these factors. First, the oral bioavailabilities of FP, CIC, and FF are similarly low (<1%), whereas those for BUD and BDP are much higher (11% and ~15%, respectively),^{31,32} meaning more drug can enter the circulation unchanged via this route. Second, FF and FP have higher lipophilicity and longer lung residence time than BUD

TABLE III. Correlation coefficients (*P* value) of iAUC of participants using FP, BUD, and BDP inhalers and markers of asthma

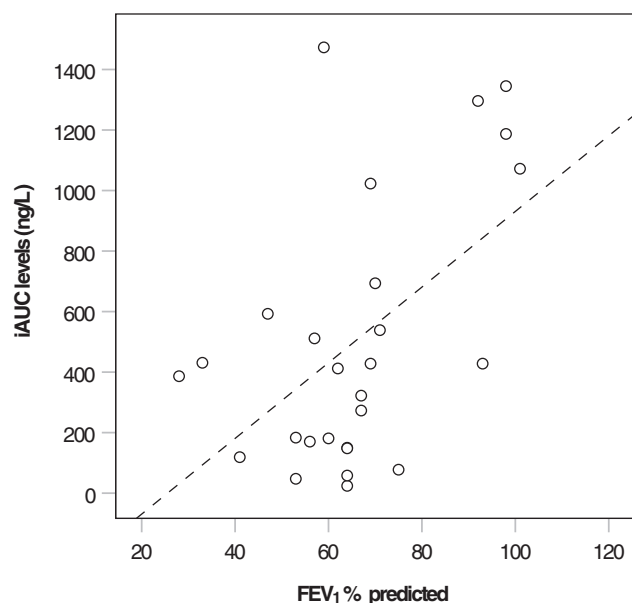
	All		FP	BUD	BDP
	Unadjusted	Adjusted*			
Age	0.191 (<i>P</i> = .174)		0.352 (<i>P</i> = .072)	0.456 (<i>P</i> = .185)	−0.307 (<i>P</i> = .266)
BMI	−0.488 (<i>P</i> < .001)	Exacerbation −0.217 (<i>P</i> = .345) ICS dose −0.308 (<i>P</i> = .029)	−0.320 (<i>P</i> = .353)	−0.613 (<i>P</i> = .079)	−0.270 (<i>P</i> = .331)
Exacerbation	−0.297 (<i>P</i> = .032)	BMI −0.232 (<i>P</i> = .105) ICS dose −0.257 (<i>P</i> = .069)	−0.320 (<i>P</i> = .104)	0.043 (<i>P</i> = .905)	0.401 (<i>P</i> = .139)
FEV ₁	0.078 (<i>P</i> = .593)		0.401 (<i>P</i> = .038)	−0.359 (<i>P</i> = .309)	−0.385 (<i>P</i> = .216)
Prednisolone dose	−0.103 (<i>P</i> = .469)		0.018 (<i>P</i> = .930)	−0.502 (<i>P</i> = .140)	0.006 (<i>P</i> = .984)
Total daily ICS dose†	−0.644 (<i>P</i> = .001)	BMI −0.422 (<i>P</i> = .002) Exacerbation −0.377 (<i>P</i> = .006)	−0.206 (<i>P</i> = .302)	NA	0.279 (<i>P</i> = .314)

NA, All patients having the same dose.

Bold indicates significant (*P* < .05) correlations.

*Partial correlation controlled by variables as shown.

†Dose converted to BDP equivalent.

**FIGURE 3.** Scatter plot showing serum iAUC against % predicted FEV₁ % for FP. The dashed line represents the line of correlation (*r* = 0.401; *P* = .038).

and BDP,^{32,33} and will therefore have lower systemic bioavailability, at least during acute dosing. These PK factors are the likely explanation for the counterintuitive finding that (when including all 3 ICSs) total daily ICS dose was inversely correlated with serum levels; our participants on FP had the highest daily dose (interquartile range, 2000–4000 µg BDP equivalent) but the lowest serum levels of drug. We did not measure any surrogate for corticosteroid toxicity, such as serum or urinary cortisol, because more than half of our patients were prescribed maintenance systemic corticosteroids, which would likely have masked any suppression attributable to ICSs.

We did not find any significant difference in PK between patients based on inhaler type (DPI or metered-dose inhaler) or spacer use. It may be that these make little absolute contribution to total systemic absorption in the context of the other PK factors, such that no difference was found in our relatively small

sample size. Although we assessed inhaler technique, a poor technique was seen in so few patients that it is difficult to draw any conclusions. It is likely that a poor technique that results in more mouth deposition would result in particularly low blood levels for FP or FP, which have almost 100% first-pass hepatic metabolism.

We found an inverse relationship between AUC concentration and annual exacerbation rate, both in the group overall (ie, including BDP, BUD, and FP) and in those taking FP alone. It is likely that the relationship with FP was dominant in the overall analysis, because in fact there was a positive relationship (albeit nonsignificant) for BUD and BDP when analyzed individually. We also noted a positive correlation between FEV₁ and serum FP, and again the opposite (nonsignificant) relationship for BUD and BDP. This may be because, as noted above, serum FP is more reflective of lung deposition and absorption than are BUD and BDP. Two possible explanations could contribute to this relationship between higher blood FP, fewer exacerbations, and better FEV₁: (1) the higher systemic ICS exposure leads to better outcomes or (2) patients with less airflow obstruction and less damaged airway epithelium absorb more drug. The first explanation may be less likely, because the relationship did not hold for BUD and BDP, which had much higher serum levels. The second is supported by previous studies that have also demonstrated a correlation between serum FP (but not BUD) levels and FEV₁,²⁸ and shown lower levels in asthma *versus* healthy controls.³⁰

Therapeutic drug monitoring is already in clinical use in many areas including epilepsy, cardiovascular disease, and infection, usually performed as in the present study by methods based on LC-MS. Although detecting drugs in this way requires time and expertise, and is potentially costly, it is still likely valuable in patients with severe disease, especially those being considered for expensive and/or potentially toxic treatments. Indeed, monitoring of oral corticosteroids (prednisolone) is already used (and recommended by the Global INitiative for Asthma⁶) as an adherence measure in severe asthma, even without any supporting data available such that we here provide for ICSs.^{34,35}

There were a number of limitations to our study. We did not achieve the target sample size of 62, because the key researcher (F.A.) involved in patient recruitment and sampling moved to a new post, and were unable to collect samples in all participants at

8 hours (samples missing in 3 participants taking FP, 5 taking BDP), potentially reducing the precision of our estimated detection rate. We were unable to recruit many patients taking CIC and FF, due to their infrequent use in the United Kingdom. In addition, we did not measure the active metabolite of CIC, desisobutyryl-CIC.^{36,37} CIC has a relatively short plasma half-life,³¹ and hence we were unable to detect it in any samples beyond 2 hours postdosing, in line with previous findings.³⁸ We were able to detect FF at later time points but not reliably, probably due to its PK properties as previously mentioned, and the relatively low total daily dose of 184 µg. The exploratory analysis investigating associations between serum ICS concentration and markers of asthma severity has generated interesting results with potential clinical implications, but first need to be replicated in a prospective study powered to control for multiple possible confounders. Finally, as noted above, a positive result confirms only short-term adherence; inference about longer term adherence would require additional information, obtained, for example, from counting prescription refills.

CONCLUSIONS

We have shown that commonly used ICSs (BUD, BDP, and potentially FP) can be reliably detected in the blood at least 8 hours after dosing and could therefore have a potential clinical application as a direct measure of adherence in severe asthma. Higher exacerbation rates and poor lung function were found in those with lower blood levels of FP, likely due to lower lung absorption in such patients. Future work should assess the ability of “random” spot-tests to detect adherence in real-world practice, compare to ICS detection in other samples such as urine, increase the sensitivity of the assay for drugs prescribed at lower doses, and include measurement of active metabolites such as desisobutyryl-CIC.

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ONLINE REPOSITORY

MEASUREMENTS

Exhaled nitric oxide

A Niox device (Aerocrine, Sweden) was used to measure fractional exhaled nitric oxide at a constant flow rate of 50 mL/s. Patients were asked to inhale deeply via a mouthpiece connected to a screen and exhale for 10 seconds, with visual feedback provided to guide exhalation flow, in compliance with the American Thoracic Society/European Respiratory Society recommendations.^{E1}

Spirometry

Spirometry was performed using a handheld digital spirometer (In2itive, Vitalograph, UK), which allows measurement of FEV₁ and forced vital capacity, stores data on the digital device, and is synchronized to Vitalograph Spirotrac software. Digital spirometers were calibrated on every study day using a 3-L syringe to within $\pm 3\%$.

ADHERENCE

Prescription refill records for all participants were collected from their general practitioner. It was calculated as the number of taken prescribed doses of medication divided by the actual number of doses prescribed and multiplied by 100. The optimal adherence rate was defined as the refill of more than 80% for the previous 12 months minimum.^{E2,E3}

Venous blood sampling and storage

Venous blood was drawn *via* a size 22G cannula, into a 5 mL EDTA tube, and then after 30 to 60 minutes centrifuged (centrifuge 5702; Eppendorf, Darmstadt, Germany) for 10 minutes at a speed of 3500 rpm at 20°C. Extracted serum was transferred into 1.5-mL cryotube vials and stored in -80°C freezer before analysis.

ICS analysis by LC-MS/MS

Sample preparation. Steroid stock solutions (Alsachim, Illkirch-Grafenstaden, France) of 1 mg/mL were prepared by dissolving BDP, BUD, and CIC in ultrapure methanol, and FP and FF in dimethylsulfoxide (Fluka, Poole, UK), and stored at -20°C for up to 6 months. Separate stock solutions were prepared for standards and quality controls (QCs). Working standards and weighed-in QCs were prepared by diluting the stock solution with PBS, pH 7.4 (Sigma, Poole, UK), containing 0.1% (wt/vol) BSA (Sigma) to give concentrations of 0 to 10,000 ng/L. Aliquots (300 μL) of these were stored at -30°C for up to 6 months. In addition, serum QCs (12.5, 50, 200, 800 ng/L) were used to assess imprecision in matrixed samples. Deuterated steroids for use as internal standards (Alsachim, Illkirch-Grafenstaden, France) were prepared at a working concentration of 10 $\mu\text{g/L}$ each in ultrapure methanol.

Standard, QC or sample (250 μL), 20 μL of working internal standard and 100 μL deionized water were added to a supported liquid extraction plate (SLE+ Biotage, Uppsala, Sweden). After elution with dichloromethane into a deep well plate, the solvent was evaporated to dryness and the extract was reconstituted with 75 μL of 70% ultrapure methanol/water. The plate was heat-sealed and transferred directly to the Acquity autosampler (Waters, Manchester, UK) for analysis; 30 μL of sample was injected.

Chromatography and solid-phase extraction. The mobile phases used were (A) distilled water with 2 mmol/L ammonium acetate containing 0.1% formic acid (Sigma) and (B) methanol (ultrapure grade, Fluka). The analytical column used was a 2.1×50 mm $1.8 \mu\text{m}$ Waters Acquity UPLC HSS C18 SB column coupled to a Waters Vanguard filter. Steroids were eluted from the analytical column with a linear gradient starting from 50% mobile phase B at a flow rate of 0.4 mL/min, rising to 90% over 2.5 minutes. After 2.5 minutes, the composition was stepped up to 100% B for 0.6 minutes before returning to starting conditions. Both guard and analytical columns were maintained at 50°C . Run time injection to injection was 4.3 minutes.

Mass spectrometry

The eluate was injected directly into a XEVO TQ-XS tandem mass spectrometer (Waters, Manchester, UK), using MassLynx NT 4.1 software (Waters, Manchester, UK) for system control. The mass spectrometer was operated in electrospray positive mode, the capillary was maintained at 1.0 kV, and the source temperature was 150°C . The desolvation temperature and gas flow were 600°C and 1000 L/h, respectively. The source offset was maintained at 50 V. The quantifier transitions identified were m/z 431.2 > 323.3, m/z 465.2 > 279.2, m/z 501.2 > 313.2, m/z 539.2 > 313.2, and m/z 541.3 > 147.1 for BUD, BDP, FP, FF, and CIC, respectively. The internal standards had transitions of m/z 439.2 > 323.1, m/z 470.3 > 279.2, m/z 506.2 > 313.2, m/z 543.2 > 293.2, and m/z 548.2 > 323.2 for D8 BUD, D5 BDP, D5 FP, D5 FF, and D7 CIC, respectively. Transitions were monitored in multiple reaction monitoring mode, with a dwell time of 0.025 seconds.

Elution times were as follows, BUD 2.2 minutes, BDP and FP 2.4 minutes, FF 2.6 minutes, and CIC 3.05 minutes. Matrix effects were minimal, and recoveries were 93%, 113%, 97%, 102%, and 103% for BUD, BDP, FF, FP, and CIC, respectively. LOD, and lower limit of quantitation, was 10 ng/L for all steroids except for CIC, for which it was 50 ng/L. All steroids were linear to at least 10,000 ng/L. Between-batch imprecision showed CV less than 8% for all steroids except CIC at a concentration of 50 ng/L; CIC gave a CV of 6.6% at 200 ng/L.

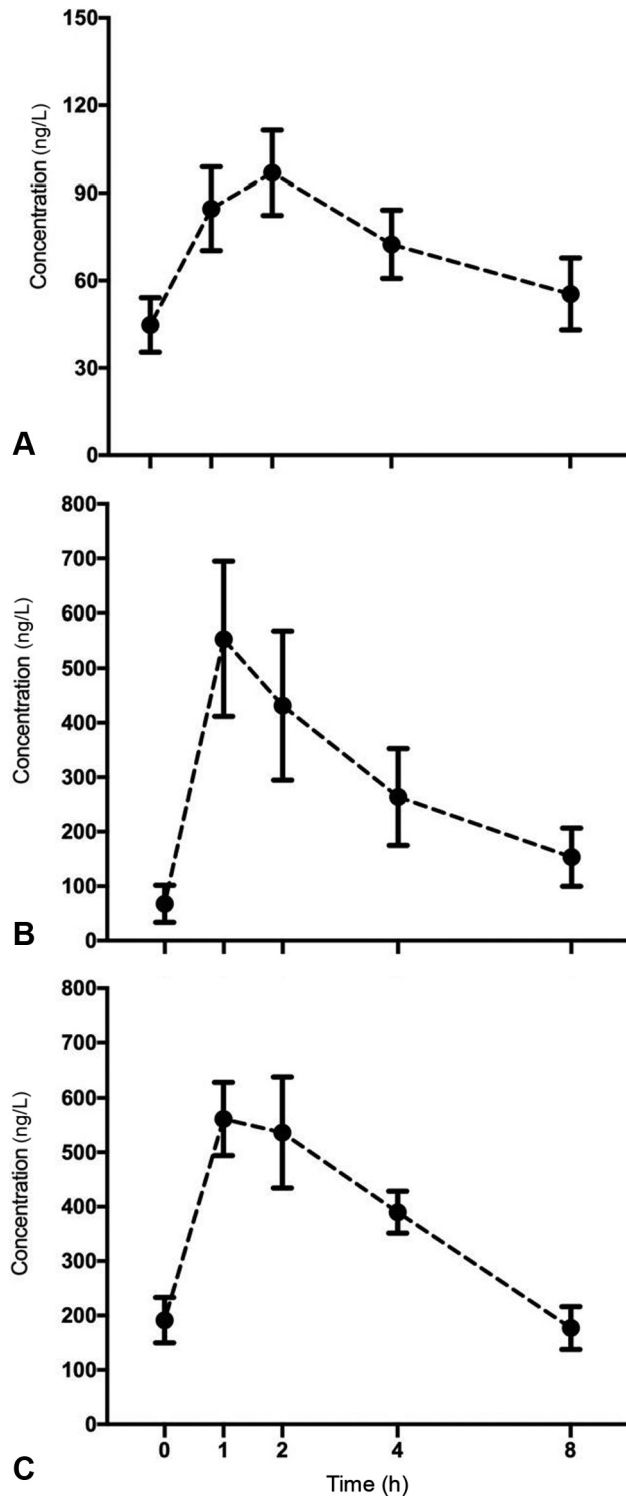


FIGURE E1. Mean (SE) serum concentration-time graph of (A) FP, (B) BUD, and (C) BDP in subjects with severe asthma.

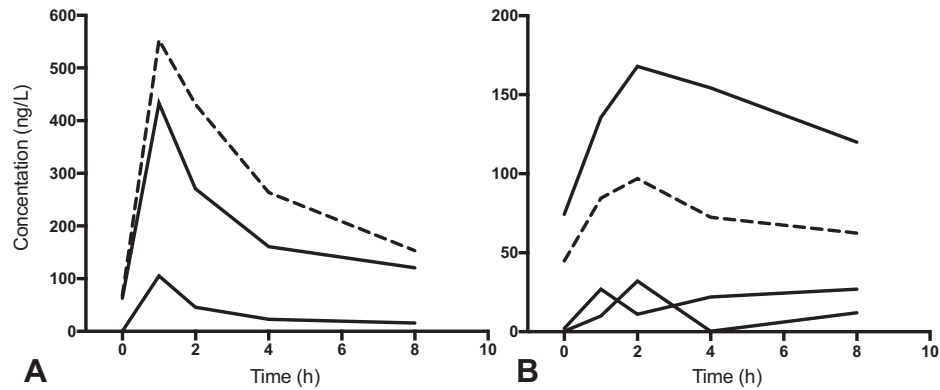


FIGURE E2. Serum concentration-time graph for patients with poor inhaler technique: (A) FP and (B) BUD. Solid lines represent the ICS concentration of individual patients with poor technique and dashed lines the mean concentration for all patients.

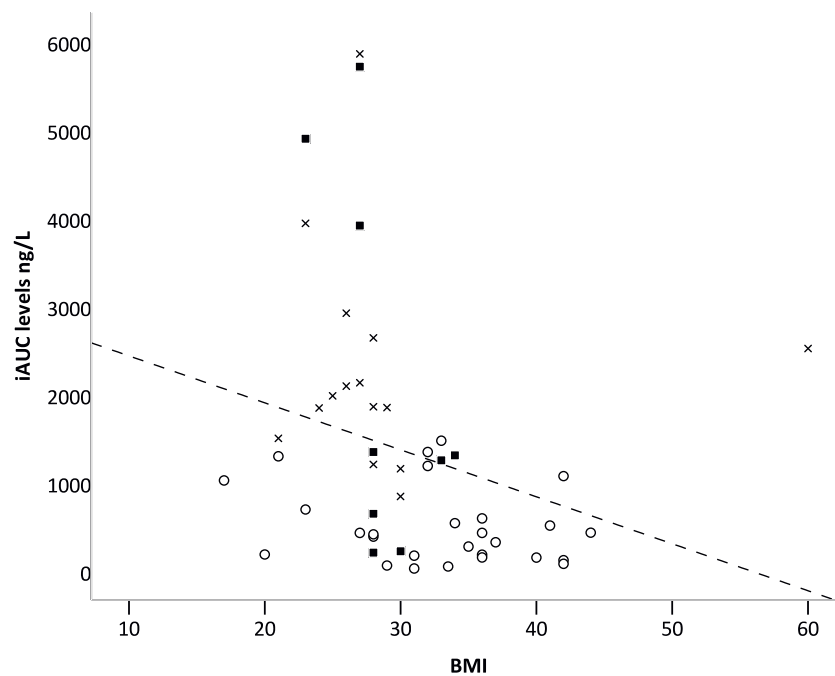


FIGURE E3. Scatter plots showing the iAUC against BMI for FP (open circle), BUD (closed black square), or BDP (X). Dashed line represents line of correlation, $r = -0.488$ ($P < .001$).

TABLE E1. FP group: Detection over time, adherence level, and inhaler technique (n = 27)

FP dose	Age (y)	Sex	PR %	Technique	Times (h)				
					0	1	2	4	8
250 µg	59	M	+	+	+	+	+	+	+
	45	M	+	+	+	+	+	+	+
	53	F	±	+	+	+	+	+	NA
	22	F	±	+	—	+	+	+	+
	60	F	+	+	+	+	+	+	+
	64	F	±	+	+	+	+	+	+
	30	F	±	+	—	+	+	+	+
	64	F	±	+	+	+	+	+	+
	50	F	+	+	+	—	+	+	NA
	24	F	—	+	+	+	+	+	+
	46	F	±	+	+	+	+	+	+
	54	M	+	+	+	+	+	+	+
	22	F	+	+	—	+	+	+	+
	53	M	—	+	+	+	+	+	+
	49	M	+	+	+	+	+	+	+
500 µg	54	M	±	+	+	+	+	+	+
	69	F	±	—	+	+	+	+	+
	60	F	±	+	+	+	+	+	+
	29	F	—	+	+	+	+	+	NA
	35	F	+	+	—	+	+	—	—
	30	F	±	+	—	+	—	—	+
	45	F	—	+	+	+	+	+	+
	64	M	+	+	+	+	+	+	+
	50	M	+	—	+	+	+	+	+
	52	F	+	—	—	+	+	—	+
	44	M	+	+	+	+	+	+	+
	58	F	+	+	+	+	+	+	+
% patients with detectable ICS					77%	96%	96%	89%	96%

F, Female; LOQ, limit of quantitation; M, male; NA, missing; (+), detected ICS; (—), undetected ICS; inhaler technique: (+) good; (—) poor; prescription refill (PR) %: (—) <50%; (±) 50%-79%; (+) >80%.

In 2 participants, FP was detected at 8 h but not at 4 h; possible explanations include the possibility of an extra undeclared dose being taken between the 2 sample collection points, or the concentration falling just below the LOQ at 4 h but being just detectable at 8 h (the respective concentrations at 8 h were 10 ng/L and 12 ng/L; the LOQ was 10 ng/L).

TABLE E2. BUD group: Detection over time, adherence level, and inhaler technique (n = 10)

BUD dose	Age (y)	Sex	PR %	Technique	Times (h)				
					0	1	2	4	8
400 µg	46	F	—	+	—	+	+	+	+
	57	F	+	—	—	+	+	+	+
	47	F	±	+	—	+	+	+	+
	42	F	+	+	+	+	+	+	+
	58	F	+	+	+	+	+	+	+
	63	M	—	+	+	+	+	+	+
	54	M	+	+	+	+	+	+	+
	58	F	+	+	+	+	+	+	+
	52	F	+	+	+	+	+	+	+
320 µg	50	F	±	+	+	+	+	+	+
% patients with detectable ICS					70%	100%	100%	100%	100%

F, Female; M, male; (+), detected ICS; (—), undetected ICS; inhaler technique: (+) good; (—) poor; prescription refill (PR) %: (—) <50%; (±) 50%-79%; (+) >80%.

TABLE E3. BDP group: Detection over time, adherence level, and inhaler technique (n = 15)

BDP dose	Age (y)	Sex	PR %	Technique	Times (h)				
					0	1	2	4	8
100 µg	33	M	+	+	+	+	+	+	+
	52	M	+	+	—	+	+	+	+
	48	F	±	+	+	+	+	+	+
	52	F	±	+	+	+	+	+	NA
	54	M	—	+	+	+	+	+	NA
200 µg	18	F	±	+	+	+	+	+	+
	30	F	—	+	+	+	+	+	+
	48	F	+	+	+	+	+	+	+
	51	F	±	+	+	+	+	+	NA
	58	M	+	+	+	+	+	+	NA
	48	F	±	+	+	+	+	+	+
	60	F	+	+	+	+	+	+	+
	54	F	—	+	+	+	+	+	+
	63	M	+	+	+	+	+	+	NA
400 µg	60	F	+	+	+	+	+	+	+
% patients with detectable ICS					93%	100%	100%	100%	100%

F, Female; M, male; NA, missing; (+), detected ICS; (—), undetected ICS; inhaler technique: (+) good; (—) poor; prescription refill (PR) %: (—) <50%; (±) 50%-79%; (+) >80%.

TABLE E4. CIC group: Detection over time, adherence level, and inhaler technique (n = 7)

CIC dose	Age (y)	Sex	PR %	Technique	Times (h)				
					0	1	2	4	8
160 µg	45	F	+	+	—	—	—	—	—
	57	F	+	+	—	+	+	—	—
	50	F	±	+	+	—	—	—	—
	53	F	±	+	—	—	—	—	—
	60	F	+	+	—	+	—	—	—
	35	F	±	+	—	+	—	—	—
80 mg	60	F	±	+	—	—	+	—	—
% patients with detectable ICS					14%	43%	28%	0%	0%

F, Female; (+), detected ICS; (—), undetected ICS; inhaler technique: (+) good; (—) poor; prescription refill (PR) %: (—) <50%; (±) 50%-79%; (+) >80%.

TABLE E5. FF group: Detection over time, adherence level, and inhaler technique (n = 4)

FF dose	Age (y)	Sex	PR %	Technique	Times (h)				
					0	1	2	4	8
184 µg	54	F	+	+	—	+	—	+	—
	45	F	+	+	—	—	+	+	+
	57	M	±	+	—	+	+	+	+
	29	F	±	+	—	+	+	—	—
% patients with detectable ICS					0%	75%	75%	75%	50%

F, Female; M, male; NA, missing; (+), detected ICS; (—), undetected ICS; inhaler technique: (+) good; (—) poor; prescription refill (PR) %: (—) <50%; (±) 50%-79%; (+) >80%.

TABLE E6. PK comparison between participants with a high and low rate of PR (FP, BDP, BUD inhalers)

	All (n = 52)			FP (n = 27)			BUD (n = 10)			BDP (n = 15)		
	PR ≥80% (n = 26)	PR <80% (n = 26)	P value	PR ≥80% (n = 13)	PR <80% (n = 14)	P value	PR ≥80% (n = 6)	PR <80% (n = 4)	P value	PR ≥80% (n = 7)	PR <80% (n = 8)	P value
PR, %	92 (80-100)	58 (33-75)	<.001	88 (80-100)	58 (38-66)	<.001	80 (80-83)	51 (36-70)	.010	100 (92-100)	70 (36-75)	<.001
C _{base} (ng/L)	37 (7-125)	42 (13-158)	.713	30 (1-53)	28 (12-101)	.488	55 (6-194)	5 (0-49)	.257	203 (22-260)	214 (37-401)	.536
AUC (ng/L)	833 (256-2474)	1202 (427-1668)	.784	285 (151-642)	517 (200-1326)	.280	2955 (723-5802)	1130 (384-1569)	.357	2462 (2210-3365)	2025 (1538-2989)	.281
C _{max} (ng/L)	202 (65-684)	273 (85-420)	.621	66 (35-144)	122 (47-196)	.350	693 (256-1211)	305 (138-419)	.257	655 (497-881)	482 (404-724)	.281

AUC, Area under the curve; C_{base}, baseline concentration; C_{max}, maximum concentration; PR, prescription refill.
Data presented as median (interquartile range).

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