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Original Research

Developments in the Analysis of Biological Volatile Organic Compounds: Breast cancer and Cystic Fibrosis Using Gas Chromatography-Mass Spectrometry

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Abstract:

The technique of gas chromatography-mass spectrometry (GC-MS)-based metabolomics is highly suitable for the identification and quantification of small molecular metabolites (with a molecular weight of less than 650 daltons). These metabolites encompass small acids, alcohols, hydroxyl acids, amino acids, sugars, fatty acids, sterols, catecholamines, drugs, and toxins. In order to make these compounds volatile enough for gas chromatography, chemical derivatization is frequently employed. The purpose of this unit is to demonstrate that GC-MS-based metabolomics makes it possible to simply integrate targeted tests for absolute quantification of certain metabolites with untargeted metabolomics in order to find novel substances. GC-MS is able to detect and semi-quantify approximately 200 substances per study in human body fluids (such as plasma, urine, or stool) samples. This capability is complemented by database annotations that make use of huge spectral libraries and validated, standardised standard operating procedures. Similar to liquid chromatography-mass spectrometry (LC-MS) untargeted profiling, deconvolution software enables the detection of more than 300 extra unidentified signals. These signals can be annotated by using precise mass instruments and proper data processing methods. Because of this, gas chromatography-mass spectrometry (GC-MS) is a well-established technique that not only makes use of traditional detectors (quadrupole), but also target mass spectrometers (triple quadrupole) and accurate mass instruments (quadrupole-time of flight).

Keywords: Volatile Organic Compounds, Breast cancer, Cystic Fibrosis

Introduction:

There are almost fifty years of established protocols for metabolite analyses using gas chromatography–mass spectrometry (GC-MS), which is the most standardised method in the field of metabolomics. These protocols include sugars, amino acids, sterols, hormones, catecholamines (Anggard & Sedvall, 1969), hydroxyl acids, fatty acids [1], aromatics, and many other intermediates of primary metabolism. In point of fact, the exciting idea of combining targeted analysis of certain constituent classes into profiling tests for vast swaths of metabolism was first realised by GC-MS in the 1970s. This included the utilisation of such profiles to improve the diagnosis of human disorders, with at least 140 people being involved. Today, we refer to this type of profiling as "metabolomics" to emphasise the necessity of identifying and quantifying all of the tiny molecules that are present in a particular biological scenario. Additionally, we use these profiles as output of the cellular machinery in response to genetic or environmental perturbations.

The NIST Mass Spectral Library collection of the United States National Institute of Standards and Technology (NIST) is the most prominent example of the accumulation of mass spectra and chromatographic retention times in publicly accessible libraries under standardised conditions of 70 eV electron ionisation energy. However, there are also larger versions that are less well curated, such as the Wiley registry [2], the open-access MassBank database, and the Golm repository. This process has been going on for more than four decades. In a similar vein, the endeavours to computationally match mass spectral records to experimental data and to interpret mass spectra for the purpose of compound identifications began in the 1960s and continue to this day. The NIST collection contains GC-MS mass spectra for different compounds, of which about one third have recorded standardised retention durations. This makes it possible to identify compounds by utilising two orthogonal criteria, namely mass spectral matching and retention index matching. With only distinct compounds in the NIST library and 12,099 unique compounds in the Metlin LC-MS/MS library (which lacks retention information), LC-MS/MS spectrum libraries are much less in size when compared to other types of libraries.

In addition to these standardised libraries, GC-MS possesses a number of specific advantages that have led to the technology being referred to as the "gold standard" in the field of metabolomics. This means that it is the method that newer approaches should be compared to in terms of the breadth, sensitivity, and specificity of metabolite detections. The most important thing to note is that electron ionisation results in intricate and abundant fragmentation patterns. These patterns can be utilised to enhance the specificity of mass spectral matching. This is especially true if the mass spectra are recorded in large user libraries that have standardised protocols for data acquisition, such as the Fiehnlib libraries and the BinBase databases [3-6]. Peak picking is consequently complemented by genuine mass spectrum deconvolution, which summarises all fragment ions into pure mass spectra. This is in contrast to LC-MS techniques, which use MS and data dependent MS/MS fragmentations independent of one another. Automated mass spectral deconvolution software, also known as AMDIS, has been openly accessible for use with gas chromatography-mass spectrometry (GC-MS) since 1998. Since that time, it has been successfully utilised for metabolomics. These kinds of attempts to couple ionisation with fragmentation in LC-MS in an untargeted manner have recently begun, for instance by utilising SWATH approaches. However, as of right now, there is no open-access software for LC-MS that is as capable of purifying mass spectra of co-eluting mass spectra in GC-MS (for example, AMDIS) or its commercial versions (for example, ChromaTOF).

Metabolomics:

There has been a tremendous increase in the amount of attention paid to metabolomics in clinical research, which has provided insights into

the pathological processes of a variety of diseases. Untargeted and targeted techniques are the two primary categories that can be used to classify these investigations under. In the field of metabolomics, the non-biased technique known as untargeted metabolomics seeks to investigate as many metabolites as possible in order to identify changes that occur among the groups of samples. On the other hand, targeted metabolomics concentrates on particular metabolites, which provides improved sensitivity and specificity. Using these methods together makes it possible to test hypotheses.

Advances of chromatography analysis-comprehensive two-dimensional gas chromatography as a powerful tool for metabolomics studies:

When applied to the study of extremely complex biological samples, the typical capillary columns with one dimension demonstrate inefficiency in separation. For the purpose of enhancing column separation through the prevention of coelutions in complicated matrix analyses, Liu and Phillips92 devised a comprehensive two-dimensional gas chromatography (GCGC) method by which they achieved this. The GCGC technique involves the coupling of two columns in line through a modulator. Within a short amount of time, the eluate that has emerged from the first dimension (1 D) is concentrated while maintaining the separation that was achieved, and then it is transferred to the second dimension (2 D). The entire transfer of the sample from one dimension to two dimensions is made possible by these distinct separation processes. When compared to the one-dimensional gas chromatographic approach, the GCGC technique offers a number of advantages.92: The normal dimensions of the first column in a gas chromatograph is typically 30 metres in length, with an internal diameter of 0.20 millimetres and a thickness of roughly 0.2 or 0.3 micrometres for the stationary phase. Nevertheless, the second column is characterised by its short and narrow length, similar to the columns utilised in fast gas chromatography. Its length can reach up to 2 metres, its internal diameter is roughly 0.10 millimetres, and the thickness of the stationary phase is approximately 0.1 micrometres. Therefore, the elution in two-dimensional chromatography takes place in a few seconds prior to the injection of the subsequent fraction by the modulator, and this occurs without the total run time being extended.

If possible, the columns should be orthogonal, and the separation principles should be independent and distinct. For example, a low polarity column should be used in one dimension, while a high polarity column should be used in two dimensions.(93) The modulator, which is considered to be the central component of the GC×GC system, is responsible for facilitating the concentration of effluent from the main column and its subsequent injection into the second column at regular intervals. Furthermore, as a result of this effluent focus, the peaks that are obtained at the conclusion of the second dimension are narrower, which results in an improvement in the technique's sensitivity [7]. A number of modulators are currently available for commercial use. These modulators include those that are based on temperature and have interfaces that are either heating or cryogenic, as well as valve-based modulators that use pneumatic means to execute the modulation. Cryogenic modulators, on the other hand, are the most commonly employed because of their capacity to produce very narrow peak widths and their successful application for a wide variety of substances [8]. The duration of a full modulation cycle is equivalent to the modulation period, which is the same as the separation period in the second dimension. According to a one-dimensional analysis, the total amount of time required for analysis is identical to the amount of time that was spent on separation in the first column. This is because all of the effluent from the first column is transferred into the second column at the same time. One of the most well-established analytical techniques for elucidating complex matrices, such as those utilised in metabolomic research, is the gas chromatography-gas chromatography (GCGC) approach [9]. By using this method, it is possible to obtain a detailed profile of known substances,

also known as targeted analytes, which is two to five times larger than the profiles obtained using one-dimensional chromatography. In addition, two-dimensional chromatograms are extremely accurate and make it possible to obtain a fingerprint that is unique to a particular sample. When you want to uncover alterations that have occurred as a result of changes in metabolism over the years or as a result of exogenous means, the fingerprint of a sample is vital. Using the power of a thorough two-dimensional gas chromatography analysis, it is possible to investigate the metabolite profiles of mouse spleen tissue, which can be a very efficient alternative in the metabolomic investigation of damaged skin [10]. It was demonstrated by a GC×GC-MS technique that the GC×GC exhibited enhanced resolving power, which resulted in an improvement in the quality and sensitivity of the mass spectrum. In contrast to the 500 molecules that were discovered by one-dimensional chromatography, this investigation discovered 1200 different metabolites (metabolites). In addition, the findings demonstrated that the compounds that were discovered were strikingly comparable to those that had been reported in the past in mammalian tissues using NMR research and other investigation techniques.

Some limitations based on metabolomics by GC-MS:

The gas chromatography-mass spectrometry (GC-MS) technique is an effective method for the study of many chemicals; nevertheless, it does have several limitations that must be taken into consideration in metabolomics research, particularly in situations where the metabolomics approach is untargeted. The most significant drawback is on account of the fact that it is dependent on the analysis of tiny compounds that are both volatile and thermally stable. As a result, additional issues may emerge as a consequence of the derivatization process. These issues can range from the preparation of the sample itself, which takes a considerable amount of time, to erroneous quantifications as a result of incomplete derivatization of the analytes. Another reason is that during the derivatization reaction, distinct forms of metabolite derivatives are converted into different forms. This results in the production of a sample that contains different forms of the same original metabolite. Using standard derivatized chemicals and data correction procedures to normalise this bias is one element that can help lessen these inconveniences. This bias can be normalised by using these strategies.

Data Interpretation:

It is necessary to have a high capacity for investigating the biochemical status of cells, tissues, or organisms in order to get the most out of the large amount of data that is produced by metabolomic studies. It is possible for there to be two independent stages of data processing. Over the course of the preprocessing, the raw data are filtered, identified, aligned, and normalised in order to make the future analysis more straightforward. The data that has been processed is next interpreted by the analysis stage, which makes use of either univariate or multivariate artefacts [11]. When dealing with chromatographic data derived from mass spectrometry, the primary objective of the preprocessing stage is to enhance peak visualisation by eliminating noise. During the detection process, all signals that have relative intensities that are higher than the noise are recognised. The alignment process seeks to correct discrepancies in retention durations between runs and merge data from a variety of samples. Following the completion of the pre-processing step, the data will be subjected to statistical analysis in order to display the pertinent biological information. The statistical method that is utilised is determined by the kind of information that is being gathered. Methods that do not require supervision can be utilised in situations where the purpose is to summarise, investigate, and find information when there is no prior information available regarding the identity of the sample. The procedures that are utilised the most frequently include the hierarchical cluster analysis (HCA), principal component analysis (PCA), clustering, independent component

analysis (ICA), or a type of neural network such as the self-organization map (SOM) [12]. As an illustration, principal component analysis was utilised by Randhawa et al. 17 in order to draw a comparison between the global pattern of metabolites found in skin biopsies acquired from sun-exposed and shielded locations. The findings unequivocally revealed that exposure to the sun had an effect on the metabolic profile of skin biopsies that were exposed to the sun. When comparing the two sets of samples, a subset of 122 metabolites was found to be substantially different (p-value < 0.05) with a false discovery rate threshold of less than or equal to 5%. On the other hand, when comparing biopsies taken from sun-exposed sites to those taken from sun-protected regions, 46 of the 122 metabolites were found to be minor, while 76 were found to be major. There were a total of 52 biological pathways that the detected metabolites belonged to, and a subset of 42 of those pathways had one or more metabolites that were significantly different from one another. A number of different metabolic pathways are included in this category, including amino acids, nucleotides, sugars, peptides, cofactors, and lipid metabolism.Unsupervised approaches, on the other hand, are utilised in situations when the identity of the sample is already known. The objective of these methods is to categorise the biomarkers according to the features that they possess.

Analysis of variance (ANOVA), partial least square (PLS), discriminant analysis of partial least square (PLS-DA), discriminant analysis of partial orthogonal least squares (OPLS-DA), and support vector regression (SVR) are some of the statistical methods that have been utilised in metabolomic research [13]. Through the use of the OPLS-DA approach, for instance, the profiles of primary metabolites in mouse skin that were produced in response to UVB irradiation were analysed in order to discover variables that might be used to differentiate between the experimental groups. A number of different substances, including amino acids, chemical compounds, fatty acids, lipids, carbohydrates, and cis- and trans-urocanic acid (UCA), have been discovered as discriminators that characterise the distinctions between the groups. Additionally, the same procedure was utilised to evaluate and analyse the duration of radiation exposure during the study. Additionally, machine learning can be utilised in multi-omic approaches, which involve the integration of many methods, such as genomics, proteomics, and metabolomics. In these cases, the amount of data is significantly greater, and the integration process is more complicated [14]. Additionally, this artefact has the potential to promote integrative analysis by efficiently resolving issues such as data heterogeneity, missing data, class imbalance, scalability of problems, and the curse of dimensionality.

The identification of biomarkers and the interpretation of biological data:

There is a vast quantity of information regarding metabolic activities that is contained within the chemical composition of the matrix component of the skin. The majority of these metabolic pathways indicate an increase in the production of reactive oxygen species (ROS), which resulted in increased oxidative stress, which may be responsible for changes in the phenotypic appearance of sun-exposed skin. Furthermore, as was previously presented, recent studies using GC-MS showed that exposure to solar radiation had an effect on a number of metabolic pathways. The differences between the groups were characterised by a number of metabolites, including amino acids, chemical compounds, fatty acids, lipids, nucleotides, carbohydrates, and cis- and trans-UCA. These metabolites were identified as discriminators. Nevertheless, it has been noted that the UCA metabolite was the one that demonstrated the most significant difference between skin that had been subjected to photons and skin that had not been exposed to photons in the majority of these studies [15].

A significant amount of UCA, in its trans-UCA form, can be discovered in the stratum corneum. This particular isomer has been suggested as the primary acid-base regulator of the epidermis [16], and it plays a significant part in the protection

from the sun [109]. Over the past few years, there has been a rise in interest in UCA not only because of its advantageous qualities, but also because of the negative effects it has on the skin, which are brought about by the photoisomerization of the cis-UCA isomer by exposure to ultraviolet light. In contrast to the trans isomer, cis-UCA causes pro-apoptotic intracellular acidification, oxidative damage to deoxyribonucleic acid (DNA), and particular immunological responses, all of which have the potential to neutralise any protective impact [17]. The fact that this is the case suggests that UCA could be a signalling pathway that leads to the discovery of a biomarker that identifies damage induced by exposure to ultraviolet light. Having said that, it is important to point out that their responsibilities in maintaining the homeostasis of the skin are still somewhat complicated and call for additional research. In addition, the tricarboxylic acid (TCA) cycle was found to be related with the metabolite alterations that were brought about by skin photoaging.

Dihydrolipoyl dehydrogenase, often known as DLD, was the only TCA cycle protein that exhibited a decrease in expression in mice after being subjected to ultraviolet light. Furthermore, in order to validate their findings, they utilised GC-TOF/MS to identify two metabolites, namely malic acid and fumaric acid, which were found to be downregulated following the induction of UVB. Based on the findings, the scientists came to the conclusion that a decrease in the DLD enzyme can be associated with an increase in oxidative damage, a reduction in energy metabolism, a regulation of the Fe metabolism, and metabolic acidosis. As a result, the modulation of DLD might be a significant target for the treatment of skin photodamage [18]. In addition, GC-MS analyses were utilised in the metabolomic investigation of volatile organic chemicals in order to identify potentially useful biomarkers for skin cancer. Melanoma was shown to have raised amounts of lauric acid (C12:0) and palmitic acid (C16:0), which were found to correspond with increased oxidative stress and also as a consequence of unregulated lipid synthesis, which is a characteristic that is known to be associated with cancer [19].

The parameters that were investigated in this work had an effect on the systems that were primarily responsible for energy metabolism, lipid metabolism, reactive oxygen species defence, and DNA repair. Taking this into consideration, it is reasonable to make the observation that a variety of research studies have been conducted in order to find potential markers of damaged skin based on metabolomics. On the other hand, they are still in the preliminary stages and continue to place a significant emphasis on cancer and photoaging. Therefore, more efforts need to be made to obtain more concrete answers for the metabolomic analysis of damaged skin. This damage can be caused by UV radiation or cancer, but it can also be caused by other areas of clinical experience, such as dermatitis, psoriasis, eczema, changes caused by environmental pollutants, chemical agents, and other conditions.

Cancer of the stomach It has been determined by the World Health Organisation (WHO) that gastric cancer (GaC) will be one of the five most lethal forms of cancer in the year 2020. Smoking, eating a lot of meat, drinking alcohol, being overweight, and having an infection with Helicobacter pylori are the primary risk factors for GaC, which is a completely stochastic condition that accounts for 90% of cases. Atrophy, metaplasia, dysplasia, and carcinoma are the precursor lesions that are associated with GaC. Chronic inflammation arises as a result of persistent H. pylori infection, which produces chronic inflammation. Upper endoscopy, which is followed by a biopsy, is considered the diagnostic approach of choice, despite the fact that it is invasive and requires the participation of specialists. Despite the fact that countries with high incidence have already adopted screening programmes, countries with low incidence require options that are more cost-effective.

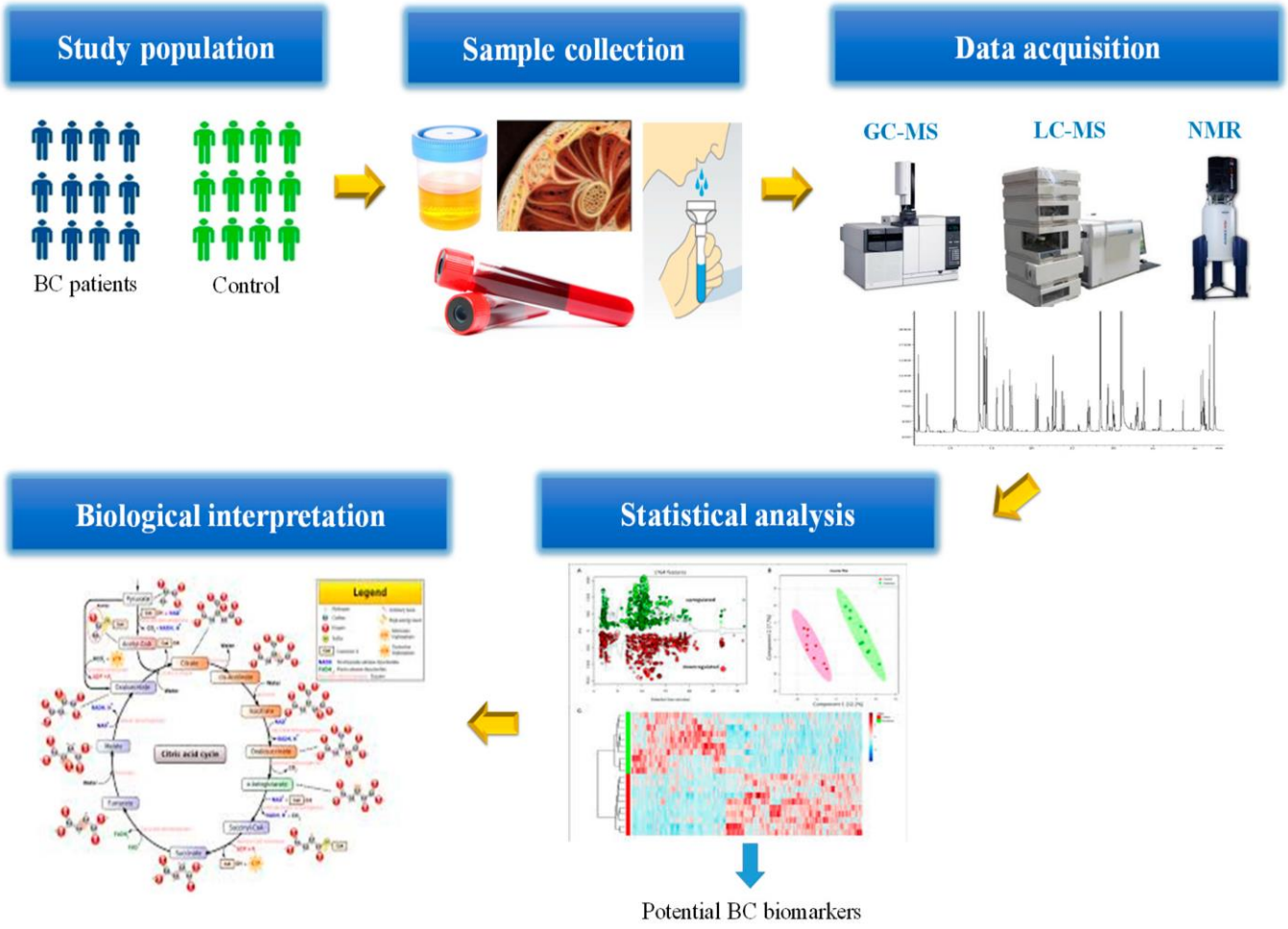
Carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), and carbohydrate antigens (CA19-9 or CA72-4) are examples of serum biomarkers that have been utilised for early diagnosis. However, due to their lack of specificity, these biomarkers have resulted in low positive rates and have been unable to detect precancerous lesions.

Breast cancer:

The type of cancer that is diagnosed the most frequently is breast cancer (BC), which also ranks as the fifth leading cause of death from cancer. When identified at a later stage (2012–2019), the relative survival rate reduces substantially to 31%, despite the fact that it now stands at 90.8% based on the 5-year percentage. Among the screening modalities that are currently considered to be the gold standard, annual mammography and clinical breast examination are recommended for women who are above the age of 40. It is unfortunate that physical breast examinations, even when carried out by a physician, do not achieve the desired effect of reducing mortality. In terms of mammography, the sensitivity is reduced due to the density of the breasts, and the procedure necessitates an X-ray examination. Additionally, the method has the potential to result in an overdiagnosis, which can lead to unneeded procedures and treatments. A number of alternative screening methods, including digital breast tomosynthesis (DBT), ultrasonography, magnetic resonance imaging (MRI), and positron emission tomography/computed tomography (PET/CT), are limited by a number of factors, including high costs, discomfort, the demand for experienced technicians, and radiation exposure. Therefore, there is an immediate need for creative screening methods that are capable of overcoming these disadvantages, and breath tests show promise as a potential approach that could be taken. In the Supplementary Tables S1–S3, a summary of four research that focused on possible biomarkers for breast cancer is presented. The same geographical region, China, served as the location for the execution of these investigations. BC showed a significant increase in the expression of all of the targeted aldehydes, while BNMD showed an increase in the expression of hexanal, both in comparison to HC. Furthermore, when compared to BNMD, the amount of nonanal was found to be higher in BC. When it came to distinguishing early-stage breast cancer from head and neck cancer, the combination of these volatile organic compounds (VOCs) demonstrated a sensitivity of 91.7% and a specificity of 95.8% (0.934 area under the curve). Furthermore, the prediction model attained an accuracy of 80.4% following leave-one-out cross-validation (LOOCV).

In addition to the reorganisation of metabolic networks that are involved in the adaptation to sustain cell growth and survival, the remodelling of tissues, and metastasis, cancer cells have distinctive characteristics of their metabolic status that contribute to the reorganisation of these networks. The phenomenon known as "aerobic glycolysis" was initially found by Warburg in cancer cells. This phenomenon helps to prevent the unneeded catabolic oxidation of nutrients and encourages anabolism in cells that are already in the process of multiplying [20]. This special characteristic of cancer has been actively utilised

in study ever since it was discovered. The research has demonstrated that the dynamic portrayal of the metabolic pattern of individuals offers fresh insights into a better understanding of the molecular changes, even in the most heterogeneous diseases, such as breast cancer [21].



In this context, metabolomics refers to the quantitative study of the full metabolic profile obtained from a biological sample. This study is a component of the large-scale "omics" techniques, and it reflects the ongoing cellular activities. It is possible that this high-throughput measurement technology has the potential to enrich the common gold-standard imaging-focused techniques and provide novel comprehensions of carcinogenesis. In the field of cancer, this technology has a significant advantage and the ability to provide a global snapshot of tumour biology, which includes development and progression. Real-time results of the metabolomic profile of an individual with a specific disease or with cancer can be obtained through the quantification of metabolites in circulating samples, such as blood-derived specimens. The comparison of these results with the metabolome of a disease-free control can provide valuable insights regarding the altered signalling pathways involved in the pathophysiology of a particular disease or tumour biology of cancer. This technique is minimally invasive and can provide these results. There is a substantial possibility that the downstream-affected metabolites, which exhibit large variations in concentrations between the cases and the controls, could be utilised in the future as non-invasive biomarkers for the purpose of accurate and timely diagnosis. These metabolomic fingerprinting techniques have already been investigated in Chronic Obstructive Lung illnesses

(COLD) as well as in a variety of auto-immune illnesses (such as rheumatoid arthritis, vitiligo, and inflammatory bowel disease) [22]. On the other hand, metabolomics is just a research lab-based approach. This is due to the fact that the technological and computational restrictions make the translation of metabolomics into clinical practice particularly challenging.

The metabolic profiling approach has been shown to be applicable in a growing number of applications for breast cancer screening in particular, including early disease detection, the discovery of novel biomarkers, the monitoring of disease progression and occurrence, and the analysis of altered metabolic pathways. This can be demonstrated by the accumulation of evidence that has been accumulated over the past few years. Metabolomics has been shown to be an effective method for distinguishing samples from patients with breast cancer from samples from healthy individuals in a significant number of studies [23-25]. Using an ultra-high-performance liquid chromatography/electrospray ionisation tandem mass spectrometry (UHPLC/MS) with an additional gas chromatography-mass spectrometry (GC/MS) platform, Dougan et al. (2018) [26] investigated the metabolomic profile from pre-diagnostic plasma samples of 45 patients diagnosed with breast cancer and 45 controls. They discovered a statistically significant case-control difference (greater than 20%) in 24 metabolites. This is an interesting study on the subject.

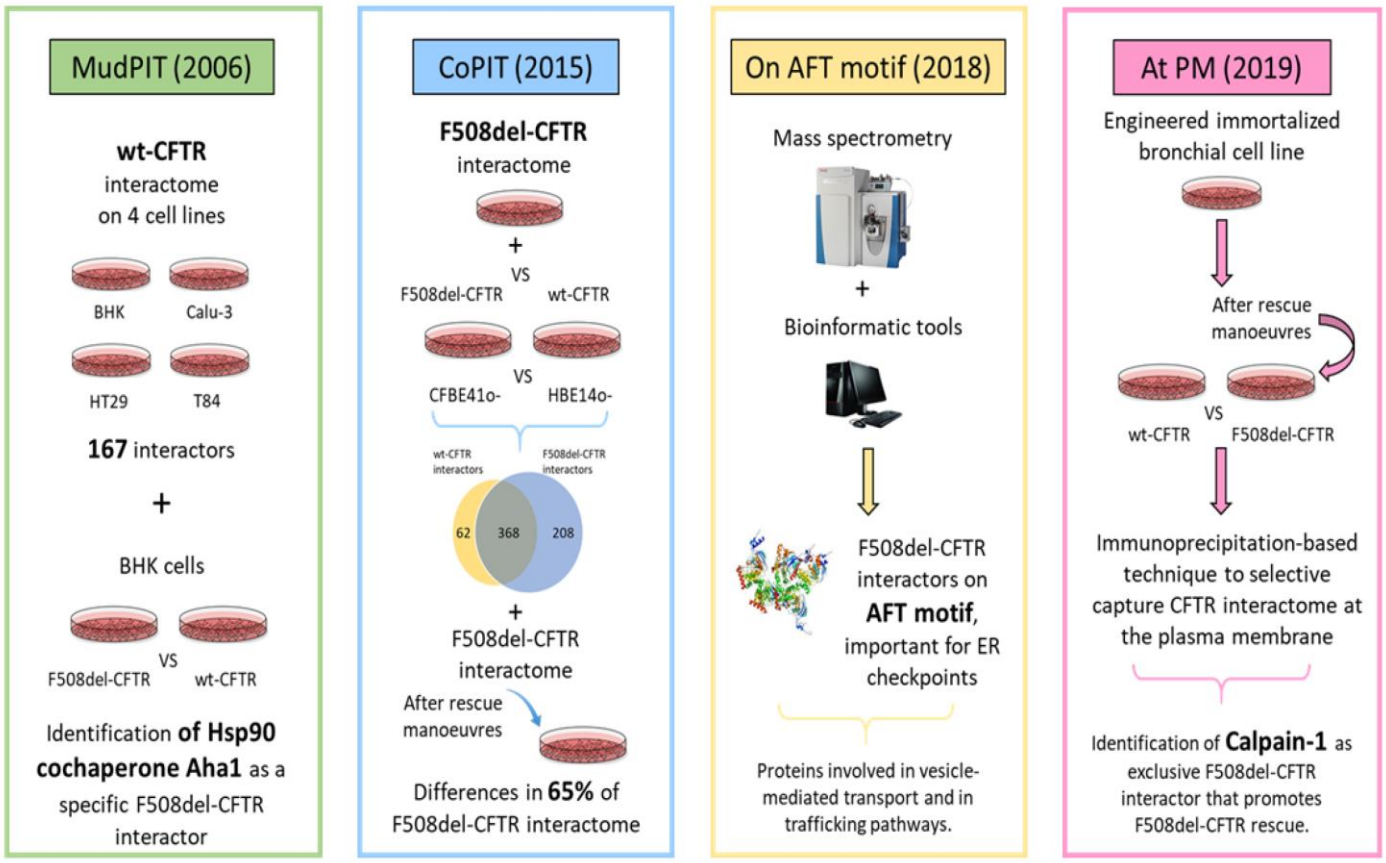
Cystic Fibrosis:

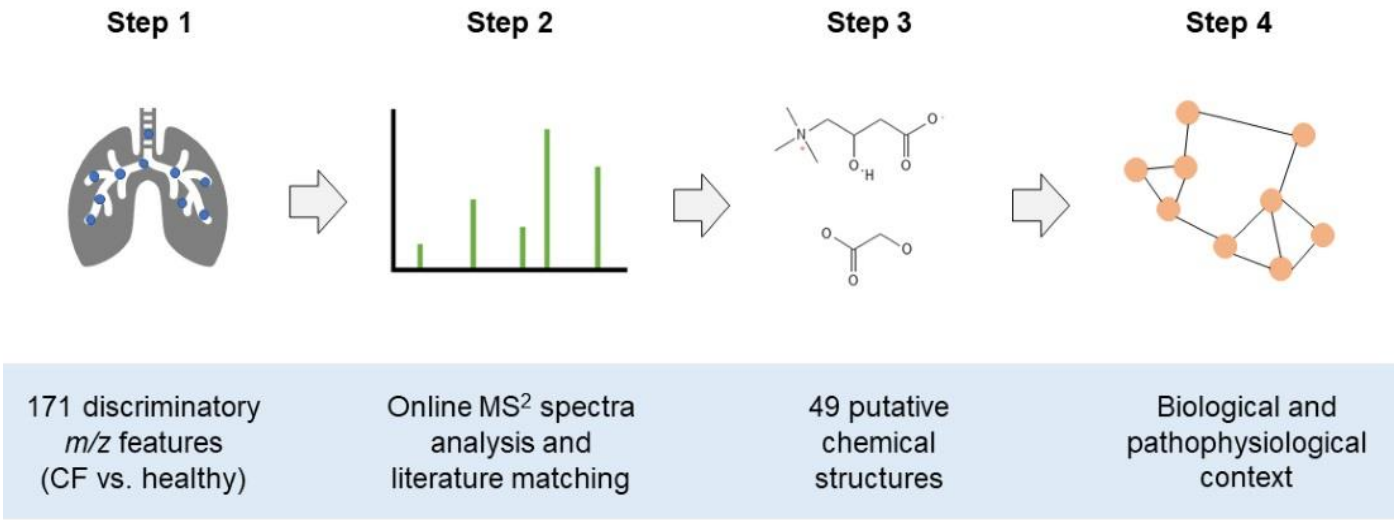
The genetic disorder known as cystic fibrosis (CF) is one of the most prevalent life-shortening conditions that is inherited and causes chronic progressive lung damage. The progression of the disease is characterised by bouts of acute or subacute clinical deterioration, which are known to as pulmonary exacerbations (PEx). These episodes manifest themselves as a result of increasing airway infection and inflammation. Examples of symptoms that are commonly associated with PEx include an increased productive cough as well as systemic symptoms such as fatigue [27], lack of appetite, and weight loss. It is possible for the clinical presentation of PEx to be modest, particularly in the early stages of the disease's progression. As a result, people may miss opportunities to act with antibiotics in order to preserve lung function. Improvements in patient outcomes are possible through the utilisation of biomarkers for the purpose of predicting the likelihood of imminent exacerbation [28] or facilitating earlier diagnosis.

A mutation in the gene that codes for the cystic fibrosis transmembrane conductance regulator (CFTR) is the root cause of cystic fibrosis (CF), which is a genetic disorder that is inherited in an autosomal recessive manner. Due to the fact that this mutation causes disruptions in the electrolyte transport system of the cells, it primarily affects organs that are responsible for secretion, such as the reproductive system, the lungs, and the pancreas. The abnormal absorption of salt in the lungs leads to the formation of thick and rigid secretions, which in turn raises the risk of respiratory infections, inflammation, and oxidative stress. Pulmonary exacerbations, also known as PEx, are common occurrences that occur during the evolution of the illness. They have the potential to result in the loss of lung function permanently, a deterioration in quality of life, and a reduction in survival. The treatment for PEx comprises antibiotics; however, the outcomes are worsened when symptoms are delayed.

Symptomatology, clinical evaluation, and the measurement of variations in forced expiratory volume in one second (FEV1pp) using spirometry equipment are the three components that are necessary for the detection of PEx. On the other hand, when UVA was applied to patients with stable and CF PEx, there was not a single VOC that was observed to be significantly affected. At the time of breath collection, it was discovered that four volatile organic compounds (VOCs) were connected with forced expiratory volume in one second (FEV1pp). Among these four VOCs, two of them, namely 4-methyl-octane and 3,7-dimethyldecane, were found to be further correlated with changes in FEV1pp. In addition, it

was discovered that there were four volatile organic compounds (VOCs) that were significantly different between patients with CF baseline and patients with CF PEx. PEx patients had an upregulation of 2,4,4-trimethyl-1,3-pentanediol 1-isobutyrate, whereas 3,7-dimethyldecane, durene, and 5-methyltridecane were found to be downregulated. Despite the fact that both investigations sought to find different volatile organic compounds (VOCs) between individuals with stable cystic fibrosis and those with CF PEx, none of the observed VOCs were same.





The present understanding of CF-associated metabolism was supported by the findings of several metabolite changes that were evaluated between cells with cystic fibrosis and those that did not have CF. During the course of our metabolomic investigation, we discovered that the levels of both reduced and oxidised glutathione in CF cells were dramatically reduced. It was noted that there was a drop in S-lactoylglutathione, which is a metabolite that is formed from glutathione detoxification, as well as S-nitrosoglutathione and ophthalmate, which is a marker for glutathione formation. These findings were consistent with the decreased amount of glutathione. It has been demonstrated through research that S-nitrosoglutathione and S-nitrosylating substances have the ability to stimulate the activation of the CFTR channel and boost the expression of CFTR on the surface of CF epithelial cells [29]. It has been demonstrated that mutations in the CFTR gene can cause disturbances in the amounts of GSH in both the intracellular and extracellular milieu [30]. Patients who have cystic fibrosis have lower amounts of glutathione (GSH) in the fluid and blood that line the lung epithelial lining, but the GSH levels in the lung itself appear to be unaffected. Due to the fact that CFTR is located on the apical side of lung epithelial cells, it has been hypothesised that CFTR may transport glutathione into the epithelial lining, where it may perform a variety of functions. These functions may include breaking disulfide bonds to reduce mucus viscosity, influencing mucus hydration, and playing a role in the inflammatory response to infection [31-34].

Although it has been demonstrated that CFTR is capable of transporting GSH, it is also plausible that CFTR interacts with other GSH transporters and, as a result of these interactions, influences the transport of GSH for some reason. It has been demonstrated that CFTR is physically and functionally associated with MRP4, which is a cAMP transporter that is also a potential GSH transporter [35, 36]. Patients with cystic fibrosis have lower amounts of glutathione (GSH) in their neutrophils, which may be a contributing factor to aberrant function and accelerated degeneration of neutrophils [37]. There is a possibility that the failure to raise GSH levels in response to infection is something that contributes to the low response rate to infection. In addition to being the most prevalent cellular redox molecule, glutathione is also an essential component in the process of keeping the redox status in cells stable. The presence of oxidative circumstances, which further contribute to the pathogenesis of cystic fibrosis, may be the result of decreased glutathione levels. Consequently, elevating the amounts of glutathione within cells has been proposed as a potential therapeutic approach for cystic fibrosis [38]. Both inhaled GSH and N-acetylcysteine are now being evaluated in clinical settings [39].

It has been demonstrated that patients with cystic fibrosis experience a rise in oral N-acetylcysteine, as well as increase in whole blood and neutrophil concentrations of glutathione, as well as an increase in extracellular glutathione in induced sputum [40]. Through the pentose phosphate route, the majority of the NADPH that is produced by cells comes from the cellular source. Both reduced glutathione and thioredoxin are two of the most important antioxidants, and NADPH is the source of the reducing equivalents that are necessary for their regeneration. It is possible that a reduction in the metabolic flux along the pentose phosphate pathway will increase the vulnerability of the cell to oxidative stress. Secondly, our findings indicate that the metabolism of tryptophan was changed in CF cells, which led to the buildup of kynurenine and anthranilate. Kynurenine has been linked to the production of oxidative stress as well as circumstances that are associated with disease.

Conclusion:

The purpose of this in-depth review is to evaluate the possibility of using gas chromatography-mass spectrometry (GC-MS) to analyse volatile organic compounds (VOCs) in breath as biomarkers for serious diseases, such as cancer, pulmonary diseases, and infectious diseases. Some of the most important aspects of the workflow are carefully considered and discussed. These aspects

include the type of exhaled breath, collection devices, pre-concentration techniques, and analysis, as well as the experimental designs, statistical analysis, identification strategies, and potential VOCs biomarkers that are proposed. With that being said, the selection of the kind of breath sample was more varied, ranging from mixed breath to alveolar breath. This is an essential factor to take into account when attempting to accurately evaluate and establish levels of endogenous volatile organic compounds (VOCs). The lack of standardisation in the methodological approach and the limited absolute quantification of possible biomarkers both contribute to the delay in the transfer of these biomarkers to clinical settings, despite the abundance of research that have been conducted already. In addition, the identification of a single pathology-specific volatile organic compound was hampered by relatively small cohorts, which had only a limited model validation in an independent cohort. Furthermore, there was a lack of unanimity in the findings among the many research.

To have a complete awareness of the significance of each volatile organic compound (VOC) in distinguishing between healthy and diseased states, it is essential to have a more in-depth comprehension of the endogenous origin of VOCs. We are well on our way to developing personalised and non-invasive diagnostic procedures that have the potential to revolutionise the identification and management of pathology, which will ultimately be beneficial to public health. This is because we are delving deeper into the intricacies of volatile organic compounds (VOCs) in exhaled breath.

References:

1. Niehaus TD, Nguyen TND, Gidda SK, ElBadawi-Sidhu M, Lambrecht JA, McCarty DR, Downs DM, Cooper AJL, Fiehn O, Mullen RT. Arabidopsis and maize RidA proteins preempt reactive enamine/imine damage to branched-chain amino acid biosynthesis in plastids. The Plant Cell Online. 2014;26:3010–3022.
2. Roessner U, Wagner C, Kopka J, Trethewey RN, Willmitzer L. Simultaneous analysis of metabolites in potato tuber by gas chromatography–mass spectrometry. The Plant Journal. 2000;23:131–142.
3. Skogerson K, Wohlgemuth G, Barupal DK, Fiehn O. The volatile compound BinBase mass spectral database. BMC bioinformatics. 2011;12:321.
4. Altomare, D. F., Di Lena, M., Porcelli, F., Trizio, L., Travaglio, E., Tutino, M., et al. (2013). Exhaled volatile organic compounds identify patients with colorectal cancer. Br. J. Surg. 100, 144–150.
5. Beale, D. J., Jones, O. A. H., Karpe, A. V., Dayalan, S., Oh, D. Y., Kouremenos, K. A., et al. (2016). A review of analytical techniques and their application in disease diagnosis in breathomics and salivaomics research. Int. J. Mol. Sci. 18, 24.
6. Fortes, P. R., Petruci, J. F. S., and Raimundo, I. M., Jr. (2017). Optical gas sensors for exhaled breath analysis. SPIE Press.
7. Cazzola, M., Segreti, A., Capuano, R., Bergamini, A., Martinelli, E., Calzetta, L., et al. (2015). Analysis of exhaled breath fingerprints and volatile organic compounds in COPD. COPD Res. Pract. 1, 7.
8. Sumner, L. W., Amberg, A., Barrett, D., Beale, M. H., Beger, R., Daykin, C. A., et al. (2007). Proposed minimum reporting standards for chemical analysis chemical analysis working group (CAWG) metabolomics standards initiative (MSI). Metabolomics 3, 211–221.
9. Kuruvilla, M. E., Lee, F. E. H., and Lee, G. B. (2019). Understanding asthma phenotypes, endotypes, and mechanisms of disease. Clin. Rev. Allergy Immunol. 56, 219–233.

10. Tang, Z., Liu, Y., and Duan, Y. (2015). Breath analysis: technical developments and challenges in the monitoring of human exposure to volatile organic compounds. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1002, 285–299.
11. Dudzik, D., Barbas-Bernardos, C., García, A., and Barbas, C. (2018). Quality assurance procedures for mass spectrometry untargeted metabolomics. a review. J. Pharm. Biomed. Anal. 147, 149–173.
12. Fowler, S. J., Basanta-Sanchez, M., Xu, Y., Goodacre, R., and Dark, P. M. (2015). Surveillance for lower airway pathogens in mechanically ventilated patients by metabolomic analysis of exhaled breath: a case-control study. Thorax 70, 320–325.
13. Kuo, T. C., Tan, C. E., Wang, S. Y., Lin, O. A., Su, B. H., Hsu, M. T., et al. (2020). Human breathomics database. Database (Oxford) 2020, baz139.
14. Lawal, O., Ahmed, W. M., Nijsen, T. M. E., Goodacre, R., and Fowler, S. J. (2017). Exhaled breath analysis: a review of "breath-taking" methods for off-line analysis. Metabolomics 13, 110.
15. Bayrakli, I., Öztürk, Ö., and Akman, H. (2016). Investigation of acetone, butanol and carbon dioxide as new breath biomarkers for convenient and noninvasive diagnosis of obstructive sleep apnea syndrome. Biomed. Chromatogr. 30, 1890–1899.
16. Chen, S., Mahadevan, V., and Zieve, L. (1970). Volatile fatty acids in the breath of patients with cirrhosis of the liver. J. Lab. Clin. Med. 75, 622–627.
17. Furge, L. L., and Guengerich, F. P. (2006). Cytochrome P450 enzymes in drug metabolism and chemical toxicology: an introduction. Biochem. Mol. Biol. Educ. 34, 66–74.
18. Basanta, M., Koimtzis, T., Singh, D., Wilson, I., and Thomas, C. L. P. (2007). An adaptive breath sampler for use with human subjects with an impaired respiratory function. Analyst 132, 153–163.
19. Netzker, T., Shepherdson, E. M. F., Zambri, M. P., and Elliot, M. A. (2020). Bacterial volatile compounds: functions in communication, cooperation, and competition. Annu. Rev. Microbiol. 74, 409–430.
20. Hirschey M.D., DeBerardinis R.J., Diehl A.M.E., Drew J.E., Frezza C., Green M.F., Jones L.W., Ko Y.H., Le A., Lea M.A., et al. Dysregulated metabolism contributes to oncogenesis. Semin. Cancer Biol. 2015;35:S129–S150.
21. Vander Heiden M.G., Cantley L.C., Thompson C.B. Understanding the Warburg effect: The metabolic requirements of cell proliferation. Science. 2009;324:1029–1033.
22. Jové M., Collado R., Quiles J.L., Ramírez-Tortosa M.C., Sol J., Ruiz-Sanjuan M., Fernandez M., de la Torre Cabrera C., Ramírez-Tortosa C., Granados-Principal S., et al. A plasma metabolomic signature discloses human breast cancer. Oncotarget. 2017;8:19522–19533.
23. Jasbi P., Wang D., Cheng S.L., Fei Q., Cui J.Y., Liu L., Wei Y., Raftery D., Gu H. Breast cancer detection using targeted plasma metabolomics. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 2019;1105:26–37.
24. Wang X., Zhao X., Chou J., Yu J., Yang T., Liu L., Zhang F. Taurine, glutamic acid and ethylmalonic acid as important metabolites for detecting human breast cancer based on the targeted metabolomics. Cancer Biomark. 2018;23:255–268.

25. Shen J., Yan L., Liu S., Ambrosone C.B., Zhao H. Plasma metabolomic profiles in breast cancer patients and healthy controls: By race and tumor receptor subtypes. Transl. Oncol. 2013;6:757–765. doi: 10.1593/tlo.13619.

26. Dougan M.M., Li Y., Chu L.W., Haile R.W., Whittemore A.S., Han S.S., Moore S.C., Sampson J.N., Andrulis I.L., John E.M., et al. Metabolomic profiles in breast cancer:a pilot case-control study in the breast cancer family registry. BMC Cancer. 2018;18:532.

27. Ferkol T, Rosenfeld M, Milla CE. Cystic fibrosis pulmonary exacerbations. J Pediatr. (2006) 148:259–64.

28. Shoki AH, Mayer-Hamblett N, Wilcox PG, Sin DD, Quon BS. Systematic review of blood biomarkers in cystic fibrosis pulmonary exacerbations. Chest. (2013) 144:1659–70.

29. Servetnyk, Z., Krjukova, J., Gaston, B., Zaman, K., Hjelte, L., Roomans, G. M., and Dragomir, A. (2006) Respir. Res. 7, 124 44. Hudson, V. M. (2004) Treat. Respir. Med. 3, 353–363

30. Hudson, V. M. (2001) Free Radic. Biol. Med. 30, 1440–1461

31. Gao, L., Kim, K. J., Yankaskas, J. R., and Forman, H. J. (1999) Am. J. Physiol. Lung Cell. Mol. Physiol. 277, L113–L118

32. Roum, J. H., Buhl, R., McElvaney, N. G., Borok, Z., and Crystal, R. G. (1993) J. Appl. Physiol. 75, 2419–2424

33. Ballatori, N., Krance, S. M., Notenboom, S., Shi, S., Tieu, K., and Hammond, C. L. (2009) Biol. Chem. 390, 191–214

34. Li, C., Krishnamurthy, P. C., Penmatsa, H., Marrs, K. L., Wang, X. Q., Zaccolo, M., Jalink, K., Li, M., Nelson, D. J., Schuetz, J. D., and Naren, A. P. (2007) Cell 131, 940–951

35. Day, B. J., van Heeckeren, A. M., Min, E., and Velsor, L. W. (2004) Infect. Immun. 72, 2045–2051

36. Tirouvanziam, R., Conrad, C. K., Bottiglieri, T., Herzenberg, L. A., Moss, R. B., and Herzenberg, L. A. (2006) Proc. Natl. Acad. Sci. U.S.A. 103, 4628–4633

37. Ciofu, O., Riis, B., Pressler, T., Poulsen, H. E., and Høiby, N. (2005) Antimicrob. Agents Chemother. 49, 2276–2282

38. Visca, A., Bishop, C. T., Hilton, S. C., and Hudson, V. M. (2008) J. Cyst. Fibros. 7, 433–436

39. Bishop, C., Hudson, V. M., Hilton, S. C., and Wilde, C. (2005) Chest 127, 308–317

40. Dauletbaev, N., Fischer, P., Aulbach, B., Gross, J., Kusche, W., ThyroffFriesinger, U., Wagner, T. O., and Bargon, J. (2009) Eur. J. Med. Res. 14, 352–358.