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Noninvasive Disease Diagnostics: The Swiss Contribution Highlights to Breath Analysis Research

Stefan James Swift[1] | Kseniya Dryahina[2] | Patrik Španěl[2] | Flora Kontopidou[3] | Christos Kokkotis[4] | Renato Zenobi[1] | Stamatios Giannoukos[1]

[1]Laboratory of Organic Chemistry, ETH Zurich, Zurich, Switzerland | [2]J. Heyrovsky Institute of Physical Chemistry of CAS, Prague, Czechia | [3]Mitera Childrens' Hospital, Athens, Greece | [4]AIDEAS OÜ, Tallinn, Estonia

Correspondence: Stamatios Giannoukos (stamatios.giannoukos@org.chem.ethz.ch)

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ABSTRACT

To celebrate the 125th anniversary of the Swiss Chemical Society, we present a review and perspective to highlight the recent research in breath analysis that has been conducted in Switzerland, with a particular focus on secondary electrospray ionization mass spectrometry (SESI-HR-MS). We focus on breath analysis research from 2019, the publication year of the last major review. We highlight where improvements are needed in experimental and clinical protocols and outline the current gaps in the field, to support the implementation of breath analysis into the clinical domain.

1 | Introduction

Even since the days of Hippocrates (estimated 460 BCE to 370 BCE), the concept of diagnosing ailments using breath was around [1, 2]. In more recent history, dogs have been reported to have identified incidences of melanoma in their owner [3] and have been trained to help identify patients with specific diseases [4–9]. The fundamentals behind this concept are associated with the smell of volatile organic compounds (VOCs) emitted from diseased persons. In recent years, this idea has expanded to using mass spectrometry as a more precise sniffing tool in the effort to standardize the diagnostic process of "smelling disease." By using qualitative and quantitative techniques such as mass spectrometry, further understanding and useful information can be gathered regarding the health and metabolic processes in an individual, thereby advancing biomarker discovery and the field of clinical diagnostics [10–12].

The human body undergoes a range of metabolic reactions to maintain a person's health and normal functioning. These biochemical reactions lead to the production of volatile metabolites, which are eventually secreted by the body in some form of biofluid. On the incidence of disease, these metabolic processes are altered, inevitably changing the VOCs produced by the body's biochemical processes due to changes in the cell's metabolic processes [13–16]. As a result, VOC fingerprints within biofluids may be used to help identify a healthy or a sick individual, with specific profiles that may show the presence of particular diseases [4, 12, 17, 18], as well as monitor environmental exposure, microbiota, bacterial activity, pharmacokinetics, toxicokinetics, and other metabolic processes, for use in medical diagnostics [14, 22].

In addition to the routine biofluids analyzed in biomedical science (blood, urine, feces, etc.), human breath is also a biofluid matrix that contains a wealth of information, mirroring the body's health [23]. Although the main constituents of breath are the same as those of air (primarily N_2, O_2, and CO_2) [24], it also contains hundreds [16, 17, 25–27], and according to some sources even thousands [28], of low-boiling-point [16, 18] VOCs [19, 20]. These occur in concentrations ranging from ppt_v to ppm_v [22], along with non-volatile compounds present

in microdroplets and breath aerosol [10], together carrying a wealth of chemical information [11]. The VOCs and semi-VOCs released from metabolic reactions occurring within cells in the body are transported in the blood around the body to the lungs, where they cross the blood–air barrier and enter the breath of a human [17, 20, 29–31]. Human breath is therefore another biofluid that, although rarely applied in routine clinical diagnostics to date [10, 32], may provide valuable insights into an individual's metabolic [29, 33] and physiological [27] state, as well as systemic metabolic processes [17]. Monitoring VOCs in breath can also provide insights into diurnal metabolic patterns and is being used in research to identify unknown human breath biomarkers [30]. Many biomarkers remain undiscovered because certain VOC species have very short lifetimes [22] and/or occur only at very low concentrations [22].

The advantages of using human breath as an analytical medium to monitor human health and diagnose disease are manifold. The nature of breath analysis which requires a subject to breath into an offline sampling bag or a device to capture exhaled breath condensate (EBC), or an online inlet to an instrument [4, 9, 11, 34, 35]. This immediately makes this technique harmless [36], noninvasive [9, 11, 12, 14, 16, 18, 25, 27, 34, 37–39], and avoids the need for intrusive clinical practices for disease diagnosis which is especially attractive in pediatrics [34, 37]. The ability to perform online sampling further renders this technique rapid [14, 25, 39], both in sample collection and results retrieval, as it typically eliminates the need for sample preparation [16] thereby underscoring its potential for clinical implementation. Furthermore, due to the ease of sampling, its rapid nature, and the absence of specialized personnel required to operate instruments [40], the prospect of continuous health monitoring becomes a real possibility, which allows for [11] early disease diagnostics [39] and timely treatment decisions [21, 39]. The alteration of metabolic processes starts at disease onset, well before any symptoms appear, and typically only at later stages of a disease when the illness is much more difficult to treat.

One particular mass spectrometric technique, which has been extensively used in breath analysis research in Switzerland, is secondary electrospray ionization high-resolution mass spectrometry (SESI-HR-MS). This soft ionization method can be applied to both online and offline analyses, offering high sensitivity. When coupled to high-resolution mass analyzers (e.g., Orbitrap, TOF), it enables high-resolution [13] untargeted *m/z* feature detection [26], with thousands of features detected across entire breath profiles [12, 37]. It is semi-quantitative and may also be used in targeted VOC analyses, where compound identification provides valuable insights into the biochemical [25, 31] and metabolic processes underlying diseases [12, 41]. Furthermore, SESI-HR-MS is particularly effective for the analysis of polar molecules [21].

With SESI-HR-MS and breath analysis as an entity, there are, however, also limitations and challenges. These revolve around the differentiation between endogenous and exogenous [14] compounds [24, 36], the general accuracy in compound identification [40], and quantification with different breath analysis instrumentation techniques [26]. This is further intertwined with a lack of understanding of the metabolic processes leading to the detected VOCs [14]. The lack of validation studies [4], standardization [13, 36], and the practicalities surrounding instrumental mobility pose further challenges [18]. The advantages and limitations of breath analysis research are therefore explored within this review. Nonetheless, breath analysis is an emerging [12] and rapidly growing field in medical diagnostics [11], with great potential for the introduction of breath analysis into the clinical environment in the future [19, 42].

2 | Breath Analysis and Disease Research in Switzerland

The field of breath analysis is undoubtedly expanding. To illustrate Switzerland's position within this research domain, we conducted a bibliometric analysis using the Scopus database [43]. As of 4 September, 2025, a search for the term "Breath Analysis" returned 43,926 publications. After refining the results to include only journal articles at the final publication stage, 35,366 records remained, spanning global research outputs since 1930. Of these, 15,273 originated from European institutions and 1021 from Swiss institutions. An overview of the different research institutes that have contributed in some way to breath analysis research since 1930 has been outlined below and mapped onto a map of Switzerland (Figure 1). The research institutes have been listed by canton. The number of publications by researchers from the respective Swiss institutes is indicated by the number after the institute's name. Agroscope and Vetsuisse-Fakultät are both inter-cantonal institutions, with multiple sites.

Finally, a Scopus-based analysis was performed to assess the contributions of funding agencies to successful breath analysis projects. Figure 2 presents the number of publications acknowledging each funding body. Since 2019, the Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung (Swiss National Science Foundation) has been the most prominent funding agency.

It should however be noted that different publications will have had varying holistic amounts associated with them. The data in Figure 2 are from 2019 and therefore represent the most recent contributions. The year 2019 was also the year of the last comprehensive review on breath analysis research by Bruderer et al. [1], from which the highlights mentioned in this review are based (Figure 3).

3 | SESI-HR-MS: A Key Technology in Swiss Breath Research

SESI-HR-MS is an instrumental technique used for the monitoring of VOCs and semi-VOCs [39], as well as non-volatile gaseous species [33], and is most well-known for its application in breath analysis research. It is popular among the breath analysis community in Switzerland, due to its soft-ionization nature, high resolution (when coupled to a high-resolution mass analyzer), and high sensitivity [1, 44, 45]. It is a direct infusion technique [46], which, in recent years, has evolved from an experimental technology in the laboratory into a profiling tool for the clinical setting [20, 47], used for biomarker discovery in breath research [48]. It is an emerging technology with increasing popularity [47] for VOC metabolite analyses [49], enabling real-time breath

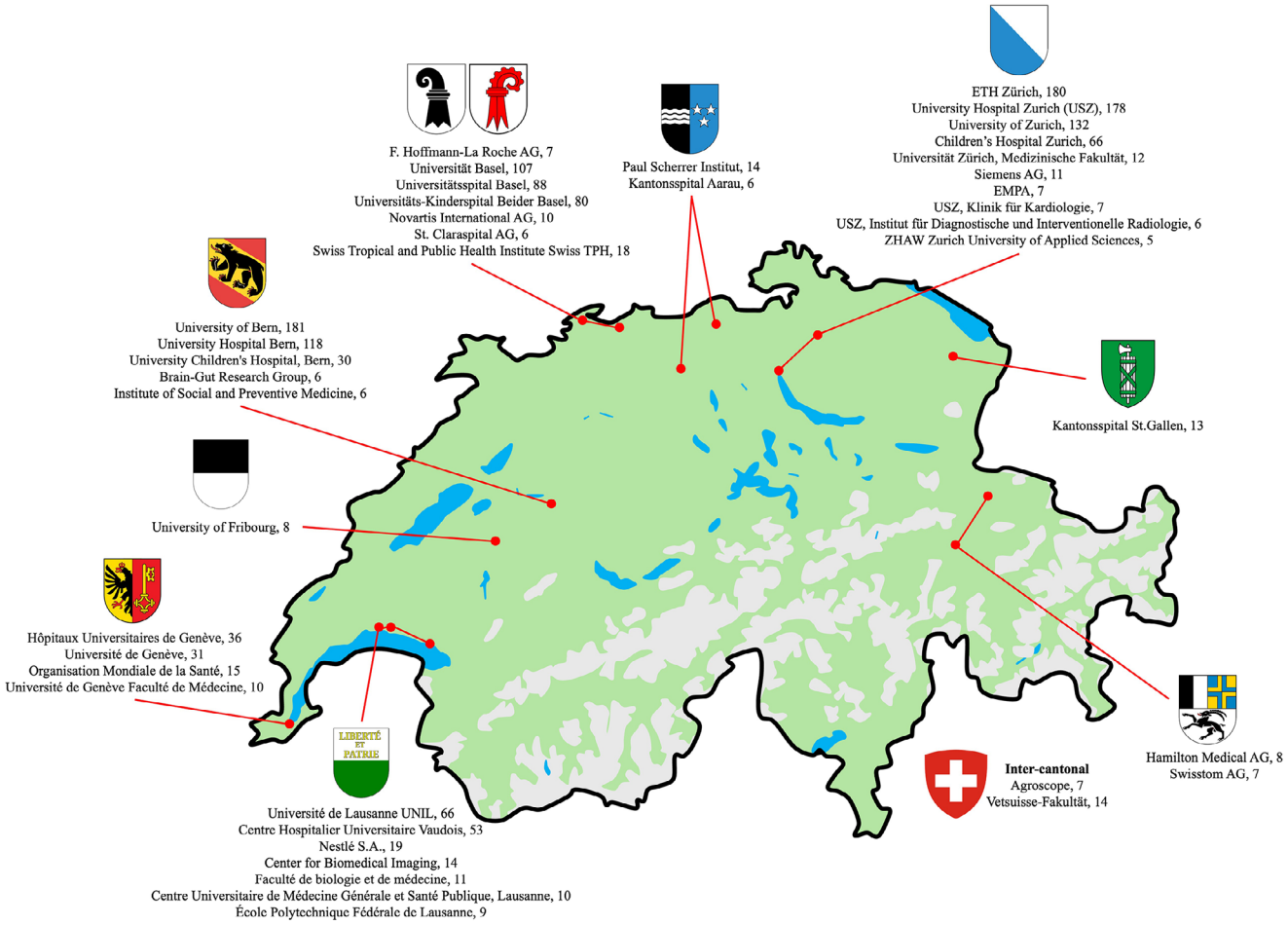

FIGURE 1 | Research institutes across Switzerland that have contributed to breath analysis research since 1930.

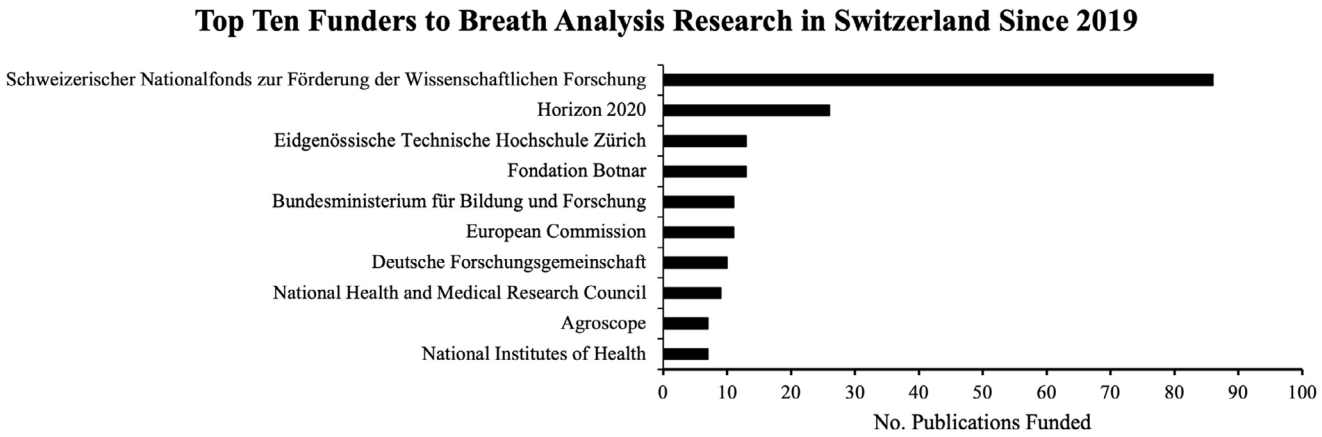

FIGURE 2 | Bar chart showing the top ten funders of breath analysis research by the number of papers published.

monitoring through repeated full mass scans, allowing for a breath sample to be tracked and monitored [49].

3.1 | Development of SESI-HR-MS

SESI-HR-MS is one of the most recent advancements in breath analysis research technology. The evolution of modern breath analysis began with the seminal work of Pauling et al. [50], who detected over 250 compounds in a breath condensate sample using offline GC-MS. Subsequently, John B. Fenn and colleagues developed electrospray ionization (ESI), demonstrating the ionization of neutral molecules in both liquid and gaseous phases [33, 51]. Building on this, Hill and co-workers later introduced the concept of "Secondary Electrospray Ionization (SESI)" [33, 52, 53], first exploited by Wu et al. [53] and Chen et al. [54]. A

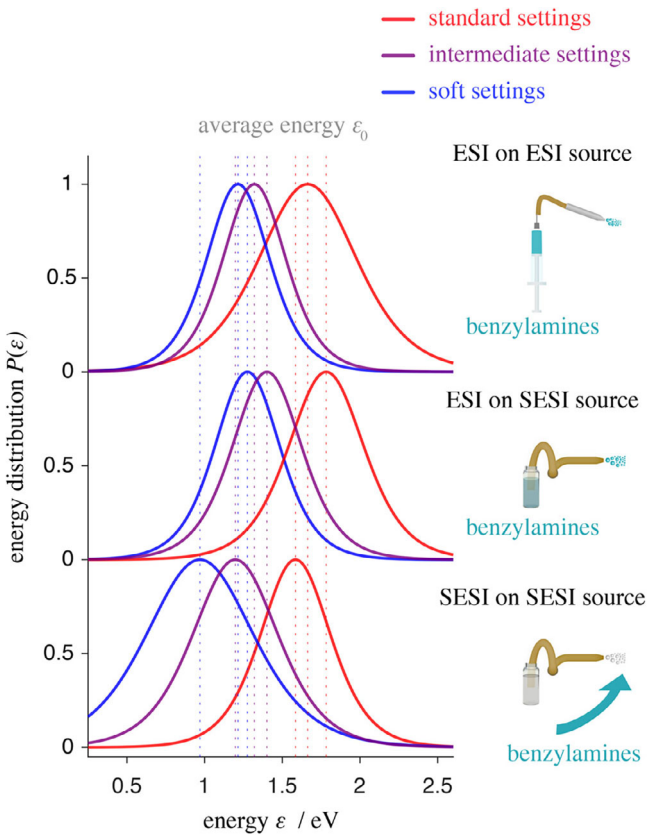

FIGURE 3 | Comparison of the internal energy distribution $P(\varepsilon)$ for different ionization setups and different settings. The dotted line represents the average energy for a distribution. Reproduced from Kaeslin et al. with permission from the American Chemical Society.

major breakthrough occurred with the coupling of SESI to high-resolution mass spectrometry (e.g., Orbitrap systems) [49, 55], first demonstrated in Switzerland by García-Gómez et al. [56]. This integration significantly improved the sensitivity, resolution, selectivity, and compound coverage for VOC analysis [39, 41, 49, 57]. Given the chemical complexity of exhaled breath, accurate VOC identification requires high-resolution mass spectrometry to resolve isobaric species and enable confident annotation of metabolites and biomarkers [29, 49]. Consequently, SESI-HR-MS has become a powerful tool for breath-based biomarker discovery and clinical decision support [39, 41, 47, 56]. The capacity of SESI-HR-MS to detect thousands of features, which are essentially unidentified signals in the mass spectrum [55], strengthens its use for biomarker identification when conducting untargeted analyses [46, 55]. Further technical improvements, such as the incorporation of an ion funnel by Meier et al. [35], extended the accessible mass range up to 500 m/z [33] for online breath profiling. As an ion source, SESI is compatible with any atmospheric pressure ionization mass spectrometer [19, 58] and can also operate within ion mobility systems [39, 52].

Despite its relatively recent emergence in analytical science, SESI-HR-MS has already been applied across diverse domains. These include clinical and biomedical research [29, 39], exhaled breath studies [1, 22, 46, 59], disease detection and monitoring [29, 39, 60], and assessment of therapeutic interventions and health status [33, 60]. Beyond clinical contexts, SESI-HR-MS has been employed to analyze VOC emissions from plants and animals [22, 39], monitor outdoor and indoor air quality [22], investigate cell culture headspace [22], and study pharmacokinetics [20, 60–62].

3.2 | Ionization Mechanisms

SESI-HR-MS detects analyte molecules within a fluid through an ionization process. When analyte molecules enter the SESI source, they interact with the electrospray plume composed of charged droplets, leading to the formation of gas-phase charged species that are subsequently detected by the HR-MS system [39, 46, 48]. Although the detailed ionization mechanism and ion-molecule reaction pathways in SESI are not yet fully understood [23, 57], and only a few fundamental investigations of SESI are available [57], two main models have been proposed. One hypothesis suggests that analyte molecules are solvated within charged electrospray droplets, where ion-molecule reactions occur before the charged products are released back into the gas phase for detection [33, 46], a mechanism which would be largely dependent on analyte solubility. Alternatively, Rioseras et al. [33] proposed that reagent ions evaporate from the electrospray droplets into the gas phase and subsequently react with neutral analyte molecules, forming product ions via gas-phase ion–molecule reactions, a pathway which would likely be influenced by the thermochemistry of the reacting species.

In either case, the principal product ions are protonated molecules (MH^+) [57] in positive mode [39, 48], and deprotonated molecules ($M\text{-}H^-$) in negative mode [39, 48], with ligand or ion switching believed to play a major role [23]. When the electrospray consists of only water, the reactive species are thought to be $(H_2O)_nH^+$ clusters [39, 63]. Unlike the direct proton transfer reactions [46, 64, 65] seen in selected-ion flow-tube mass spectrometry (SIFT-MS) and proton-transfer-reaction mass spectrometry (PTR-MS) in the presence of H_3O^+ reagent ions forming MH^+, or the direct hydrogen abstraction process in SIFT-MS producing $M\text{-}H^-$ species by the newly achieved negative reagent ions (OH^-, O_2^-, O^-, NO_2^-, and NO_3^-), Dryahina et al. [66] proposed a ligand switching pathway for SESI. This would involve a water molecule from a $(H_2O)_nH^+$ cluster exchanging with the analyte molecule to produce a charged analyte-water cluster $M(H_2O)_nH^+$. Within the pathway to the mass spectrometer, water molecules evaporate from this cluster, leaving behind the residual MH^+ (or $M\text{-}H^-$ in the negative mode) [39]. The analyte solvation and ligand switching theory also supports the softness of SESI, consistent with collision-induced dissociation (CID) experiments [46]. At higher analyte concentrations, protonated dimers have also been reported to be produced [48, 67].

3.3 | Electrospray Solution and System Optimization

The composition of the electrospray solution strongly influences the ion–molecule chemistry and, consequently, the nature of the product ions generated in SESI-HR-MS. Adjusting the spray solvent and dopants may therefore enhance ionization efficiency and improve data quality. Most SESI studies employ water [39] as the primary solvent, which has repeatedly been shown to be highly effective for ionization [39, 64]. Some investigations,

however, have utilized methanol-water mixtures (typically 1:1, v/v) [39, 53] or combinations with other organic solvents such as acetonitrile and isopropanol, either individually or mixed [48, 68]. To promote ionization, various dopants have been added to these solvents, including formic acid [20, 39], acetic acid [39, 48, 69–71], and the ammonium formate salt [39, 72], at varying concentrations [39]. The most widely used solution remains 0.1% formic acid in water [48, 73]. Other studies explored more unconventional dopants, including metal salts, which can form characteristic adduct ions and extend the detectable range of compounds [39, 74–76].

A recent study by Wüthrich et al. [48] investigated metal salt doping for untargeted breath metabolomics using SESI-HR-MS. The authors tested both NaI and $AgNO_3$ dopants in a proof-of-principle study using a single human subject. NaI showed fewer and similar metabolite features compared to the standard formic acid solution. $AgNO_3$, however, produced a wider variety of metabolite species in the mass spectra compared to when using the standard formic acid solution, including increased detection of sulfur compounds, primary amines, and unsaturated hydrocarbons. These findings align with previous reports showing a strong affinity of $AgNO_3$ for sulfur-containing species in breath [10, 48]. These results demonstrate that modifying the spray solution and, consequently, the ion chemistry may reveal new metabolic features and potential biomarkers, thus opening new avenues for SESI-HR-MS breath analysis. Nonetheless, formic acid solutions still provide the most consistent signal intensity, sensitivity, and economic practicality, and thus remain the preferred choice for most applications.

Beyond the spray solution, several hardware parameters also critically influence SESI performance. The type of capillary—commonly fused silica, its inner diameter, and the alignment between the sample inlet, the electrospray capillary, and the mass spectrometer inlet are all important optimization factors [39]. Recent developments include the integration of interfaces that measure breath flow rates and CO_2 concentrations, thereby improving standardization and reproducibility in online breath sampling [47].

3.4 | Comparison of SESI-HR-MS With Other Techniques

The most commonly employed and sensitive analytical methods [60] for breath analysis include SESI-HR-MS, SIFT-MS, and PTR-MS for online [19, 57] analysis, and GC-MS for offline [38, 49] analysis. For breath analysis, SESI-HR-MS offers several advantages over SIFT-MS and PTR-MS. It has been reported to achieve higher sensitivities [23], particularly for polar (i.e., amines) and higher m/z compounds [57], compared to the other online methods [44, 48]. Like SIFT-MS and PTR-MS, SESI-HR-MS not only requires no-sample pre-treatment which simplifies workflows but also precludes sample pre-concentration, a process that is feasible with GC-MS [47].

Unlike SIFT-MS, which provides absolute quantification based on well-characterized ion–molecule reaction kinetics, both PTR-MS and SESI-HR-MS are semiquantitative [33]. The kinetic rate constants and reaction mechanisms for SIFT-MS and PTR-MS are well established [44], whereas the fundamental ionization chemistry of SESI-HR-MS remains less understood. Consequently, SESI ionization efficiencies are matrix- and compound-dependent, often requiring external gas standards for accurate quantification [55]. This makes SESI-HR-MS somewhat more labor-intensive than the other online MS techniques.

An advantage of the SESI-HR-MS system, however, compared to SIFT-MS or PTR-MS, is its ability to ionize species at atmospheric pressure [60], whereas SIFT-MS and PTR-MS have flow tubes that operate at much reduced pressures [19, 47]. Operating at atmospheric pressure enhances ionization efficiency and enables straightforward coupling to commercial high-resolution mass spectrometers, such as Orbitrap mass spectrometers [47] which can routinely achieve mass resolutions exceeding 140,000 and thus provide exceptional selectivity. Nonetheless, these advanced analytical systems rely on sophisticated and costly instrumentation, limiting their routine clinical deployment [47].

Recent work compared SESI-HR-MS with plasma ionization (PI) [23] and PTR-HR-MS [57]. To conduct a direct comparison, Zeng et al. [23] separately coupled both a commercially available PI source and a SESI source to the same HR-MS instrument and measured the breath of two healthy individuals longitudinally, in a proof-of-principle study. In total, 58 breath samples were collected on both ion sources, yielding 2296 and 2209 m/z features for PI and SESI, respectively. Although each system achieved rich spectral profiles, only 60% of the features overlapped between the two techniques. The signal-to-noise ratio (S/N) was markedly higher for SESI-HR-MS (median 115, IQR 408) than for PI (median 5, IQR 5). Both achieved ppt-level detection limits for some species, but SESI-HR-MS offered superior S/N performance. The discrepancy in spectral fingerprints likely reflects differences in ionization chemistry: SESI primarily produces protonated or deprotonated species, whereas PI tends to yield oxygenated plasma adducts, complicating direct comparison of results.

To compare SESI-HR-MS with PTR-HR-MS, Bruderer et al. [57] simultaneously measured the exhalations and examined the spectra from 14 healthy subjects, using 97 reference gas standard species from 9 chemical classes produced from a liquid evaporation system. For this experiment, a Vocus PTR-TOF instrument (TOFWERK AG, Thun, Switzerland) was employed as the PTR system. This portable device was temporarily installed at the University Children's Hospital Zurich to allow a direct comparison with a Super SESI ion source (FIT, Málaga, Spain) coupled to a Q-TOF mass spectrometer (Triple TOF 5600+, SCIEX, Toronto, Canada). Both systems provided a similar resolving power of approximately $m/\Delta m \geq 15{,}000$. While both methods mainly produced protonated analytes in positive mode, their ionization conditions differed substantially: SESI operates at atmospheric pressure in a compact ion source, whereas PTR-HR-MS ionization occurs in a 10 cm drift tube at a pressure of 1–3 mbar. The study found that SESI-HR-MS outperformed PTR-HR-MS at higher mass ranges (m/z 150–250 and up to 500), while PTR-HR-MS was more sensitive at lower masses (m/z 50–150). This distinction is important because higher-mass features are often associated with more specific metabolic biomarkers, whereas low-mass VOCs tend to be common compounds that may overlap due to the presence of fragments. SESI-HR-MS detected 828 total spectral features versus 491 for PTR-HR-MS,

with 797 unique to SESI and 374 unique to PTR. However, PTR-HR-MS was more efficient at ionizing compounds with lower proton affinities. These findings confirm that SESI-HR-MS and PTR-HR-MS are complementary: SESI is advantageous for higher-mass, polar compounds and broader *m/z* coverage, while PTR-HR-MS excels for small, low-proton-affinity VOCs.

Gisler et al. [60] assessed reproducibility by analyzing exhaled breath before and after ingestion of a peppermint oil capsule at two sites using identical SESI-HR-MS platforms. They observed 57 additional features significantly associated with peppermint ingestion, and repeated measurements indicated a core set of approximately 35–40 VOCs that were consistently detected across sessions. The study supports a more complex peppermint metabolism than previously assumed and shows that observed differences were driven largely by time, regarding post-ingestion and inter-individual physiology, for example, metabolism and absorption, rather than instrumental effects.

Because untargeted SESI-HR-MS yields many unresolved features, Wüthrich et al. [59] compared SESI-HR-MS direct breath measurements with dynamic headspace vacuum in-tube extraction gas chromatography mass spectrometry (DHS-V-ITEX-GC-MS) and LC-MS [2] EBC measurements, in 16 participants. GC-MS provided robust structural annotations but mostly detected exogenous compounds, for example, oral-care additives. LC-MS [2] detected numerous features, especially with DIA, with chemical classes assigned in silico; in positive mode, amino acids and amines were common, and in negative mode, carboxylic acids predominated. Overlap with SESI features was approximately 25% for LC-MS and around 5% for GC-MS, with coverage spanning the SESI *m/z* range. This highlights that combining online SESI-HR-MS with offline orthogonal methods provides the most reliable attribution and broader chemical coverage. Table 1 compares key analytical platforms for breath VOC analysis in terms of ionization, detection limits, resolution of isomers, quantification, and clinical suitability.

3.5 | Beyond Gas-Phase: Aerosol Sampling in SESI-HR-MS

Aerosolized formulations are increasingly used for drug and supplement delivery, so reliable chemical characterization and quantification of species within these aerosols is essential to screen for constituents, by-products, and impurities [45]. Although most SESI-HR-MS work targets gas-phase measurements, Semren et al. [45] investigated direct aerosol analysis with SESI-HR-MS in two settings: (i) exhaled breath from volunteers after inhaling aerosols from commercial devices, and (ii) programmable syringe-pump introduction of aerosols that mimic breathing patterns. In these experiments, SESI-HR-MS detected, and MS/MS-confirmed, several compounds, including caffeine, melatonin, cannabidiol, chloroquine, and hydroxychloroquine. In contrast, azithromycin and vitamin B_{12} were not detected in the generated aerosols; however, the vitamin B_{12} breakdown product 5,6-dimethylbenzimidazole was observed, consistent with thermal degradation during aerosolization. The study highlights that device conditions, for example, temperature and residence time, can influence aerosol chemistry. The research therefore demonstrates the feasibility of using SESI-HR-MS to profile aerosolized active pharmaceutical ingredients and their transformation products in real-time.

4 | Advantages and Limitations: SESI-HR-MS in Context

Like any analytical platform, SESI-HR-MS possesses notable strengths along with inherent limitations. In this section, we summarize advantages and constraints as reported in the recent literature, and emphasize research priorities necessary to improve performance and facilitate clinical translation.

4.1 | SESI-HR-MS Advantages

SESI-HR-MS excels at trace-level VOC analysis in exhaled breath [55], enabling applications in therapeutic drug monitoring (TDM) and pharmacokinetics [20], interrogation of metabolic processes [20] and clinical use cases [60] such as continuous health monitoring [24, 59], as well as disease detection. These capabilities arise from features detailed below, including real-time online sampling with minimal preparation, broad chemical coverage particularly for polar species, and coupling to high-resolution mass analyzers that afford exceptional selectivity.

4.1.1 | An Online Technique

A key advantage of SESI-HR-MS as a real-time online method, compared with offline chromatographic approaches such as GC-MS and GC × GC—ToF [47, 77], is the provision of instant results [30] with minimal sample preparation, eliminating chromatographic separation and extensive handling steps [22, 23, 29, 33, 39, 58, 60, 78]. This reduces lengthy analysis procedures and sample storage, and as a result, the introduction of artifacts or other contaminants [29], which may complicate data processing and compound identification [22, 32, 47, 79]. Work has also been done by Fido et al. [80] to investigate the presence of positive and negative artifacts from sampling bags. The online format also supports high throughput and time-resolved, for example, breath-by-breath measurements, aligning with prospects for continuous health monitoring in clinical settings [39]. Furthermore, as with SIFT-MS and PTR-MS, another advantage of SESI-HR-MS for its use in breath analysis is its non-invasive nature [24, 35, 48, 55, 78]. This greatly improves patient experience during metabolomic analyses, disease detection, or drug monitoring [20, 39, 60]. The wide detection range and metabolome coverage [20, 32] exhibited by SESI-HR-MS make this technique particularly adept for targeted and untargeted analyses [47] of metabolites and thus for biomarker identification.

4.1.2 | Soft Ionization Technique

The ability of SESI-HR-MS to be used for a myriad of applications stems from its soft ionization properties [39, 46], which reduce fragmentation because product ions are formed with low excitation and low internal energies [46, 60, 81]. This simplifies mass spectral data interpretation and therefore, compound identification [46, 48]. The extent of this softness was investigated by

TABLE 1 | Comparative overview of SESI-HR-MS, PTR-MS, SIFT-MS, and GC–MS for breath VOC analysis.

Attribute/ Method	SESI-HR-MS	PTR-MS	SIFT-MS	GC–MS
Ionization	Secondary electrospray ionization; cluster-/ligand-switching and protonation/deprotonation pathways; ambient	Proton-transfer (typically H_3O^+; sometimes NO^+/O_2^+ variants) in drift tube	Selected reagent ions (H_3O^+, NO^+, O_2^+) with known kinetics in flow tube	EI (70 eV) most common; CI optional; preceded by GC separation
Pressure/source conditions	Atmospheric-pressure ion source; coupled to HRMS (vacuum downstream)	Reduced pressure (~1–3 mbar), controlled E/N in drift tube	Reduced pressure (~0.3–1 mbar) in flow tube	GC at ambient carrier flow; MS source under high vacuum
Pre-treatment/separation	None (online, direct breath)	None (online)	None (online)	Yes (offline/near-online): bags/TD tubes/cryo-traps; optional derivatization; chromatographic separation
Typical LoD	Low-ppb to ppt (analyte and humidity dependent)	ppt–low-ppb (best for high proton-affinity species)	ppt–low-ppb (for many targets with known kinetics)	Low-ppt to ppb with preconcentration; typically ≥ppb without preconcentation
Isomers/isobars	HR helps isobars; isomers not resolved without MS/MS/IMS or prior separation	No chromatographic separation; limited isomer discrimination (some via reagent/kinetics), isobars unresolved at unit-res	Kinetic channels help some cases, but isomers generally not resolved; unit-res limits isobar separation	GC resolves many isomers (column dependent); MS libraries aid confirmation
Quantification	Semi-quantitative; external gas standards needed; matrix & humidity dependent	Quantitative with calibration; absolute quant feasible where rate constants known	Absolute quant from known kinetics for many species; calibration still beneficial	Quantitative with calibration/IS; accuracy depends on trapping & recovery
Typical analyzers	Orbitrap HRMS, Q-TOF/TOF; optional IMS coupling	TOF (PTR-TOF) dominant; some quadrupole	Quadrupole most common; some TOF implementations	Quadrupole (workhorse), TOF/Q-TOF; high-res GC–Orbitrap available
Temporal resolution	Seconds (breath-by-breath feasible)	Seconds	Seconds	Minutes to hours per run
Clinical practicality	Noninvasive, high throughput; needs calibration & robust SOPs	Noninvasive, quantitative; specialized instrument	Noninvasive, absolute quant; specialized instrument	Widely available; slower, prep-intensive; strong identification confidence

Kaeslin et al. [46], who claimed the SESI ionization procedure to be even softer than ESI under specific settings and conditions [39, 46, 48]. The authors characterized the softness of SESI by using thermometer ions to obtain a clear understanding of the internal energy distribution of ions and the fundamental ion chemistry occurring within SESI [46]. Despite previous reports of significant in-source fragmentation of analytes [23, 46, 60, 82] and the standard settings used within the SESI community to date being relatively harsh; if settings are properly fine-tuned, the system's full soft ionization potential may be exploited [46]. Volatile benzylamines, which have known bond dissociation enthalpies and identical dissociation pathways, were used to form differently

substituted benzylammonium ions. It was found that the SESI mechanism may be both analyte and temperature-dependent and that to achieve softer ionization, proper instrument tuning is necessary. Kaeslin et al. [46] found that lower S and RF voltages and a lower transfer capillary temperature encouraged the solvation of analytes, and therefore much softer ligand switching mechanisms as opposed to a harder gas-phase proton transfer collisional activation mechanism [46]. The authors also found that these settings came at the expense of sensitivity.

4.1.3 | High-Resolution and Sensitive Technique

When SESI is coupled to high-resolution mass analyzers, breath analysis can achieve a resolving power of over 100,000 [22, 60], improving the discrimination of isobaric interferences and overall sensitivity that is essential for confident VOC assignment in clinical contexts. High resolution and exact mass substantially aid annotation, although unambiguous identification typically still benefits from MS/MS and/or orthogonal evidence. SESI-HR-MS also offers high sensitivity, with low-ppb to ppt detection demonstrated for many compounds [22, 29, 60]. Sensitivity is analyte- and matrix-dependent and is influenced by gas-phase properties and ion chemistry: responses often correlate with proton affinity, gas-phase basicity, polarity, and dipole moment [39, 48, 67]. Humidity and other sampling conditions can further modulate signal levels and adduct patterns [39, 78]. Optimized source settings and solvent/dopant choices are therefore important to balance softness, signal intensity, and selectivity.

4.2 | SESI-HR-MS Limitations and Challenges

Despite the strengths of SESI-HR-MS and its promise for routine applications in clinical monitoring and disease detection, translating breath analysis into clinical practice remains challenging [32, 47].

4.2.1 | Identification and Quantification

Because the SESI-HR-MS mechanism and the ion-molecule reactions discussions are still under debate [33, 39, 44, 46, 66, 83], accurate and unambiguous biomarker identification [29, 33] and quantification [33] of VOC species is challenging. Most SESI-MS studies rely primarily on MS1 data, which are susceptible to misassignments [55] and spectral overlaps [55], and have a limited ability to differentiate isomers [59].

Although SESI-HR-MS exhibits very high-resolution capabilities, making the separation of isobars possible, unlike SIFT-MS, SESI-HR-MS is unable to separately quantify isomers [58]. This renders the interpretation of the underlying metabolic processes and biological pathways complicated [59]. In addition, SESI-HR-MS does not involve a prior chromatographic or separation step [29, 48, 58, 59]. The flow-injection nature of SESI-HR-MS further hinders robust feature annotation, often requiring complementary methods, such as GC-MS and LC-MS, to confidently identify compounds [59, 84].

Clinically meaningful interpretation requires accurate quantification. SIFT-MS can achieve absolute quantification based on established ion-molecule rate coefficients; PTR-MS is often quantitative when calibrated to appropriate standards, though rate constants are also universally available. In contrast, SESI-HR-MS is generally semi-quantitative and requires external calibration with gas-phase standards, procedures that are time-consuming and labor-intensive. Gas standards are typically generated via a dynamic dilution of reference vapors or controlled liquid evaporation [39]. Examples of this are the internal standard addition system described by Wüthrich et al. [85] and the gas-phase standard delivery system as described by Streckenbach et al. [86] (Figure 4).

An example of the use of reference standards is found in the work of Liu et al. [22] who developed a quantitative method for a SESI-HR-MS system using a Q-Exactive quadrupole Orbitrap HR-MS. The authors investigated eight representative VOCs, which are typically of interest in studies using SESI-HR-MS, and were able to demonstrate that their system could produce calibration curves with R^2 values reaching $R^2 = 0.993–0.999$ for each of the eight VOCs between the concentrations of 0–10 ppb_v, by using a dynamic dilution calibrator, in N_2 as the carrier gas. They also demonstrated LODs which ranged from 3 to 15 ppt_v, sensitivity values between 3.82×10^5 and 2.70×10^7, and coefficients of variation of ≤6% and ≤10% for intra- and inter-day measurements. Similar to this work, Wüthrich et al. [78] developed a modular dynamic vapor generator that could produce gas standards at varying concentration levels under controlled conditions. They tested the use of this system for the quantification of short-chain fatty acids: hexanoic acid, pentanoic acid, butyric acid, propionic acid, and acetic acid—and demonstrated excellent linearity ($R^2 = 0.97–0.99$) with low limits of detection and quantification in the ppb-ppt ranges under dry and humid (RH 95%) conditions [78, 87, 88]. An increased humidity generally demonstrated better LOD and LOQ values, with the lowest detection limit of the fatty acids analyzed being 0.71 ppt at RH% = 95% for butyric acid. Another observation by the authors was that the longer the fatty acid chain length, the better the sensitivity obtained, which was inversely proportional to the compound's Henry's constant. Even when the fatty acid species were mixed, the sensitivity remained constant for the fatty acids. These authors were therefore able to develop a robust calibration unit.

Challenges in SESI-HR-MS VOC identification and quantification were highlighted by Käser et al. [55] who investigated methods for improved accuracy. They evaluated full-scan (FS), selected-ion monitoring (SIM), and parallel reaction monitoring (PRM) on exhaled breath from 12 adults, focusing on pyridine, monoterpenes (e.g., limonene, α/β-pinene), and other endogenous compounds, for example, C_5-C_{10} aldehydes. Limonene and pyridine were identified and quantified, supported by MS^2, whereas α/β-pinene remained challenging to quantify separately. The aldehydes were difficult to measure due to their low abundance and overlapping features, such as ketone interferences. The combination of SIM and PRM improved selectivity and quantitative accuracy. The compounds tested were also shown not to be affected by ion competition. The study concludes that reliable identification and quantification rarely come from m/z alone; orthogonal methods remain pivotal,

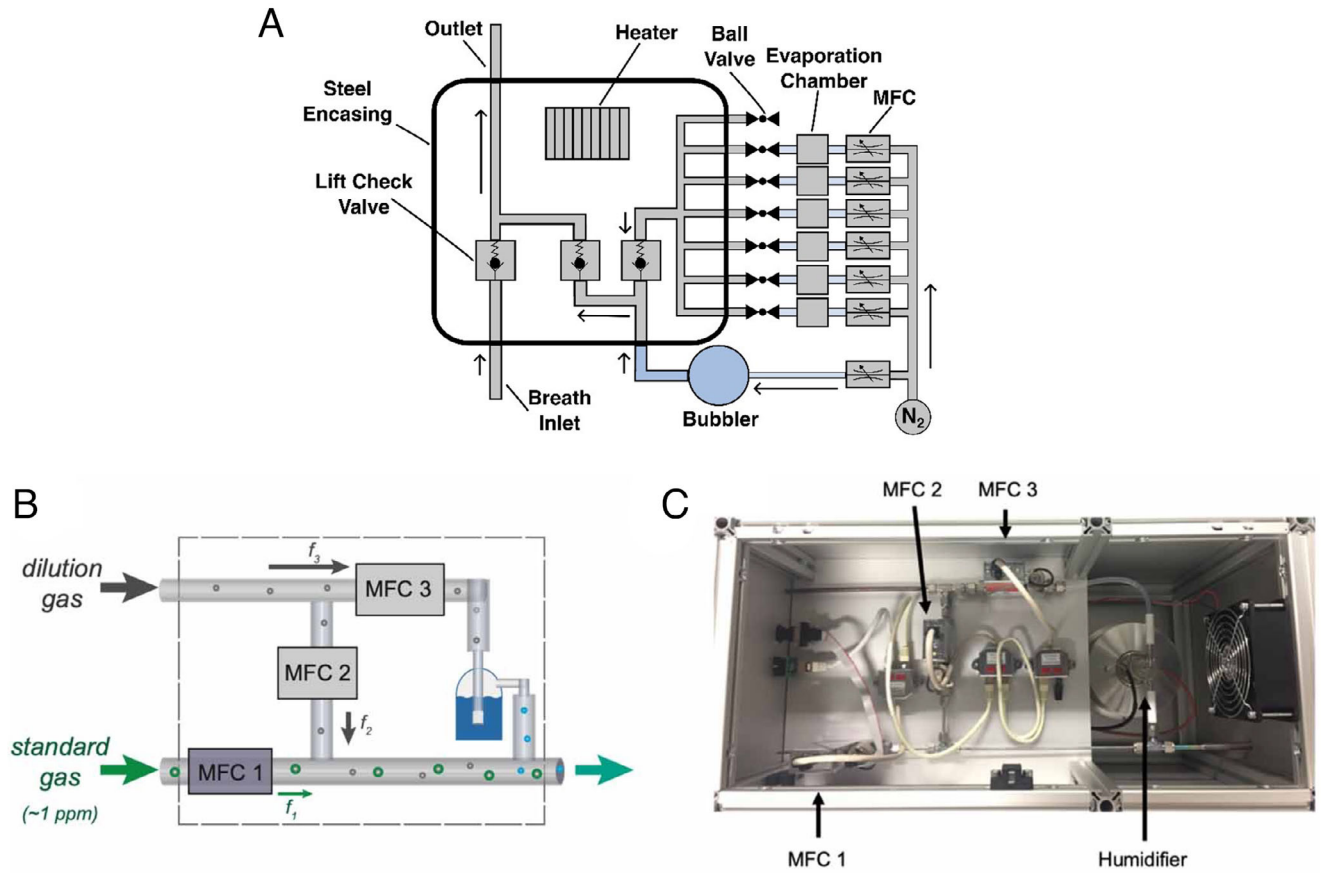

FIGURE 4 | The internal standard addition and gas delivery systems used in recent breath analysis work. Reproduced from Wüthrich et al. [85] with permission from ACS Publications, and Streckenbach et al. [86] with permission from IOP SCIENCE.

and absolute quantification without external standards is a key research priority[22].

4.2.2 | Matrix Effects

Although calibration units may be used for accurate quantification of specific substances in an ideal setting, the reality is that the breath matrix is fairly complex, and accurate identification and quantification of VOC species in real breath samples are influenced by matrix effects such as ion suppression and ion competition, leading to errors [39, 44].

Ion suppression is a matrix effect in which contaminants and other species present in a sample reduce the efficiency of ionization for target compounds by competing for reagent charge carriers [49, 89]. This results in decreased signal response, and has been widely reported across analytical methods [44, 57, 90–97], including SESI-HR-MS [49], although a fundamental understanding of the ion-suppression mechanism in SESI-HR-MS is still lacking [44]. It is suspected that the ion suppression effect is more prominent for online techniques, because the sample is not pre-treated and there is no effort to remove any matrix compounds which could induce suppression [44]. Ion suppression causes issues with VOC quantification, sensitivity and reproducibility, limiting the potential of SESI-HR-MS in untargeted VOC profiling for metabolomic studies [49].

To gain further understanding of ion-suppression mechanisms within the SESI-HR-MS, Wüthrich et al. [44] investigated this phenomenon. The authors captured, thawed, and injected EBC into the previously described gas-phase calibration evaporation chamber unit, along with other VOC standards, which were thought to induce ion suppression. These spiked VOCs included pyridine, deuterated acetic acid, deuterated acetone, and acetone. Acetone was suspected of causing a significant ion-suppression effect, due to its high abundance in breath. When tested, a 30% reduction was observed in many EBC m/z features when 1 ppm of acetone, which is around the concentration expected in breath, under humid conditions, was simultaneously injected into the SESI-HR-MS. This highlighted the importance of accounting for acetone-induced ion suppression in SESI-HR-MS studies. A mechanistic hypothesis proposed by the authors is based on the ligand switching ion-molecule process previously described, when charge transfer to the analyte molecule is mediated by the exchange with a water molecule within a water cluster. They argued that if an abundant molecule, such as acetone, could replace the analyte in the water cluster, this would reduce the efficiency of ionization for the analyte molecule of interest. Gas-phase acid-base chemistry was also suggested by the authors to be involved in ion suppression when they found that pyridine exhibited the most substantial ion suppression effect on the analyzed VOCs, which was attributed to its relatively high basicity.

The authors describe how dilution of a breath sample by an additional inert carrier gas flow could help alleviate suppression by reducing the concentration of the suppressing species. However, the success of this would also depend on the chemistry of the suppressing compounds and analytes, as well as the final concentrations. Diluting compounds would, however, inadvertently reduce the signal responses of the m/z features and therefore the sensitivity. The study also suggested that selective filtering approaches could be employed to remove unwanted suppressing compounds. Further investigations are needed to evaluate the ion-suppressive effects of humidity and to extend assessments across a broader range of compound classes. In addition, the influence of spray solution composition warrants examination as a potential strategy to enhance ionization efficiency.

Ion competition within the C-trap of an Orbitrap HR-MS is another mitigating factor which has an adverse effect on the reproducibility and sensitivity of the SESI-HR-MS system, and has been documented and investigated in the work of Lan et al. [49]. Ion competition occurs when the C-trap is overfilled with the most abundant VOC analytes, rendering the less abundant VOCs to be minimally detected [49]. However, this effect may be alleviated by applying spectral stitching [49, 98]. Lan et al. [49] found that by splitting the spectral range of m/z = 50–500, into four windows, a compromise was achieved between scanning speed and lessening of ion competition for the purposes of analyzing bacterial culture and human breath samples.

4.2.3 | Endogenous and Exogenous Compound Identification

A particular challenge in breath analysis, which encompasses all VOC analysis techniques, is deciphering whether the VOCs measured are of exogenous, that is, from the environmental background and re-exhaled, or of endogenous origin, that is, from metabolic origin within the body; of which human breath contains both [29, 99]. Interfering contaminants may also come from the analytical technique itself, such as phalates which have out-gassed from O-rings or from the laboratory such as polydimethylcyclosiloxanes, which are common plasticizers [33]. Exogenous species have also been reported to interfere in EBC, for example, in the work of Wüthrich et al. [59], who mostly found oral hygiene product additives such as menthol in their EBC samples when using GC-MS. As SESI-HR-MS can measure a broad range of masses (up to 500 m/z) [57, 99], airborne plasticizers can also contaminate spectra [99].

To alleviate the interference of exogenous VOCs on mass spectra within SESI-HR-MS, a recent study by Weber et al. [99] investigated the potential of using a VOC activated carbon filter for subjects to inhale through, before exhaling into the online SESI-HR-MS system. The authors explored the differences in breath profiles of 24 adult participants through pairwise breath analysis measurements to conduct a direct comparison of spectra, as well as the feasibility for subjects and operators to incorporate this into the clinical field. It was found that when using the VOC filter, a decrease was observed in high-intensity plasticizer contaminants, as well as some metabolites that were also present in the laboratory background air. Operational challenges were, however, also identified including the lack of feasibility for pediatric patients who were not able to inhale through the VOC filter.

4.2.4 | Lack of Validation and Standardization

Finally, a lack of standardization [47, 78], limited reproducibility [78], and a lack of validation from multicenter trials [32] for breath analysis methods [32] are perhaps the most hindering factors for this technique to enter the clinical setting. It is mainly due to most breath analysis instruments, such as SESI-HR-MS [47], still being at the prototype stage. This stems from a lack of benchmarking or comparability studies conducted, involving at least one other breath analysis technique [60]. Nonetheless, researchers from Swiss institutions have recently completed work to improve the state of SESI-HR-MS standardization, which moves the transition and integration of SESI-HR-MS into the routine clinical environment another step forward.

Gisler et al. [32] established and applied an interoperability framework for the standardized data acquisition across multiple sampling sites using SESI-HR-MS. The authors developed a quality control procedure using a gas standard containing eight compounds, for use in advance of breath sampling. The authors obtained 255 breath samples from nine healthy adult controls (30 ± 5 years) across three study sites (Zurich, Basel, and Guangzhou). They developed a standard operating procedure for data collection and analyzed data using a patented data processing pipeline. A technical variability of 20% was found from the SESI-HR-MS instrument itself. Approximately 850 core breath features were identified. High inter-subject variability was observed among certain metabolic classes, such as amino acids and fatty acids, while other regions including the TCA cycle, were relatively stable across subjects. The core breath features identified mainly corresponded to amino acid, xenobiotic, and carbohydrate metabolic pathways. For each site, however, a batch effect was observed, which was corrected using ComBat. The authors indicate that this interoperability framework will act as a steppingstone for the design and implementation of future multicenter clinical studies. Some limitations to this study should, however, be noted, such as the small number of sampling sites (three) as well as limited geographical diversity (Zurich, Basel, and Guangzhou), reducing the generalizability of the subjects studied in this work. Furthermore, the sampling took place over 6 weeks, which may have missed any long-term metabolic process variations among subjects. No information regarding the subjects' diets or lifestyles that may alter the breath metabolome from exogenous VOCs was considered either. Batch correction may also introduce unwanted artifacts into the data.

Standardization efforts were demonstrated by Singh et al. [47], who presented a collection of instrumental developments with the aim of standardizing the SESI-HR-MS analytical workflow in breath analysis. The study introduced a new interface to standardize exhalation flow rates by measuring the breath volume and CO_2 concentrations exhaled. Four healthy subjects (33 ± 8 years) were tested over a month and each gave 49 exhalations, for which the system's repeatability was assessed using gas reference standards and was found to have a coefficient of variation of 2.9%, for the standards. To assess the reproducibility of breath measurements,

three aldehyde classes were examined, which are considered indicators of oxidative stress in the body. A systematic signal decay in repeated measurements of shorter chain aldehydes was observed, for which a steady state was reached after the third or fourth subsequent exhalation. Longer-chain aldehydes showed steady-state concentration levels much sooner compared to short-chain aldehydes. The authors hypothesized that this reasoning was due to the higher blood-to-air partitioning coefficients of the shorter aldehyde chain molecules. It was also found that the exhalation flow rates, as well as the type of mouthpiece filter used, significantly impact the breath profile achieved. The authors found an intra-subject variability of 6.7% (median CV, after excluding the first three exhalations), and an intersubject variability of 48.2% (median relative difference), for the aldehydes. The study further mentions that the intersubject variability is consistent with aldehyde variability in blood. Moreover, it was found that each of the 27 aldehydes analyzed in this work correlated strongly with the aldehyde correlation network, reflecting the similar metabolic origins of these species in the body. The authors demonstrated that reproducibility is strongly dependent on the site of aldehyde exchange in the respiratory tract and that the instrumental interface achieves low intra-subject variability. Finally, the study recommends that to minimize variability within breath samples, at least six subsequent exhalations should be conducted after excluding the first three, to capture a steady state.

5 | Advances in Breath Analysis for Asthma

Asthma and other wheezing disorders are among the most common respiratory diseases in adults and children [100], with country-level prevalence ranging from 5% to 20% [11]. The World Health Organization (WHO) estimated that approximately 339 million people worldwide had asthma in 2020 [101]; up from 315 million in 2014 [102] and 334 million in 2019 [103]. Five percent of asthma cases are considered severe [1, 16, 104–109]. Asthma is a chronic, inflammatory disease [110] of the lower respiratory tract causing variable airflow limitation, airway hyperresponsiveness, and episodic symptoms such as wheezing, dyspnea, cough, and chest tightness [102–104, 106, 111–115]. Its multifactorial etiology involves genetic and environmental factors, while comorbidities and lifestyle contribute to disease burden, which significantly impairs quality of life and may be life-threatening in severe cases [107, 109, 116–118].

Identifying abnormalities in air flows, via pulmonary function testing [103], that would be indicative of asthma is more difficult in much younger—that is, pre-school age and below, or much older patients, due to overlapping symptoms of other diseases which may be similar to those of asthma [113]. A standardized diagnosis test is lacking [11], and as a result, misdiagnosis is frequent [11]. There is therefore a need to improve asthma diagnosis methods, particularly among children [16].

Although some work has been done on breath analysis using fractional nitric oxide (FENO), this method primarily reflects eosinophilic airway inflammation [16, 107, 119–121], which is associated with allergic asthma [1]. Therefore, the FENO test is limited in its diagnostic utility [121, 122]. Furthermore, different measuring instruments may yield varying FENO concentration readings [123], and external factors such as smoking can interfere with signals [124].

Although physical airflow tests are available, there is a specific need for reliable diagnosis in children, where these tests may not be so accurate [107]. Noninvasive breath analysis, which identifies VOC profiles, is therefore a promising avenue for accurate asthma diagnoses. Specific VOC profiles in the data may facilitate a deeper molecular understanding of pathophysiology and, consequently, the diagnosis of different asthma phenotypes [1]. VOC analyses would also enable early detection and have a greater predictive capability for severe attacks and uncontrollable asthma symptoms, compared to conventional FENO tests [125, 126]. In addition, analyzing multiple VOCs for disease presence may help identify external interferences such as signals from smoking, and specific profiles may in the future give rise to the understanding of what is driving the disease. There is currently no specific biomarker or VOC profile that can differentiate between asthmatic phenotypes, as of 2024 [107], although e-noses have been reported to show potential in asthma phenotype diagnosis [118]. There is therefore a substantial gap in the current state of breath analysis research regarding the crucial early detection of asthma, especially among children.

5.1 | GC-MS

A study known as the "The all age asthma cohort (ALLIANCE)," established by the German Center for Lung Research, is a long-lasting observational cohort study across seven recruiting sites, with an exceptional number of study participants [100]. In addition, high follow-up rates were reported for adults (90.5%) and children (83.9%) at the 12-month follow-up [100]. Using the ALLIANCE cohort, a study was conducted by Shahrokny et al. [107] to investigate VOCs in the breath samples of 142 children: 36 controls, 55 preschool wheezers, and 51 asthmatic patients. The study aimed to identify a VOC biomarker or biomarker profile that could potentially differentiate between patients of different phenotypes and concurrently ascertain environmental risk factors that influence the disease mechanics of asthma.

Offline samples were taken and analyzed using GC-MS within 8 days, and a target of 158 VOCs was evaluated using the Mann–Whitney U-test (MWU) and the Pearson correlation. While no disease-specific VOC profile was identified to distinguish asthma or wheeze from controls, the authors observed elevated exogenous pollutants, particularly naphthalene, in the wheeze-asthma groups. No correlation was noted between these environmental pollutants versus TH2 inflammation or lung function. The results of this study are in line with previous work [127], which also observed higher naphthalene concentrations in preschool children. Other studies have shown the correlation between asthmatic patients and an increase in PAH metabolites found in other biofluids. For example, some urinary PAH metabolites were found to be linked with the presence of asthma in children between the ages of 6 and 19 years [128]. Furthermore, naphthalene concentrations within the serum of 195 Saudi children demonstrated strong associations with asthma in the study by Al-Daghri et al. [129]. Another study by Lin et al. [130] reported an increase in the level of 2-naphthol, a metabolite of naphthalene, in the urine samples of children with asthma.

In the work of Shahrokny et al. [107], numerous air pollutants were shown to be more concentrated in breath samples of asthmatic subjects. However, naphthalene was shown to be the most prominent. Air pollutants such as PAHs are, however, well known to induce other respiratory diseases in addition to asthma and wheezing, and therefore using exogenous VOCs as markers of asthma or wheezing in patients is questionable. Additionally, the study notes that little is known about the pathophysiology of asthma and naphthalene. Although no strong associations emerged between inflammatory asthma phenotypes and specific VOC markers, the study supports breath analysis as a noninvasive approach to probe how air pollutant exposure contributes to the onset of respiratory disease.

Limitations included a restricted targeted panel of 158 VOCs, which may have missed discriminatory biomarkers; co-elution risk, increasing the chance of misidentification; and medication confounding, as 73% of asthmatic participants were on anti-inflammatories, potentially masking inflammation-related VOCs. In addition, the modest sample size ($n = 142$) and multicenter heterogeneity across seven recruiting sites reduce statistical power and complicate between-group comparisons.

Another publication, which involved a co-author from a Swiss institution, was that of Holz et al. [42], who used ALLIANCE adult data to question the robustness of breath-VOC diagnostics for asthma, despite previous positive reports—the article references Vries et al. [131] and Schleich et al. [132].

Breath VOCs were compared between the different phenotype subjects, based on FENO tests and cell counts in sputum and blood biofluids, from 133 adult participants. An offline GC-MS method was used to evaluate the state of 134 VOCs. Of these, 40 VOCs were below the LOD in 85% of participants and were therefore excluded from the study. A particular strength of this study is the use of active carbon filters for participants to inhale through. Carbon filters are widely accepted to efficiently remove organic matter and purify an air sample of VOCs [133–136], and have been used as inhaled purification systems to improve breath VOC data in other studies [99]. This would remove any background artifacts from the sampling site. A further strength of this particular methodology was the use of aluminum reservoir tubes (Tenax TA adsorption tubes), known to be inert, and therefore would have reduced the potential for positive and negative artifacts, despite being an offline sampling technique [137, 138].

The study's results, however, found no statistically significant correlations between markers of inflammation: nitric oxide levels, sputum neutrophils/eosinophils, or blood eosinophils, versus breath VOCs, as determined by the Benjamini–Hochberg method. The breath matrix, however, is known to be a vast array of thousands of VOCs and a lack of correlation may be due to the limited number of VOCs tested using GC-MS. This is a similar limitation as observed in the study of Shahrokny et al. [107].

The study by Holz et al. [42] emphasizes that no positive results of an asthma breath VOC trial were obtained in this work, nor is there yet as of 2019, an established breath VOC profile for any disease. They also suggest that substantial work needs to be invested into breath analysis research of asthma to obtain clinically valuable VOC biomarker patterns for asthma phenotypes. Additionally, they emphasize the need for standardized methodological approaches and a comparison of methods between research groups. Considering the major challenge of interfering factors, that is, background external VOCs, the study also infers the need for a greater understanding of the biochemical processes behind the production of VOC profiles for a specific disease.

Khamas et al. [125], during the SysPharmPediA (Systems Pharmacology Approach to Uncontrolled Pediatric Asthma) study, evaluated GC-MS to differentiate VOC profiles between uncontrolled and controlled asthmatic children. Out of 196 subjects, it was found that acetophenone, styrene, and ethylbenzene were promising species that were significantly different between the controlled and uncontrolled asthma cohorts. Limitations mirror prior GC–MS works, including targeted scope and a modest sample size, but the study's strength lies in its classification and prediction performance.

5.2 | Soft Ionization Mass Spectrometry

A recent promising study by Houssni et al. [11] is the first publication in which the Vocus PTR-TOF-MS instrument was used to identify VOC profile differences between 41 allergic asthmatic children and 40 healthy control children (ages 11.8 ± 2.7 years) during the EXhalomics in PEDIatric Asthma (EXPEDIA) study. The participating subjects were recruited from the respiratory outpatient clinic of the University Children's Hospital in Zurich. PTR-MS predominantly uses H_3O^+ as the reagent ion for VOC ionization, although in this work both H_3O^+ and NH_4^+ were used. Complementary to the presented PTR-MS methods, molecular identification was carried out using GC × GC-Q-TOF. Statistical analysis was performed using the Wilcoxon rank sum test and the Benjamini-Hochberg procedure.

The authors reported four confirmed exhaled VOCs, which they attributed to asthma incidence, as well as 16 novel markers; predictor candidates: three exogenous, seven likely endogenous, and four unknowns, with high confidence in their identification (Figure 5). Other interesting findings of this study included the elevation of siloxane species, such as hexamethyldisiloxane, found in the breath samples of asthmatic children. The study argues that siloxanes are not naturally occurring in the environment and that these species are not present in asthma inhalers. The levels among the asthma patients were higher compared to the healthy controls, which this study further argues rules out the possibility of contamination from the sampling system. To explain this, Houssni et al. [11] hypothesize that the reason for this is a difference in the uptake and release of these species between asthmatic subjects and healthy controls.

A particular strength of this study is the biologically relevant interpretation of the presence of identified compounds. The authors indicate that the identified aldehydes, methyl esters, and fatty acids are known markers for lipid peroxidation and enzymatic reduction. They explain how reactive oxidative stress, often associated with inflammation, damages a cells' lipid membranes, although this is not specific to asthma. They also mention

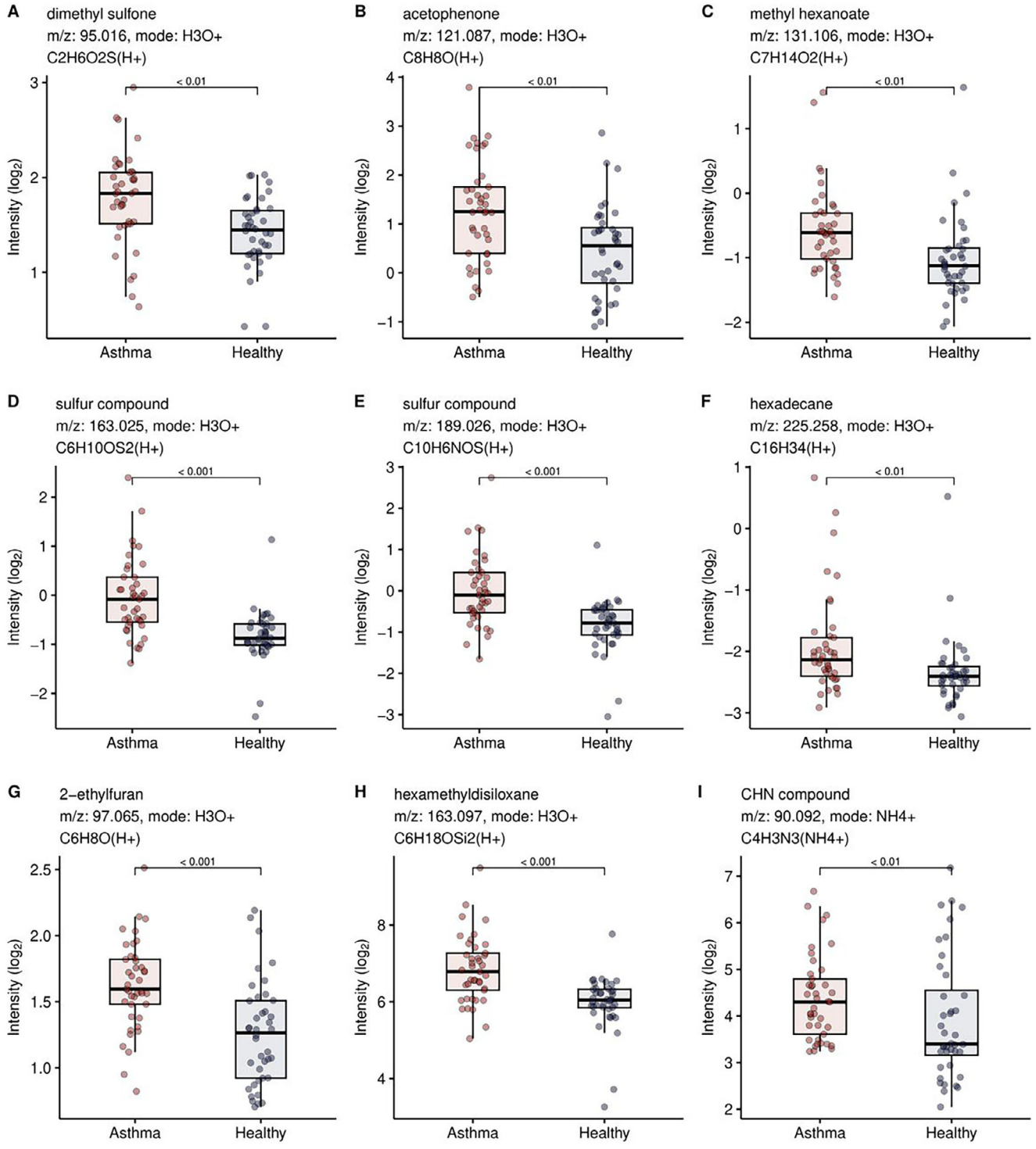

FIGURE 5 | Box and Whisker plots showing the statistical differences between asthmatic and healthy controls regarding breath concentrations of specific VOCs. Reproduced from Houssni et al. [11] with permission from BMJ Publishing Group Ltd.

that butyrate is converted into methyl butyrate in people with allergic asthma during the regulation of immune cell behavior. A further strength of this study was the comparability between the asthmatic patients and healthy controls within the sample group, which had a relatively narrow age range (11.8 ± 2.7 years), evenly distributed genders, and no significant differences in body mass index. Regarding instrumentation, using two reagent ions allows for the potential to differentiate isomers of species, dependent on ion-molecule reactions. However, more research is needed to understand the kinetics of the VOCs of interest in this context.

Limitations included the absence of objective lung-function testing, that is, spirometry, with respiratory health data drawn primarily from questionnaires. Furthermore, it is known that VOCs will only react with H_3O^+ if the exothermicity of the reaction exceeds 40 kJ/mol [139–141], potentially causing the loss

of some information. Only two reagent ions were used, both with predominant ion-molecule reactions being proton transfer, which limits the ability to separately analyze isomers. It was also reported in the manuscript that no certified reference standard was used for the GC × GC-Q-TOF analyses. The authors also compared their work to other studies [16, 142, 143], although not much overlap was seen in the detected biomarkers, which raised questions about the reproducibility between VOC analysis methods in breath analysis for asthma distinction.

SESI-HR-MS has also been used in pediatric allergic asthma studies, with the first published work being that of Weber et al. [16]. The authors aimed to identify VOC signature differences in patients with allergic asthma as well as healthy controls over a cross-sectional observational study. Asthmatic subjects were recruited from the University Children's Hospital Zurich outpatient clinic and ranged in age from 5 to 18 years: 56 healthy controls and 48 patients. As opposed to targeted analysis, *m/z* features were analyzed for their differences using the Bayes-moderated *t*-statistical test. Weber et al. [16] found 375 significant features, of which 134 were putatively identified. A particular highlight from this study was the finding that the lysine metabolism, for example, was the most elevated pathway in the asthmatic group, for which the associated evidential compounds were identified using MS^2. They identified two pathways for lysine degradation: one within the gut microbiota and another originating from a human degradation pathway. Tyrosine metabolism was also observed to be upregulated within the asthmatic cohort. This study identified several metabolite species using SESI-HR-MS, which are known to be associated with pathways linked to the pathophysiological course of asthma. The limitation of this study, however, was its sole focus on allergic asthma and therefore dismissing other phenotypes of asthma, as well as the higher proportion of males in the asthmatic group. For both the healthy controls and the asthmatic group, however, the body mass index and ages were similar.

Another study that utilized SESI-HR-MS was that of Zeng et al. [108]. As opposed to studying the VOC profile differences to differentiate between diseased groups and healthy controls, this study focused on the pharmacometabolomics of salbutamol for bronchodilation. The aim of this work was to better understand the responsiveness of children to salbutamol, a sample cohort where the responsiveness is known to be very heterogeneous [144]. Pharmacometabolomics is a very powerful tool that links therapeutics and metabolomics for the understanding of the biochemical mechanisms underlying a patient's response to a drug [145–147]. In this work, 34 pediatric asthmatics were recruited between the ages of 6 and 18 years, from the pulmonology department of the University Children's Hospital Basel (UKBB). The study specifically identified enhancements in the arginine biosynthesis and sphingolipid metabolic pathways. This was based on significant increases in the signal intensities of *m/z* features which mediate bronchodilation as a result of salbutamol inhalation, with substantial metabolic changes occurring after 1 h. A highlight of this study is the demonstration of rapid metabolic profiling for the purposes of understanding pharmacometabolic processes in real-time as a response to salbutamol inhalation. It opens up a promising sub-field of breath analysis in pharmacometabolomics for the continuous and routine monitoring of drug efficacy in asthmatic patients. The biochemical explanations provided in this study are also noteworthy. The limitations, however, surround the very small sample size of 34 subjects, and that compound identification was based purely on unambiguous molecular formula assignment. Furthermore, it is difficult for the authors to differentiate whether the changes in metabolic features are caused by increased metabolism or whether another part of the respiratory system is activated, yet unknowingly.

5.3 | E-noses

E-noses, composed of sensor arrays to detect multiple species, are a novel and increasingly used system in breath analysis. They have been used in the work of Abdel-Aziz et al. [118] to investigate the physiological changes in asthmatic patients when presented with a rhinovirus challenge—a common cold virus and a trigger for asthma attacks. The breath samples of both asthmatic patients (12 subjects) and healthy controls (12 subjects) were measured using an e-nose (SpiroNose) to obtain signals from several VOC mixtures, both before and after the challenge, at multiple time points. Data analysis was conducted using statistical pattern recognition tools such as LASSO modeling. Abdel-Aziz et al. [118] state that the breath print results from their study allow them to distinguish between the healthy and diseased populations. However, there is no mention in the work regarding specific VOCs or VOC mixtures, nor are there any biochemical explanations.

E-noses have also been used in the work of Abdel-Aziz et al. [148] to investigate subphenotyping and whether atopic and nonatopic asthma could be distinguished. A total of 655 subjects were recruited, which included 503 asthmatic adults, 54 preschool children, and 98 school children, for which exhaled breath samples were measured using either the SpiroNose or an integrated e-nose platform. Three separate machine learning algorithms were used for the supervised data analysis. An unsupervised Bayesian network was also used. The machine learning models were able to discriminate between the VOC signatures of atopic and nonatopic asthmatic groups and the study showed that different e-noses were able to distinguish between atopic and nonatopic asthma, showing the potential for the use of e-noses in asthma phenotyping. The main strengths of this study were the much larger sample size compared to the previous studies reviewed, enhancing the statistical significance and accuracy of the research findings. Furthermore, different e-nose types were used in the study, incorporating both online and offline measurements. There was also reproducibility recorded across the devices and cohorts. The limitations of the study were however that e-noses do not identify specific VOCs, and therefore, no information on the bio-metabolomics can be inferred. As a result, understanding the biomechanics behind different asthmatic phenotypes and how they occur cannot be evaluated using e-noses. Furthermore, in contract to the work of Abdel-Aziz et al. [118], only one time point was recorded in this work, which does not take into account the possible diurnal variability of breath, resulting in a lack of longitudinal tracking. The study also notes that the majority of subjects were white, reducing generalizability.

Similar to the other e-nose studies, Brinkman et al. [104] aimed to identify phenotypes of asthma based on VOC signatures given by e-noses, although they went further and evaluated

the stability of e-nose derived phenotyping on the incidence of inflammatory and clinical changes of a patient. This is a multicenter longitudinal study across seven participating sites that took breath samples from adult patients with severe asthma: 78 subjects with a median age of 55 years, all above 18 years, and of which 41 % were male from the U-IBIOPRED cohort. Breath samples were analyzed using an array of sensors, for which the data subsequently underwent Ward clustering as well as K-means clustering, using internal validation through partitioning around medoids combined with topological data analysis. The longitudinal within-patient stability was subsequently assessed by resampling patients' breath at 12–18 months. Subjects were asked to inhale through a VOC filter and exhale into Tedlar bags. The sample was then drawn through Tenax adsorption tubes from the Tedlar bag, with N_2 as the carrier gas. Offline analysis was then carried out using an array of four e-noses from different developers, exploiting different sensor technologies. The authors discovered three unbiased and unique clusters identified by the e-noses, which revealed three distinct severe asthma inflammatory phenotypes. These results demonstrate the potential of using e-noses for phenotyping patients with severe asthma phenotypes in the clinical setting, which is essential for asthma monitoring, personalized management, and for patients who are more challenging to treat. Strengths of this study included using a platform of e-noses assembled using four different brands of sensors and using different techniques for detection. Furthermore, the recruitment of patients from seven different centers allows for greater generalizability of the patient cohort. Limitations included the lack of any external validation with an independent cohort. As with the aforementioned studies, e-noses lack the capability to identify specific VOCs, inhibiting progress in understanding the biochemical processes behind the different phenotypes. Finally, 78 subjects were involved in the study, which is modest, although patients were told not to smoke, eat, or drink from only 2 h before sampling, increasing the likelihood of exogenous VOCs affecting results.

Lammers et al. [149] also used e-noses to investigate breath profiles of subjects who had been presented with a rhinovirus challenge, similar to the work of Abdel-Aziz et al. [118]. Lammers et al. [149] monitored the day-to-day fluctuations in breath profiles in both asthmatic and non-asthmatic patients, before and after the rhinovirus-16 challenge was presented to subjects. Breath analysis was conducted on 12 atopic asthmatic patients and 12 atopic healthy controls, using e-noses, consisting of seven different sensors. Breath samples were taken three times a week, 60 days before the rhinovirus-16 challenge, and 30 days afterward. The authors found that greater fluctuations in the e-nose readings occurred after the subjects were presented with the rhinovirus challenge. Substantial differences in data were observed between the asthmatic and healthy control groups, suggesting the potential use of e-noses to one day monitor unstable asthmatic episodes caused by a virus. A particular strength highlighted by the work was the long follow-up period of 3 months, for subjects.

5.4 | Summary on Asthma Diagnosis

Cohorts such as ALLIANCE provide valuable infrastructure for asthma breath studies, but small sample sizes, limited phenotype breadth, and restricted generalizability remain common. This is often due to ethical and logistical constraints that preclude inclusion of severe cases, for example, rhinovirus-triggered. Recent work has mainly relied on GC-MS and e-noses. GC-MS typically requires standards, is susceptible to co-elution, and covers only a finite analyte panel which can be insufficient for robust case–control discrimination. E-noses, while low-cost and applicable for pattern recognition, do not identify VOC structures therefore limiting biochemical interpretation across asthma phenotypes. Overall, concerns persist about the paucity of high-quality data and the lack of standardized methods in asthma breath analysis [42].

6 | Lung Diseases Other Than Asthma

6.1 | Chronic Obstructive Pulmonary Disease (COPD)

Chronic obstructive pulmonary disease (COPD) is a progressive lung disease causing persistent and progressive bronchial airflow obstruction and limitation [36, 150–155], which may be classified as either emphysema [150] or chronic bronchitis [154] or a combination of both [155]. It may be further classified by the multiple phenotypes and endotypes it features [156]. It is a pathological inflammatory condition that declines lung function [153] and exhibits symptoms, including a chronic cough, excessive mucus production [151, 154], wheezing, expectoration, and exertional dyspnea, resulting in a reduced quality of life [25, 150, 153–157]. The inflammation associated with COPD causes airway narrowing and lung parenchyma damage, reducing the lungs' ability for elastic recoil [150]. As a result, the airways struggle to remain open on exhalation [150, 158]. Further developments from COPD may include cognitive decline, lung cancer, skeletal muscle wasting, and cardiovascular disease [159]. The burden of COPD on health care expenditure has also been noted [160].

COPD is incurable and remains a major global cause of morbidity and mortality [31, 36, 156, 159, 160]. Cigarette smoking is one of the leading factors [36, 154–156, 159, 160], with additional contributions from air pollution, combustion flames, dust, and occupational chemicals [158]. Biological and developmental factors, including childhood growth disorders, asthma, frequent childhood infections, premature birth, and other previous lung infections [36, 156], also increase susceptibility. Diagnosis relies on spirometry and lung function testing, together with symptom assessment and medical history [9, 31]. These approaches, however, offer limited insight into underlying biochemical and metabolomic processes, and physiological testing can be challenging for young children and the elderly [31]. By contrast, non-invasive breath analysis may reduce patient burden and provide molecular information. Prior studies report distinct VOC signatures differentiating COPD patients with frequent exacerbations from infrequent exacerbators [25], and COPD from healthy controls.

Two breath analysis studies on COPD, conducted by researchers affiliated with Swiss institutions, are notable research highlights since 2019. Basler et al. [157] discovered particular VOC breath signatures associated with the acute exacerbation phases of COPD (AECOPD). By sampling breath using SESI-HR-MS from patients during stable COPD and AECOPD phases, they found

alterations in the VOC signatures representative of an activation in the tryptophan, tyrosine, and linoleate metabolic pathways, all of which are linked to inflammation. However, the decreased levels of several metabolites within the impaired tyrosine pathway in particular, during AECOPD episodes, could serve as a potential VOC signature for this condition. Based on these VOC signatures, the authors reported the ability to accurately predict AECOPD with a sensitivity of 82.5%, compared to the stable COPD state, and this result gives hope of accurate AECOPD clinical diagnosis using breath analysis in the future. This study was conducted at the University Hospital in Zurich between January 2020 and September 2022, in which 35 participants were successfully enrolled and completed the sampling. Similarly, Gaugg et al. [25] also investigated the VOC profile of patients undergoing exacerbations in COPD using SESI-HR-MS. This study sampled 26 frequent exacerbators and 26 non-frequent exacerbators and found a significant increase in the levels of nitro-aromatic compounds in the VOC signatures of patients undergoing COPD exacerbations. Conversely, the authors discovered a decrease in ω-oxo, ω-hydroxy, and dicarboxylic acids, inferring a reduced activity from the metabolic ω-oxidation pathway, during COPD exacerbations. Both studies shed light on the biochemical processes surrounding COPD exacerbations, although further work is needed.

Furthermore, the work of Basler et al. [157] had no healthy control group to compare results against. Another similar limitation surrounds causality. Basler et al. [157] conducted an observational study and therefore no information regarding the causal relationship between AECOPD and metabolic VOC signatures could be inferred. Gaugg et al. [25] also had no previous information regarding the initiation of exacerbations, and therefore, this also limited the biochemical understanding of how VOC signatures related to COPD exacerbations. The main future needs in COPD analysis, therefore, encompass much larger cohort studies, the involvement of healthy control groups, COPD groups, and AECOPD groups, as well as the need to further understand the biological mechanisms occurring which define the VOC signatures between these cohorts. The use of SESI-HR-MS methods which have much higher resolution power will be imperative to bring insight into AECOPD pathophysiology.

6.2 | Lung Cancer

Among all cancers, carcinogenesis of the lungs is the leading cause of cancer deaths worldwide [13, 28, 161–167]. Progressive morphological alterations develop over several years in the lungs' epithelial cells [168, 169] into a malignant neoplasm, which then may grow rapidly and uncontrollably [4, 167] as cancer cells multiply and cluster to produce tumors [170, 171]. Lung cancer develops after many years of exposure to oxidizing species of both endogenous and exogenous origin [4], of which cigarette smoke [167, 172] is the leading cause of lung cancer incidence [164, 167, 168]. Other causes include air pollution [4, 167], second-hand smoke exposure [170], asbestos [165, 168], radon gas [165, 168], ionizing radiation [167], chronic infections [165], diet [173], mitochondrial products [4], the further development of other chronic pulmonary conditions [167], resulting in epigenetic and genetic processes [172] causing mutations in the lungs or abnormal cell growth [170]. Family history and genetics also play a role in the likelihood of developing the disease [168, 170, 172].

Epidemiological studies have shown a gender disparity, with a higher mortality rate demonstrated in males compared to females, from lung cancer [167]. The high mortality rate of cancer stems from a lack of early diagnosis, mainly due to symptoms of lung cancer only arising at later stages of the disease [28, 173]. This is when treatment options are limited [166], and cancer is much more difficult to treat successfully [13, 170, 171]. Early detection using noninvasive techniques are therefore highly desired [13].

A recent study by Herth et al. [13] investigated the breath profiles of lung cancer patients before and after surgery. The authors involved 29 participants between March 2020 and January 2023, at the University Hospital of Zurich. Online breath samples were analyzed from participants using SESI-HR-MS. Among 3482 features found in the spectra, 515 m/z features differed between before and after surgery. Out of these 515 features, 154 features were likely actual differences, due to the small sampling size and a false positive rate of 0.71. Despite this, indole and 3-oxotetradecanoic acid were prospectively identified from the spectral features which may in the future help in the understanding of the biochemical and metabolic processes surrounding the development of lung cancer. The study further employed PCA, which revealed a primary cluster of subjects with recurrent disease incidence that was initially undetected.

In contrast, Kort et al. [28] used e-nose sensors in their study. In this work, the authors aimed to train and validate a prediction model based on sensor data to accurately distinguish patients with non-small cell lung cancer from healthy controls. This was based on features seen from exhaled breath, and to further assess the usefulness of other clinical variables in the diagnosis of lung cancer. The training cohort consisted of 376 subjects, with 199 forming the validation cohort. Based on the validation phase of the study, the authors were able to distinguish lung cancer patients from healthy controls, with a sensitivity and specificity of 95% and 49%, respectively. It was concluded that combining clinical variables into the predictive model increased the diagnostic ability in identifying lung cancer.

Each study has a specific strength. The work of Herth et al. [13] utilized SESI-HR-MS, which is known to have a resolving power of around 140,000, and is able to produce spectra with m/z VOC features in extreme detail. This is to the point at which specific VOCs may be proposed, allowing for the potential understanding of the metabolic and underlying physiological processes, resulting in disease incidence. The work of Kort et al. [28], on the other hand, used a much greater sample size of 576 participants for both the baseline and validation phases. The participants were also taken from multiple hospitals which further enhances the generalizability of the findings.

There were, however, also limitations to each study. Herth et al. [13] reported a very high false positive rate of 0.71, which was due to only acquiring 29 participants. This substantially reduced the number of usable features for potential diagnostic predictability and as a result, despite using SESI-HR-MS, reduced the VOC identification potential and therefore the physiological understanding of the causation of disease. Furthermore, this inhibited the possibility of identifying specific cancer phenotypes and histologies. By using only sensors in the work of Kort et al. [28], there is no possibility of identifying the structures

of VOC metabolites associated with lung cancer incidence, and therefore, no further understanding of the metabolic processes governing lung cancer incidence may be inferred from this study. Furthermore, the work reports that from some participating hospitals, insufficient data analysis was caused by a limited number of positive or negative diagnoses in lung cancer, resulting in a reduction in the accuracy of the study.

6.3 | Cystic Fibrosis

Cystic fibrosis is a currently incurable, progressive, autosomal recessive, and inherited lung disease [174] that is caused by alterations in the cystic fibrosis transmembrane conductance regulator gene (CFTR) [15, 175–177], specifically involving mutated alleles associated with chromosome 7 [15, 178]. This mutation causes a defective function in the production of CTFR proteins which are responsible for carrying chloride ions across lung epithelial cell membranes [175, 178, 179]. As a result, electrolyte regulation surrounding the mucosal epithelium is irregular causing excessive mucus build-up in which pathogens may harbor, causing infections. This usually occurs deeper down the respiratory tract [178], resulting in a cycle of infection and inflammation and as a consequence, a decline in lung function [15, 26, 37, 174, 175, 177–183], bronchiectasis development, and inevitably respiratory failure [181, 184]. Cystic fibrosis also affects the sinuses, reproductive tract, gastrointestinal tract, intestines, liver, pancreas, and sweat glands [15, 178, 180, 181, 183]. The disease affects approximately 1 in every 2000–3000 births, with the highest prevalence among subjects of Caucasian descent [181]. Current diagnostic methods are slow [21], and early detection of airway infections is crucial to increase the survival rates of cystic fibrosis patients by starting antimicrobial treatments early [37]. A noninvasive breath analysis method would therefore be invaluable for cystic fibrosis patients [37].

Staphylococcus aureus is a particular pathogen that may grow in the mucus of cystic fibrosis patients and lead to infection. Seidl et al. [15] conducted a longitudinal study using an e-nose to observe whether breath profiles of cystic fibrosis patients would change for different infection statuses of the disease. The authors found differences in the responses from the e-noses and therefore the VOC composition, for children who were *S. aureus* positive, compared to negative. This study acquired 72 pediatric subjects. In contrast, the work of Weber et al. [26] used SESI-HR-MS to annotate as many differing m/z features between healthy controls (49) and patients with cystic fibrosis (52), using literature comparisons and on-line MS^2 spectra. The authors were able to putatively identify 45 discriminatory exhaled VOCs, for which xanthine, glyceric acid, and glycolic acid were elevated in subjects with cystic fibrosis, compared to the healthy control group. In addition, the study reports a decrease in a group of aldehydes and acylcarnitines in cystic fibrosis patients, compared to the healthy controls. This work is a development from the author's previous study, Weber et al. [37]. In the study by Weber et al. [37], the authors found 171 m/z features that were significantly different in pediatric subjects with cystic fibrosis, compared to the healthy controls. As pathogenic bacterial infection is known to exacerbate cystic fibrosis through the infection and inflammation cycle which causes the decline in lung function, Kaeslin et al. [21] investigated whether different breath VOC patterns could be identified in patients hosting different bacterial infections, using SESI-HR-MS and principal component analysis. The authors were able to distinguish between six pathogens based on VOC profiles and putatively assigned VOC identifications, using 180 headspace samples.

Strengths in data quality assurance were explicitly addressed across these studies. In the study by Seidl et al. [15], a longitudinal design was implemented, and monthly quality-control gases were used to verify instrument performance and to check for sensor drift. In the study by Weber et al. [26], building on Weber et al. [37], MS^2 spectra together with literature matching were used for putative VOC identification. A similar approach was applied by Kaeslin et al. [21], who used SESI-MS/MS to further characterize VOCs. Such SESI-HR-MS-based assignments, even when putative, have the potential to advance breath analysis by linking observed signals to biochemical processes relevant to cystic fibrosis and to pathogen-specific signatures, an analytical depth that e-noses (as in Seidl et al. [15]) cannot provide. A notable limitation, however, is that in the three recent Swiss CF studies, most VOC identifications remain putative rather than confirmed.

There is, nevertheless, a clear need to refine our understanding of CF-associated breath profiles and the contributions of individual pathogenic infections that exacerbate disease. Priorities include improving confidence in VOC identification and clarifying the metabolomic pathways underlying changes in VOC patterns with CF and co-occurring infection. Distinguishing inflammatory from non-inflammatory VOC responses in CF is also important [15], as is assessing the influence of inhaled medications on measured breath signatures [26]. Although more work-intensive, orthogonal off-line methods such as GC×GC-TOF-MS and LC-MS/MS will be valuable to verify and refine the putative assignments reported to date [21]. Finally, as in many breath studies, larger cohorts and standardization across sites and protocols [37] are essential for robust cross-comparison and to support the translation of SESI-HR-MS into routine clinical practice.

6.4 | Obstructive Sleep Apnea (OSA)

A common breathing condition experienced during sleep, which is particularly prevalent at an advanced age, is obstructive sleep apnea (OSA) [17, 27, 185, 186]. OSA is caused by the narrowing and collapsing of the soft palate [27, 186], causing complete or temporary obstruction of breathing during sleep [187, 188], which therefore limits oxygen intake [189]. This results in recurrent apnea and hypopnea events [17, 187, 189]. The reduced quality of sleep [17] and oxygen intake leading to blood oxygen desaturation and in turn, increased blood CO_2 levels, during sleep significantly increase the development of further diseases [186] as well as daytime sleepiness [187] and headaches [188].

OSA is often underdiagnosed [17], and current methods to detect it are costly, time-consuming, and inconvenient for the patient, as they involve polysomnography in the hospital sleep lab [17, 186, 188, 189]. Furthermore, night-to-night variability is often a diagnostic challenge for OSA, requiring several nights for an accurate diagnosis of OSA [17]. Screening questionnaires may also be used, but these are not very accurate [188]. There is therefore a

lack of a reliable and fast diagnosis method for OSA [27], which a breath analysis method could one day suffice.

Work has already started on finding VOC patterns in OSA. Using SESI-HR-MS, Nowak et al. [17] confirmed clear differences in the VOC profiles of 149 subjects with and without OSA and found that biomarker levels were connected with diseases severity. The study also presented a list of 33 VOC biomarkers for OSA diagnostics, which the authors concluded to be robust enough despite inter-individual variability. In particular, numerous furanes, unsaturated aldehydes, and benzothiazole were identified. Furthermore, 32 of these species were found to correlate with the oxygen desaturation index, a well-known marker for OSA. Further work, however, is needed to understand the metabolomics behind this correlation. Following on from this work, Streckenbach et al. [27] were able to confirm 42 previously reported biomarkers associated with OSA, of which nine VOCs, including 4-(hexyloxy) phenol and 2-butylfuran, were significantly increased in subjects who did not receive OSA treatment, compared to patients who did. The findings were in agreement with previous work of Nowak et al. [17] and Schwarz et al. [190], despite changes in instrumental set-up and diversified cohorts, demonstrating promise for the eventual OSA diagnosis using VOC biomarkers.

Some future needs in research surrounding OSA are highlighted in both the work of Nowak et al. [17] and Streckenbach et al. [27]: the need for larger patient cohorts [17, 27], a significant need for further validation studies [17], as well as a standardized methodology for using SESI-HR-MS in the clinical field [17]. The quantification of such VOCs [27] will also be essential to define thresholds for the presence of disease, and enable continuous health monitoring of patients [27]. The addition of patient history as well as qualitative data such as screening questionnaires [17] could also enhance the accuracy of future studies. Accurate compound identification [27] and collaboration with clinicians and biomedical scientists would also help to better understand the association of metabolites with in vivo biochemical processes.

6.5 | Further Studies Involving Exhaled Breath

Since 2019, authors from Swiss institutions have also been involved in other breath analysis studies, not necessarily associated with disease but also focused on method development. The work of Decrue et al. [191], for example, evaluated the feasibility of using offline sampling using nalophan bags to be able to analyze the breath of infants, who are unable to breathe into a mass spectrometer. Offline sampling, which could also be beneficial for immobile, elderly, very weak individuals, or intensive care patients, is less ideal than online due to artifacts. This particular study investigated the accuracy of metabolites analyses in the breath of infants. The authors first tested an adult population of 13 subjects who provided 176 pairs of offline samples (into nalophan bags) and online real-time measurements, for detecting the presence of artifacts in the sampling bags. In parallel, nitric oxide which is an inflammation marker, and lung function which is an indicator of breathing patterns, were also measured. The authors found that the *m/z* features from the SESI-HR-MS using offline nanolophan bag sampling caused a reduction in signals, indicating the loss and adsorption of metabolites to the walls of the nanolophan bag, with a dependence on the functional groups within the metabolites. Some artifacts were found to be introduced from the material of the sampling bags and were ignored in the data analysis. Only *m/z* features with a Lin's CCC > 0.6 threshold were considered for further analysis. Furthermore, the authors were able to correlate 4-hydroxynonenal, a biomarker for oxidative stress, with nitric oxide and lung function. As a result, the authors were able to successfully apply breath sampling in Nalophan bags to study 16 infants.

Particle pollution is known to exacerbate lung diseases and is widely acknowledged to be connected to adverse respiratory conditions [192–196], a major cause of premature deaths. Air pollution caused an estimated 4.2 million deaths globally in 2019 [197]. Epidemiological studies have associated air pollution exposure with adverse respiratory [198] and cardiovascular conditions [199, 200], which may be induced by oxidative stress from air pollution exposure [201–203]. Oxidative stress may be induced by the exposure of a cell to organic compounds as well as transition metals present in particulate matter, causing cytotoxicity and inflammation [204–206]. It is currently measured by in vitro assays, although these types of clinical examinations often are limited to single time points and labor-intensive. Samples also cannot be reused [204]. To improve such tests, noninvasive, fast, and routine methods such as breath analysis would be ideal. A VOC analysis experiment was therefore completed by Cassagnes et al. [204], which investigated the oxidative stress induced by air pollution on cultured bronchial epithelial cells, by observing the released VOCs with PTR-MS. In this work, the cells were exposed to Cu(II), 1,4-naphthoquinone as single entities, as well as aerosol filter extracts from wood burning and secondary organic aerosol (SOA) originating from α-pinene. The authors found that dimethylbenzaldehyde, benzaldehyde, and acetonitrile were released by the cells upon exposure to biomass burning pollutants, α-pinene SOA, and 1,4-naphthoquinone, respectively. It was also noted that no VOCs were emitted when the cells were presented with Cu(II) and that the emission of benzaldehyde was associated with cell death. As a result, the authors suggest that their study paves the way for determining biomarkers as an indication of pulmonary damage as a result of exposure to particle pollution.

The work of Gaugg et al. [207] examined the VOC patterns in the breath of patients with idiopathic pulmonary fibrosis (IPF). This disease involves the gradual decline in lung function leading to worsening dyspnea and patients have an average life expectancy of only 2.5–5 years after diagnosis. The pathway to IPF development is poorly understood, although many with the disease have a medical history of smoking. By using SESI-HR-MS, Gaugg et al. [207] analyzed the breath of 21 IPF patients and 21 healthy controls, with a particular focus on the levels of proline and other amino acids, with the aim of validating the previous results obtained by Kang et al. [208]. Gaugg et al. [207] found an increase in the concentrations of allysine, leucine/isoleucine, valine, alanine, 4-hydroxyproline, and proline in the breath of patients with IPF (Figure 6). The study obtained a cross-validated area under the receiver operating characteristic curve of 0.86, indicating that the increases in these amino acids in breath could potentially be used as IPF biomarkers in breath samples.

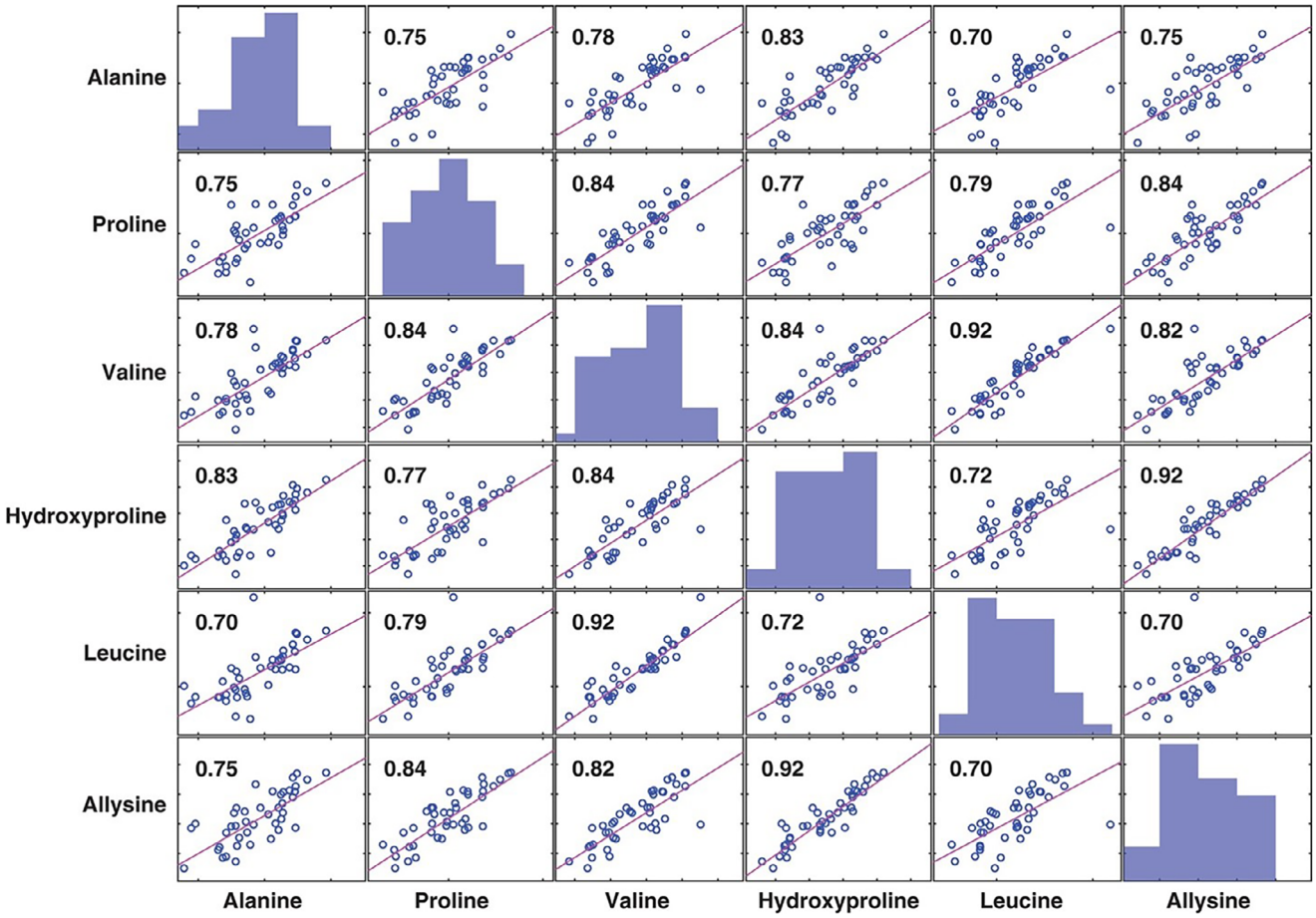

FIGURE 6 | Correlation matrix across all subjects for all amino acids that were found to be significantly increased in the exhaled breath of IPF patients. Numbers represent the pairwise Pearson correlation coefficients. Intensity distributions are shown on the diagonal. Reproduced from Gaugg et al. [207] with permission from John Wiley & Sons, Inc.

A common limitation of all studies reviewed in this section is the small sample size. Further validation is also required in the future to enable definitive statements regarding specific VOC patterns and biomarkers for particular disease incidence. A comprehensive investigation of other VOCs that produce the observed *m/z* spectral features, making up most of the data collected in many of these types of studies, is also lacking. Decrue et al. [191] also highlight the different limitations with online and offline sampling, including the presence of artifacts from the use of nalophan bags, and the issue of not being able to distinguish isomers with SESI-HR-MS.

7 | Diabetes and Other Metabolic Diseases

Due to the health and socioeconomic impact of diabetes, early diagnosis is crucial [209]. In recent years, authors affiliated with Swiss institutions have investigated the potential use of breath analysis for diabetes diagnostics. Nicolier et al. [18], for example, investigated the breath VOC signatures from type 1 diabetic subjects, with the aim of trying to identify unique biomarker VOCs associated with hypoglycemic episodes. Ten subjects with type 1 diabetes were induced with hypoglycemia. Frequent breath samples were taken between every 10 and 15 min and were analyzed using GC coupled to ion mobility mass spectrometry (GC-IMS). The authors found modest correlations between the concentrations of certain VOC species, for example, acetone and isoprene, and blood glucose levels. Machine learning models, including support vector machine classifiers as well as partial least squares discriminant analysis (PLS-DA), were used to map VOC signatures over different glycemic states. The models demonstrated a PLS-DA accuracy of 93% for categorizing different glycemic states. The authors conclude that measuring VOC profiles using GC-IMS may be a viable method in the future for monitoring and managing diabetes and emphasize that VOC profiles, as opposed to individual biomarkers, are essential for accurate analysis and classifying glycemic states. This study also highlights some limitations. A very small sample size of only 10 subjects was included in this work, limiting the generalizability of the study. The use of GC-IMS also restricted the number of VOCs that could be analyzed, compared to a GC-MS system, which has a comparatively higher sensitivity. Furthermore, systems such as GC-IMS are unsuitable for measuring small alkanes, such as propane, due to their lower proton affinity compared to water, rendering their analysis challenging.

A further serious complication of diabetes is diabetic ketoacidosis (DKA), which is an accumulation of ketonemia, acidosis, and hyperglycemia. Episodes of DKA occur when insulin levels are insufficient and require immediate medical attention. By

rehydrating a patient and providing insulin, metabolic processes are re-balanced, avoiding further risk of other complications [12, 210, 211]. The work of Awchi et al. [12] studied the breath VOC signatures of subjects to investigate the metabolic changes occurring throughout rehydration and insulin therapy of patients suffering from DKA. The study sampled 30 patients, of which 5 suffered a DKA episode. Offline breath samples were taken from patients and ran on a SESI-HR-MS. This study was longitudinal, that is, breath samples of DKA patients were taken during therapy and after recovery. The study detected acetoacetate, pyruvate, and acetone from SESI-HR-MS measurements, which were identified in the breath samples of the DKA patients. Furthermore, analysis of the collected spectra identified 665 *m/z* features that correlated with the metabolic progression toward a stable state of the patient. The authors suggest that their study offers a promising prospect for real-time monitoring of DKA in an ICU setting and provides further insight into the metabolic changes that occur during DKA therapy. The limitations of this work, however, include the interfering factors such as blood acidity induced by excessive aspirin use, diarrhea, or sultiame. Although the study was able to identify some VOC structures, the majority of VOCs were not identified. To further develop this work, the study highlights the need to identify additional VOCs from breath samples using specialized methods and to utilize these identified VOCs to gain a deeper understanding of the metabolic processes governing DKA.

Continuing the theme of metabolic illness, the release of bioactive compounds by adipocytes, that is, fat tissue, may be dysregulated in people suffering from obesity, leading to alterations in the normal functioning of metabolic and physiological processes. This leads to conditions such as cardiovascular and kidney diseases, as well as diabetes [14, 212, 213]. Furthermore, there is mounting evidence indicating that obesity induces chronic low-grade inflammation, which is linked to systemic metabolic dysfunction [213]. By understanding the metabolic dysregulation and the associated VOC signatures within adipose cells, there is the potential to apply this knowledge to the clinical field for early clinical diagnosis of metabolic disorders. This is especially true for type 2 diabetes, which is closely associated with adipose tissue metabolic dysfunction [14].

In order to investigate the metabolic processes within human fat cells, Mochalski et al. [14] used human Simpson-Golabi-Behmel syndrome (SGBS) adipocytes, the commonly used model cells, [214] to investigate the VOC signatures associated with metabolic processes occurring within fat cells [14]. To investigate the VOC signatures, the authors used GC-MS as well as head-space needle trap extraction for up-concentration. The study found 16 compounds emitted from a cultivation flask which were dependent on the presence of adipose cells. This included ethyl acetate, *n*-heptane, isoprene, 2-pentanone, acetone, 2-pentylfuran, 2-methyl-5-(methyl-thio)-furan, 2-ethylfuran, dimethyl disulphide, ethyl methyl sulphide, dimethyl sulphide, carbon disulphide as VOCs given off by the cells. Four more aldehydes were found to be metabolized and consumed by the cells: hexanal, pentanal, butanal, and 2-methyl-propanal. This was mostly attributed to the oxidation of these species by aldehyde dehydrogenases, to their corresponding carboxylic acids [14, 215, 216], with a potentially small fraction of aldehydes being reduced by alcohol dehydrogenases to alcohols [14, 217]. It is suggested in the work of Mochalski et al. [14] that the metabolism of cysteine and methionine, may produce sulfur-containing VOC products. The authors also suspect that the very high dimethyl sulfide (DMS) levels (median of 317 ppb) recorded may be due to reactive sulfur-containing compounds being detoxified by enzymes, producing species of reduced toxicity. In summary, the authors produced the first VOC signatures associated with human adipocytes and explored different possible reasons for the VOC patterns observed. As a result, this work demonstrates the potential for early detection of metabolic changes using VOCs, which could be achieved through breath analysis.

TDM is a branch of clinical chemistry that monitors medication levels in the blood for the purpose of achieving the correct dosage required for an individual. Some drugs have a narrow therapeutic window, which, in addition to patient inter-variability in response to a drug, renders the correct dose calculation challenging. Currently, blood-based TDM methods are used routinely. Noninvasive methods such as breath analysis would however be more attractive, especially for pediatric patients, due to its non-invasiveness [34]. In addition, patients incapable of proving an online sample at a sampling site, that is, babies, could still be sampled offline. The risk with offline sampling, however, is the potential occurrence of artifacts. The work of Awchi et al. [34] therefore set out to investigate the potential of using custom-made Nalophan bags for offline breath sampling, running the sample on a SESI-HR-MS within 30 min of collection. Based on previous work [218], the authors focused on volatile breath metabolites that were associated with valproic acid, a medication used for epilepsy, and monitored their concentration stabilities in connection to offline sampling with Nalophan bags. The work of Awchi et al. [34] developed on the work of Decrue et al. [191] by aiming to predict VPA concentrations in blood, based on offline breath VOC concentrations.

In this work, 40 pediatric patients (mean age of 11.5) who were taking VPA for epilepsy were sampled. Similar to Decrue et al. [191], the Lin's concordance correlation coefficient (CCC) was evaluated to assess the agreement between offline and online sampling methods. This was achieved by using a SESI-HR-MS, in which the authors focused on *m/z* features which were associated with VPA response and side-effects, and how suitable offline analysis using nalophan bags would be. Awchi et al. [34] found a Lin's CCC value of above 0.6 for all of the analyzed VPA features, except for two isotopic peaks of low signal intensity. As a result, the authors demonstrated the potential of using offline breath analysis to predict the VPA free fraction in blood. This is despite protonated heptanedione and protonated 3-heptanone being negative and positive artifacts, respectively.

8 | Conclusion and Perspectives

Clinical breath research has shown strong potential for non-invasive disease diagnosis and monitoring through advanced VOC detection methods such as SESI-HR-MS, PTR-MS, GC-MS, and e-noses. These technologies have provided early evidence of disease-specific metabolic patterns and predictive capabilities for conditions like asthma, COPD, diabetes, and lung cancer. Recent progress, particularly in Switzerland since 2019, underscores the field's proximity to clinical translation; however, significant challenges remain before breath analysis can be integrated into

routine practice. Current limitations include small and demographically narrow cohorts, inconsistent sampling and analytical protocols, and a lack of standardized calibration, validation, and external benchmarking systems. Cross-study variability still hampers reproducibility and regulatory acceptance.

To achieve clinical readiness, future studies must assemble large, well-characterized, and longitudinal patient cohorts across multiple centers, supported by harmonized workflows and validated methods for VOC collection, quantification, and identification. Greater collaboration between hospitals and research institutes is essential to increase patient access and enable multi-site standardization. Moreover, integrating healthy and disease controls, multi-omics data, and biochemical pathway analysis will strengthen mechanistic interpretation and clinical relevance. Coordinated benchmarking, transparent data sharing, and methodological standardization will ultimately be critical to establish robust, disease-specific VOC signatures and move breathomics from research to routine clinical application.

Author Contributions

Stefan James Swift, Renato Zenobi, and Stamatios Giannoukos discussed and defined the initial structure of the manuscript. Stefan James Swift prepared the first draft of the current publication. Kseniya Dryahina and Patrik Španěl contributed expertise on PTR-MS and SIFT-MS technologies, Flora Kontopidou on diagnostic aspects, and Christos Kokkotis on the statistical components of the reviewed studies. Stamatios Giannoukos and Stefan James Swift jointly prepared the second version of the manuscript, and Stamatios Giannoukos secured funding for this work. All authors critically evaluated and revised the manuscript and approved the final version, agreeing to be accountable for all aspects of the work.

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Conflicts of Interest

The authors declare no conflict of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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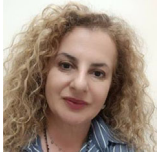
Biographies

Dr. Stefan James Swift, MRSC, is a postdoctoral research fellow at ETH Zurich, working on breath analysis for disease detection. He completed his PhD in Chemistry at the University of York, where he investigated $PM_{2.5}$ in Asian megacities. Following this, he undertook his first postdoctoral position at the J. Heyrovsky Institute of Physical Chemistry in Prague, where he studied ion-molecule reaction kinetics using SIFT-MS. He then became a visiting researcher at the University of Oslo, working on the NASA ASIA-AQ campaign, where he measured particulate pollution using a CHARON-PTR-ToF-MS around Asia on the NASA DC-8 research aircraft.

Kseniya Dryahina is a scientist at the J. Heyrovsky Institute of Physical Chemistry, Czech Academy of Sciences, in Prague. She earned her PhD from Charles University after completing her studies in physics at Sumy State University in Ukraine. Her research focuses on ion chemistry involved in the quantification of volatile trace compounds in air, exhaled breath and in the headspace of biological samples. Over the past two decades, she has contributed to interdisciplinary studies in clinical diagnostics, food science, and environmental chemistry.

Patrik Španěl serves as Vice-Director for Science and heads the Department of Gas-Phase Ion Chemistry at the J. Heyrovský Institute of Physical Chemistry, part of the Czech Academy of Sciences in Prague. He studied plasma physics at Charles University and earned his Dr. rer. nat. in ion physics from the University of Innsbruck. His research centers on ion-molecule reactions in the gas phase and the development of mass spectrometric techniques for analyzing trace gases and VOCs. He co-developed SIFT-MS, established kinetic data for specific reagent ions, and demonstrated real-time breath analysis for clinical and environmental use.



Dr Flora Kontopidou is a physician specialized in Internal Medicine, Infectious Diseases, and Public Health, with extensive experience in managing infections by multidrug-resistant (MDR) pathogens in high-AMR healthcare settings. She spent over a decade at the Greek National Organization of Public Health, coordinating national AMR and HAI control interventions under the One Health framework. Her PhD focused on implementing antibiotic stewardship in hematology units. She currently leads Infection Prevention and Control and Antibiotic Stewardship at MITERA General Hospital in Athens and serves as the Scientific Coordinator in Greece for the European REVERSE Project on antimicrobial stewardship and infection prevention.

Christos Kokkotis, PhD, is a Physicist and Machine Learning Engineer specializing in motion analysis and healthcare-related predictive modeling. He holds a PhD from the University of Thessaly, where he worked on applying machine learning algorithms to assess quality of life, focusing on conditions such as Knee Osteoarthritis, Stroke recovery, and ACL rehabilitation. He has contributed to research projects at CERTH and continues as a postdoctoral fellow at the Democritus University of Thrace. His research interests include time-series analysis, feature engineering, and developing ML and deep learning models.

Renato Zenobi is Professor for Analytical Chemistry at ETH Zurich. He is best known for using modern mass spectrometry methods to solve problems in the life sciences, for his contributions to understanding ion formation mechanisms in laser mass spectrometry, and for the invention of tip-enhanced Raman spectroscopy, a method that allows one to gain detailed molecular information on the nanometer scale. Together with clinicians at the University Hospital Zurich, he is developing a mass spectrometric "chemical nose" that could revolutionize medical diagnosis. He is the recipient of numerous fellowships and awards.

Stamatios Giannoukos is a Senior Scientist/Lecturer at the Department of Chemistry and Applied Biosciences at ETH Zurich. Trained in chemical engineering and holding a PhD in electrical engineering/electronics (University of Liverpool), he specializes in analytical chemistry, ambient ionization, and high-resolution mass spectrometry. His work spans real-time breath metabolomics (clinical diagnostics, NutriExhalome), animal health monitoring, microbial volatilomics, and environmental/atmospheric field studies. He has contributed to the development of portable mass spectrometry systems, led/participated in multiple EU and Swiss projects, and authored over 50 journal papers and 80 conference publications.