

1 **Assessment of adherence to corticosteroids in asthma by drug monitoring or fractional exhaled nitric**
2 **oxide: a literature review.**

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25 **ABSTRACT**

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Background: Although the efficacy of corticosteroid treatment in controlling asthma is widely accepted, its effectiveness is undermined by poor adherence. However, the optimal method for measuring adherence to asthma therapy remains unclear.

Objective: To perform a review of the literature reporting biological, objective methods for assessing adherence to inhaled or oral corticosteroids in asthma; we included studies reporting direct measurement of exogenous corticosteroids in blood, or the effect of adherence on exhaled nitric oxide.

Design: We searched three databases MEDLINE (using both PubMed and Ovid), the Cumulative Index of Nursing and Allied Health Literature (CINAHL), and Web of Science for articles published between January 1975 and July 2020. Quality of the studies was assessed using the National Institute of Health checklist.

Results: From 2850 screened articles, 26 fulfilled the inclusion criteria. Measurement of blood prednisolone with or without cortisol was used in eight studies as a measure of oral corticosteroid adherence, and fractional exhaled nitric oxide (FeNO) in 17 studies for inhaled corticosteroid adherence. Inhaled corticosteroids were measured directly in the blood in one study. By direct measurement of drug levels in the blood, adherence rates to oral corticosteroids ranged from 47 to 92%, although the performance and timing of the test was often not known, making interpretation of findings and serum prednisolone concentrations difficult. FeNO is generally lower in adherent than non-adherent patients, but no absolute cut-off can be proposed based on the available data. However, a fall in FeNO following supervised inhaled corticosteroid dosing could indicate previous poor adherence.

Conclusions and Clinical Relevance: Despite prednisolone and cortisol levels commonly being used as adherence markers in clinical practice, further work is required to assess the influence of the dose and timing on blood levels. The promising findings of FeNO suppression testing should be explored in further prospective studies.

53 INTRODUCTION

54 Asthma is the most common chronic inflammatory airway disease, affecting more than 300 million people
55 worldwide (1). Inhaled corticosteroids (ICS) are the foundation of daily asthma control medication, and in
56 severe asthma are needed at high doses, often in combination with daily oral corticosteroids (OCS) (2). A
57 high prevalence of poor adherence has been identified in all asthma severities, which impacts
58 exacerbation and hospitalisation rates, quality of life, and pulmonary function. The impact on healthcare
59 costs is complex, with non-adherence resulting in lower direct spending on inhalers, but potentially higher
60 costs associated with exacerbations, systemic steroid toxicity, and escalation to biologic therapy (1, 3-6).

61
62 Measuring adherence to asthma medications is recommended in national and international guidelines (2,
63 7), but represents a significant challenge. Methods of monitoring adherence should be accurate, easy-to-
64 use and cost-effective. They can be classified into objective and subjective methods, but all have
65 drawbacks and none would be considered as a 'gold standard'. The most common subjective methods of
66 adherence include self-report questionnaires, patient diaries, and assessment by healthcare providers.
67 This is known to be unreliable, as patients particularly tend to overestimate their adherence (3). Objective
68 assessments, which include pill counting, prescription pick-ups, and electronic device monitoring (smart
69 inhalers) may also overestimate adherence where patients pick up or actuate their inhalers without
70 inhaling the drug as prescribed. The effect of corticosteroid medications (pharmacodynamics) on blood
71 biomarkers such as blood eosinophils (8-10) or cortisol (11) may be useful in ascertaining efficacy and
72 toxicity, but this may not directly reflect adherence. The fractional exhaled nitric oxide test (FeNO) is an
73 alternative biological non-invasive method used as a surrogate marker of ICS adherence (12-14), due to
74 the effect of ICS on nitric oxide synthase (15).

75
76 Direct drug monitoring in biological fluids is probably considered the most reliable and accurate method,
77 as it at least provides evidence that the patient has taken the medication sometime in the recent past,
78 according to the specific assay and pharmacokinetics. Numerous studies have used blood assays to
79 identify nonadherence in asthma patients regarding prescribed daily OCS by measuring prednisolone and
80 cortisol levels (9, 16, 17), and more recently a similar approach has been reported for ICS (18).

81
82 We have conducted a review aiming to identify and summarise the available literature on two objective
83 biological methods that have been used to assess the adherence to either ICS or OCS in adults and
84 children with asthma: 1. detection of inhaled or oral corticosteroids in body fluid, and 2. level of exhaled

85 nitric oxide. Due to the heterogeneity of the studies within each adherence method included, a meta-
86 analysis was not conducted.

87 METHODS

88 **Search strategy**

89 This review was reported according to PRISMA (the preferred reporting items for systematic reviews and
90 meta-analyses) guidelines (19). Although this was undertaken according to the standards of a systematic
91 review in terms of search strategy, quality assessments, and data extraction, PICO (Population,
92 Intervention, Comparison and Outcome) reporting criteria were not suitable, due to multiple
93 interventions of interest, and the lack of availability of a robust “gold standard” comparator for adherence
94 in the vast majority of literature reviewed. The search was completed by 6 July 2020. The databases
95 searched were MEDLINE (using both PubMed and Ovid), the Cumulative Index of Nursing and Allied
96 Health Literature (CINAHL), and Web of Science. The terms used were (asthma OR asthmatic) AND
97 (adherence OR compliance OR concordance) AND oral corticosteroids OR inhaled corticosteroids) AND
98 (exhaled nitric oxide OR FeNO). We screened the entire reference list from each eligible study, and Google
99 Scholar was used to find any relevant citing articles. The study selection was performed based on the
100 following inclusion and exclusion criteria.

101

102 **Inclusion criteria**

- 103 • A clear diagnosis of asthma based on a physician’s diagnosis or international guidelines.
- 104 • Studies published in full, between January 1975 and July 2020
- 105 • Primary research involving direct corticosteroid monitoring or the level of exhaled nitric oxide as a
106 marker of adherence, or at least was investigated with an assessment of the rate of adherence.

107

108 **Exclusion criteria**

- 109 • Other indirect methods of adherence (e.g., blood eosinophils or measures of adrenal
110 suppression).
- 111 • Non-human studies.
- 112 • Case series or case report.
- 113 • Review articles.
- 114 • Research in abstract form only.
- 115 • Non-English language publications.

116

117 **Study identification and data extraction**

118 The first author screened all the titles and abstracts to exclude non-relevant articles. All the selected
119 studies were screened in full-text to assess the eligibility. The last author (SF) confirmed the eligibility of
120 studies, and verified appropriate data extraction. Any disagreement between the authors in study
121 selection and data extraction was resolved by discussion.

122

123 **Quality assessment**

124 We used the National Heart, Lung, and Blood Institute of the National Institutes of Health (NIH) quality
125 assessment tools for observational, cohort and cross-sectional studies, and controlled intervention studies
126 (20). The first (FA) and the second (AP) reviewers evaluated the included studies individually.

127

128 **Synthesis of results**

129 Due to the different methodologies between the included studies, a meta-analysis was not conducted.

130 RESULTS

131 We identified 4603 studies through all databases. After removing duplicates, the titles and abstracts of
132 2850 articles were screened. The full texts of 40 potential articles were retrieved for further evaluation;
133 21 fulfilled the inclusion criteria. Reference list checking and screening Google Scholar added five studies,
134 resulting in 26 studies included in total (Figure 1). Excluded studies are detailed in online supplementary
135 table S1.

136
137 The relevant characteristics and findings of the selected articles are reported in Tables 1-5. The studies
138 were conducted in 10 countries: 15 in the United Kingdom and Ireland, three in the Netherlands, two in
139 the USA, and one each in Germany, Denmark, Spain, Finland, Greece, and China. No relevant studies were
140 found before 1998. The sample size of the included studies ranged from 17 to 529 participants. 13 studies
141 included only children, 12 adults, and only one both.

142

143 **Quality assessment**

144 Cohort and cross-sectional designs were most common ($n = 22$); the remaining four studies were
145 randomised controlled trials (RCTs). Generally, most of the studies ($n = 14$) were ranked as poor quality
146 (see online supplementary tables S2 and S3). Of the observational cohort and cross-sectional studies,
147 seven were rated fair (9, 14, 21-23, 39, 41), three good (24, 25, 37), and 12 poor-quality (16-18, 26-34).
148 Among the randomised controlled studies, one was rated as good (35), one fair (38), and two poor (12,
149 36).

150

151 **Adherence methods**

152 Oral corticosteroid adherence was assessed by direct measurement of blood levels in eight studies (9, 16,
153 17, 26-28, 30, 37), and ICS levels in one (18) (tables 1 and 2). Seventeen studies (12, 14, 21-25, 29, 31-39)
154 used the FeNO test to indirectly assess adherence (tables 3-5).

155

156 **Corticosteroid adherence: assessment by direct monitoring of drug levels**

157 *Methods used for corticosteroid detection*

158 Oral prednisolone was the only OCS used among the included studies, which typically reported blood
159 prednisolone level in addition to cortisol (9, 16, 17, 27, 28, 30, 37), applying various cut-offs to each as
160 shown in tables 1 and 2. One study reported ICS levels over eight hours after witnessed inhalation (40).
161 The analyses of prednisolone and cortisol concentrations were done by either high-performance liquid
162 chromatography (HPLC) or liquid chromatography-mass spectrometry LC-MS, although important

163 analytical parameters such as the limits of detection (LoD) or quantification (LoQ) were not mentioned in
164 all but one (37), as shown in tables 1 and 2. George *et al.* reported serum ICS levels measured using liquid
165 chromatography-tandem mass spectrometry (LC-MS/MS) with a LoQ of 25 ng/L (18).

166

167 *Adherence rates by direct corticosteroid detection*

168 Four studies investigated a daily dose of 40mg prednisolone given as an 'interventional' drug (table 1).
169 Overall poor adherence rates were between 8% and 25% (16, 17, 27, 30). Four further studies reported
170 'real-world adherence' in patients who were prescribed regular daily prednisolone at enrolment (9, 26,
171 28, 37). Stirling *et al.* reported three of 15 (20%) participants had undetectable prednisolone in the blood
172 two hours after dosing (26), although the daily prescribed dose was not reported. Mean (SD) prednisolone
173 levels were 344 (285) nmol/L (approximately equivalent to 124 mcg/L). Robinson *et al.* assessed
174 adherence in patients referred with "difficult" asthma and compared adherence rates between those with
175 and without a subsequently confirmed diagnosis. They found that half of the participants (9/18) with
176 confirmed asthma were non-adherent, while none of the 10 patients in the non-asthma group were
177 considered as having poor adherence (28). Mean (SD) prednisolone concentration in the adherent
178 patients prescribed at least 15 mg/d was 616 (143) mcg/L, and 465 (90) mcg/L in those prescribed less
179 than 15 mg/d). In a cross-sectional study in Northern Ireland, the prevalence of poor adherence to OCS
180 was 23/49 (47%). Of those with undetected prednisolone, 35% had a good level of adherence based on
181 ICS prescription refill >80% in the last six months (9). In this study mean (SD) prednisolone levels of the
182 adherent group (mean daily dose 15.6 mg) was 194 (160) mcg/L. Mansur *et al.* measured serum
183 prednisolone and cortisol, estimated 5-8 hrs post prednisolone dosing (unwitnessed) in severe asthmatics.
184 Good adherence (defined as suppressed morning cortisol) was identified in 40/67 subjects; median (IQR)
185 serum cortisol was 27 (48) nmol/L and 259 (622) nmol/L for serum prednisolone.

186

187 In an ICS study (18), all participants were witnessed using their inhaler and had inhaler technique rated.
188 One patient observed to have a poor inhaler technique did not have a detectable level of ICS. Eighteen of
189 19 (95%) participants had detectable ICS (budesonide or fluticasone propionate) in the serum eight hours
190 after witnessed inhalation.

191

192 **Adherence to inhaled corticosteroids and exhaled nitric oxide**

193 There was marked heterogeneity in the 17 studies that measured FeNO levels. Three different designs
194 were used: 1. studies investigating the association between a single FeNO measurement and ICS
195 adherence (n=7); 2. studies examining the association between repeated FeNO measurements and ICS

196 adherence (n=8); and 3. studies using the “FeNO suppression test” as a surrogate marker of adherence in
197 patients using ICS (n=2). All studies used the FeNO test accompanied by different objective adherence
198 methods, for example prescription refill, pill count for a specific period, or self-reported questionnaires.

199

200 *Single FeNO test and adherence*

201 Seven studies measured FeNO level at a single time point and reported FeNO in adherent and non-
202 adherent patients (22, 23, 31, 32, 34, 39, 41). Methods such as self-reported questionnaires, electronic
203 devices, and patients’ diaries were used in assessing the adherence rate. ICS dose and route were not
204 controlled (i.e. participants used their usual medication). Cano *et al.* considered adherence satisfactory if
205 parents reported that at least 75% of prescribed doses were taken (31), and found that adherence was
206 the only independent predictor of FeNO in patients treated with ICS after adjusting for age, gender, BMI,
207 and ICS dose. Scott *et al.* defined poor adherence where the patient missed at least two doses of ICS per
208 week (32); they found no difference in FeNO by adherence levels. A validated medication adherence self-
209 report scale (MARS), reported by the parents of participants, was used to identify poor adherence to ICS
210 in two studies (22, 23). Both of the studies reported an association between adherence and FeNO, Koster
211 *et al.* (22) noted that FeNO was lower in adherent compared to non-adherent patients. Vijverberg *et al.*
212 investigated several confounding factors for FeNO, and found that adherence was an independent factor
213 in patients with high FeNO (>25 ppb) (23). One study evaluated adherence over six to 12 months by smart
214 inhalers and reported an inverse correlation between adherence and FeNO ($r = -0.41$; $p = 0.001$) (34). Two
215 recent studies used prescription records with $\geq 80\%$ cut-off point for good adherence in adult asthmatics
216 (39, 41), both found no difference in FeNO levels between groups with good and poor adherence.

217

218 *Repeated FeNO measurements and adherence*

219 Longitudinal FeNO measurements and adherence were reported in eight studies conducted over 6 weeks
220 to 12 months (12, 21, 24, 29, 33, 35, 36, 38). Beck-Ripp *et al.* measured FeNO three times over 16 weeks
221 and compared with estimated adherence by dose-counting (12). Any patients with a history of suboptimal
222 adherence or poor inhaler technique were excluded. In the first four weeks, children were prescribed high
223 dose inhaled budesonide, which was then withheld between the fifth and the eighth week (the washout
224 period). In the last eight weeks, patients were randomised to 200mcg daily inhaled budesonide *versus* no
225 ICS and a correlation was identified ($r^2 = 0.59$; $p=0.0003$) between ICS dose counts and percentage
226 reduction in FeNO. Another study measured FeNO in 30 children and evaluated adherence by checking
227 the diaries of the participants (number of daily doses missed) in each visit for six months (29). FeNO levels

228 were significantly lower in those regularly using ICS *versus* the poorly adherent group (34 vs 130 ppb,
229 $p=0.001$).

230

231 Similarly, in 2006, children used a data logger attached to their pMDI for one month and attended four
232 visits to test the level of FeNO in each visit (21), but no link was demonstrated. Strandbygaard *et al.* (36),
233 reported data from pharmacy records alongside FeNO for 12 weeks and randomised patients to short
234 message services (SMS) daily intervention reminder, or control (no intervention). However, despite the
235 non-SMS group having poorer adherence over 12 weeks, both groups of patients (SMS and non-SMS) had
236 significant improvements in FeNO. A large cohort study in adults and children found that those with a
237 high FeNO had poor adherence by measuring the medication possession ratio (MPR) at baseline, but that
238 this improved at 12 months (33). One good-quality RCT compared the FeNO level with adherence.
239 Adherence was evaluated at each visit by counting the missed doses in a Diskus device and adherence
240 was considered poor if <50% was taken during the treatment trial (35). A recent longitudinal
241 observational study assessed the level of adherence in children by attaching an electronic monitoring
242 device (Smartinhaler) to the participants' inhalers and by MARS questionnaire (24). There was an
243 improvement in FeNO at the end of the trial in all participants, but no difference between the good and
244 poorly adherent groups. Likewise, Koumpagioti *et al.* have used Smartinhalers and found a general
245 improvement in FeNO levels in both interventional and non-interventional groups after six-weeks of ICS;
246 comparative FeNO levels between patients with good *versus* poor adherence were not reported (38).

247

248 **FeNO suppression test as a marker of adherence:**

249 Two studies reported an application for FeNO testing in assessing adherence to ICS in patients with severe
250 or uncontrolled asthma (14, 25). This method measures the daily level of FeNO along with direct
251 observation or monitoring of participants using a high daily dose of ICS over five to seven days, or one
252 month. McNicholl *et al.* first reported the feasibility of using the FeNO suppression test to predict non-
253 adherence in patients with "difficult asthma" and high FeNO levels (≥ 45 ppb) (14). They determined a
254 positive FeNO suppression test where the FeNO level dropped beyond a threshold ($\log_{10} \Delta \text{FeNO} \geq 0.24$),
255 calculated by change from mean $\log_{10}$ FeNO (day 0 – 1) to mean $\log_{10}$ FeNO (day 6 – 7). This threshold
256 demonstrated 78% sensitivity and 92% specificity for non-adherence, determined by ICS prescription
257 refill-rates over the previous six months (adherent if >80%). They noted a high reduction of FeNO levels
258 among the poorly adherent group (<70% of prescription refill), and the mean level of FeNO suppressed
259 from 79 to 47 ppb ($p=0.003$) after seven days of observed ICS. Likewise, Heaney *et al.* used the FeNO
260 suppression test and the same threshold for FeNO suppression testing over seven days and four weeks

261 and monitored adherence by a smart-inhaler device attached to Diskus inhaler, which recorded the sound
262 of the inhaler opening alongside the date, time, and technique (25). FeNO in 65% (130/201) of the
263 participants was suppressed (>42% reduction from baseline) after one week of ICS. However, data from
264 almost 40% of participants were excluded, due to them either forgetting to measure daily FeNO, or
265 missing daily ICS doses.

266 DISCUSSION

267 We have reviewed studies using objective biological methods for adherence monitoring in asthma,
268 specifically through drug concentration assays in blood or measurement of exhaled nitric oxide. Our key
269 findings are: 1. measurement of blood prednisolone levels, with or without serum cortisol, is commonly
270 used as an adherence measure in studies, and if accurate suggests adherence to oral prednisolone is often
271 poor, even within clinical trials; 2. whilst exhaled nitric oxide typically falls with inhaled corticosteroid use,
272 and is generally found to be lower in adherent *versus* non-adherent patients, there appears to be no
273 reliable cut-off that can be used to classify adherence; 3. it may be that a fall in FeNO following monitored
274 ICS use can identify previous non-adherence, although this needs to be prospectively validated.

275
276 Although prednisolone detection was commonly used as marker of adherence, sometimes with
277 simultaneous measurement of cortisol, none of the included papers cite data that support this
278 assumption over the range of prednisolone doses used, and the timing of the sample post-dose. Indeed
279 many studies did not report the dose or timing. Although prednisolone can likely be reliably detected for
280 at least eight hours following a 40mg dose (42), this cannot be assumed to hold for the lower doses that
281 are commonly used for maintenance in severe asthma (43). If this measure is to be used in clinical
282 practice, further dose ranging pharmacokinetic studies are required in this patient group. One study
283 reporting the level of ICS within an eight-hour window in serum as a marker of adherence (18) found that
284 they could be detected in all but one of 19 patients. Whilst promising, this study was performed in
285 patients taking only budesonide or fluticasone, and similar studies are needed for other commonly used
286 ICS. Corticosteroids in biological samples were typically detected by either LC-MS or HPLC. The mass
287 analytical detector in LC-MS gives a much higher sensitivity (~ about 1000-fold) with a LoD in the low
288 ng/mL range (44, 45), although it is relatively high-cost and requires a skilled operator. In many of the
289 included studies we found that key methodological details and performance parameters, such as LoD or
290 LoQ, were not given, thus making the application and generalisability of the findings difficult.

291
292 Exogenous corticosteroids have an adrenal suppressive effect (16, 46), and cortisol level was reported in
293 several studies as a surrogate for adherence to oral corticosteroids (9, 16, 17, 27, 28, 30), although using
294 varied and non-validated cut-offs. Many factors can influence cortisol levels, such as circadian rhythm,
295 ICS use, and the variability of cortisol values between individuals (47, 48), and so (as for prednisolone
296 levels above) studies are required to validate timings and cut-offs to support their clinical use in patients
297 with severe asthma.

298

Most of these studies reporting direct serum monitoring were of poor or fair quality, with small sample sizes and cross-sectional designs. Prednisolone administration was almost always unsupervised, and so it is unknown whether lack of detection represents non-adherence or failure of the test. As expected, low adherence rates were more common in real-world study designs (20% to 50%) when compared to interventional studies (8% to 25%). Whilst most guidelines acknowledge that adherence should be assessed, they do not suggest a cut-off for “good” adherence. A cut-off of 80% has been suggested for example by McNichol *et al* (14) and adopted widely (in the UK at least) as the standard required before escalation to biologics, with little supporting evidence. It may be that some patients “self titrate” their treatment to maintain good control, and apparent “low adherence” may not be very important in such cases. However when a patient with asthma presents with an exacerbation or poor control, the investigation must include an appraisal of whether the current treatment plan is working, and for that of course we need to know whether it is being followed.

Most studies reporting a single measurement of FeNO (22, 23, 31, 34), or repeated measures (12, 29, 33, 35), found higher levels in poorly adherent subjects. There are not enough data available to be able to propose a single cut-off, or a clinically meaningful change (fall) over time, for clinical use. This may be in part because important confounding factors such as age, height, gender and atopy were not taken into consideration (49). In addition the “gold standard” adherence in these studies often had significant limitations. A number of studies measured the level of adherence by diaries or self-report questionnaires (22, 23, 29, 31, 32). The accuracy and reliability of such methods are low (50), with patients typically over-estimating their adherence (5, 51, 52). The FeNO suppression test may be able to identify patients with previous poor adherence (14), and may be useful in a clinical setting at least in patients with elevated FeNO levels (>45ppb) (14, 25), where adequate resources are available. Further, non-suppressors may be identified with relative corticosteroid insensitivity who may require alternative therapeutic strategies.

Given the costs and time associated with adherence monitoring in asthma, it may be worth considering applying these methods only in selected patients where financial and clinical value will be most likely. The most obvious area would be in those with apparent severe disease who are being considered for biologics, where better ICS adherence may improve disease control without the need for such high-cost therapies. In such patients a relatively comprehensive assessment including pick-up checking, direct monitoring (blood ICS/OCS levels), smart inhalers (when available) and FeNO suppression (where relevant) may be justified. Conversely, it may also prove useful at start of asthma therapy, where guidelines suggest a trial of ICS therapy as part of the diagnostic workup (2); interpreting response without any

332 knowledge of adherence is impossible and may lead to underdiagnoses or inappropriate therapy
333 escalation (where there appears to be no response, but in fact enough ICS has not been taken). It is here
334 that the possibilities raised by the emergence of smart inhalers offer promise both in helping the clinician
335 assess adherence patterns but also potentially in helping the patient to establish adherent behaviours.
336
337 Several limitations of this review need to be considered. The included studies were selected based on the
338 method of adherence from either direct drug measurement or measuring the FeNO level, and these
339 methods cannot be compared to each other. Most studies were low-quality with high heterogeneity
340 across the studies, including patient characteristics (in particular asthma severity and age), and cut-off
341 values of both adherence methods. Methods not included in the review, but of potential use, include the
342 detection of corticosteroid metabolites in the urine, as reported in (53). In this study fluticasone
343 propionate-17 β -carboxylic, was found in the urine of all 30 included subjects 16 to 24 hours after
344 witnessed inhalation of fluticasone propionate. A further single case report also found that fluticasone
345 propionate and beclomethasone dipropionate were detectable in an induced sputum sample (54).
346 Further improvements to methods that assess the hypothalamic-pituitary-adrenal (HPA) axis may also
347 prove useful; Smy *et al.* found that hair cortisol during ICS treatment was reduced, and proposed that this
348 as a useful surrogate marker of adherence (55). Finally, the robustness of this review would have been
349 improved by independent searching, study selection and data extraction involving at least two
350 researchers; here one author performed these duties and the results of each were subsequently verified
351 by a second author.

352
353 In conclusion we have reviewed the use of serum exogenous corticosteroid and cortisol detection, and of
354 FeNO, as biological measures of adherence in asthma. Further work is required to adequately define the
355 influence of drug dose- and timing-dependent factors on prednisolone and cortisol levels, in order to
356 support their use in clinical and research practice. Although FeNO is usually lower in adherent patients,
357 there are no data available to allow a single cut-off to be proposed. FeNO suppression testing merits
358 further investigation both as a marker of adherence and steroid insensitivity.

359
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364

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Table 1. Interventional studies reporting adherence to corticosteroids by direct serum measurement.

First author, year	Participants* and asthma severity (ICS dose)	Study description	Intervention, control and timing (where given)	Adherence measure and cut-off	Adherence results
Payne et al. 2001 (16)	n=27, children Budesonide ≥ 1000mcg/day or equivalent	Assessing adherence to prednisolone in patients treated with two weeks of OCS by measuring serum prednisolone and cortisol levels.	Oral prednisolone, 40mg daily; no control The time between the last dose and OCS measurement was variable.	Serum prednisolone and cortisol level by HPLC. Good adherence: Last dose < 24 hours: detectable prednisolone + cortisol level <100nM. Last dose > 24 hours: cortisol level <100nM (no LoD given)	Two patients (2/27) had undetectable prednisolone or high cortisol levels. Prednisolone and cortisol levels not reported.

Payne et al. 2001 (27)	n=31, children Budesonide $\geq$ 1600 mc/day or equivalent	Assessing prednisolone adherence in patients treated with two weeks OCS by measuring serum prednisolone and cortisol levels.	Oral prednisolone, 40mg daily; no control The time between the last dose and OCS measurement was variable.	Serum prednisolone and cortisol level by HPLC. Good adherence: Detectable prednisolone + cortisol level <100nM. (no LoD given)	Non-adherence to prednisolone was identified in five patients. Prednisolone and cortisol levels not reported.
Lex et al. 2007 (30)	n=12, children and adolescents Budesonide $\geq$ 1600 mcg/day or equivalent	Assessing prednisolone adherence in patients treated with two weeks OCS by measuring serum prednisolone and cortisol levels.	Oral prednisolone, 40mg daily; no control The time between the last dose and measurement was variable.	Serum prednisolone and cortisol level by HPLC. Good adherence: Detectable prednisolone + cortisol level <100nM. (no LoD given)	Three patients were considered as non-adherent. Prednisolone and cortisol levels not reported.
Bossley et al. 2009 (17)	n=65, children Budesonide $\geq$ 800 mcg/day or equivalent μ cg	Asthmatic patients referred to difficult asthma clinic; response (lung function, inflammation, symptoms) to corticosteroids (oral or	Oral prednisolone, 40mg daily; no control The time between the last dose and	Serum prednisolone and cortisol level; analytical method analysis not given Good adherence:	12 patients (12/65) were non- adherent. Prednisolone and cortisol levels not reported.

		IV) measured after two weeks. Adherence to OCS was assessed by the detection of prednisolone and cortisol in serum blood.	measurement was variable.	Last dose < 24 hours: detectable prednisolone + cortisol level <100nM. Last dose > 24 hours: cortisol level <100nM (no LoD given)	Adherence was confirmed in all children who a clinical corticosteroid response.
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*We only considered those who have participated in the adherence measurements (not all the participants of the study). HPLC: High Performance Liquid Chromatography; LoD: Level of Detection; Oral corticosteroids; ICS: Inhaled corticosteroids.

Table 2. Studies reporting adherence to corticosteroids by direct serum drug measurements in patients on long term treatment.

First author, year	Participants * and asthma	Study description	Corticosteroid route, dose, duration and	Adherence measure and cut-off	Adherence results
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	severity (ICS dose)		timing (where given)		
Stirling, 1998 (26)	n=17, adult. BDP $\geq$ 2000 mcg/day or equivalent	Assessing the level of adherence to OCS by serum prednisolone level.	Levels measured 2 hours after prednisolone dosing (dose not reported).	Serum prednisolone by HPLC. Good adherence: detectable prednisolone (no LoD given).	Prednisolone level in three patients (3/15) was undetectable. Mean (SD) levels 344 (285) nmol/l.
Robinson, 2003 (28)	n=18 “Confirmed asthma” n=10 “unconfirmed asthma” BDP $\geq$ 1000 mcg/day or equivalent.	An observational study in uncontrolled patients on prednisolone attending the ‘difficult asthma’ clinic.	Mean (range) dose of prednisolone in ‘confirmed asthma’ 15 (12.5-125) mg/day. Mean (range) daily dose of prednisolone in ‘unconfirmed asthma’ 30 (2-60) mg. Levels measured 2	Serum prednisolone and cortisol by HPLC. Good adherence: detectable prednisolone AND ‘normal’ cortisol in patients on at least 15 mg/day. (Reference range and LoD not given).	Confirmed asthma: nine patients “poorly adherent” (six with undetectable OCS and normal cortisol; three with detectable OCS and ‘normal’ cortisol); mean (SD) prednisolone concentration ($\geq$ 15mg/d) 616 (143) mcg/L, and (<15mg/d) 465 (90) mcg/L. Unconfirmed asthma: none were classed as non-adherent; mean

			hours after prednisolone dosing (unsupervised).		(SD) prednisolone concentration ($\geq$ 15mcg/d) 624 (125) mcg/L, and (<15mg/d) 397 (136) mcg/L.
Gamble, 2009 (9)	n=51, adult Mean (SD) BDP equivalent 1388 (550) mcg/day.	Measuring the prevalence of non-adherence to OCS among severe asthma and its relationship with asthma outcomes	Mean (SD) dose prednisolone 15.6 (11.1) mg/day. Levels measured 2-4 hours after prednisolone dosing (unsupervised).	Serum prednisolone and cortisol (methods of analysis not given). Good OCS adherence: detectable prednisolone and undetectable cortisol (LODs not given; no reference given for cortisol level). Good ICS adherence: prescription refill $\geq$ 50%	Poor OCS adherence in 45% (23/51). Mean (SD) prednisolone level of the adherent group was 194 (160) ng/ml with undetected cortisol. Poor ICS adherence in 35% (8/23) Eight patients (8/26) who were adherent to OCS were filling <50% or fewer ICS prescriptions. Note: QoL score and hospital anxiety mean scores are higher in patients filling $\leq$ 50% in the preceding 12 months

George, 2017 (18)	n=19, adult. BDP $\geq$ 1000 mcg/day or equivalent.	Determining the feasibility of liquid chromatography-mass spectrometry in detecting ICS over eight-hours post-dosing.	Inhaled fluticasone propionate dose range: 1000 to 1600mcg; budesonide dose range: 400-800 mcg Directly observed and inhaler technique noted	Prescription refill for ICS in the last 6 months. ICS level measured by LC-MS/MS. Good ICS adherence: prescription refill >80%; detectable ICS (LoD=10 ng/ml)	18 subjects (18/19) had detectable serum ICS 8-hours post-dosing. ICS levels not reported. ICS adherence rate 'undetectable baseline drug' in 52% (10/19).
Mansur, 2020 (37)	n=67, adult. BDP $\geq$ 1600/day or equivalent.	Evaluating the utility of liquid chromatography-mass spectrometry in detecting the adherence to OCS.	Median (IQR) dose of prednisolone 10 (15) mg/day. Levels measured 5 - 8 hours post-dose (unsupervised)	OCS level measured by LC-MS/MS. Good OCS adherence: Suppressed cortisol level by <100 nmol/L and detectable prednisolone (LoD=20 nmol/L).	Cortisol suppressed in 40 subjects, from those 34 had detectable prednisolone levels (adherent), while undetectable prednisolone identified in the rest 6. Unsuppressed cortisol level with undetectable prednisolone was reported in 22 (non-adherent). 5 subjects had prednisolone detected with unsuppressed

					cortisol. The median (IQR) cortisol and prednisolone of the adherent group was 27 (48) nmol/L and 259 (622) nmol/L respectively. In the non-adherent group was 211 (130) nmol/L.
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*We only considered those who participated in the adherence measurements (not all the participants of the study). BDP: beclomethasone dipropionate; HPLC: high performance liquid chromatography; LC-MS/MS: liquid chromatography-tanden mass spectrometry; QoL: quality of life; LoD: Level of detection; OCS: oral corticosteroids; ICS: inhaled corticosteroids; SD: standard deviation

Table 3: Changes in FeNO (cross-sectional) in observational studies monitoring adherence by other methods

First author, year	Participants* and asthma severity (ICS dose)	Brief study description	Adherence measure and cut-off	Adherence results
Cano, 2010 (31)	n=67, children. Budesonide 200-800mcg/day or equivalent.	Identifying the main determinants of airway inflammation.	Self-reported by patient or parents. Good adherence: if over 75% of the prescribed doses taken.	Good adherence rate: 83%. Lower FeNO with ICS <i>versus</i> no ICS, [median (IQR) 27.5 (15.0-51.5) <i>versus</i> 41.8 (19.9-62.5) ppb. FeNO independently predicted adherence to ICS.
Scott, 2010 (32)	n=65, adult. ICS dose not reported.	Assessing the effect of atopy on FeNO.	Self-reported. Good adherence: if participants reported forgetting ICS once a week or less.	Good adherence rate: 50% (30/59). No difference between atopic and non-atopic asthma adherence vs good adherence.
Koster, 2011 (22)	n=527, children. ICS dose not	Investigating factors affecting adherence.	MARS questionnaire completed by parent.	Good adherence rate: 57%. Factors associated with good adherence: low FeNO and

	reported.		Good adherence: cut off ≥ 21 .	young age.
Vijverber, 2012 (23)	n=529 children. ICS dose not reported.	Assessing FeNO as a marker of uncontrolled asthma and adherence.	MARS questionnaire completed by parent. Good adherence: cut off ≥ 21 .	No overall adherence rate reported. Children with FeNO >25 ppb had lower adherence. FeNO measurements not reported for both groups (adherent and non-adherent).
Klok, 2016 (34)	n=60, children. Low-moderate fluticasone propionate.	FeNO level assessed as a marker for ICS adherence.	Smartinhaler-recorded dosages Good adherence: If over 80% of the prescribed doses taken.	Overall median (IQR) adherence: 82 (56-90)%. Low FeNO associated with good adherence. High FeNO (>25ppb) predicted poor adherence with a sensitivity of 41% and specificity of 94% when ICS adherence < 80%.
Yuan, 2019 (39)	n=222, adult ICS (dose not reported)	Assessing the association between asthma exacerbations in 12 months follow-up	Prescription records Good adherence: if > 80% of the prescribed dose taken.	Good adherence rate: 53% Patient without exacerbations had better adherence rate (p=0.004).

		and baseline FeNO.		No difference in the mean (IQR) of FeNO levels between good <i>versus</i> poor adherence groups: 25 (10-47) <i>versus</i> 29 (12-63) ppb.
Vähätalo, 2020 (41)	N=181, adult Budesonide equivalent 400-1000 mcg/day.	Assessing the variability of long-term adherence within 12-year follow-up.	Prescription record (checking both prescribed and dispensed) Cut-point of 80% was used to determine good adherence for both 12 year-adherence and annual adherence.	The average adherence rate over 12-years was 69%. Only 9% of patients had good annual adherence rate during the 12-year. 37% of patient had at least one period of non-adherence during 12-year. No difference was found in FeNO between the good and poor adherence groups.

*We only considered those who have participated in the adherence measurements (not the whole participants of the study). ICS: Inhaled corticosteroids; IQR: interquartile range; FeNO: fractional exhaled nitric oxide; MARS: medication adherence response scale.

Table 4: Repeated FeNO measurements in studies monitoring adherence by other methods

First author, year	Participants* and asthma severity (ICS dose)	Brief study description	Adherence measures and cut-off	Adherence results
Beck-Ripp, 2002 (12)	n=15, children. Budesonide 200-400mcg/d	Measuring FeNO to determine if it could be used as a potential marker of inflammation	Dose counting, calculated as doses are taken/doses prescribed×100 (%). Good adherence: If over >65% of the prescribed doses taken.	Mean (SD) FeNO pre-ICS 14.0 (1.2) ppb; post-ICS 7.7 (2.5) ppb. Positive correlation between ICS adherence and % reduction in FeNO ($r^2 = 0.59$; $p=0.0003$). Adherence rate after eight weeks of ICS not reported.
Claudia, 2004 (29)	n=30, Children ICS dose estimated range (84-2000)mcg BDP equivalent	To determine the utility of FeNO in assessing asthma control and ICS adherence.	Diaries (number of doses per day and number of days used as prescribed) then recorded as a percentage. Good adherence: if self-reported adherence was >75% of their prescribed regime.	Poorly adherent patients had high FeNO compared to adherent patients (130 vs 34 ppb, $p=0.001$) Negative correlation between FeNO and adherence ($r=-0.76$, $p=0.001$).

Katsara, 2006 (21)	n=20, children ICS (dose not reported)	Assessing the relationship between FeNO levels and ICS compliance in asthmatic children.	A data logger was attached to the inhaler (recorded time and date of all actuations) Good Adherence: Day compliance >60%.	No correlation between adherence and mean FeNO ($r = 0.05$, $p = 0.67$). No overall adherence rate reported. No repeated FeNO measurements reported
Szeffler, 2008 (35)	n=276, adolescent and adult. Fluticasone dose range: 100-500mcg/day	Determine if FeNO could increase the effectiveness of asthma treatment	Dose counter. Good adherence: if 50% of the prescribed dose taken.	Mean (SD) adherence rate in the study was 89 (28) %. FENO lower in adherent group (geometric mean 23.9 vs 30.8).
Strandbygaard, 2010 (36)	n=26, adult. Fluticasone ICS (dose not reported) GINA 2 (n=8) GINA 3 (n=16)	The impact of daily SMS text message reminder on asthma treatment and change of FeNO over 12 week period	Dose counter. Good adherence: Dose count on Seretide Diskus within week 4 until week 12. Pharmacy record (cut-off not	In the intervention group adherence rate did not change; in the control group adherence decreased from 84 to 70%. FeNO fell in both groups. With no between-group difference Data on pharmacy records not given.

	GINA 4 (n=2)		reported)	
Price, 2013 (33)	n=226, adult and children. BDP 100-400 mcg/day or equivalent	Evaluating FeNO in predicting steroid-responsiveness.	Medication possession ratio. (Defined as a number of days' supply of therapy/number of days in the total prescribing period × 100%) Good adherence: Unknown	Low baseline adherence to ICS associated with high FeNO (adult >50 ppb, children >35 ppb) In the following year, participants with high FeNo had improved adherence.
Jochmann, 2017 (24)	n=93, children and adolescents ICS dose range: 100-3200 mcg BDP/day or equivalent	Electronic ICS monitoring devices in identifying non-adherence.	Smart inhaler attached to ICS Good adherence: if $\geq 80\%$ of the prescribed dose taken. MARS-5 (parent-reported). Good adherence (MARS): Unknown	39/93 (42%) participants adherent. Baseline visit: no difference between adherent and non-adherent groups in FeNO. Improvement in FeNO in all participants at the end of monitoring. (34 vs 21ppb; p= 0.001). Patients with good adherence and asthma control test >20

				showed a significant reduction in FeNO in the follow-up visit (median: 18 vs 11ppb). No correlation identified between MARS questionnaire and adherence.
Koumpagioti, 2020 (38)	n=39, children and adolescent. ICS (dose not reported)	To investigate the impact of asthma care educational program on adherence.	Smart inhalers attached to ICS. Adherence was categorised as good ($\geq 80\%$), moderate (60-79%), poor ($< 60\%$).	Median (IQR) adherence rate 80 (73-85)% for the interventional group, with 51% of those reported as good adherers. Overall significant improvement in FeNO levels after ICS administration in interventional and non-interventional groups.

*We only considered those who have participated in the adherence measurements (not the whole participants of the study). ICS: Inhaled corticosteroids; FeNO: fractional exhaled nitric oxide; SD: standard deviation; BDP: beclomethasone dipropionate; MARS: medication adherence response scale.

Table 5: FeNO as marker of adherence (cross-sectional and suppression studies)

First author, year	Participants* and asthma severity (ICS dose)	Brief study description	Adherence measure and cut-off	Adherence results
McNicholl 2012, (14)	n=146, adult. BDP 800-2000mcg/day equivalent.	Assessing daily level of high FeNO suppression after using high dose of ICS for seven days as marker in identifying non-adherence.	Good ICS adherence: prescription refill $\leq$ 80% ICS. Good OCS adherence: detectable prednisolone and undetectable cortisol (LODs not given; no reference given for cortisol level).	At baseline: no correlation between baseline FeNO level and adherence; poor adherence rate: 76%. Five days of observed ICS: 13/40 of the participants were non-adherent. 42% fall in FENO over five days of treatment with high dose ICS indicated a previous non-adherence. Seven days of observed ICS: the non-adherent group (n=13) had a reduction in FeNO compared to the adherent group (p =0.003).
Heaney, 2018 (25)	n=290, adult BDP 800-2000mcg/day	The utility of FeNO suppression testing in clinical care using remote monitoring	Diskus inhaler connected with INCA device. Good adherence: defined as $\geq$ 70%	FeNO after seven days: Positive FeNO suppression identified in 130 patients [median FeNO= 88 ppb (64-127) vs 32ppb (21-46)] 45 (63%) patients from the negative FeNO suppression

	equivalent. OCS (n=129)	technologies.	FeNO levels: reduction by 42% from baseline FeNO level day 7 (positive FeNO suppression test).	group (n=70) on daily prednisolone. FeNO after four weeks: Positive FeNO suppression group (n=85); good adherence rate: 63% Negative FeNo suppression group (n=40); good adherence rate: 67% FeNO level at 7 days associated with FeNO after four weeks in good adherence group
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*We only considered those who have participated in the adherence measurements (not the whole participants of the study). OCS: Oral corticosteroids; ICS: Inhaled corticosteroid; BDP: beclomethasone dipropionate; INCA: INhaler Compliance Assessment; IQR: interquartile range; FeNO: fractional exhaled nitric oxide.

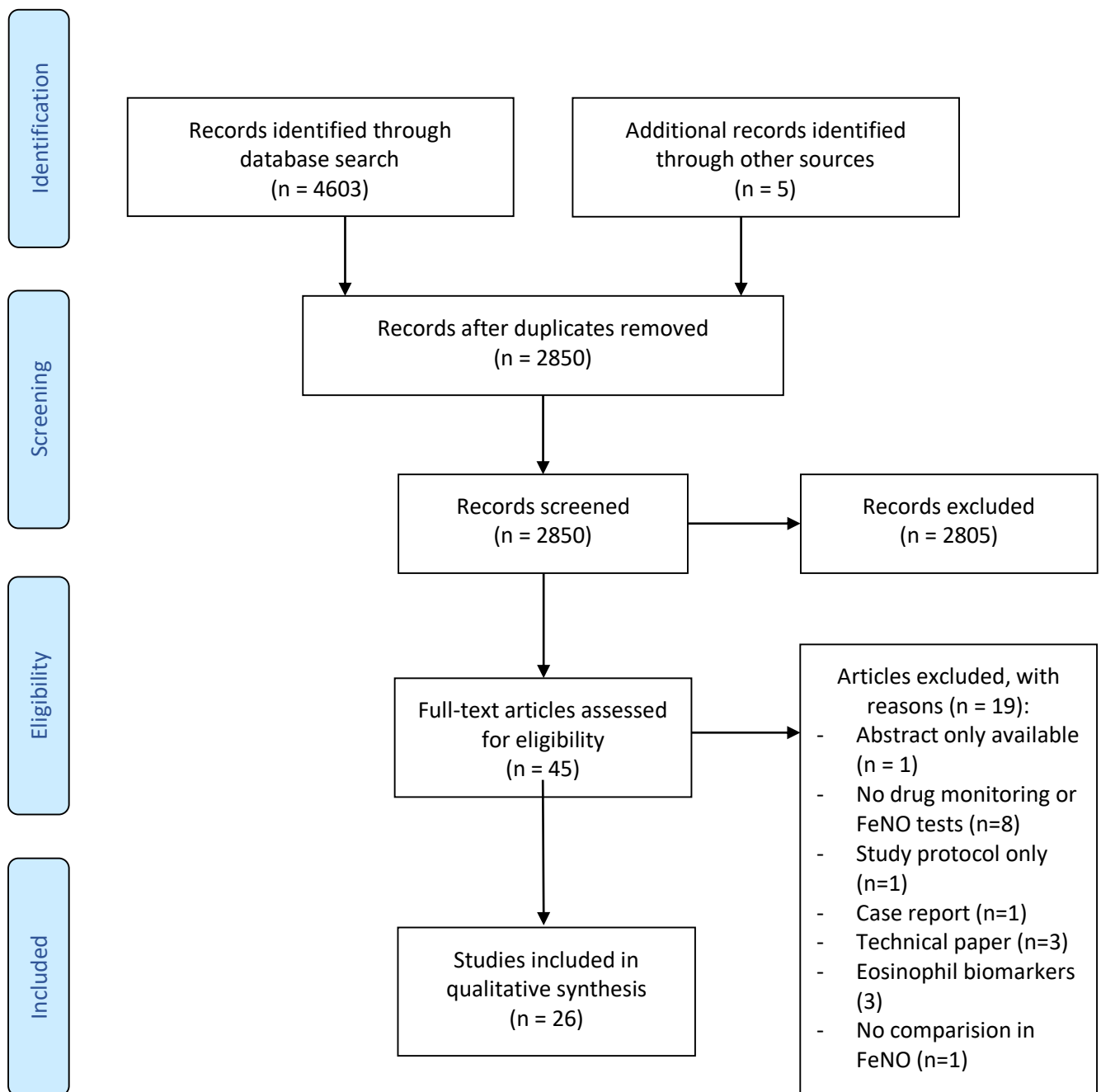


Figure 1. PRISMA flow diagram summary of study selection process.

Assessment of adherence to corticosteroids in asthma by drug monitoring or fractional exhaled nitric oxide: a literature review.

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Table S1: Excluded Studies

Ref	Author	Title	Reason
1	Knoery, 2015	Non-adherence to inhaled corticosteroid therapy as an explanation for persistently elevated exhaled nitric oxide levels in patients with asthma: Effect of directly observed therapy with inhaled corticosteroids (DOT-ICS)	Abstract only
2	Agache, 2012	Predictive Value of Lung Function Trend and FeNO for Difficult Asthma in Children	No direct measurement or not mentioning any correlation between FeNO and adherence level
3	Hederos, 2009	Six-year follow-up of an intervention to improve the management of preschool children with asthma	No direct measurement or not mentioning any correlation between FeNO and adherence level
4	Vijverberg, 2013	Inflammatory phenotypes underlying uncontrolled childhood asthma despite inhaled corticosteroid treatment: rationale and design of the PACMAN2 study	Study design and protocol
5	Hill, 1990	Monitoring glucocorticoid therapy: a pharmacokinetic approach.	Direct biological measurements were done for the purpose of assessing the pharmacokinetics of oral steroids only. No adherence data were reported
6	Smy, 2016	Hair cortisol as a novel biomarker of HPA suppression by inhaled corticosteroids in children	No biological body fluids or FeNO levels were assessed in term of adherence.
7	Zöllner, 2011	Hypothalamic-pituitary-adrenal axis suppression in asthmatic children on inhaled and nasal corticosteroids--more common than expected?	No direct biological body fluids or FeNO levels were assessed in term of adherence.
8	Zöllner, 2012	Hypothalamic-pituitary-adrenal axis suppression in asthmatic school children.	No direct biological body fluids or FeNO levels were assessed in term of adherence.
9	Gamble, 2011	A study of a multi-level intervention to improve on-adherence in difficult to control asthma	No direct biological body fluids or FeNO levels were assessed in term of adherence.
10	Hagan, 2011	Assessment of synthetic glucocorticoids in asthmatic sputum	Case report.
11	Hagan, 2011	Analysis of fluticasone propionate in induced sputum by mass spectrometry.	Technical study to assess the detection of ICS in whole sputum, no adherence assessment were reported.
12	Hagan, 2011	Urinary fluticasone propionate-17beta-carboxylic acid to assess asthma therapy	Technical study to assess the detection of ICS in Urine.

		adherence	
13	Korpi-Steiner, 2010	Liquid chromatography-tandem mass spectrometry analysis of urinary fluticasone propionate-17beta-carboxylic acid for monitoring compliance with inhaled-fluticasone propionate therapy	Technical study on non-asthmatic subjects.
14	Murphy, 2012	The relationship between clinical outcomes and medication adherence in difficult-to-control asthma	Sputum eosinophil counts was investigated with adherence rate.
15	Papi, 2018	Relationship of Inhaled Corticosteroid Adherence to Asthma Exacerbations in Patients with Moderate-to-Severe Asthma	Blood eosinophil counts was investigated with adherence rate.
16	Pijnenburg, 2005	Titration steroids on exhaled nitric oxide in children with asthma: a randomized controlled trial	No comparison between adherent and nonadherent groups and also in FeNO levels.
17	Berthon, 2015	Effects of short-term oral corticosteroid intake on dietary intake, in adults with asthma randomized controlled trial.	Association between blood eosinophil and adherence level were tested only.
18	Shimoda, 2017	Lung sound analysis can be an index of the control of bronchial asthma.	Lung sounds were correlated to adherence levels and FeNO. No data reported the
19	Price, 2020	Treatment adherence in adolescents with asthma	Review article

Table S2: results of quality assessment of included observational studies

Year	Author (ref)	Q1	Q2	Q3	Q4	Q5	Q 6	Q 7	Q 8	Q 9	Q 10	Q 11	Q 12	Q 13	Q 14	QUALITY
1998	Stirling (20)	Y	Y	Y	Y	N	CD	Y	N	N	N	Y	CD	Y	N	POOR
2001	Payne (21)	Y	Y	CD	Y	N	NA	Y	N	Y	N	Y	Y	Y	N	POOR
2001	Payne (22)	Y	Y	CD	CD	N	CD	Y	N	Y	N	Y	CD	Y	N	POOR
2003	Robinson (23)	N	Y	CD	Y	N	NA	Y	N	N	N	N	CD	Y	N	POOR
2004	Delgado (24)	Y	N	CD	Y	N	Y	Y	Y	N	Y	Y	CD	CD	Y	POOR
2006	Katsara (25)	Y	N	CD	Y	N	Y	Y	Y	Y	Y	Y	CD	Y	N	FAIR
2007	Lex (26)	Y	Y	CD	Y	N	NA	Y	N	Y	N	Y	NA	Y	N	POOR
2009	Bossley (27)	Y	Y	Y	Y	N	CD	Y	N	Y	N	Y	CD	Y	N	POOR
2009	Gamble (28)	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	CD	Y	Y	FAIR
2010	Cano (29)	Y	Y	Y	Y	N	N	Y	N	N	N	Y	Y	Y	Y	POOR
2010	Scott (30)	Y	N	Y	Y	N	CD	Y	N	N	N	Y	CD	NA	Y	POOR
2011	Koster (31)	Y	Y	Y	Y	N	CD	Y	N	Y	Y	Y	CD	Y	Y	FAIR
2012	Vijverberg (32)	Y	Y	CD	Y	N	NA	Y	NA	Y	NA	Y	NA	Y	Y	FAIR
2012	McNicholl (33)	Y	Y	N	NA	N	N	Y	N	CD	Y	Y	CD	Y	N	FAIR
2013	Price (34)	Y	N	CD	CD	N	Y	CD	Y	Y	Y	Y	NA	NA	N	POOR
2017	Klok (35)	Y	N	CD	Y	N	Y	Y	Y	Y	Y	Y	CD	CD	Y	POOR
2017	George (36)	Y	N	CD	Y	N	NA	Y	N	N	N	N	CD	Y	N	POOR
2017	Jochmann (37)	Y	Y	CD	Y	N	Y	Y	Y	Y	Y	Y	CD	Y	Y	GOOD

2018	Heaney (38)	Y	Y	Y	Y	N	N	Y	N	Y	Y	Y	CD	Y	N	GOOD
2019	Yuan (39)	Y	Y	CD	Y	N	Y	Y	Y	Y	Y	Y	CD	Y	Y	FAIR
2020	Vähätalo (40)	Y	N	CD	Y	N	Y	Y	Y	Y	Y	Y	CD	N	Y	FAIR
2020	Mansur (41)	Y	Y	Y	Y	N	NA	Y	Y	NA	Y	Y	CD	Y	Y	GOOD

Abbreviation: Y: Yes; N: No; : CD: cannot determine; NA: Not applicable.

Q1. Was the research question or objective in this paper clearly stated?

Q2. Was the study population clearly specified and defined?

Q3. Was the participation rate of eligible persons at least 50%?

Q4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?

Q5. Was a sample size justification, power description, or variance and effect estimates provided?

Q6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?

Q7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?

Q8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?

Q9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?

Q10. Was the exposure(s) assessed more than once over time?

Q11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?

Q12. Were the outcome assessors blinded to the exposure status of participants?

Q13. Was loss to follow-up after baseline 20% or less?

Q14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?

Table S3: results of quality assessment of included randomised control studies

Year	Author	Q 1	Q 2	Q 3	Q 4	Q 5	Q 6	Q 7	Q 8	Q 9	Q 10	Q 11	Q 12	Q 13	Q 14	QUALITY
2002	Beck-Ripp (42)	Y	CD	CD	N	CD	Y	Y	CD	CD	Y	Y	Y	Y	N	POOR
2008	Szeffler (43)	Y	Y	Y	N	CD	CD	N	N	CD	Y	Y	Y	Y	Y	GOOD
2010	Strandbygaard (44)	N	Y	Y	N	N	Y	N	Y	Y	Y	Y	N	Y	N	POOR
2020	Koumpagioti (45)	Y	Y	CD	N	CD	Y	Y	Y	CD	Y	Y	N	Y	N	FAIR

Abbreviations: Y: Yes; N: No; : CD: cannot determine; NA: Not applicable.

Q1. Was the study described as randomized, a randomized trial, a randomized clinical trial, or an RCT?

Q2. Was the method of randomization adequate (i.e., use of randomly generated assignment)?

Q3. Was the treatment allocation concealed (so that assignments could not be predicted)?

Q4. Were study participants and providers blinded to treatment group assignment?

Q5. Were the people assessing the outcomes blinded to the participants' group assignments?

Q6. Were the groups similar at baseline on important characteristics that could affect outcomes (e.g., demographics, risk factors, co-morbid conditions)?

Q7. Was the overall drop-out rate from the study at endpoint 20% or lower of the number allocated to treatment?

Q8. Was the differential drop-out rate (between treatment groups) at endpoint 15 percentage points or lower?

Q9. Was there high adherence to the intervention protocols for each treatment group?

Q10. Were other interventions avoided or similar in the groups (e.g., similar background treatments)?

Q11. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?

Q12. Did the authors report that the sample size was sufficiently large to be able to detect a difference in the main outcome between groups with at least 80% power?

Q13. Were outcomes reported or subgroups analyzed prespecified (i.e., identified before analyses were conducted)?

Q14. Were all randomized participants analyzed in the group to which they were originally assigned, i.e., did they use an intention-to-treat analysis?

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