Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 22 May 2026
Revised 26 June 2026
Accepted 18 July 2026

Natural Resources for Human Health 2026, Vol. 6(7s): 1162–1173

KEYWORDS:
medication adherence, asthma, inhaled corticosteroids, long-acting β2-agonists, hair analysis, biomarker, systematic review

Hair Analysis as an Objective Biomarker for Medication Adherence to Inhaled Corticosteroid/Long-Acting β_2-Agonist Therapy in Asthma: A Systematic Review

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ABSTRACT:

Background: Poor adherence to inhaled corticosteroid/long-acting β2-agonist (ICS/LABA) therapy remains a leading, modifiable cause of poor asthma control, exacerbations and excess healthcare expenditure, yet the tools routinely used to measure it are either subjective and prone to overestimation or logistically demanding for long-term outpatient use.

Objective: To synthesise the published evidence on hair analysis as an objective, minimally invasive biomarker of medication exposure, to describe the biological and analytical basis for its application to inhaled respiratory medications, and to critically evaluate its potential role and limitations as a measure of ICS/LABA adherence in asthma.

Methods: A structured systematic review was conducted, informed by the principles of the PRISMA statement for transparent reporting. Peer-reviewed literature indexed in PubMed/MEDLINE-linked sources and other academic databases was searched using combinations of terms relating to asthma, ICS/LABA adherence, medication-adherence measurement, hair analysis, drug incorporation into hair, and hair glucocorticoid/bronchodilator quantification, supplemented by hand-searching of reference lists and authoritative guideline documents. Because the available evidence in this specific application is heterogeneous in design and outcome reporting, findings were synthesised narratively rather than pooled statistically.

Results: Adherence to ICS/LABA therapy is estimated at well below 80% in routine care, and is associated with a two- to three-fold increase in exacerbations, hospitalisation and healthcare cost. Conventional adherence measures (self-report, pill/canister counts,

pharmacy refill ratios, electronic monitoring) each carry recognised biases. Hair analysis, already validated for antiretroviral, antihypertensive and immunosuppressant therapy, offers a centimeter-by-centimeter retrospective record of systemic drug exposure spanning weeks to months from a single, low-burden sample. Direct evidence for inhaled respiratory medications is still emerging, constrained by low and variable systemic drug incorporation, analytical sensitivity requirements, and confounding by hair colour, cosmetic treatment and external contamination.

Conclusion: Hair analysis is analytically feasible and clinically promising as a complementary, objective adherence biomarker for ICS/LABA therapy, but current evidence is insufficient to support its use as a stand-alone measure. Well-designed pharmacokinetic and validation studies, of the type proposed in ongoing institutional doctoral research, are needed before clinical translation.

1. INTRODUCTION

Asthma is one of the most common chronic non-communicable respiratory diseases worldwide, affecting an estimated 280 million people and contributing to roughly half a million deaths annually [1,7]. India carries a disproportionate share of this burden: population-based estimates attribute approximately 13% of the global asthma caseload and over 40% of global asthma deaths to India, with disability-adjusted life-year rates markedly higher than the global average [2,3,4,6]. The Global Burden of Disease analyses of Indian states similarly show that chronic respiratory disease, including asthma, remains a leading contributor to national mortality, with substantial heterogeneity across regions of differing socio-demographic development [5].

Combination therapy with an inhaled corticosteroid (ICS) and a long-acting β2-agonist (LABA) is recommended by the Global Initiative for Asthma (GINA) as a cornerstone of maintenance treatment from Step 3 upward, because it combines anti-inflammatory control of airway inflammation with sustained bronchodilation [1]. When taken as prescribed, ICS/LABA therapy reduces exacerbation frequency, improves lung function and quality of life, and lowers mortality risk [1,8,11]. In practice, however, real-world adherence to maintenance ICS/LABA regimens is frequently well below the levels achieved in closely monitored clinical trials: routine-care adherence in the region of 20-40% has been reported, compared with 80-90% under trial conditions [11]. Suboptimal adherence has been linked to a two-to-three-fold increase in the odds of exacerbation, greater use of oral corticosteroid bursts, more emergency department visits, and materially higher direct and indirect healthcare costs [8,9,14,24,25,26].

The reasons for non-adherence are multifactorial and are conventionally divided into unintentional barriers (forgetfulness, poor inhaler technique, complex regimens, cost) and intentional barriers (concerns about side effects, perceived lack of necessity given the episodic nature of asthma, stigma, and dissatisfaction with treatment) [16,18,19,20,21,22,23]. Because both categories can coexist in the same patient and can fluctuate over time, accurately identifying non-adherence rather than assuming it is a prerequisite for any meaningful clinical or research intervention aimed at improving outcomes.

Unfortunately, the adherence measurement tools in widest clinical and research use are imperfect. Self report, whether unstructured or captured through validated instruments such as the 8 item Morisky Medication Adherence Scale (MMAS-8) or the Test of Adherence to Inhalers (TAI), is convenient and inexpensive but is vulnerable to recall bias and social desirability bias, and has been repeatedly shown to overestimate adherence relative to objective measures [17,20,39,40,41]. Pill or canister counts and pharmacy refill based metrics such as the proportion of days covered (PDC) or medication possession ratio (MPR) are more objective but reflect acquisition rather than actual ingestion or inhalation, and cannot capture within day dosing patterns [30,32,34,35,36,37]. Electronic monitoring devices provide detailed time stamped dosing data but are costly, require patient cooperation with an unfamiliar device, and are not yet widely deployed outside research settings [13,17,18,38].

Biological (direct) methods measurement of the drug or its metabolites in blood, urine, or other body fluids offer the most objective confirmation of recent exposure, but are limited by short detection windows that reflect only the period immediately preceding sampling, and by the invasiveness or inconvenience of repeated sampling for a chronic, self-administered inhaled therapy [17,18]. Hair analysis has emerged over the past two decades as a complementary direct method that addresses several of these shortcomings simultaneously. Because scalp hair grows at an average rate of approximately one centimeter per month and incorporates circulating drugs and metabolites into its keratin matrix as it forms, segmental analysis of a single hair sample

can reconstruct a multi-month, retrospective record of systemic drug exposure from one low burden, non-invasive collection [29,47,48,51]. The approach is already established for monitoring long term exposure to antiretroviral therapy in HIV and pre exposure prophylaxis programmers, where hair drug concentration is recognized as one of the strongest independent predictors of virological response [43,44], and has been explored for antihypertensive medication [46], immunosuppressant's after solid-organ transplantation [65,66,67,68], and psychotropic agents including clozapine [45].

Extending this approach to inhaled respiratory medications is conceptually attractive but analytically more demanding, because systemic absorption of most inhaled corticosteroids and long acting β2-agonists is deliberately minimised by design, leaving smaller and more variable quantities available for incorporation into hair compared with orally or parenterally administered drugs [42]. A recent narrative review has begun to map the mechanistic and analytical groundwork for this specific application, while explicitly cautioning that clinical validation remains incomplete [42]. This gap is directly relevant to ongoing doctoral research at our institution assessing medication adherence in asthma patients treated with ICS/LABA combinations using hair analysis, and motivates the present review.

This review therefore aims to (i) summarise the burden of ICS/LABA non-adherence in asthma and the strengths and limitations of currently available adherence-measurement tools; (ii) describe the biological mechanisms by which systemically absorbed drugs enter the hair shaft and the analytical methods used to quantify them; (iii) synthesise the existing clinical evidence for hair analysis as an adherence biomarker, both in inhaled respiratory medication specifically and in analogous therapeutic areas; and (iv) critically appraise the confounding factors and methodological limitations that must be addressed before this approach can be translated into clinical or research practice.

2. METHODS

This article was prepared as a structured narrative review informed by the reporting principles of the PRISMA 2020 statement [69], adapted to a topic for which the primary clinical literature is still sparse and methodologically heterogeneous, precluding formal meta-analysis. A structured narrative synthesis, rather than a strictly quantitative systematic review with pooled effect estimates, was therefore judged the most appropriate and transparent format; this decision, and its rationale, is stated explicitly here in the interest of methodological honesty.

Literature was identified through iterative searches of peer reviewed, PubMed/MEDLINE indexed sources and other major academic databases, using combinations of the following concept groups: (i) asthma, ICS, LABA, inhaled corticosteroid, combination inhaler; (ii) medication adherence, non-adherence, compliance, persistence; (iii) adherence measurement, self-report, Morisky scale, Test of Adherence to Inhalers, pharmacy refill, proportion of days covered, electronic monitoring; (iv) hair analysis, hair testing, segmental hair analysis, drug incorporation into hair, hair cortisol, hair glucocorticoid, LC-MS/MS hair; and (v) analogous applications in antiretroviral therapy, antihypertensive therapy and transplant immunosuppression. Reference lists of retrieved articles and relevant authoritative guideline documents (GINA 2024) were hand searched for additional sources.

Articles were considered eligible for inclusion if they were published in a peer reviewed journal (or, for background epidemiological and guideline context, an authoritative institutional or governmental source), were available in English, and addressed at least one of: the epidemiology or economic burden of asthma or ICS/LABA non adherence; the validity or performance characteristics of an adherence measurement method; the biological mechanism of drug incorporation into hair; the analytical chemistry of drug or corticosteroid quantification in hair; or clinical/observational evidence on hair analysis as an adherence or exposure biomarker in any therapeutic area. Conference abstracts without full text, non-English sources, and material that could not be independently verified were excluded.

Because this synthesis draws on a heterogeneous evidence base spanning pharmacology, forensic toxicology, respiratory medicine and health services research, no formal risk of bias score was applied uniformly across all included sources; instead, the design and limitations of each key primary study are described narratively at the point of citation, consistent with the conventions of published narrative reviews in this field [42,46]. The review does not report a numerical PRISMA study selection flow diagram, since no single bibliographic database was queried through a fully reproducible Boolean search protocol; this is stated here explicitly rather than implied, and should be read as a limitation of the present synthesis (see Section 6).

3. THE BURDEN OF ICS/LABA NON-ADHERENCE IN ASTHMA

Multiple large retrospective cohorts using pharmacy claims and electronic monitoring data converge on the same conclusion: adherence to maintenance ICS/LABA therapy in routine practice is poor, and its consequences are clinically and economically substantial. A large US claims based analysis found that patients with a proportion of days covered below 0.8 experienced significantly more overall and severe (inpatient/emergency department) exacerbations than adherent patients [8]. Electronic inhaler-sensor studies have shown that patients on twice daily fluticasone propionate/salmeterol achieved a sufficient daily dose only about one third of the time, compared with approximately 60% of the time for once-daily fluticasone furoate/vilanterol, suggesting that dosing frequency itself is an important, modifiable determinant of adherence [13]. The pragmatic, open label Salford Lung Study similarly found that a substantial minority of patients randomised to once daily fluticasone furoate/vilanterol switched away from or discontinued their assigned medication during real world follow up [11].

Persistence, a related but distinct construct describing continuation of therapy over time (as opposed to the completeness of dosing while on therapy), is also modest: a Spanish retrospective cohort found twelve month persistence rates across several ICS/LABA combinations clustering around 60 - 68%, with medication possession ratios in the 74 - 81% range [15]. Longer term data extending to twelve years after diagnosis show that a meaningful proportion of patients with persistently uncontrolled asthma have high measured adherence, underscoring that non adherence, while important, is not the sole explanation for poor control and must be distinguished from other causes such as incorrect inhaler technique or corticosteroid resistant phenotypes [14].

The downstream cost of this gap is considerable. In the United States, uncontrolled asthma has been projected to generate over $300 billion in direct costs over twenty years [25], and severe, exacerbation prone asthma alone has been associated with several thousand dollars in additional annual per patient cost driven predominantly by hospitalisation and emergency care [24,28]. A systematic review of the global economic burden of asthma identified hospitalisation and medication as the largest drivers of direct cost, and work or school absence as the largest driver of indirect cost, across sixty eight included studies spanning more than four decades [27]. A broader narrative synthesis of non-adherence in asthma and chronic obstructive pulmonary disease similarly concluded that indirect costs from lost productivity often exceed direct medical costs, and that adherence rates above 80% are consistently associated with improved clinical and economic outcomes [26]. Access and affordability further compound the problem in resource limited settings, where cost has been identified as a major, and largely modifiable, barrier to consistent inhaler use [20,29].

4. CURRENT METHODS OF MEASURING MEDICATION ADHERENCE: STRENGTHS AND LIMITATIONS

Adherence measurement methods are broadly classified as indirect (self report, pill/canister counts, pharmacy refill data, electronic monitoring) or direct (detection of the drug or a metabolite in a biological specimen) [18]. Each method occupies a different position on the trade-off between objectivity, cost, feasibility and the length of the retrospective window it can capture, and no single method has been shown to be universally superior; this has led to repeated calls in the literature for multi-method or triangulated approaches to adherence assessment [18,19].

Self report remains the most widely used method in both clinical practice and research because of its low cost and ease of administration. Validated instruments such as the MMAS-8 and the disease specific Test of Adherence to Inhalers (TAI) have demonstrated acceptable internal consistency and criterion validity against outcomes such as the Asthma Control Test and quality of life scores [39,40,41]. Even so, systematic comparisons consistently find that self-reported adherence exceeds adherence measured by objective means: in one contraceptive adherence study, 76% of participants self-reported high adherence compared with only 68% by pharmacy claims based proportion of days covered, with only 54% classified as highly adherent by both methods simultaneously [37]. Comparable overestimation by self report relative to electronic monitoring has been documented across conditions as varied as malaria treatment, rheumatoid arthritis and HIV [22,31,38].

Pill or inhaler canister counts and weight based methods are objective in the sense that they do not rely on patient recall, but they measure medication removed from packaging rather than medication actually inhaled, and are similarly prone to overestimation: an early comparative study found that medication-log and canister weight estimates were consistently higher than those obtained from electronic monitors in the same patients [17,30]. Pharmacy refill based metrics (proportion of days covered, medication possession ratio) are attractive for large scale retrospective research because they can be derived from routinely collected administrative data without direct patient burden, and are endorsed by pharmacy quality organisations as a

preferred adherence metric for chronic disease [37]; however, they capture possession rather than consumption, cannot detect within day timing errors, and are unavailable in health systems without integrated electronic pharmacy records a significant constraint in many Indian clinical settings. Electronic monitoring, whether through medication event monitoring systems (MEMS) for oral therapy or inhaler embedded sensors for respiratory disease, provides the most granular indirect data, including the exact time and frequency of device actuation, and has been used as a reference standard against which other indirect methods are validated [13,17,32,38]. Its principal limitations are cost, the need for patient consent to continuous monitoring, the possibility of "dumping" (activating the device without actually inhaling the dose), and limited availability of validated sensors for many inhaler devices marketed in India. Direct biological methods plasma, urine or saliva drug level testing offer unambiguous, objective confirmation that a dose has entered systemic circulation, and have long been used for therapeutic drug monitoring of narrow therapeutic index agents such as tacrolimus after organ transplantation [65,66,67]. Their principal limitation for chronic, self-administered outpatient therapy is temporal: plasma and urine concentrations reflect only the hours immediately preceding sampling, so a single clinic day sample can be confounded by short term "white coat adherence", in which patients take their medication conscientiously in the days before a scheduled review [17,18]. This temporal limitation is precisely the gap that segmental hair analysis is positioned to address, and is discussed in detail in the following section.

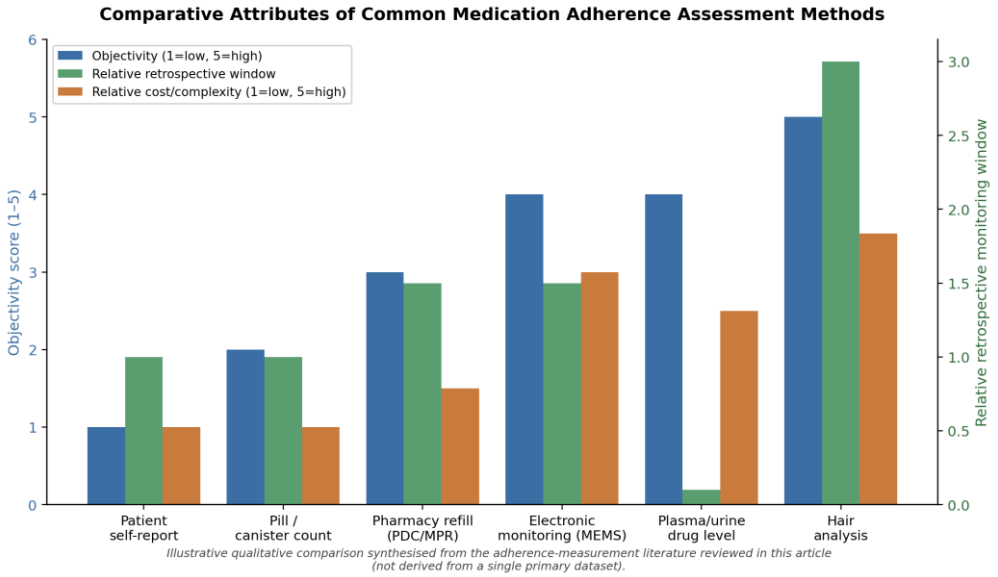

Figure 2. Illustrative qualitative comparison of the objectivity, retrospective monitoring window, and relative cost/complexity of commonly used medication-adherence assessment methods, synthesised from the literature discussed in Sections 4 and 6 of this review. Scores are qualitative and relative, intended to illustrate the trade-offs discussed in the text rather than to represent a validated measurement instrument or a single primary dataset. Original figure prepared for this review.

5. HAIR AS A BIOLOGICAL MATRIX FOR DRUG MONITORING

5.1 Mechanisms of drug incorporation into hair

Human scalp hair grows continuously from follicles anchored in the dermis, at an average rate widely cited as approximately one centimeter per month, though individual and regional variation exists [29,47,51]. As the hair shaft forms and keratinises, it can incorporate xenobiotics including corticosteroids, bronchodilators, and a wide range of other therapeutic and illicit drugs through several overlapping pathways [29,51]. The best characterized of these are: (i) an endogenous pathway, in which drug molecules circulating in the blood diffuse from the dermal papilla capillary network into the actively growing hair matrix; (ii) an endogenous exogenous pathway, in which drug is transferred to the hair surface via sweat and sebaceous secretions after systemic absorption and dermal excretion; and (iii) a purely exogenous, external pathway, in which airborne particulates, environmental residues or topically applied products contaminate the hair surface without any systemic drug exposure having occurred [29,51]. Distinguishing genuine systemic incorporation (relevant to adherence) from external contamination (a false signal of exposure) is one of the central analytical and interpretive challenges of the field, discussed further in Section 6. Micro segmental analyses using single hair, sub millimeter sectioning combined with liquid chromatography tandem mass spectrometry

(LC-MS/MS) and, in some studies, mass spectrometry imaging, have shown that after a single oral dose, drug first appears concentrated near the hair root and bulb, subsequently moving distally along the shaft at a rate consistent with the individual's hair growth velocity [47,48]. Because incorporation occurs close to the time of systemic exposure and the labelled segment then moves outward as the hair continues to grow, a hair sample of several centimeters in length effectively encodes a chronological, month by month exposure history, in a manner directly analogous to tree rings recording annual growth [47,50,51].

5.2 Analytical methods for corticosteroid and bronchodilator quantification in hair

Because inhaled medications and endogenous glucocorticoids are present in hair at very low (pictogram to nanogram per milligram) concentrations, sensitive and selective analytical platforms are required. LC-MS/MS, typically preceded by hair washing/decontamination, pulverization, and methanol or acid based extraction with solid phase clean up, has become the reference method for both illicit and therapeutic drug quantification in hair, owing to its combination of high sensitivity, chromatographic separation and structural specificity via multiple reaction monitoring [29,33,51,52]. Validated LC-MS/MS methods now exist for a wide range of corticosteroids (cortisol and cortisone) in human hair, with limits of quantification in the sub picogram per milligram range and demonstrated linearity, accuracy and precision suitable for clinical use [33,52,54]. Related methods, including a lower cost high performance liquid chromatography with fluorescence detection (HPLC-FLU) approach, have been developed and cross validated against mass spectrometric reference methods, offering a potentially more accessible option for laboratories without ready mass spectrometry access [36]. The same underlying methodology sample decontamination, segmental cutting, extraction, and LC-MS/MS or immunoassay based quantification has already been adapted for a wide range of drug classes across doping control and forensic toxicology, including bronchodilators, and multiplexed dynamic multiple reaction monitoring panels capable of screening several therapeutic and illicit drug classes from a single hair sample in one analytical run [51]. This existing analytical infrastructure provides a directly transferable technical foundation for quantifying ICS and LABA compounds, although method specific validation (selectivity, matrix effects, stability) for each individual molecule of interest remains necessary before clinical application [42].

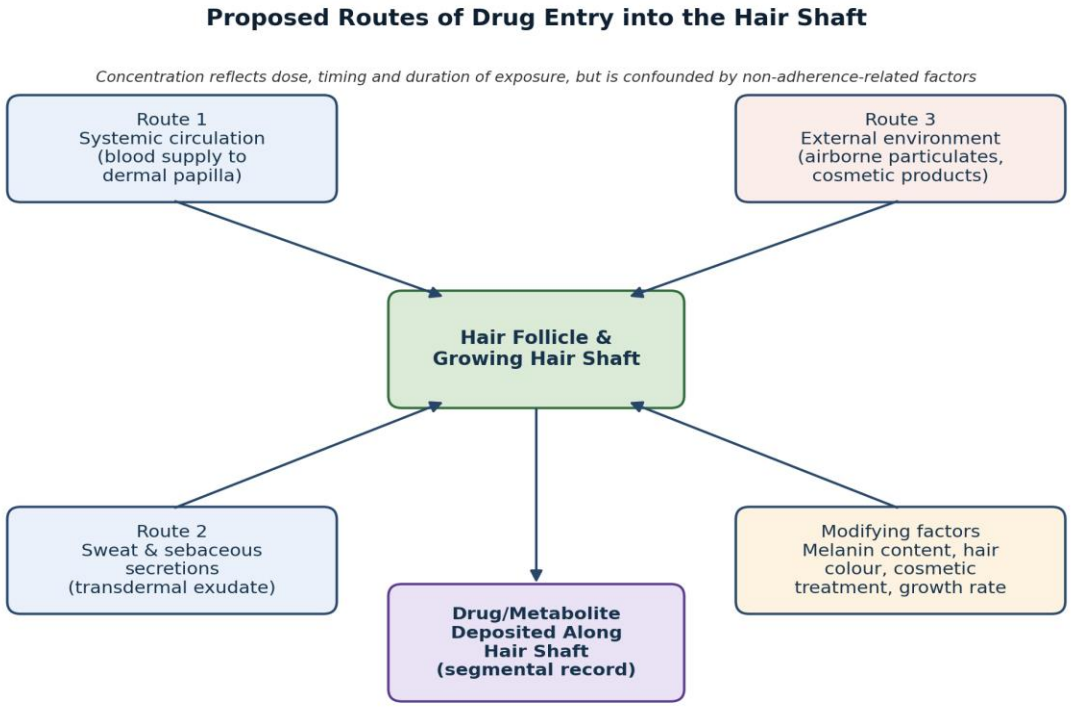

Figure 1. Conceptual schematic of the principal routes by which systemically and externally derived drug reaches the hair shaft. Route 1 (systemic circulation) and Route 2 (sweat/sebaceous secretion) reflect genuine drug exposure and are the basis for adherence inference; Route 3 (external environmental contamination) and hair-colour/cosmetic modifying factors are potential sources of false signal that must be controlled for through sample decontamination and documentation of hair treatment history. Original figure prepared for this review; not reproduced from any copyrighted source.

6. CLINICAL EVIDENCE FOR HAIR ANALYSIS AS AN ADHERENCE BIOMARKER

6.1 Antiretroviral therapy: the most mature application

The strongest clinical validation of hair analysis as an adherence biomarker comes from HIV medicine. A systematic review of thirty one studies concluded that hair antiretroviral concentration correlates meaningfully with other pharmacokinetic exposure measures (plasma, peripheral blood mononuclear cell and dried blood spot drug levels), is one of the strongest independent predictors of virological suppression, and for tenofovir specifically is also associated with drug related renal toxicity in large cohorts, indicating that hair concentration reflects a clinically meaningful cumulative exposure rather than a laboratory curiosity [43,44]. This body of evidence is the principal reason hair analysis is now considered a credible, if still specialized, tool for long term medication exposure monitoring, and it establishes the general proof of concept that the present review extends to a new therapeutic area.

6.2 Antihypertensive and other chronic oral therapies

A recent review addressing antihypertensive therapy noted that, despite forty five years of literature on measuring adherence in hypertension, none of the forty seven review articles it screened had previously considered hair based therapeutic drug monitoring as an adherence method, identifying this as an explicit evidence gap that mirrors the gap in respiratory medicine addressed here [46]. This parallel is instructive: both fields possess mature indirect adherence measurement literatures, but only recently have investigators begun to ask whether a hair based direct method could add discriminating value beyond existing tools.

6.3 Solid organ transplantation: hair analysis for narrow therapeutic index drugs

Immunosuppression after kidney transplantation offers one of the most clinically pressing use cases for objective adherence monitoring, because non adherence to calcineurin inhibitors such as tacrolimus is a recognized driver of late graft rejection, while both under and over immunosuppression carry serious risk [65,66,68]. A recent feasibility study demonstrated that tacrolimus can be reliably quantified in the scalp hair of kidney transplant recipients by LC-MS/MS, with hair concentrations correlating with prescribed dose and blood trough levels, and with a rapid "wash in" pattern consistent with genuine incorporation from systemic exposure rather than surface contamination [65]. This is a particularly relevant precedent for ICS/LABA hair analysis, since tacrolimus, like inhaled corticosteroids, is dosed chronically at doses producing relatively low absolute systemic concentrations, and yet was still successfully and reproducibly quantified in hair.

6.4 Psychotropic and hormonal applications

Hair analysis has also been explored for antipsychotic medications such as clozapine, where positive correlations between daily dose and hair concentration across multiple hair segments have been reported, supporting its use as a semi quantitative adherence marker in psychiatric populations who are often difficult to monitor by other means [45]. In parallel, an extensive and separate literature has validated hair cortisol and cortisone measurement using broadly the same analytical pipeline proposed for exogenous corticosteroids as a biomarker of chronic endogenous glucocorticoid exposure in conditions ranging from Cushing syndrome and adrenal incidentaloma to chronic stress and circadian rhythm research [52,54,56,57,58]. Although these hormonal studies measure endogenous rather than inhaled exogenous corticosteroid, they provide directly relevant, well validated analytical groundwork sample preparation, extraction chemistry, and LC-MS/MS quantification protocols for measuring exogenous ICS compounds in the same hair matrix.

6.5 Inhaled respiratory medications specifically

Direct evidence in the inhaled medication population is the least mature branch of this literature. A dedicated narrative review of hair analysis for inhaled respiratory medication adherence concluded that, while several inhaled drugs are detectable in hair and the general analytical and mechanistic groundwork from other drug classes is transferable, the number of clinical studies directly validating hair analysis against other adherence measures in asthma or COPD populations remains small, and clinically meaningful cut off concentrations distinguishing adherent from non-adherent patients have not yet been established [42]. The review explicitly concluded that hair analysis should not, at the present stage of evidence, be relied upon as a sole measure of inhaled medication adherence, but rather as a promising complementary tool pending further validation [42]. This conclusion directly frames the evidence gap that further primary clinical research of the kind represented by the ongoing hair analysis based ICS/LABA adherence study referenced in the introduction of this article is positioned to help close.

7. CONFOUNDING FACTORS AND ANALYTICAL LIMITATIONS

Several biological and methodological factors complicate the interpretation of hair drug concentrations and must be accounted for in any adherence-validation study.

Hair colour and melanin content. Basic, lipophilic drugs bind preferentially to melanin, and multiple studies have reported higher concentrations of certain drugs (for example codeine, cocaine and amphetamine) in darker, more heavily pigmented hair compared with blond or red hair from individuals with comparable exposure, raising the possibility of a systematic "colour bias" that could disadvantage or advantage specific patient groups if not accounted for analytically [55,59,61,62,64]. The magnitude and clinical significance of this effect remains debated: some authors argue the evidence for a consequential melanin driven bias is limited and that drugs also bind substantially to non-melanin keratin protein, while others have found that normalizing for melanin content only partially removes the observed disparity [59,60,61]. For a validation study in an Indian population where hair pigmentation is relatively homogeneous compared with multi ethnic Western cohorts this potential confounder is somewhat attenuated, but should still be explicitly considered and, where feasible, statistically adjusted for in the analysis plan.

Cosmetic treatment and external contamination. Chemical treatments such as bleaching, dyeing, perming and thermal straightening open the hair cuticle, increase susceptibility to both external contamination and loss of previously incorporated drug, and have been shown to reduce measurable drug and alcohol marker concentrations by 30 - 80%, in some cases down to non-detectable levels [59,60,63]. Ethnic and cosmetic hair care products containing water, humectants or oils have also been shown experimentally to increase surface contamination and produce false positive drug signals unrelated to true systemic exposure, a phenomenon sometimes termed "cultural bias" in the forensic toxicology literature [55]. Robust, standardised decontamination protocols (typically sequential organic solvent washing prior to extraction) are therefore essential, although no single decontamination method has achieved universal consensus, and residual uncertainty about the completeness of external contaminant removal remains an acknowledged limitation of the field as a whole [61,63].

Hair growth rate variability and segmental resolution. Because the temporal interpretation of segmental hair analysis depends on an assumed, relatively constant growth rate (commonly approximated at 1 cm/month), individual variation in growth rate influenced by age, sex, nutritional status, hormonal factors, and hair cycle phase can shift or blur the correspondence between a given hair segment and the calendar period it is assumed to represent, particularly for micro segmental (sub centimeter) analyses [47,50]. For adherence monitoring purposes using centimeter scale segments over a period of several months, this source of error is generally considered acceptable, but should still be disclosed as a limitation when interpreting individual level results.

Analytical sensitivity for inhaled medications specifically. Because inhaled corticosteroids and long acting β2 agonists are formulated and delivered specifically to minimise systemic absorption (their therapeutic action being local, in the airway), the fraction reaching the systemic circulation and therefore available for incorporation into hair is inherently smaller and more variable than for orally administered drugs of the same or related classes [42]. This raises the analytical bar for limit of detection and limit of quantification performance and increases the risk that non detection in hair may reflect low systemic absorption rather than true non adherence, a distinction that validation studies must explicitly address, for example by correlating hair concentrations with device confirmed dosing (electronic monitoring) rather than assuming perfect specificity from the outset [13,38,42].

Absence of validated adherence thresholds. Unlike some analogous applications (for example, tenofovir hair concentration thresholds validated against HIV virological outcomes), no consensus concentration cut off currently distinguishes "adherent" from "non-adherent" patients for any ICS or LABA compound in hair. Establishing such thresholds will require prospective studies that compare hair concentrations against a robust reference standard most plausibly, electronic dose counter or inhaler sensor data in adequately sized, well characterized cohorts [13,42].

8. DISCUSSION

This review brings together three previously separate literatures the epidemiology and cost of ICS/LABA non adherence in asthma, the comparative performance of existing adherence measurement tools, and the biological/analytical basis of hair analysis as validated in other therapeutic areas to evaluate a single applied question: can hair analysis meaningfully improve how ICS/LABA adherence is measured in asthma care and research? The synthesis suggests a cautiously optimistic answer. The core rationale is sound and empirically supported in analogous drug classes: hair analysis in HIV medicine is validated against

hard clinical outcomes, and pilot data in kidney transplant immunosuppression demonstrate that even chronically dosed drugs producing modest systemic concentrations can be reliably quantified and shown to correlate with prescribed dose and blood levels [43,44,65]. The analytical infrastructure required LC-MS/MS-based extraction, decontamination and quantification is mature, transferable, and already partly validated for structurally related corticosteroid compounds through the hair cortisol literature [33,52,54].

At the same time, the review identifies a genuine and specific evidence gap for inhaled respiratory medications: systemic absorption is deliberately minimised by inhaler design, direct clinical validation studies in asthma populations remain few, and no adherence defining concentration threshold yet exists for any ICS or LABA molecule in hair [42]. This is precisely the gap that a well-designed, adequately powered clinical validation study correlating segmental hair concentrations of the relevant ICS/LABA molecule with a robust reference standard such as inhaler-sensor-confirmed dosing, alongside self-reported adherence instruments such as the MMAS-8 or TAI for triangulation is positioned to address [13,39,40,42]. Embedding such a study within an interdepartmental collaboration between pharmacy/pharmacology and pulmonary medicine, with careful attention to the confounders discussed in Section 7 (decontamination protocol, documentation of any cosmetic hair treatment, and correlation against an objective reference standard rather than self-report alone), would directly respond to the limitations this review has identified in the existing literature.

From a health systems perspective, an adherence biomarker that requires only a single, low burden, non-invasive hair sample rather than repeated blood draws, expensive electronic sensors, or reliance on self report would be particularly well suited to resource constrained outpatient settings such as those serving much of India's asthma population, where the burden of disease is high, follow up visit frequency is often limited, and cost is a recognized barrier both to treatment and to intensive monitoring [2,4,20,29]. Even short of full clinical translation, objective, quantitative confirmation of adherence status could meaningfully improve clinical decision making in a currently ambiguous but common scenario: the patient whose asthma remains poorly controlled despite apparently appropriate prescribing, where distinguishing true non adherence from other causes of poor control (incorrect inhaler technique, under treatment, or a corticosteroid refractory phenotype) has direct implications for the next step in management [14,18].

9. STRENGTHS AND LIMITATIONS OF THIS REVIEW

This review synthesises evidence across several normally separate literatures (respiratory epidemiology, adherence measurement science, analytical/forensic toxicology, and hair based biomarker validation in other chronic diseases) to construct a focused evaluation of a specific, clinically motivated question, which we believe is a novel contribution given that no prior review has, to our knowledge, brought these strands together specifically for ICS/LABA therapy in asthma.

Several limitations should nonetheless be acknowledged. First, as noted in Section 2, this synthesis was conducted as a structured narrative review rather than a fully reproducible, multi database systematic search with independent dual screening and a quantitative PRISMA flow diagram; while the search strategy and eligibility criteria are reported transparently, formal replication of the search is not guaranteed. Second, direct clinical evidence on hair analysis for inhaled respiratory medication adherence specifically is genuinely limited in the primary literature, meaning that much of this review's argument for feasibility necessarily rests on analogical evidence from other drug classes rather than a large body of directly applicable clinical trials; this is stated as an evidence gap rather than papered over. Third, some included sources are institutional, guideline, or industry/clinical education material (for example GINA strategy reports and adherence education resources) rather than original peer reviewed research; these are used only for epidemiological or practice context, and are clearly distinguishable in the reference list from primary research articles. Finally, because no formal quantitative meta-analysis was performed, this review cannot provide a pooled estimate of, for example, the correlation coefficient between hair ICS concentration and adherence a gap that future systematic reviews, once a larger body of primary studies exists, should aim to fill.

10. CONCLUSION

Non adherence to ICS/LABA therapy is common, costly, and difficult to measure reliably with the tools presently in widespread use. Hair analysis offers a biologically plausible, analytically mature, and already partly validated approach to objective, longitudinal medication exposure monitoring in other chronic disease contexts, and the same underlying mechanisms and laboratory methods are, in principle, transferable to inhaled corticosteroids and long acting β2 agonists. However, the specific clinical evidence base for this application remains immature, constrained by the low systemic bioavailability inherent to inhaled therapy, unresolved questions about hair colour and cosmetic treatment confounding, and the absence of validated adherence

defining concentration thresholds. Prospective, adequately designed clinical studies that correlate segmental hair ICS/LABA concentrations against robust reference standard adherence measures are needed before this promising biomarker can be recommended for routine clinical or research use a need that the present review identifies as both scientifically justified and directly actionable through ongoing institutional research.

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