Chapter 13
Metabolomics in Asthma

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Abstract Asthma and airway inflammation are responses to infectious stimuli and the mechanisms of how they are mediated, whether by the innate or adaptive immune response systems, are complex and results in a broad spectrum of possible metabolic products. In principle, a syndrome such as asthma should have a characteristic temporal-spatial metabolic signature indicative of its current state and the constituents that caused it. Generally, the term metabolomics refers to the quantitative analysis of sets of small compounds from biological samples with molecular masses less than 1 kDa so unambiguous identification can be difficult and usually requires sophisticated instrumentation. The practical success of clinical metabolomics will largely hinge on a few key issues such as the ability to capture a readily available biofluid that can be analyzed to identify metabolite biomarkers with the required sensitivity and specificity in a cost-effective manner in a clinical setting. In this chapter, we review the current state of the metabolomics of asthma and airway inflammation with a focus on the different methods and instrumentation being used for the discovery of biomarkers in research and their future translation into the clinic as diagnostic aids for the choice of patient-specific therapies.

13.1 Introduction

As discussed in other chapters (e.g., Brasier and Zhao, Chap. 15), asthma is characterized by episodic airway inflammation coupled with reversible obstruction that is oftentimes linked to specific environmental influences such as

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aero-allergen exposure or respiratory virus infection. Clinicians generally choose therapies for the control of an asthmatic episode based on the patient's symptoms, most of which are primarily due to the airway inflammation. The mechanisms of how asthmatic airway inflammation is mediated, whether by the innate or adaptive immune responses, are complex and involve a number of different cell types and numerous pathways and results in a broad spectrum of metabolic products. The infectious stimuli, in principle, shape the response into a set of symptoms with a characteristic temporal–spatial metabolic profile that could be potentially used as a source of biomarkers or as a spectral "finger print" to identify the stage and trajectory of the episode, which would aid in the choice of therapeutic action.

Thus, being able to determine the degree of inflammation by a readily available and robust laboratory test to identify the presence and relative amounts of biomarkers for inflammation should have a great deal of value in the clinic. Unfortunately, such a system does not currently exist, but there are sophisticated research instrumental analysis methods that have the potential to do so, if they can be modified for use in clinical practice. One candidate Omics technique being intensely studied for this purpose is metabolomics. Metabolomics is interesting to us because metabolism has been scientifically studied for well over two centuries, is well understood, many metabolic pathways have been described in great detail, and a large number of metabolites and their precursors have been chemically identified. Generally, the term metabolomics refers to the quantitative analysis of the full range of metabolites found in a biological specimen. Metabolites are usually (but not always) considered to have molecular mass less than 1 kDa so unambiguous identification can sometimes be challenging. Thus, full detection and identification can be very difficult in practice and many investigations instead have chosen to focus on subsets of metabolites that have well-identified spectral properties that are known to be associated with a specific metabolic state (e.g., a set of symptoms). Others have chosen to use differential multivariate analysis (e.g., by Principle Components Analysis, PCA) to identify spectral patterns that have features characteristic of the specific and instantaneous metabolic state of the donor when the sample was acquired. Though this can be done without identifying each metabolite contributing to the characteristic spectral signature, it has become generally accepted that this should be done in order to validate the biomarker pattern and establish biological context through pathways analysis and other data mining techniques.

Metabolism itself generically covers all the chemical reactions that occur in living organisms and is divided into anabolism, which are the constructive processes where complex molecules are synthesized, and catabolism, those processes that break down large molecules such as proteins into metabolites. Metabolites are thus small molecules, which are the end products of the catabolic digestion of larger precursor molecules. Every protein or other molecule in the body has a metabolic fate such that there is a panel of metabolites that remain after its inevitable degradation, thus in principle, a syndrome such as asthma should have a metabolic signature indicative of its current state and the constituents that caused it.

Metabolomics thus searches for small-molecule metabolite profiles that describe a metabolome, which is the set of all metabolites that characterize the specific state of an organism at a specific point in time. Because different metabolites arise from different processes within specific cells and tissues at a specific time, the metabolome gives us a detailed temporal-spatial description of the current state of the organism, which can then be compared against other states (e.g., the presence or absence of airway inflammation). If we discover a metabolite profile whose signature is diagnostic for a specific physiological state such as early airway inflammation, we call this set of metabolites a panel of biomarkers.

Metabolomics is also attractive because we should be able to rationalize the metabolic signature of an airway inflammatory state according to the regulatory pathways characteristic of that inflammatory state. Metabolic regulation allows organisms to respond to signals and to interact with their environments. Metabolic pathways and reactions are highly regulated to maintain a constant set of cellular conditions in homeostasis. Many diseases, including airway inflammation where a cascade of biochemical events mediates the inflammatory response, are characterized by a disturbance of homeostasis. Inflammatory mediators are generally short lived and are quickly degraded leaving a trail of metabolites, some of which will be potential biomarkers.

The practical success of metabolomics, in asthma specifically and airway inflammation in general, will largely hinge on a few key issues. One is the ability to capture a readily available and reliable biospecimen (e.g., a biofluid) from a patient that can be processed in such a way as to make metabolomic analysis possible. Samples that are currently being used to study the metabolomics of asthma include induced sputum, bronchoalveolar lavage fluid (BAL), blood, urine, and exhaled breath condensate (EBC). Another key issue is the ability to reduce to practice a metabolite instrumental analysis method that can identify metabolite biomarkers with the required sensitivity and specificity in a cost-effective manner in a clinical setting. Several different methods are being actively pursued for this purpose and we discuss them here.

13.2 Current Literature on the Metabolomics of Asthma

At least ten metabolomics review articles have appeared in the literature over the last few years, several focusing on airway diseases, and these have been summarized briefly in Table 13.1. Collectively these contain over 700 (not necessarily unique) references and the number of references in each article is noted in the table for convenience. Adamko et al. (2012) is the most recent as of this writing that focuses specifically on the metabolomics of asthma. Note that the articles that have a download charge were not reviewed for this chapter and are only included in the References and Table 13.1 for completeness. For practical reasons, none of the reviews in the table are discussed in detail here and the reader is encouraged to access them online if interested in the particular subject matter presented therein.

Table 13.1 Recent metabolomics reviews with emphasis and number of references

Year (#Refs)	References	Title	Emphasis
2012 (57)	Adamko DJ, Sykes BD, Rowe BH., Chest. 141(5) (2012 May) 1295–302	The metabolomics of asthma: novel diagnostic potential	Outlines recent work to develop the metabolomic profile of asthma
2012 (124)	Cathcart MP, Love S, Hughes KJ, Vet J. 2012 Mar;191(3):282–91	The application of exhaled breath gas and exhaled breath condensate analysis in the investigation of the lower respiratory tract in veterinary medicine: A review	Discusses EBC analysis in veterinary medicine with potential application in monitoring disease progression and treatment response in animals
2012 ($60)	Snowden S, Dahlén SE, Wheelock CE. Bioanalysis. 2012 Sep;4(18) 2265–90	Application of metabolomics approaches to the study of respiratory diseases	An in-depth discussion of metabolomics for the study of respiratory diseases, including experimental design, choice of clinical material to be collected, and potentially confounding experimental factors as well as particular challenges in the field
2012 (£43)	Zhou B, Xiao JF, Tuli L, Ressom HW. Mol Biosyst. 2012 Feb;8(2):470–81	LC-MS-based metabolomics	Presents a workflow for LC-MS metabolomic analyses for the identification and quantitation of metabolites discussing challenges and solutions in each step of the workflow
2011 (100)	Dunn WB, Broadhurst D, Begley P, Zelena E, Francis-McIntyre S, Anderson N, Brown M, Knowles JD, Halsall A, Haselden JN, Nicholls AW, Wilson ID, Kell DB, Goodacre R; Human Serum Metabolome (HUSERMET) Consortium., Nat Protoc. 2011 Jun 30;6(7):1060–83	Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry	Describes the detailed workflows for long-term and large-scale metabolomic studies involving thousands of human samples with data acquired for multiple analytical batches over many months and years. Protocols for serum- and plasma-based metabolic profiling by GC-MS and UPLC-MS are described including biofluid collection, sample preparation, data acquisition, data preprocessing, and quality assurance
2011 (38)	Sofia M, Maniscalco M, de Laurentiis G, Paris D, Melck D, Motta A., J Biomed Biotechnol. 2011 2011:403260	Exploring airway diseases by NMR-based metabonomics: a review of application to exhaled breath condensate	Reviews the use of EBC as a matrix for NMR metabonomic studies in airway diseases, including separating specific EBC profiles with implications for disease phenotyping and personalized therapy

2010 (£43)	Junot C, Madalinski G, Tabet JC, Ezan E., Analyst. 2010 Sep;135(9) 2203–19	Fourier transform mass spectrometry for metabolome analysis	FT/MS provides accurate mass measurements with ppm to sub-ppm errors and high to ultra-high resolving power. Evaluates the improvements to metabolomics by different types of FT/MS instruments including various analytical requirements, e.g., global metabolite profiling, absolute quantification and structural characterization, and data preprocessing
2010 (79)	Want EJ, Wilson ID, Gika H, Theodoridis G, Plumb RS, Shockcor J, Holmes E, Nicholson JK, Nat Protoc. 2010 Jun;5(6) 1005–18	Global metabolic profiling procedures for urine using UPLC-MS	Describes UPLC-MS for urinary metabolite profiling, including sample preparation, stability/storage and the selection of chromatographic conditions to balance metabolome coverage, chromatographic resolution and throughput. Discusses QC, metabolite identification, and multivariate data analysis
2007 (145)	Dettmer K, Aronov PA, and Hammock BD, Mass Spectrom Rev.; 26(1) (2007) 51–78	Mass spectrometry-based metabolomics	Describes sample preparation, separation, and MS analysis with examples for metabolic fingerprinting, which requires the analyses of all detectable analytes in a sample
2007 (164)	Metz TO, Zhang Q, Page JS, Shen Y, Callister SJ, Jacobs JM, and Smith RD, Biomark Med. 2007 June ; 1(1): 159–185	The future of liquid chromatography-mass spectrometry (LC-MS) in metabolic profiling and metabolomic studies for biomarker discovery	Describes NMR, GC-MS, and LC-MS metabolomics for biomarker discovery with emphasis on LC separations and ESI approaches with their advantages and disadvantages

Amounts in $US or £UK in parenthesis indicate an article which must be purchased and the cost

13.3 Sample Collection and Preparation Methods

Most of the review articles in Table 13.1 have excellent sections on sample collection so this is not discussed here with the exception that due to the increasing interest and potential importance of EBCs to the metabolomics of airway inflammation field, it is worthwhile to discuss it in more detail here.

13.3.1 Exhaled Breath Condensates

Exhaled air contains mixtures of gases, volatile organic compounds (VOCs), nitric oxide (NO), and nonvolatiles such as peptides and cytokines, so differential metabolomic profiling of EBCs is emerging as an increasingly useful method for the determination of airway disease state. The collection of EBC samples is noninvasive, reproducible, and well tolerated by patients. EBC samples are collected by having patients exhale through a mouthpiece where the breath is then cooled resulting in a condensed fluid sample. Further processing may be necessary depending upon the instrumental method of choice. Details of EBC sample collection and preparation, especially for Gas Chromotography-Mass Spectrometry (GC-MS) and Nuclear Magnetic Resonance (NMR), are included in several reviews and articles referenced here and references therein (Sofia et al. 2011; Fens et al. 2009; Loukides et al. 2011; Carraro et al. 2007). Results of EBC analyses are very encouraging and in an early proof of principle study, Carraro et al. (2007) demonstrated that EBC analysis by NMR can provide airway metabolomic fingerprints of low molecular weight (MW) analytes in EBCs that are able to successfully discriminate between asthmatic and healthy children. Recently, the NMR of EBCs has also been shown to discriminate between patients with stable vs. unstable cystic fibrosis (Montuschi et al. 2012). Virtually all these studies have been done at very low statistical power and the use of NMR for the accurate and reproducible determination of the constituent compounds in EBCs is not without some controversy (Izquierdo-García et al. 2011; Motta et al. 2012; Sinha et al. 2011) and this is discussed later. EBCs are clearly very encouraging for use as a biological fluid for metabolomic studies of airway inflammation and asthma. Its use beyond that of a research medium has been hindered by the lack of standardized protocols and reference values but that effort is gathering momentum as interest expands (Loukides et al. 2011).

13.3.2 Sample Preparation

Sample preparation techniques are generally extensible across the range of instrumentation used in metabolomic studies and are chosen as much for the sample type and the type of metabolites to be observed as anything else. A typical biospecimen is a complex mixture of cells, large and small molecules, and salts common to biological fluids, so the successful extraction of metabolites from one is challenging. It

is, however, clearly one of the most crucial steps in the process of conducting a metabolomics experiment. This is particularly true for samples common to asthma studies such as BALs and EBCs which can contain many low abundance metabolites and VOCs. Important metabolite physicochemical characteristics include volatility, hydrophilicity, lipophilicity, polarity, thermal stability, the presence of active hydrogens in functional groups and lability. Thus, choosing the proper sample preparation technique to be used is exceedingly important for achieving high quality and reproducible quantitative results from the instrument. The procedure chosen must be appropriate for both the sample and the instrument and requires a skilled practitioner for the results to be reproducible. This is especially important for low abundance and chemically labile metabolites. Most metabolomics journal articles give reasonably good details of their sample preparation with references and it behooves any investigator to follow the literature in this regard as the field moves forward rapidly. A general review of sample preparation methods and best practices is well beyond the scope of this article and there are several recent and thorough reviews and articles of metabolomics with good discussions of sample preparation (see refs in Table 13.1 and Fens et al. 2009; Loukides et al. 2011; Carraro et al. 2007).

13.3.3 Separation Methods

Liquid chromatography (LC) and gas chromatography (GC) are commonly used methods to serially resolve complex mixtures of compounds commonly found in biological samples into either very small packets of coeluting molecules with similar physicochemical properties, or ideally, as discrete molecules in order to reduce spectral complexity and background noise and to improve detection limits and data quality. It is the mobile phase which is either a liquid or gas and both use capillary columns with varying stationary phases depending on the physicochemical properties of the metabolites of interest. In the case of NMR studies, the chromatographic separation is done offline, but for MS studies it is done in-line with the spectrometer. Besides general high-performance liquid chromatography (HPLC), LC variations increasingly being used in metabolomics studies include reverse phase LC (RPC), which typically uses narrow bore columns with 3–5 μm particle sizes, and ultra performance LC (UPLC) which uses very small, sub-2 μm particle sizes but requires specialized, very high-pressure UPLC equipment (Want et al. 2010; Dettmer et al. 2007). Capillary electrophoresis (CE) is an excellent method for the high-resolution separation of charged metabolites and CEMS should also see more use in metabolomic studies (Dettmer et al. 2007). These methods are almost always performed inline with the mass spectrometer. There are numerous variations in use or emerging in LC and GC practice and one should consult any of the many excellent texts or reviews (Dettmer et al. 2007) on the subject for details, much of which is readily available online.

GC-MS is widely used in metabolomics studies because it is an excellent method for the separation, detection, and identification of small compounds and especially for VOCs. The technique is rapid and the instrumentation has become well suited

for clinical use. GC-MS for urine testing is widely used in forensics, for the detection of illegal or performance enhancing drug use, and increasingly in medicine for the detection of congenital metabolic diseases. This makes it very suitable for airway inflammation studies because small molecules are often found in BAL and urine samples and VOCs are commonly found in EBC samples (Dettmer et al. 2007). Metabolic activity can also be measured using GC-MS by isotopically labeling metabolic compounds then calculating the isotopic ratios (e.g., ^{12}C–^{13}C).

13.4 Instrumental Methods

There are two major instrumental methods being actively pursed as a means of performing metabolite measurements suitable for asthma samples that have the requisite sensitivity and specificity to be useful. These are nuclear magnetic resonance spectrometry (NMR) and mass spectrometry (MS). MS can be further subdivided according to the applied molecular separation technique used as being either LC-MS or GC-MS or Capillary Electrophoresis/Mass Spectrometry (CE-MS) (Dettmer et al. 2007). Each has its advantages and disadvantages and no single one of them is appropriate under all sample types or conditions. Currently, all of them are performed on extremely complex, sophisticated, and expensive research instruments that require highly trained experts to run them and skilled bioinformaticists to analyze the results. A third, emerging nanosensor technique, which has promise to be very cost-effective and suitable for clinical use is the eNose (Adamko et al. 2012; Fens et al. 2009; Dutta et al. 2002; Ideo 2012; The eNose Company 2012).

A commonality across all these methods is that due to the broad range of chemical species represented by even a modest set of naturally occurring metabolites that might be found in an analytical sample, the experimentalist is presented with an equally broad range of chemical and physical properties that can be exceedingly challenging to approach from an analytical chemistry standpoint. The requirement for metabolite identification and quantification across orders of magnitude differences in abundance increases these challenges considerably. Many metabolites are either naturally labile to oxidation, hydrolysis, or other chemical transmutation or become so under the conditions of the instrumental analysis. Thus, samples must be handled appropriately as soon as they are obtained and much care must be taken in the sample preparation phase regardless of the spectroscopic method of choice. This takes both skill and experience and inadvertently mishandling a sample often becomes an insidious source of indeterminate error.

13.4.1 Nuclear Magnetic Resonance Spectrometry

NMR spectrometry is a research method that exploits the behavior of certain atomic nuclei in a strong magnetic field when hit by a high-frequency pulse of radio waves. For the purposes of metabolomics, the primary nucleus of interest is usually the

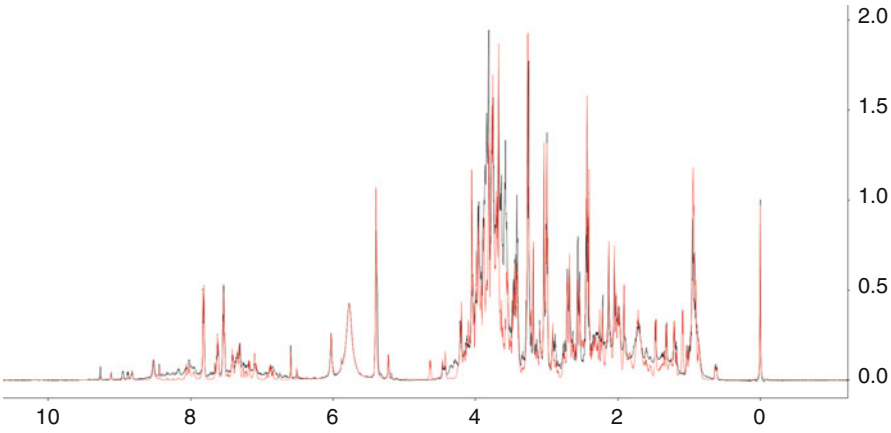

Fig. 13.1 1D proton NMR spectrum. Rat urine *metabolomics* NMR sample showing the original spectrum (*black*) and the computer-generated deconvoluted spectrum (*red*)

hydrogen-1 proton (^{1}H), but carbon-13 (^{13}C) and nitrogen-15 (^{15}N) nuclei are also used. Depending on the chemical structure of a molecule, these nuclei will be arranged by covalent bonds into characteristic spatial patterns that are to varying degrees unique. This spatial organization of the NMR nuclei gives rise to a characteristic spectral pattern, i.e., spectrum for each molecule. NMR spectra for metabolomics studies are generally always taken on samples in solution, which contain from several to many different molecules, each of which has its own characteristic spectral pattern. The resulting NMR spectra of a solution sample will therefore be comprised of the superposition of the unique molecular spectral patterns for each molecule contained in the sample. Thus, each sample spectrum is characteristic of the molecules contained within the sample.

Once you have the specimen's experimentally derived NMR spectrum, the challenge is to deconvolute the composite spectrum into its constituent molecular signature spectra. This is done using knowledge base driven software such as Chenomx NMR Suite (Chenomx Inc. 2012) to identify and quantify the small molecule content of the sample. It does this by searching its database of small molecule metabolite spectra to find one that overlays to a portion of the sample spectrum. It continues to do this until it has accounted for the entire spectrum. The software then superimposes the metabolite spectra from the database onto the experimental spectrum (Fig. 13.1) then produces a list of potential metabolites in the sample solution and their relative concentrations. In practice, this works well if the metabolites within your sample are represented in the spectral knowledge base. You also have the option of running stock samples of specific metabolites yourself and adding these spectra to the knowledge base. Completely unknown metabolites can be challenging, however, and can take some time, skill and effort to resolve. There are also

other software packages available and many of them, including ones for mass spectrometry metabolomics, are described on the Metabolomics Society's Metabolomics Software and Servers webpage (Metabolomics Society 2012).

NMR is being used with increasing success in the determination of asthma and airway inflammation metabolomics. In Adamko et al. (2012), they reviewed several such studies, all preliminary, but highly supportive that the use of NMR in metabolomics studies warrants further investigation. One study they reviewed showed that the NMR analysis of EBCs could discriminate between children with or without asthma (Carraro et al. 2007). Another study used urine samples to discriminate between groups of children with stable asthma vs. those with unstable asthma that were seen in the emergency department (Saude et al. 2011). Future studies will require a significantly larger number of subjects to be statistically conclusive, but these results are very promising.

13.4.2 NMR Studies on Exhaled Breath Condensates

As mentioned earlier, there has been some controversy over the use of NMR for the analysis of EBC samples due to suspected contamination and the inherent insensitivity of NMR. In a 2011 study by Izquierdo-Garcia et al., they concluded that previous studies, which used an EBC sample collection apparatus with reusable condenser parts, showed artificial peaks in the spectrum. It was concluded that the primary source of these peaks was from contamination by residual disinfectant used in cleaning the apparatus. The removal of this contamination appeared to be refractory to standard cleaning protocols. This produced an artificial metabolic fingerprint not related to the endogenous metabolic pathway of the lungs. Furthermore, they posited that because NMR does not have the sensitivity "required to observe the endogenous metabolites presented in the EBC," it should not be used to analyze EBC samples. Based on their study, they then proposed that UPLC-MS and nonreusable collection devices be used as a standard method for metabolomic studies of EBC.

The controversy in their statements arose because in an earlier 2007 study, Carraro et al. were able to clearly discriminate between children with asthma vs. healthy children with a success rate of approximately 86 % using selected signals from EBC NMR spectra using a different reusable sample collection device than the 2011 study (Izquierdo-García et al. 2011).

Furthermore, a 2011 study by Sinha et al., subsequent to the 2011 Izquierdo-Garcia et al. study, used disposable EBC collection tubes to demonstrate reproducible and useful metabolomic fingerprinting using NMR on EBC samples that was able to distinguish clearly between asthmatic and normal patients. They stated in conclusion that their study removed the need for further debate and that the suitability of NMR for the metabolomic analysis of EBC samples was firmly established.

In a very recent study, Motta et al. (2012) assessed the effects of a cleaning procedure, different from that used in the 2011 Izquierdo-Garcia et al. study, on a reusable-part condenser on EBC metabolomics. They determined that when the

same collection device is used repeatedly and using Milton (Milton Pharmaceutical UK Ltd, Gloucester, UK), a pure sodium hypochlorite solution cleaning product, the metabolic profiles of EBC were not altered. Furthermore, to appraise the ability of NMR spectroscopy on EBC samples to discriminate between chronic obstructive pulmonary disease (COPD) patients and healthy subjects, they collected 54 EBC samples from 27 patients with COPD (16 males and 11 females) and 27 HS (healthy subjects; 16 males and 11 females) using an EcoScreen condenser. Using principal components analysis and partial least squares discriminant analysis, NMR-based metabolomics of EBC successfully and decisively discriminated between COPD patients and HS with $r^2 = 99.9$ %. They concluded that EBC NMR is suitable and useful for characterizing the metabolic fingerprints of patients with respiratory diseases. They also proposed that the combination of several different techniques, including NMR and MS, might establish "breathomics" as a new noninvasive approach for the assessment of patients with respiratory diseases.

Considering these findings, it seems clear that technique and procedure are exceedingly important in the study of EBCs using NMR or any other method. Thus, NMR has many advantages as a metabolomics method, but there are several drawbacks which require serious consideration as well.

13.4.3 NMR Advantages

NMR samples are relatively fast and easy to run if you have an experienced NMR spectroscopist on your team and access to a modern high-field NMR instrument in the range of 400–800 MHz. Cost of the sample run is relatively low with the single largest material expense being the NMR tube, which is less than $20. It is possible for experienced operators to run 60+ samples in a day.

NMR actually collects the response of each single proton on each molecule in the sample so NMR is inherently highly quantitative. Thus, it is fairly easy to determine relative metabolite concentrations within a sample and absolute concentration quantitation can be improved by spiking samples with a known concentration of a standard compound.

13.4.4 NMR Disadvantages

NMR is an inherently insensitive technique and requires fairly high concentrations of each molecule in the solution to adequately collect a high-quality spectrum across all the metabolites present. In practice, metabolite concentrations in a sample range from fairly high to vanishingly low so NMR will generally find the mid- to high-level metabolites, but the low-level metabolites will be lost in the baseline. If your metabolites of interest are reasonably highly abundant, then you will do well but if they are not you will not see them and need to use an alternate method such as MS.

High-field NMR instruments and highly trained and skilled NMR spectroscopists are not trivial assets and are exceedingly expensive to acquire and sustain so you will have to have access to an established NMR facility. Unless you have both of these your experiment will not be successful. The hourly cost for the use of an NMR instrument varies widely depending on the facility and whether it is a commercial facility or an academic facility. There are commercial services that will run and analyze your samples for you, but these change frequently so they are not referenced here and can be found fairly easily by searching the internet.

13.5 Mass Spectrometry

Mass spectrometry (MS) is an enabling technology for the high-resolution investigation of extremely small samples and for low-abundance molecules. Experimentally, MS measures the mass-to-charge ratio of charged molecular ions or their fragments in order to determine their chemical structures and identities by using a software search engine and database tables containing the spectra of known compounds. In metabolomics, the molecules of interest are usually (but not always) small, typically <1 kDa, and include a wide variety of chemical species.

A mass spectrometer is technically a highly sophisticated, complex, and expensive instrument. It works by ionizing chemical compounds to generate charged molecules or molecule fragments and measuring their mass-to-charge ratios (m/z) using magnetic and electrical fields in a high vacuum. MS instruments consist of three modules:

1. An ion source to convert gas phase molecules into molecular ions.
2. An analyzer, which uses electromagnetic fields to sort the resulting ions according to their mass and charge.
3. A detector, which provides data for quantitating each ion present.

The resulting signals comprise the mass spectrum, which is typically a graph in which the mass to charge m/z ratio increases along the abscissa (horizontal axis) and the relative abundance or intensity increases on the ordinate (vertical axis). Oftentimes, the m/z vs. intensity pattern or "fingerprint" is diagnostic for a specific metabolite but not always and generally needs to be verified, especially for the identification of biomarkers.

13.5.1 MS Advantages

The primary advantage of mass spectrometry in metabolomics is the ability to measure accurately and quantitatively low-abundance metabolites. With recent advances in mass spectrometer technology, this already impressive capability is improving substantially with Fourier transform MS (FT-MS) and FT ion cyclotron resonance MS (FT-ICR-MS), which achieves both high resolution and mass accuracy. 2D

FT-ICR MS is also in development. With these advances, the future of high-resolution MS-based metabolomics is very bright.

13.5.2 MS Disadvantages

The main disadvantages of mass spectrometry are similar to NMR. MS requires very expensive, highly sophisticated instruments that require highly skilled and well-trained operators to run the experiments. MS can be very time-consuming and experiments take substantial preparation. Instrumental results are challenging to analyze and require substantial computational and bioinformatics support. Data management concerns and computational demands can also be significant. Taken altogether, the effort and cost of MS experiments must be carefully considered, but when properly carried out, the results will be worth the time and expense.

13.6 eNose

The electronic nose (eNose) is emerging as having the potential for practical clinical use. It is a portable device that uses an array of composite nanosensors with embedded algorithms to analyze the different fractions of the VOC mixture in exhaled breath to identify a pattern that is diagnostic for the disease phenotype. Both Adamko et al. (2012) and Fens et al. (2009) discuss the use of the eNose in asthma and airway inflammation in more detail. In Fens et al., they used the Cyranose 320 eNose (Dutta et al. 2002; Ideo 2012) (Smiths Detection, Pasadena, CA, USA) to differentiate COPD from asthma subjects and was consistent with their GC-MS findings. An eNose is also being developed by The eNose Company, a small R&D company in the Netherlands (2012). Medically relevant areas of application include the detection of metabolites for medical diagnostics applications such as tuberculosis, asthma, diabetes, and head and neck cancer among many others. If successful in clinical trials, the eNose has the potential to provide an appropriately scaled, simple to use, and highly cost-effective clinical diagnostic aid.

13.7 Conclusions and Projections

Metabolomics studies of asthma and airway inflammation and associated diseases hold tremendous promise. While currently confined to the laboratory, with the substantial amount of talent and resources being increasingly focused on the field, it is virtually inevitable that metabolomics will have significant future impact in the clinic (Adamko et al. 2012; Atzei et al. 2011) as a component of systems and translational medicine. It has the potential to considerably enhance clinical chemistry diagnostics in this area as well as other pathologies.

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