

Letter to the Editor

A pilot study to investigate the use of serum inhaled corticosteroid concentration as a potential marker of treatment adherence in severe asthma

To the Editor:

Inhaled corticosteroids (ICSs) are the mainstay of asthma management. Despite this, adherence is known to be very poor.¹ Measuring adherence to ICSs presents its own challenges and currently there is no way to directly measure it. Good adherence to ICSs is associated with improved outcomes and can reduce the need for treatment to be escalated and therefore potential exposure to unnecessary and harmful side effects.² The primary aim of this pilot study was to determine whether ICSs could be directly measured in serum using LC-MS/MS and whether this could be used as a marker of adherence. A secondary aim was to see whether serum concentration levels correlated with markers of asthma severity.

We approached inpatients with severe asthma treated with at least 1000 µg beclomethasone dipropionate equivalent for inclusion in this study. The patients were taking a stable dose of either inhaled fluticasone propionate (FP) or budesonide (BUD) and were willing to have multiple blood samples taken over an 8-hour period. The only exclusion criterion was if they were unable to understand the patient information sheet. Ethical approval for the study was authorized through the Manchester Respiratory and Allergy Biobank (ManRAB) (REC reference 10/H1010/7) and all participants gave written informed consent.

Five blood samples were collected from each participant via a peripheral venous cannula over an 8-hour period. One baseline sample was taken (between 8 and 10 am) before they were directly observed taking their inhaler; then, further samples were taken 1, 2, 4, and 8 hours postinhalation. Inhaler technique was assessed by the researcher using an inhaler checklist. In addition to the blood sample, the following data were recorded for each patient: patients' demographic characteristics, comorbidities, medications, annual exacerbations, annual hospital admissions, and FEV₁% predicted.

Within 1 hour following collection, samples were centrifuged for 10 minutes at 3500 rpm at 20°C. Serum was then transferred into 1.5-mL cryotube vials. The cryotubes were labeled with a unique ManRAB ID number and time and date stamped. The samples were stored in a -80°C freezer until analysis. Samples were analyzed by LC-MS/MS. Full details are given in this article's [Online Repository](http://www.jacionline.org) at www.jacionline.org.

Nineteen patients were recruited, 13 on FP and 6 on BUD inhalers. The mean (range) age of the participants was 50 (22-64) years; 12 were women. The mean FEV₁ was 70 ± 19.2% predicted; mean (range) BMI was 31 (20-42) kg/m². Nine patients were taking maintenance prednisolone at the time of sampling, with mean (range) dose 20 (10-60) mg daily. All patients were taking the ICS in the form of a combination inhaler. For FP, there were 6 using a dry powder inhaler (DPI) and 7 using a metered dose inhaler. All the patients on BUD were taking a DPI. Of the patients on FP, 10 patients were taking 500 µg twice a day and 3 patients were taking 1000 µg twice a day. In the BUD cohort, 5 were taking 800 µg twice a day, with 1 patient taking 400 µg

TABLE I. Number (%) of patients with detectable serum ICS levels (excluding 2 participants with poor technique) at each time point for FP and BUD

Inhaled steroid	No. (%) of participants with detectable serum ICS levels				
	Baseline	1 h	2 h	4 h	8 h
FP (n = 11)	6 (55%)	10 (91%)	10 (91%)	9 (82%)	8 (73%)
BUD (n = 6)	3 (50%)	6 (100%)	6 (100%)	6 (100%)	6 (100%)
Total (n = 17)	9 (53%)	16 (94%)	16 (94%)	15 (88%)	14 (82%)

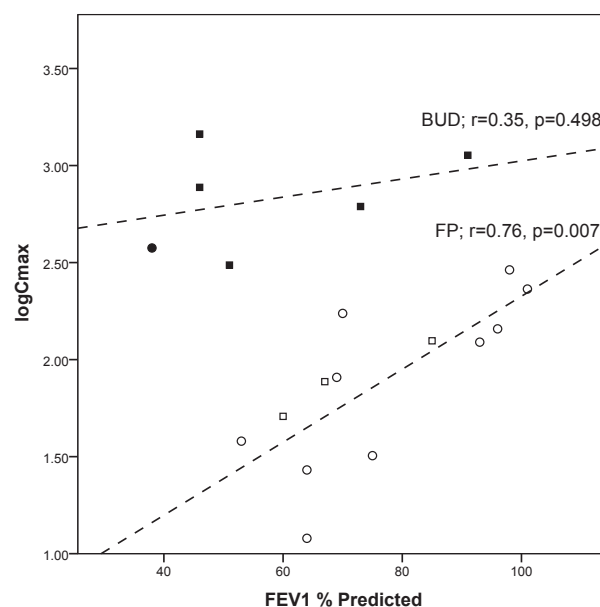


FIG 1. Scatter plot showing FEV₁% predicted versus maximum serum concentration of drug along with correlation line of best fit for BUD (solid symbols, top) and FP (open symbols, bottom). Circles represent medium-dose ICSs (500 µg twice a day for FP; 400 µg twice a day for BUD) and squares represent high-dose ICSs (1000 µg twice a day for FP; 800 µg twice a day for BUD).

twice a day. Two patients were noted to have inadequate inhaler technique.

Serum concentration levels were successfully detected in 18 of 19 patients and were still detected above the lower limit of detection for BUD after 8 hours in all patients and for FP in 8 out of 13 patients (see [Table I](#)). Two of the 3 patients with the lowest serum concentrations of FP were those noted to have poor inhaler technique; data from these 2 patients were excluded from further analyses. To explore the possibility that poor adherence may have accounted for differences in baseline drug levels, we collected prescription information from the patients' general practitioners. *Low adherence* was defined as collection of less than 80% of expected prescriptions in the preceding 6 months. Of the 10 patients with undetectable baseline drug levels, 2 were those with poor inhaler technique and a further 6 were low-adherent, versus 2 of 7 patients with detectable baseline levels (we were unable to obtain prescription records for the 2 other patients).

Correlations between $\log_{10}$ ICS concentration and markers of severity were evaluated (exacerbation rate, number of hospital admissions, and $\log_{10}$ FEV₁ predicted). Of these, $\log_{10}$ FEV₁ correlated with $\log_{10}$ serum FP (Pearson $r = 0.76$; $P = .007$), but not with serum BUD ($r = 0.35$; $P = .498$) (see Fig 1). Subgroup analysis indicated that the correlation for FP was not dependent on whether drug levels were detectable ($n = 6$; $r = 0.81$; $P = .051$) or undetectable ($n = 7$; $r = 0.708$; $P = .075$) at baseline, although these correlations did not reach statistical significance likely due to low numbers. There was no correlation with any of the other measured markers of disease severity.

This pilot study is the first to describe measurement of serum ICSs as a potential marker of treatment adherence in asthma. We were able to detect both FP and BUD in the serum in all but 1 of the participants with good inhaler technique at 2 hours postinhalation and in all but 3 at 8 hours. The 2 patients with obviously poor inhaler technique were found to have very low concentration levels (peak concentrations of 27 and 32 ng/L). Hence, this test merits further investigation as a potential marker of nonadherence, intentional or otherwise. Our methodology is being further developed to reduce the lower limits of detection and quantification, which should allow us to detect lower concentrations more accurately and perhaps beyond 8 hours.

There was large variability in the baseline serum drug levels, including some where the drug was undetectable. It is not possible to determine from the data whether this was due to prior poor adherence or whether serum concentration levels drop below detectable levels in between daily dosing, or even both.

Fluticasone propionate has very low oral bioavailability; therefore, serum levels are predominantly from lung deposition and subsequent absorption.³ The strong correlation between FEV₁% predicted and peak concentration for FP could be explained by worse airway obstruction, limiting the peripheral deposition of the inhaled steroid; it could also be attributed to the impact of airway remodeling on absorption. The possibility of reverse causation, whereby prior low adherence could have resulted in a low FEV₁, is less likely as we demonstrated that the correlation remained regardless of whether levels were detectable or not before directly observed drug inhalation. Although this correlation between airway obstruction and ICS

concentration levels has been previously demonstrated,⁴ the question as to why has not been answered. The evidence from this pilot study is insufficient to reliably answer this question and further work is required.

In conclusion, we have developed a methodology for serum detection of both FP and BUD by LC-MS/MS in clinical practice. This has the potential to be used as a direct measure of adherence. Further work with increased patient numbers and alternative ICSs (drugs and devices) is now warranted. Finally, further work is needed to explore the impact of airway obstruction on serum concentrations of ICSs, and in particular whether this may have an impact on drug efficacy in patients with severe obstructive lung disease.

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HPLC

The mobile phases used were (A) distilled water with 2 mmol/L ammonium acetate containing 0.1% formic acid (Sigma, Poole, United Kingdom [UK]) and (B) methanol (ultrapure grade, Fluka, Poole, UK). The analytical column used was a 2.1×50 mm $1.8\text{-}\mu\text{m}$ Waters Acquity UPLC HSS C18 SB column coupled to a Waters Vanguard filter. FP and BUD 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% B 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.

TANDEM MASS SPECTROMETRY

The eluate was injected from the LC directly into a XEVO TQS tandem mass spectrometer (Waters, Manchester, UK). MassLynx

NT 4.1 software was used for system control and the MassLynx TargetLynx program allowed data processing. This software used the area of the detected peaks, $1/x$ weighting, and linear least squares regression to produce a standard curve. The $1/x$ weighting gives greater accuracy at lower concentrations. The mass spectrometer was operated in the 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 and 501.2>313.2 for BUD and FP, respectively. The internal standards had transitions of m/z 439.2>323.1 for D8 BUD and 506.2>313.2 for D5 FP. Transitions were monitored in the multiple reaction monitoring mode, with a dwell time of 0.025 seconds.

Both BUD and FP eluted at 2.2 minutes and were linear to at least 4000 ng/L. The lower limit of detection and quantification was 25 ng/L for both FP and BUD. This analytical method is highly selective and not affected by the presence of prednisolone (or other corticosteroids) in the samples.