Exploring the complex relationship between metabolomics and breast cancer early detection (Review)

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Abstract. An overview of metabolomics in cancer research, focusing on the identification of biomarkers, pharmacological targets and therapeutic agents, is provided in the present review. The fundamentals of metabolomics, the role of metabolites in cancer emergence and the methods used in metabolomic analysis, are reviewed. The applications of metabolomics in cancer therapy and diagnostics, as well as the challenges encountered in metabolomic research, are discussed. Finally, the potential clinical uses of metabolomics in cancer research and its future possibilities are explored, emphasising the importance of non-invasive diagnostic and monitoring techniques. The present review highlights the significance of metabolite-based metabolomics as a specialised tool for illuminating disease processes and identifying treatment potentials. The malfunctioning of metabolomic pathways and metabolite accumulation or depletion is caused by metabolomics abnormalities. Metabolite signatures close to a subject's phenotypic informative dimension can be used to monitor therapies and disease prediction diagnosis and prognosis. Non-invasive diagnostic and monitoring techniques with high specificity and selectivity are urgently needed. Metabolite-based metabolomics is a specialised metabolic biomarker and pathway-analysis technique, illuminating the putative processes of numerous human illnesses and determining treatment potentials. Locating biochemical pathway modifications that are early warning signs of pathological malfunction and illness is possible by identifying functional biomarkers linked to phenotypic variance. Scientists generated numerous metabolomics profiles to disclose the underlying processes and metabolomics networks for therapeutic target research in biomedicine. The metabolomic analysis of the potential utility of metabolites as biomarkers for clinical events is summarised in the present review. The significance of metabolite-based metabolomics as a specialised tool for illuminating disease processes and identifying treatment potentials is highlighted.

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1. Introduction

The metabolome, a final product of the transcriptome, genome and proteome, contains small-molecule metabolites correlated with specific metabolic phenotypes. It provides insights into the pathophysiology and therapeutic targets of numerous illnesses (1). The metabolome has previously shown considerable advantages for identifying biomarkers, diagnosing and treating illnesses, and defining metabolic-control mechanisms. Comprehensive metabolic fingerprints can identify

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treatment targets and infer potential illness mechanisms. In the rapidly expanding science of metabolomics, tiny molecules in biological processes known as metabolites undergo comprehensive investigation. As a potential method of identifying new biomarkers, pharmacological targets and therapeutic agents, metabolomics in cancer research has attracted considerable attention (2).

The results of protein translation, gene transcription, or structural modifications to the proteome, genome, or transcriptome are called metabolites. Metabolites have the potential to play a significant role in the interaction between genotype and environment and give a clearer picture of the final phenotype. A publicly available human metabolome primarily includes comprehensive data on 41,993 small-molecule metabolites (1-3). In addition to acting as cofactors, energy producers' storage units, signalling molecules, metabolites may also start regulatory processes. Compared with other omic methods, metabolomics focuses on metabolites and has several benefits. Although metabolomics may directly identify the biochemical reaction to a stimulus, genomics may not have considerable influence on how a protein's expression induces its function (3). The present review aims to cover various aspects of metabolomics in the context of cancer research, including fundamentals, the role of metabolites in cancer development, analysis methods, applications in cancer detection and diagnostics, comparison of metabolomic analysis instruments, and potential clinical uses in cancer and breast cancer (BC) research (4). Overall, a comprehensive overview of metabolomics in cancer research is provided, highlighting its potential as a powerful tool for understanding cancer biology and improving clinical outcomes through early detection and personalised treatment strategies. Finally, we discuss metabolomics' possible clinical uses in cancer and BC research. It is aimed to identify and discuss the possibility of metabolites' early detection through metabolomics research.

2. Discussion

BC. Malignant tumours are divided into locally malignant at the same organ or tissue without spreading and tumours spreading to other organs or parts of the body, which is called metastasis. Not all tumours arrive at the metastatic stage, especially if diagnosed early. The tumour derives nutrients from other surrounding healthy cells. As a result, the healthy cells die, which allows the tumour cells to grow even faster. The process of spreading the cancer cells to other body parts and growing continuously in those locations is known as metastasis. BC is a type of cancer affecting one in every eight women in high-income nations by the age of 85, and it will continue to be the primary source of disease burden for women (5). BC remains a severe health issue despite significant advancements in the field of cancer research and is now a high focus for biomedical research. The most frequent disease amongst women worldwide is BC, and its incidence and mortality rates are predicted to rise sharply in the coming years (6). With over 1,700,000 new cases each year, the frequency of this aggressive illness remains disturbingly high, and these numbers point to decreased progress in the preventative field (7). The estimated number of deaths globally in 2020 according to Globocan 2020 (WHO) is 684,996 cases, which comprises 15.5% of the total worldwide death percentage. Genes, the fundamental building blocks of inheritance, can change in ways that lead to cancer (8). Genetic changes that cause cancer can happen because of errors that occur as cells divide or DNA damage inflicted by harmful environmental substances (such as the chemicals in tobacco smoke and ultraviolet rays from the sun). Such changes can also be inherited from parents (9). In elderly people, whose bodies become less capable of eliminating damaged and old cells, the chance of developing cancer later increases. The genetic mutations in every individual cancer differ from one another. Further changes occur when the cancer spreads. Several cells in the same tumour may have distinct genetic changes (10).

Types of BC. BC is categorized into several types based on the characteristics of the cancer cells and their behaviour (11-14). The major types of BC are as follows: i) Ductal carcinoma *in situ* (DCIS); DCIS is a non-invasive cancer where abnormal cells are found in the lining of a breast duct. While it is not life-threatening, it can increase the risk of developing invasive BC later. ii) invasive ductal carcinoma (IDC); IDC is the most common type of BC, accounting for ~70-80% of cases. It begins in the milk ducts and invades surrounding breast tissue. Symptoms may include a lump or changes in breast shape. iii) invasive lobular carcinoma (ILC); This type starts in the lobules (milk-producing glands) and accounts for ~10-20% of invasive BCs. ILC may present as a thickening or swelling rather than a distinct lump, making it harder to detect via mammograms. iv) human epidermal growth factor receptor 2 (HER2)-positive BC; HER2-positive BC tests positive for excess HER2 proteins, which promote cell proliferation. This type tends to be more aggressive but responds well to targeted therapies that inhibit HER2. A total of ~15-20% of BCs are HER2-positive. v) triple-negative BC (TNBC); TNBC lacks three common receptors: Estrogen, progesterone and HER2. This type is more prevalent among younger women and tends to be more aggressive with fewer treatment options available compared with other types.

Metabolites. The metabolism or metabolic reaction can be defined as the sum of all biochemical reactions carried out by an organism. Metabolites have various roles, including those related to energy, structure, signalling, catalysis, defence and interactions with other organisms. Plants, humans and microbes, all produce metabolites. Metabolites can be divided into two different types, namely, primary metabolites and secondary metabolites. Metabolites are the intermediates or final products of metabolic reactions, which are typically limited to small molecules and are catalysed by several enzymes that naturally exist within cells (15,16). The cell produces primary metabolites and typically participates in respiration and photosynthesis, the two main metabolic activities. Primary metabolites can keep the body's physiological processes running smoothly. Considering this function, it is often referred to as the central metabolite. Amino acids, alcohols, polyols, organic acids, vitamins (B2 and B12), inosine-5'-monophosphate, and guanosine-5'-monophosphate are notable examples of primary metabolites. Ethanol, citric acid, lactic acid and acetic acid are primary metabolites necessary for healthy development, growth and reproduction.

Cells utilise primary metabolites, intermediate by-products of anabolic metabolism, to create necessary macromolecules (17).

Secondary metabolites are substances an organism produces that are not necessary for primary metabolic activities but may serve crucial ecological and other purposes. Secondary metabolites are not involved in cell proliferation and development and are synthesised at or near the end of the stationary growth phase (18). Given that secondary metabolites are produced by the same metabolic pathways that primary metabolites use, secondary metabolites are known as the final products of primary metabolites. Primary metabolites are present in every living cell with the ability to divide. Secondary metabolites are present merely incidentally and are not crucial to an organism's survival (16). However, secondary metabolites are produced from primary metabolites, which do not constitute the organism's fundamental molecular structure. Primary metabolites' absence does not immediately shorten an organism's lifespan; instead, survival is compromised to a greater extent. Within a phylogenetic group, its existence and synthesis are found in ecologically disadvantageous species (19). Drugs, flavours, scents, dyes, pigments, insecticides and food additives are examples of secondary metabolites used in pharmaceuticals, industries and agriculture (20).

Numerous intermediates in primary metabolism overlap with the intermediates of secondary metabolites, thus distinguishing between primary and secondary metabolites is not easy. Amino acids, considered primary metabolites, are also unquestionably secondary metabolites (16), in contrast to the claim that sterols are secondary metabolites essential to numerous cellular structural frameworks. The mosaic structure of an intermediate suggests that primary and secondary metabolism share the same metabolic pathway. Adding extra nitrogen and carbon can be directed into the secondary metabolites, which operate as a buffer zone to produce an inactive primary metabolism. When needed, the metabolic disintegration of secondary metabolites can convert the stored carbon and nitrogen back into primary metabolites. The primary and secondary metabolisms are dynamic and in a delicate balance with the growth, tissue differentiation, and development of the cell or organism, as well as external influences, all impacting. Secondary metabolites, also known as natural products or heterogeneous groups of natural metabolic products, are considered to play adaptive roles in ecological interactions, symbiosis, metal transport, competition and other processes even though they are not required for the vegetative growth of the producing organisms (21). For instance, they may act as defence compounds or signalling molecules.

According to Jones *et al* (23), a comprehensive analysis reveals that a typical human body contains ~2,500 metabolites. Arachidonic acid is a metabolite of prostaglandin, and the two compounds share numerous of the same functional groups, physical characteristics and formulae. Additionally, a specific sequence of enzyme-catalysed reactions that follow a rational path of chemical change connects both chemicals (24). Tyrosine is an amino acid that produces catecholamines, whereas cholesterol creates steroid hormones. By making only minor modifications to the cholesterol ring's superstructure, steroid hormones that differ biochemically from the cholesterol source molecule can be produced (2). Tyrosine is the starting point for an irreversible route that leads to catecholamines, such as norepinephrine or dopamine. Moreover, all precursors of catecholamine must pass through a tyrosine intermediate owing to biochemical principles (3). According to the free-energy exchange theory, inosine-5'-monophosphate is a metabolite that develops from the one-way condensation of two or more intermediates, specifically glutamine and phosphoribosyl-pyrophosphate (4). Small molecules are complex to define precisely because they quickly diverge from their parent structure. A metabolite may also be a component of a larger structure or a degraded product that needs to be disposed. A freely available electronic database including comprehensive data on metabolites discovered in the human body is known as the Human Metabolome Database (3-5).

BC metabolomics. In attempts to discover potential biomarkers that can be used to detect cancer cells in their earliest stages, numerous studies have been performed on the biological samples of patients with BC. Samples from patients such as tissues, blood and urine have been collected and examined to obtain the best results that can benefit individuals. Tumour DNA is the element that has been most thoroughly evaluated, including DNA concentrations, integrity, mutations and methylation status. The main aim is to gauge its potential clinical relevance (24,25). Cancer cells also have the exact needs and capacities for energy as regular cells. It has been demonstrated that most cancer cells produce energy through cytoplasm glycolysis. Energy generation is typically utilised by several contemporary technologies to detect malignancy. The rate of protein turnover and lipolysis, which is the breakdown of fat stored in fat cells, increases in cancer cells (26).

Cancer cells undergo significant metabolic changes compared with normal cells. These changes are critical to cancer cells' survival and proliferation, providing a unique opportunity to differentiate cancer cells from normal cells. Metabolomics can be used to identify these metabolic changes and thus help diagnose and treat cancer. It can also help in the discovery of new biomarkers and therapeutic targets. Some researchers have focused on potential indicators found in urine samples of patients with BC. The metabolomics approach is used for the test and research, which involves running tests on technologies such as nuclear magnetic resonance (NMR), high-performance liquid chromatography (HPLC), gas chromatography (GC)-mass spectrometry (MS), or other suitable analytical tools to obtain the most accurate results. In total, 44 pair-wise rates of RNA metabolites exist for BC urinary tests. Numerous different indicators or biomarkers can be found in the urine samples of patients with BC. Based on a study by Nam *et al* (27), homovanillate, 4-hydroxyphenylacetate, 5-hydroxyindoleacetate and urea are all found in the urine samples of patients with BC.

The main contributing compounds in the urinary metabolomics for BC include formate, succinate and nucleoside uracil. Succinate, a metabolite of the tricarboxylic acid (TCA) cycle and a marker for the Warburg effect, is also highlighted by another MS investigation (28). With reasonable specificity and sensitivity, the panel of succinic acid and dimethyl-heptanoyl-carnitine is used to distinguish between BC and healthy controls. According to research looking at nucleosides in urine, 5-hydroxymethyl-2'-deoxyuridine,

8-hydroxy-2-deoxyguanosine and succinyl adenosine are all shown to be more common in patients with BC (29-31).

Patients with BC have higher amounts of glucose, creatinine, glutamine, glutamate, arginine, lysine and valine than healthy controls. These metabolisms are closely linked to a higher risk of BC. Moreover, it has been found that those with greater levels of 5-amino valeric acid, tryptophan, phenylalanine, y-glutamyl threonine, valine, or iso-glutamine are more likely to be diagnosed with BC (23,33). A recent study predicted that 2-o-methylcytidine and 5-methylthioadenosine levels in patients with BC will rise (34). Based on the same research, hierarchical analysis reveals 71 out of 168 differentially expressed metabolites.

Analysing urine metabolomics biomarkers often uses analytical techniques such as NMR and MS (35). By identifying the distinctive electrochemical environment of each constituent proton, the urine NMR readings of molecules can be identified using NMR. Low levels of several metabolites including succinate have been found in the urine of patients with epithelial ovarian cancer and BC according to research on urinary-metabolite modifications (36,37). A total of nine metabolites significantly differ in a study comparing the urinary proton NMR metabolomic profiles of BC (n=48) and ovarian cancer (n=50) based on Wilcoxon's rank-sum test. The metabolites involved are acetone, allantoin, carnitine, urea, 1-methyl nicotinamide and levoglucosan. Slupsky *et al* (39) discovered that the amount of several high-level metabolites including glucose and creatine, which are high in cancer tissue, decreases in the urine of patients with BC (38).

Moreover, patients with BC have lower urine succinate levels compared with healthy controls (39). The urine samples of patients with BC have decreased glutamine level, which is typically high in breast tissue. This discovery is also validated by additional research that produces comparable outcomes (38). Urine of patients with BC has lower threonine levels than controls as well (40). Changes can further be observed in metabolites such as choline and 2-hydroxybutyrate. These two metabolites have higher levels in BC samples than in healthy control samples (41,42). Valine and lysine also rise (43). Furthermore, patients with BC have lower amounts of melatonin and indole-3-acetate in their urine tests.

A study on BC indicators in urine examines metabolic differences between patients with BC and healthy volunteers. The investigation identified12 metabolites including amino acids, organic acids and nucleosides as possible biomarkers (30). In a separate study, (27) used a LC-ion trap MS to analyse urine samples from 85 patients with BC and corresponding controls. A total of 44 pairwise ratios of metabolite characteristics were effectively examined by computational analysis, with a sensitivity and specificity of 83.5 and 90.6%, respectively, for the best BC prediction. S-Adenosylhomocysteine and a few other methylated nucleosides significantly dominate the classification performance. In another study, a capillary electrophoresis (CE) MS was used to examine urine samples from 21 patients with advanced BC before and after receiving chemotherapy, as well as samples from the general population (44). The aforementioned study found that metabolite levels decrease by 30% in chemotherapy-sensitive patients compared with the control group. Specifically, glycine, cysteine, histidine, cysteine, and tryptophan levels are affected. Those who are resistant to treatment have 9% changes in metabolite levels. Meanwhile, the amounts of succinate increases and the levels of chromium considerably drop, whereas most amino and organic acids do not show any apparent alterations. In another study, urine samples from 22 healthy controls were compared with those from 10 patients with BC, 9 with ovarian cancer and 12 with cervical cancer. The cancer biomarkers were found to comprise 5-hydroxymethyl-2-deoxyuridine and 8-hydroxy-2-deoxyguanosine (45).

The identification of BC biomarkers in urine samples of patients with BC is also influenced by environmental factors. Cadmium is markedly more prevalent in urine of patients with BC (46). The same applies to increasing chromium and arsenic. Moreover, it was revealed that patients with BC have a general decrease in amino acids, nucleotides and TCA cycle intermediates (40). The marker results from previous studies based on different sample types, such as tissue, serum, plasma and urine samples, are included in Table I.

Role of metabolites in cancer development. A complex network of chemical processes is responsible for metabolism within cells, which supports healthy development and reproduction. Metabolism involves catabolism and anabolism. The former provides energy and generates the cellular building blocks required for cell division. Uncontrolled cell proliferation and a diverse microenvironment are characteristics of cancer. According to Cairns *et al* (71), cancer cells alter their preferred metabolic pathway to balance their energy requirements with their need to produce biosynthesis precursors for development (69) and to survive in low nutrient areas and low oxygen concentrations (72). By changing the functions of current metabolic pathways or rewiring new connections, cancer cells experience widespread metabolic modifications, notably in glycolysis, mitochondrial biogenesis, lipid metabolism and the pentose phosphate pathway (73). Through various processes, metabolic reprogramming in cancer cells causes the accumulation or depletion of intermediate metabolites (74). The first and foremost one is an alteration in the activity of metabolic enzymes. Since the 1920s, the Warburg effect has been recognised as a distinctive feature of cancer. It is a change in metabolic state wherein cells show an enhanced conversion of glucose into lactate even in highly oxygenated areas (75-77). For instance, activating glycolysis-related enzymes results in the build-up of several glycolytic intermediates during glycolysis, the preferred method by which cancer cells receive energy and biosynthesis building blocks. Conversely, the build-up of succinate and fumarate is caused by a decrease in succinate dehydrogenase and fumarate hydratase activities, respectively.

Since the discovery of oncogenic functions of various mitochondrial metabolites such as 2-HG, succinate and fumarate, researchers have become increasingly interested in the functions of these 'oncometabolites' in cancer. Oncometabolites affect signal transduction, post-transcriptional modifications, and epigenetic changes. The inactivation of tumour-suppressor genes and the promotion of carcinogenesis are caused by metabolic remodelling, which can encourage DNA hypermethylation and histone hyperacetylation (78,79). Numerous intermediate metabolites, in addition to oncometabolites, can bind directly to proteins or nucleotides and cause them to

Table I. Metabolomics in studies of human BC: Comparison between blood, tissue and urine samples.

First author/year	Sample type and sample size	Method	Results/Markers	Population	(Refs.)
Asiago *et al*, 2010	257 serial blood serum samples: 116 samples with recurrent BC, 141 samples with no sign of recurrence	NMR and GC-MS	11 markers (NMR: formate, His, Pro, Cho, Tyr, 3-HB, Lact; GC-MS: Glu, N-acetyl-Gly, nonanedioic acid, 3-hydroxy-2-methyl-butanoic acid	Houston, Texas	(48)
Oakman *et al*, 2011	Pre- and post-operative blood serum samples from 44 patients with early BC and 51 metastatic patients	NMR	Metastatic samples: Higher values of Pro, Phe, Gluc, Lys and N-acetyl-Cys and lower values of lipids.	Prato, Italy	(49)
Tenori *et al*, 2012	Blood serum samples from 579 women with metastatic BC randomized to paclitaxel plus either anti-HER2 (lapatinib) or placebo	NMR	Gluc higher in the patients with longer time to progression. Glutamate and Phe higher in patients with shorter time to progression in on-treatment samples	Italy	(50)
Wei *et al*, 2013	Serum samples from 28 patients with different response rates to NAC	NMR/ LC-MS	Three metabolites (Ile, Thr, Gln) from NMR and linolenic acid from LC-MS were significantly different when comparing response to chemotherapy	Germany	(51)
Jobard *et al*, 2014	Blood serum from 197 patients with early BC and 90 metastatic patients	NMR	Ala, His and betaine were higher in the serum of patients with early BC; end products of lipid degradation and β-oxidation (Acac and 3-HB) (glycerol), Pyr, NAC glycoproteins, lipids, or Phe, Glu and mannose concentrations increased for metastatic BC	France	(52)
Tenori *et al*, 2015	Blood serum from 80 patients with early-stage BC and 95 patients with metastatic BC	NMR	Significantly lower levels of His and higher serum levels of Gluc, Tyr, Lact and lipids in metastatic patients	New York and Italy	(53)
Henneges *et al*, 2009	Urine samples from 85 patients with BC and 85 HC	LC-MS	44 pairwise ratios of metabolite features had distinct predictive capacity; S-adenosylhomocysteine as main identifiers; various methylated nucleosides	Germany	(28)
Nam *et al*, 2009	Urine samples from 50 patients with BC and 50 HC	GC-MS	Homovanillate, 5-hydroxyindoleacetate, 4-hydroxyphenylacetate and urea were identified to be different in normal subjects and cancer	Korea	(27)
Woo *et al*, 2009	Urine samples from 10 patients with BC, 9 patients with OV, 12 patients with cervical cancer and 22 normal controls	GC-MS/ LC-MS	BC samples contain 2-hydroxymethyl-2-deoxyuridine and 8-hydroxy-2-deoxyguanosine	Korea	(46)

Table I. Continued.

First author/year	Sample type and sample size	Method	Results/Markers	Population	(Refs.)
Kim *et al*, 2010	Urine samples from 50 patients with BC and 50 controls	GC-MS	Five potential urinary biomarkers for BC; metabolites were not identified	Korea	(54)
Slupsky *et al*, 2010	Urine samples from 48 patients with BC, 50 patients with OV and 73 healthy volunteers	NMR	67 metabolites identified; amino acids, tricarboxylic acid cycle and metabolites relating to energy metabolism, and gut microbial metabolism	Edmonton, Canada	(39)
Yu *et al*, 2013	Urine samples from 21 patients with advanced or locally advanced BC before and after chemotherapy and 21 healthy volunteers	CE-MS	In chemotherapy-sensitive patients: Cys, Gly, cystine, His and Trp were significantly decreased after chemotherapy; In chemotherapy-insensitive patients: few obvious differences between patients before and after chemotherapy (Succ increased while Cr decreased)	China	(45)
Chen *et al*, 2009	Urine samples from 20 patients withBC and 18 HC	LC-MS	12 metabolites as potential biomarkers including organic acids, amino acids, and nucleosides; elevated Trp and nucleosides metabolism and protein degradation in patients with BC	China	(31)
Bathen *et al*, 2013	228 BC tissues	NMR	The loading profiles from both PCA and PLS-DA analyses revealed choline-containing compounds as the key indicators for tumour content, with phosphocholine being more abundant in tumour tissue. Glycine, taurine and glucose are also suggestive metabolites.	Trondheim (Norway)	(55)
Borgan *et al*, 2010	46 BC tissues	NMR	One of the categories, A2, had samples with markedly lower glucose and higher alanine levels than the other luminal A samples, indicating that these tumours were more glycolytically active. Additionally, this group was enriched for genes having Gene Ontology concepts associated with DNA repair and cell cycle.	Trondheim (Norway)	(56)
Debik *et al*, 2019	118 BC tissues and serum	NMR	PLS-DA multilevel analysis revealed significant changes in blood metabolite levels following therapy (P=0.001), including unfavorable alterations in lipid levels. PLS-DA detected metabolic differences between survivors and non-survivors in tissue samples received 12 weeks into therapy with an accuracy of 72% (P=0.005), but not in serum samples	Oslo (Norway)	(57)

Table I. Continued.

First author/year	Sample type and sample size	Method	Results/Markers	Population	(Refs.)
Haukaas *et al*, 2016	228BC tissues	NMR	Among the most notable changes were Mc1's high amounts of GPC and phosphocholine (PCho), Mc2's high levels of glucose, and Mc3's high levels of lactate and alanine	Oslo (Norway)	(58)
Chae *et al*, 2016	60 BC tissues	NMR	The GPC/PC ratio, as well as the concentrations of myo-inositol and succinate, were greater in the pure DCIS group than in the DCIS with invasive cancer group (P=0.004, Bonferroni-corrected P=0.064). The OPLS-DA models generated using HRMAS MR metabolic profiles could clearly distinguish between pure DCIS and DCIS of myo-inositol and succinate with concomitant invasive cancer using multivariate analysis.	Seoul (South Korea)	(59)
Euceda *et al*, 2019	122 BC tissues	NMR	Linear mixed-effects models revealed a significant interaction between time and bevacizumab for glutathione, indicating higher levels of this antioxidant in chemotherapy-only patients than in bevacizumab receivers after treatment	Trondheim	(60)
Cala *et al*, 2019	Plasma 58 (29BC; 29HC)	NMR	Particularly, the understanding of the up regulation of long chain fatty acyl carnitines and the downregulation of cyclic phosphatidic acid. In addition, the mapped metabolic signatures in BC were similar but not identical to those reported for non-Hispanic women, despite racial differences.	Bogotà (Colombia)	(61)
Lécuyer *et al*, 2018	Plasma 602 (206BC; 396HC)	NMR	Women characterized by higher fasting plasma levels of valine, lysine, arginine, glutamine, creatine, creatinine and glucose, and lower plasma levels of lipoproteins, lipids, glycoproteins, acetone, glycerol-derived compounds, and unsaturated lipids had a higher risk of developing BC.	France	(62)

Table I. Continued.

First author/year	Sample type and sample size	Method	Results/Markers	Population	(Refs.)
Suman *et al*, 2018	Plasma 122 (72BC;50HC)	NMR	The levels of hydroxybutyrate, lysine, glutamate, glucose, NAC glycoprotein and lactate were highly distinguished in BC stages and showed a favorable biomarker potential using receiver-operating curves based diagnostic models. Furthermore, the significant modulation and favorable diagnostic performances of glutamate, NAC glycoprotein and Lactate in LBC as compared with EBC give their significance in the BC progression.	Lucknow (India)	(63)
Louis *et al*, 2015	Plasma 145 (73BC;72HC)	NMR	The levels of hydroxybutyrate, lysine, glutamate, glucose, NAC glycoprotein and lactate were highly distinguished in BC stages and showed a favorable biomarker potential using receiver-operating curves based diagnostic models. Furthermore, the significant modulation and favorable diagnostic performances of glutamate, NAC glycoprotein and lactate in LBC as compared with EBC give their significance in the BC progression.	Hasselt (Belgium)	(64)
Vignoli *et al*, 2020	Plasma 43 BC	NMR	ER status in patients with HER2-positive BC was found to induce significant changes in the host circulatory metabolome with important implications for the pCR to NACT and for the overall clinical outcome	Aviano (Italy)	(65)
Jobard *et al*, 2021	Plasma 1582 (791BC;791HC)	NMR	The concentration of NAC glycoproteins, ethanol, hypoxanthine and dimethylamine, were positively associated with BC. The concentration of 10 metabolites were increase in premenopausal group after FDR adjustment. The strongest association: histidine. Borderline inversely associated with BC are LDL and VLDL (fatty acids)	Lyon (France)	(1)

Table I. Continued.

First author/year	Sample type and sample size	Method	Results/Markers	Population	(Refs.)
McCartney *et al*, 2019	115 Serum BC	NMR	Metabolomic signature between patients with early BC and metastatic BC is similar and would be predictive of cancer recurrence. Low Random Forest score: Disease free at follow-up. High Random Forest score: One relapse case among seven patients.	NewYork (USA)	(66)
Jiang *et al*, 2018	29 Serum BC	NMR	NMR spectra containing signal from a variety of amino acids (isoleucine, valine, leucine, alanine, threonine, lysine, glutamine, glycine, ornithine, phenylalanine, tyrosine, histidine), amino acid derivative (creatinine, creatine, betaine), variety moieties of lipids, ketone bodies (acetone, 3-D-hydroxybutyrate, aceto-acetate), choline metabolites, carbohydrate metabolism related metabolites, NAC glyco-proteins and organic acids.	Singapore	(67)
Wojtowicz *et al*, 2020	Serum 95 (9BC;86HC)	NMR	31 metabolites, four unknown signals, and nine ranges of chemical shift regions assigned to different lipid types. Levels of citrate, gluta-mine, creatinine, acetoacetate, acetate, glucose, betaine, glycerol, leucine, choline and lysine were upregulated in TNBC Lipid levels, lactate, acetone, alanine, glutamate, tyrosine, pyruvate and isoleucine were down regulated in TNBC.	Wroclaw (Poland)	(68)
Men *et al*, 2020	Urine 144 (106BC;38HC)	NMR	Heavy metals in urine samples. Cd has been detected in BC tissue at high concentrations. The Cd was markedly increased in the urine of patients with BC compared with the control population (~2-fold). Numerous small molecule metabolites were altered in the urine of patients with BC compared with the control population.	Tengzhou (China)	(69)

Table I. Continued.

First author/year	Sample type and sample size	Method	Results/Markers	Population	(Refs.)
Wang *et al*, 2017	Urine 78 (40BC;38HC)	NMR	A total of 10 metabolites exhibited the highest contribution towards discriminating patients with BC from HXs (variable importance in projection (VIP) >1, $P<0.05$). The metabolomic pathway analysis indicated several metabolism pathway disruptions, including amino acid and carbohydrate metabolisms, in patients with BC, namely, glycine and butanoate metabolisms.	Funchal (Portugal)	(2)
Slupsky *et al*, 2010	Urine 170 (48BC;50OC;72 HC)	NMR	All metabolites that were significantly different between the cancers and normal controls were lower in concentration in both the EOC and BC groups as compared with normal. Intermediates of the tricarboxylic acid cycle and metabolites relating to energy metabolism, amino acids and gut microbial metabolism were perturbed.	Edmonton (Canada)	(71)

BC, breast cancer; TNBC, triple-negative HC, healthy control; OV, ovarian cancer; NMR, nuclear magnetic resonance; LC-MS liquid chromatography-mass spectrometry; NAC, N-acetylcysteine; GPC, glycerol-phosphocholine; DCIS, ductal carcinoma *in situ*.

malfunction. These intermediate metabolites can also function as transmembrane receptor ligands, triggering subsequent signalling cascades.

The phenomenon in which cancer cells enhance their intake of glucose and the formation of lactate with a significant reliance on aerobic glycolysis is described as the Warburg effect (75). Cancer cells can produce only minimal ATP during this metabolic state, and they may start to rely on glutamine as a fuel source (80). Thus, cancer therapies are intensively researching the suppression of glucose and glutamine metabolism (80,81). Under normoxic conditions, the contribution of lactate to oxidative respiration has attracted newfound attention (82). The finding that lactate is a waste product and a crucial energy source for tumours raises the possibility that metabolites other than glucose and glutamine may support an environment favourable for the proliferation and multiplication of cancer cells. Asparagine, arginine, cysteine, serine and glycine are examples of downstream amino acid by-products that have been studied for their role in the survival of cancer cells. Further research into medicines targeting each metabolic pathway is required, even if the deprivation of these nutrients is beneficial in some situations. As an alternative, several amino acids and essential vitamins such as vitamins A, B, C, D, E and K function as antitumorigenic agents and slow the spread of cancer. The same study emphasises the roles of lactate, vitamins and amino acids in advancing, inhibiting, and preventing cancer by drawing attention to these generally underestimated metabolites (Table II).

Metabolomic techniques. Under certain specific circumstances, any metabolite can be broken down into smaller product ions. Specific pressure, temperature and collisional energy are required to break down the metabolites. These processes of breaking down produce a distinctive pattern of fragmentation used as identification information. Each chemical has a unique fragmentation pattern crucial to determining a compound's retention time for intensity quantification. The methodology used in metabolomics is unique and has its own set of procedures. Each approach has a similar set-up procedure. Importantly, the samples needed must be prepared according to the desired test. Following the metabolic extraction, the collected samples are sent to metabolomics equipment for compound separation, detection and analysis (91).

Fresh tissue and cells from *in vitro* cultures are the two common sample types used to extract metabolomics data. For fresh tissue models, the tissue is collected, immediately snap-frozen in nitrogenous liquid N_2, and then homogenised in an identical mixture of solvents. This phase must maintain the effectiveness of the extraction and the biochemical integrity. Samples are centrifuged several times to guarantee that all precipitated proteins and other macromolecules are wholly removed using chemicals to help in this function. The pallets are preserved for protein concentration analysis to normalise the metabolite levels. These supernatants are collected, and then the methanol-chloroform-water mixture is removed using a speed vacuum and lyophilisation. The result is the formation of the powdered metabolites. Then, prior to metabolomics acquisition with metabolomics devices, metabolites are resuspended in a solvent combination (92-95).

Numerous options are available for selecting metabolomic equipment. Separation, detection and hyphenated techniques are the three common strategies used for categorising the instruments. Techniques including GC, CE, HPLC, ultra-performance LC and ion chromatography can be used to separate distinct metabolites that elute at varying retention durations. Regarding the method of detection, MS equipment is frequently utilised. MS equipment includes quadrupole time-of-flight (TOF) chromatography, triple quadrupole and Fourier transforms (FT) orbitrap. NMR spectroscopy is another detection method not requiring separation techniques. It is also commonly used to determine the structures of organic compounds. HPLC-MS, FT, ion cyclotron resonance (ICR)-MS and GC-MS (91).

Software programs for metabolomic analysis are required to analyse experimental metabolomic data. MS-based equipment can identify metabolites by using an internal compound standard database and MS/MS fragmentation capture under the same conditions. The sample's fragmentation should match the database's fragmentation to verify one's structure. NMR-based methods can be used to investigate the structure of compounds and isotopomers. Analyses based on NMR and MS can cross-validate and cover more metabolites overall. Planning and conducting a metabolomics study involves four significant steps. These steps include sample collection or generation, data acquisition, bioinformatics and interpretation. Based on the results, it is recommended that a hypothesis be formed or the newly discovered biomarkers to be tested in further studies. Adding quality control to obtain reproducible outcomes and generate meaningful metabolomics data during data acquisition is optimal.

MS-based metabolomics. One of the most popular analytical tools used in metabolomics applications is the MS. The primary goal of MS is the structural characterisation of significant metabolites in the search for biomarkers (96). Metabolic fingerprinting can be acquired by MS direct injection, although it has several limitations such as co-suppression and low ionisation efficiency. To avoid these issues, MS-based metabolomic techniques such as CE-MS, GC-MS, LC-MS (97) and CE are used. These tools can eliminate co-suppression whilst reducing the complexity of biological material. Adding MS to these methods increases the accuracy of compound identification, detection and quantification and shows great sensitivity, selectivity, speed and efficiency (42). The samples prepared are infused directly or by chromatography before being analysed in a MS. Data are recorded, analysed, processed and interpreted before being compared with the theoretical data.

GC-MS. GC-MS has emerged as a crucial and trusted analytical technique for the metabolomic study of separation, detection and identification (98,99). The collected samples are subjected to metabolite extraction before being injected in split-less mode. Afterwards, the high-resolution capillary column is used to propel and release the carrier gas through the sample (42). GC analysis must be performed under certain circumstances (for example, high temperatures and in an oven), and the metabolites must be volatile and thermally stable (for example, metabolites such as alkenes, organic acids, ketones and aldehydes). Non-volatile metabolites including lipids, amines, amino acids, phosphorylated metabolites and sugars must first go through derivatiza-

Table II. Metabolite contribution to tumour survival according to cancer types.

First author, year	Metabolites	Cancer type	Role in tumour progression	(Refs.)
Wang *et al*, 2021	Vitamin A	Breast	4-HPR induces cell death	(83)
Sullivan *et al*, 2016			Vitamin A and retinol reduce risk	(75)
Wang *et al*, 2021		Colon/Colorectal	4-HPR induces cell death	(83)
		Prostate	4-HPR induces cell death	
Sullivan *et al*, 2016	Vitamin B_1	Breast	Intermediate concentrations promote Ehrlich's ascites proliferation in thiamine-deficient patients; high concentrations inhibit proliferation	(75)
			Patients exhibit decreased expression of SLC9A3 transporter gene	
Doldo *et al*, 2015	Vitamin C	Breast	Low concentrations induce cell invasiveness; high doses restrict EMT	(84)
Sullivan *et al*, 2016	Vitamin D	Breast	Calcitriol and D3 analogs suppress MMP-2 and -9 and VCAM-1; low serum D3 levels are associated with high incidence	(75)
		Colon/Colorectal	Low serum D3 levels are associated with high incidence	
Zeng *et al*, 2019	Vitamin E	Breast	Tocotrienols exhibit chemotherapeutic and antitumour properties	(85)
		Colon/Colorectal	Tocotrienols exhibit antitumour properties	
		Prostate	Tocotrienols exhibit chemotherapeutic properties	
Miyazawa *et al*, 2020	Vitamin K	Breast	K_2 induces nonapoptotic cell death	(87)
	Arginine	Breast	Low plasma levels act as a prognostic biomarker	(87)
Miyazawa *et al*, 2020; Qiu *et al*, 2014			Arginine starvation is used to treat arginosuccinate synthase-deficient patients	(87,88)
Cheng *et al*, 2018		Ovarian	Cancer cells are deficient in arginosuccinate synthase-1; ADI-PED-20 is used to degrade arginine	(89)
Ji *et al*, 2020	Asparagine	Breast	Maintains health of glutamine-independent cells	(90)
Sullivan *et al*, 2016	Cysteine	Breast	Inhibition of histone deacetylase-6 sensitizes TNBC cells to cysteine deprivation via cystine/glutamate antiporter-targeted therapies	(75)
		Colon/Colorectal	Starvation induces a reduction in liver-metastatic cell proliferation	
	Lactate	Breast	10 mM L-lactate acts as chemoattractant and facilitates migration	
	Serine	Breast	Cells prefer serine over glycine and exhibit a decrease in nucleic acid synthesis when starved of serine	

tion (42). The samples can be ionised by electro-impact (EI) or chemical ionisation for MS detection. The EI approach is frequently used in ionisation. The mass spectra can be revealed by molecular-ion fragmentation, which EI can offer. The three techniques most frequently used in metabolomics are quadrupole, TOF and ion trap.

Salivary volatiles are screened for potential BC by GC-quadrupole MS (qMS) as part of an exploratory investigation; geographically remote communities are included (100). It has been claimed that the metabolomic signature of human BC cell lines can be established using GC-qMS (98). Based on the urinary volatomic biosignature, it can also be utilised to distinguish amongst various cancer types (101). In contrast to GC-qMS, GC-TOFMS can assess glutamate enrichment as a potential new method of diagnosing BC. Patients with BC with oestrogen receptor (ER)-positive (ER^+) and ER-negative (ER^-) cells can be compared metabolically using a GC-TOF-MS framework (102). In a pilot investigation on patients with BC, it was found that GC-MS can be used to assess the detectability, reliability and distribution of metabolites obtained in pre-diagnostic plasma samples (103). The sensitivity, specificity, reproducibility and high-throughput technology of GC-MS-based metabolomics to handle a huge volume of samples renders it preferable to use. However, GC-MS is limited in its mass range, and because of fragmentation, molecule ions are frequently undetected. Determining unknown metabolites is difficult because of these limitations. Additionally, the required metabolites must be thermally stable and volatile (104).

HPLC-MS. HPLC-MSis a simple method of separating and characterising various metabolites, including acids, bases, salts and hydrophobic and hydrophilic metabolites. Owing to its capability to accommodate separation processes and various mass analysers, LC-MS or HPLC-MS is preferred over MS-based metabolomics because it is not restricted to volatile and thermally stable metabolites (105). Ahad and Nissar (106) used the fundamental principles of HPLC-MS, eluting the metabolites through a column based on the partition between a stationary phase and mobile liquid phase. The kind of stationary phase that the metabolites should elute through depends on their charge, size, hydrophobicity and molecular weight (106). To achieve a quicker separation of metabolites, the current HPLC technology focuses on smaller columns, miniaturisation and low solvent volumes. Thus, ultra-high-performance LC (UHPLC) replaces HPLC. UHPLC does not require large amounts of solvent and speeds up resolution within short analysis times.

NMR-based metabolomics. NMR-based metabolomics is an alternative to MS-based metabolomics. NMR spectroscopy, commonly known as NMR, is acknowledged as a promising metabolomic approach. Despite having lesser intrinsic sensitivity than MS, NMR offers a thorough metabolite fingerprinting, profiling and metabolic study under particular conditions. This drawback has limited its ability to deal with metabolites at the trace level. NMR-based metabolomics has the benefits of automation, minor or no sample preparation requirements, non-destructive, non-selectivity in metabolite detection, excellent repeatability, and the capacity to quantify numerous classes of metabolites simultaneously (106). The foundation of NMR spectroscopy is the radiation that numerous isotopes' nuclei absorb at a particular frequency when exposed to a magnetic field (104).

An NMR spectrum has been demonstrated to correspond with a particular metabolite pattern. Additionally, it offers structural details to enable easier identification of unknown metabolites, which can be accelerated by combining spin-spin coupling, chemical shift and relaxation or diffusion data. In contrast to localised early disease (EBC), a ^1H NMR-based metabolic phenotyping study to identify metabolic serum abnormalities connected with advanced metastatic BC (MBC) is conducted (51). The MBC and EBC groups are distinguished by the metabolite's acetoacetate, histidine, pyruvate, glutamate, glycoproteins (N-acetylcysteine), mannose, glycerol and phenylalanine.

The general flowchart of the details of the *in vitro* and *ex vivo* NMR spectroscopy methodology in BC study is shown in Fig. 1. The samples obtained from the patients and controls can be analysed and studied through *in vitro* or *ex vivo* NMR spectroscopy based on the suitability of the samples. This research primarily focuses on urinary samples, thus the method used is *in vitro*. Based on previous studies, if the samples used are urine, they should be collected in the morning pre-prandial period under sterile conditions after overnight fasting (107). Then, the samples should be placed on ice and frozen in liquid nitrogen before being stored at -40˚C or lower. Next, to perform NMR analysis, the samples must be diluted with sodium phosphate buffer prepared in ddH_2O at 1:2 (prepared buffer/sample). The pH of the urine sample needs to be adjusted to 7.4 constantly because it can lead to changes in the chemical shift of the samples. A total of ~3 mM sodium azide is added to prevent bacterial growth in the solution. Then, 0.5 mM TSP is added for chemical-shift referencing and concentration quantification. TSP is an internal reference for metabolites' chemical-shift calibration and quantification in tissue and urine.

Hyphenated techniques metabolomics. Hyphenated approaches, along with MS-based and NMR-based metabolomics, are eliciting attention in metabolomic investigations owing to their ability to simultaneously detect hundreds of metabolites. This is because this method can simultaneously detect hundreds of metabolites. GC-GC-MS, LC-LC-MS, LC-FT-ICR-MS, LC-MS-NMR and MALDI-FT-ICR-MS are a few examples of analytical techniques. Two-dimensional liquid-LC and gas-GC are gaining increased attention in the metabolomics field because metabolite overlapping can be avoided by redirecting each peak from one GC or LC column to a second column. These methods also increase sensitivity and complementary selectivity (108).

Comparison between MS-based and NMR-based metabolomics study. NMR and MS are the most often applied metabolomic techniques for metabolomic profiling. NMR and MS may be utilised to detect and identify metabolites whilst precisely measuring the concentration, regardless of whether the study focuses on targeted or untargeted analysis. However, each method has advantages and disadvantages. Using several complementary technology platforms to obtain the best results is optimal.

NMR is quantitative and reproducible and does not require extensive sample preparation procedures such as separation or derivation (109-111). This method supports the simultaneous

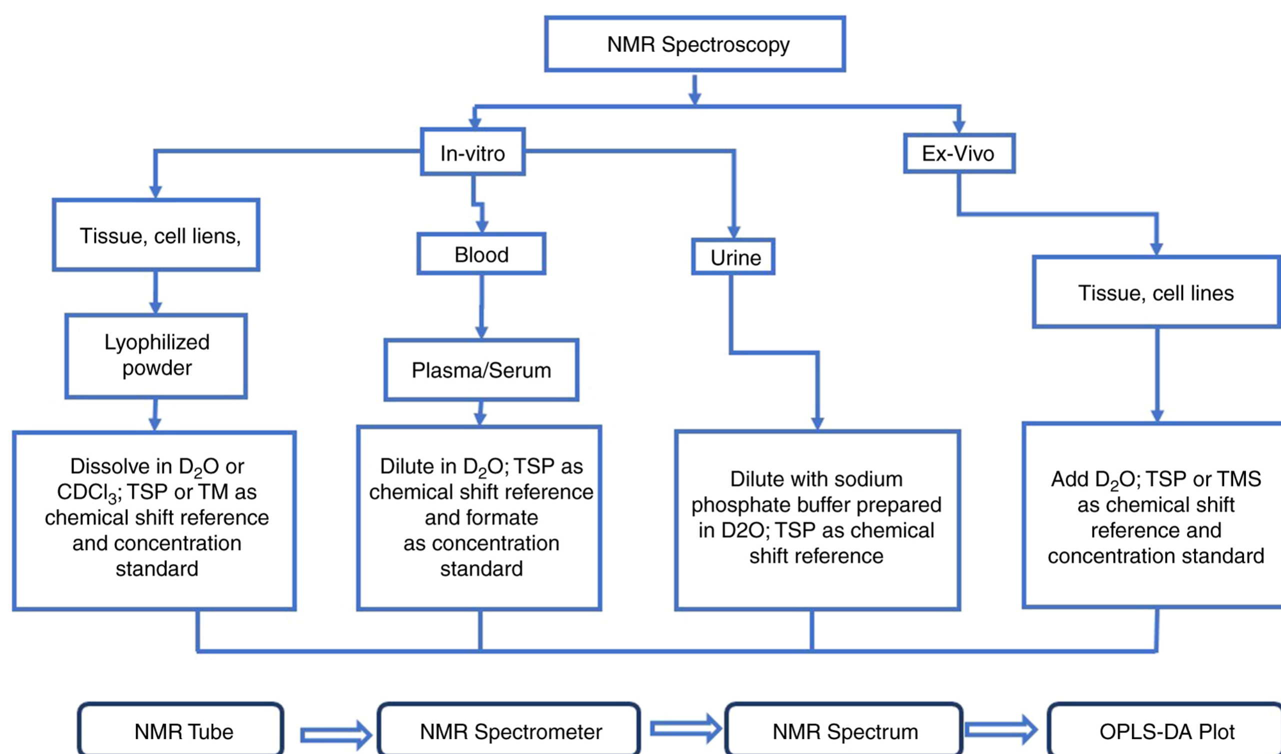

Figure 1. Flow chart depicting major procedures in the nuclear magnetic resonance spectroscopy metabolomics of breast cancer.

measurement of routine lipids, lipoprotein subclass profiling with lipid concentrations within 14 subclasses, fatty acid composition, and various low-molecular metabolites, including amino acids, ketone bodies and metabolites related to gluconeogenesis, in molar concentration units. Considering that no sample preparation is required, it is a quick analysis that requires ~5 min. The outcomes can be enhanced by running more scans and using a stronger magnetic field (111). Additionally, NMR requires a larger sample volume than MS analysis. However, the high scalability and thorough coverage of numerous chemical pathways of NMR render it ideal for the biomarker detection for chronic diseases.

A small sample quantity can be used to evaluate numerous metabolites through the compassionate MS technique. Additionally, it can quantify molecular concentrations as low as nanomolar and picomolar (109). MS can be utilised to find hundreds of metabolites in a sample when used in conjunction with chromatography, including GC and LC. If combined with chromatography (109), MS can investigate secondary metabolites even when the detection level is lower. However, a sample in MS cannot be recovered after analysis (111). In addition to requiring sample preparation and separation, MS is more expensive than NMR (112). MS is a favourable option for achieving comprehensive metabolome coverage in metabolomic profiling.

Biomarker identification

Biomarkers. A biomarker is a term that refers to a trait that is objectively assessed as an indication of normal biological processes, pathological processes, or pharmacological reactions to a therapeutic intervention (113), anticipating sickness occurrence or outcome (114). Biomarkers are used to convey information about human biology, and the discovery of new oncological biomarkers is at the top of the list of translation research goals. Diagnostic biomarkers are used to differentiate sick from healthy persons. Conversely, predictive, prognostic and therapeutic biomarkers may affect therapeutic decision-making and management techniques with the goal of personalising illness therapy (115). Prognostic biomarkers aim to predict the likelihood of a clinical event in the context of illness. Unfortunately, prognostic biomarkers are occasionally a blunt measure of stratifying outcomes, and their reliability is limited by interindividual variability (that is, varying values for a range of patients), intraindividual variability (that is, varying scoring by histopathologists providing Ki-67 measurement), and sensitivity and specificity implications (116).

BC biomarkers. Currently, biomarkers are crucial to managing patients with BC, particularly when choosing the kind of systemic treatment to be used (117). Cell receptors, one of the several varieties of biomarkers, show significant value as diagnostic, prognostic and predictive biomarkers in cancer research and therapy. Accordingly, they are incorporated into drug-development trials (118). ER, PR and HER2/neu receptors are two excellent examples of biomarkers that are prognostic of outcomes and predictive of responsiveness to specific therapy in BC (8). ERs and progesterone receptors (PR) should be assessed on all newly diagnosed invasive BCs to select patients likely to respond to endocrine therapy (117).

ER and PR. PR is a steroid receptor superfamily member that mediates progesterone's action in its target tissues. Particularly, in the mammary gland, the luminal epithelial cell compartment is the only place where PR is expressed (118). The development of sex organs, pregnancy, bone density, cholesterol mobilisation, brain function, cardiovascular system

and other biological processes are only a few of the functions regulated by steroid hormones and their receptors (119). They are essential for the development and spread of BC. Hormone receptors exist in >70% of breast tumours (120). Their cells exhibit positive ER and PR expression, which is linked to the development and spread of cancer cells. The development and spread of BC are significantly influenced by oestrogen and its receptor, ER. PR can influence how ER functions because it is an ER-upregulated target gene whose expression is regulated by oestrogen (119). In BC, PR is a useful predictive indicator of overall survival or disease-free survival (121).

The primary physiological actions of progesterone, a 21-carbon steroid, are mediated by binding to PRs A and B (PR-A and PR-B), which trigger the transcription of specific genes and change proliferative endometrium in an oestrogen-primed uterus into secretory endometrium (121). Progesterone's physiological function is essentially limited to pregnancy, the peri- and post-ovulatory periods of the menstrual cycle. The corpus luteum starts producing progesterone in the early post-ovulatory phase of the menstrual cycle (119). In the later stages of breast growth, side branching and amelogenesis, the receptor activator of nuclear factor kappa B ligand (RANKL) acts as a paracrine mediator of PR-B (119). By autocrine activation through the RANKL pathway and the activation of the downstream target Cyclin D1, the intrinsic proliferation of PR-negative luminal epithelial cells of the breast can be induced by progesterone and PR (121).

Experiments on a breast mouse model, normal human breast tissue, and clinical trials have all shown that progesterone and oestrogen are the two main proliferative steroid hormones in the mammary epithelium that signal mammary gland development (119). Early puberty requires ductal elongation but not progesterone/PR; it requires oestradiol and epithelial ER signalling (122). PR signalling is necessary for ductal elongation and side branching in the epithelial compartment in response to elevated oestrogen levels (8). Early in pregnancy, PR signalling can cause the epithelial compartment to expand rapidly. In mid-to-late pregnancy, progesterone is necessary for alveolar differentiation (117). Progesterone changes from promoting terminal differentiation to inhibiting it at term, and it must be withdrawn for lactation (119).

Progesterone has been linked to the development of BC in mechanistic investigations. However, weak epidemiologic evidence does not indicate a link between circulating levels and the risk of the disease (119). Progesterone metabolites may exert pro- and anti-carcinogenic effects, and the balance amongst these factors may affect BC risk according to data primarily from the Wiebe laboratory (120). However, population-based research pays little attention to this hypothesis primarily because assays are insufficient (117). Lastly, research links progesterone signalling to the development of BC in BRCA1 mutation carriers, raising the possibility that using chemotherapy to block downstream signalling can be beneficial (120).

3. Conclusion

According to previous studies in the manuscripts and their associations with cancer pathways and treatment, metabolomics can be used to identify new biomarkers or be one for cancer diagnosis and treatment stages and the effectivity of the medications. By comparing metabolites in patients with BC and healthy individuals, researchers may identify metabolites with high associations unique to cancer in general and specific for BC. In this review, we study the association of non-targeted and targeted metabolites pathway with patients with BC and healthy controls in numerous places and numerous publications as mentioned before. A high chance of identifying biomarkers from metabolites by conducting more studies was found.

Metabolomics face a number of challenges. i) Data analysis: Metabolomics results contain vast and complex data to analyse, which is one of the challenges. Computational tools and expertise such as websites and programs are needed to analyse the data. ii) Standardization: Metabolomic analysis involves multiple steps, including data analysis, sample preparation, and data acquisition. These steps need to be optimised to give us protocols to produce the same results accurately. iii) Analytical variability: Metabolomics is a susceptible technique, and slight variations in sample preparation or data acquisition can lead to significant differences in results. This variability can confer difficulty in reproducing results between laboratories and in developing robust and reliable biomarkers. Despite these challenges, metabolomics has the potential to revolutionise cancer research and improve patient outcomes. According to the valuable data released from the original work, it will help in accurate diagnosis and early detection of the BC. The future plan of this article aim to produce an exact phenotype for BC detection tool.

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Authors' contributions

MMBY, MM, WNBWA, RAR, WFWAR and TADAATD conceptualized the study. OMA, SSBM, NARBMR, NFABBH and LHY prepared the original draft. OMA, MMBY, MM, WNBWA, RAR, WFWAR, SSBM, NARBMR, NFABBH, LHY and TADAATD wrote, reviewed and edited the manuscript. All authors revised the manuscript. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

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Patient consent for publication

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Competing interests

The authors declare that they have no competing interests.

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