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Application of Metabolic Biomarkers in Breast Cancer: A Literature Review

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Breast cancer is the most common cancer and the second leading cause of cancer death in women worldwide. Novel biomarkers for early diagnosis, treatment, and prognosis in breast cancer are needed and extensively studied. Metabolites, which are small molecules produced during metabolic processes, provide links between genetics, environment, and phenotype, making them useful biomarkers for diagnosis, prognosis, and disease classification. With recent advancements in metabolomics techniques, metabolomics research has expanded, which has led to significant progress in biomarker research. In breast cancer, alterations in metabolic pathways result in distinct metabolomic profiles that can be harnessed for biomarker discovery. Studies using mass spectrometry and nuclear magnetic resonance spectroscopy have helped identify significant changes in metabolites, such as amino acids, lipids, and organic acids, in the tissues, blood, and urine of patients with breast cancer, highlighting their potential as biomarkers. Integrative analysis of these metabolite biomarkers with existing clinical parameters is expected to improve the accuracy of breast cancer diagnosis and to be helpful in predicting prognosis and treatment responses. However, to apply these findings in clinical practice, larger cohorts for validation and standardized analytical methods for QC are necessary. In this review, we provide information on the current state of metabolite biomarker research in breast cancer, highlighting key findings and their clinical implications.

Key Words: Biomarker, Breast cancer, Metabolite

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INTRODUCTION

Breast cancer is the most common malignancy among women globally, accounting for 24.5% of all female cancers. In 2020, approximately 2.26 million women were diagnosed as having breast cancer, contributing to 15.5% of cancer-related deaths among women. In developed countries, one in eight women (12.5%) is estimated to develop breast cancer during their lifetime [1]. Identifying risk factors and ensuring early diagnosis are crucial for reducing incidence and mortality rates.

Breast cancer is a highly heterogeneous disease characterized by multiple subtypes, each exhibiting distinct treatment responses and progression patterns based on specific molecular and cellular characteristics. This heterogeneity complicates early diagnosis, treatment strategies, and prognosis prediction [2]. Biomarker development is crucial for addressing these challenges as it would facilitate early detection, improving treatment outcomes, and enhancing survival rates. Extensive research continues to focus on identifying and validating biomarkers to address these challenges.

Metabolites, small molecules (< 1,500 Da) generated during metabolic processes, include amino acids, lipids, and organic

acids. These molecules link genotype, environment, and phenotype [3], providing metabolic insights into health conditions associated with different biological processes. Metabolic alterations caused by disease affect these metabolites, offering the potential for biomarker discovery for disease diagnosis, treatment, risk assessment, and recurrence prediction [4]. Several studies have investigated metabolites in tissues and body fluids from patients with breast cancer to identify reliable biomarkers.

Metabolite analysis primarily employs two techniques: mass spectrometry (MS) and nuclear magnetic resonance (NMR). MS provides high sensitivity and selectivity, enabling simultaneous analysis of hundreds of metabolites in biological samples. However, it requires preprocessing steps, typically involving gas or liquid chromatography (LC), and is costly. NMR, based on the energy responses of atomic nuclei to changes in external electromagnetic fields, eliminates the need for preprocessing and offers high reproducibility and cost-effectiveness but has lower sensitivity than MS [5].

In this review, we elucidate the current state of metabolite biomarker research in breast cancer, highlighting key findings and their clinical implications.

DIAGNOSTIC BIOMARKERS FOR BREAST CANCER

Mammography is widely used in large-scale breast cancer screening programs; however, its sensitivity is relatively low, emphasizing the necessity for supplementary diagnostic methods [6]. Metabolic alteration in patients with breast cancer affects metabolites in tissues, blood, and body fluids, which can be used as biomarkers for early breast cancer diagnosis [7].

Human blood contains approximately 1,200 proteins, 600 lipids, and 300 metabolites, which are actively studied to identify metabolic biomarkers [8]. Metabolite levels in the serum and plasma of patients with breast cancer are altered, suggesting their potential as diagnostic biomarkers. Table 1 summarizes studies on diagnostic biomarkers for breast cancer.

Amino acid metabolic biomarkers

Amino acid metabolites, including tryptophan and its associated pathway metabolites, arginine, proline, histidine, 5-oxoproline, kynurenine, nicotinate, and nicotinamide, have been suggested as potential early diagnostic biomarkers for breast cancer [9–17]. The catabolism of tryptophan, an essential amino acid, is associated with the immune system, and tryptophan has been studied in the context of cancer [15]. Indoleamine 2,3-dioxygenase (IDO), its splice variant IDO2, and TDO metabolize tryptophan, playing critical roles in immune regulation. Notably, IDO contributes to cancer immune evasion by depleting tryptophan, inhibiting T cell proliferation, and inducing immune tolerance. In IDO-overexpressing cancer tissues, cytotoxic T cells decrease, whereas regulatory T cells (Tregs) increase in numbers, leading to immunosuppression and treatment resistance [18]. Tryptophan depletion inactivates T cells and induces apoptosis, whereas its scarcity promotes immunosuppressive properties in dendritic cells and Treg differentiation. Kynurenine, a tryptophan metabolite, activates the aryl hydrocarbon receptor, suppressing T and natural killer cells and enhancing Treg differentiation [19, 20]. Tryptophan is a potential biomarker for breast cancer diagnosis.

Patients with breast cancer have lower plasma tryptophan levels and higher plasma kynurenine:tryptophan ratios than healthy individuals, which may aid in early diagnosis [15]. Similarly, the level of *N*-acetyl-d-tryptophan (NAT) is decreased in breast cancer, suggesting its potential as a diagnostic biomarker [13]. Other authors [16] have also reported alterations in tryptophan metabolism in patients with breast cancer, including reduced levels of metabolites such as indole and indole-3-acetate. Yuan, *et al*. [21] suggested a potential diagnostic panel for breast cancer using plasma tryptophan, glutamate, ornithine, threonine, methionine sulfoxide, and C2 and C3 metabolites. The panel effectively helped distinguish patients with primary breast cancer from healthy controls, achieving areas under the curve (AUC) of 0.87 (95% CI, 0.81–0.92) and 0.80 (95% CI, 0.71–0.87) in the training and validation cohort, respectively.

L-Arginine, a precursor to proline and glutamate, is associated with cancer development and progression, as well as with host immunity [22, 23]. It plays a crucial role in activating T cell functions. In patients with cancer, myeloid-derived suppressor cells deplete L-arginine, leading to suppressed T cell activity and reduced antitumor immune responses [24]. Therefore, L-arginine can serve as a biomarker for breast cancer diagnosis. In patients with breast cancer, arginine and proline metabolic pathways are altered, and serum arginine levels are decreased [17]. Changes in arginine/proline metabolism [16] and significantly decreased blood arginine levels [9, 25–27] have also been reported in patients with breast cancer. Conversely, Mao, *et al*. reported increased serum L-arginine levels in patients with HER-2-positive breast cancer [11].

Glutamate is a product of glutamine generated by phosphate-dependent glutaminase. In breast cancer, glutaminase activity is elevated, accelerating glutamine metabolism to glutamate

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Table 1. Studies on diagnostic biomarkers for breast cancer

Author, reference	Year	Country	Cancer cases (N)	Control cases (N)	Analytical method	Sample type	Biomarkers	Key findings
Amiri-Dashatan, *et al*. [9]	2022	Iran	22	10	LC-MS/MS	Serum	Metabolites related to amino acids and lipids; significant pathways included arginine and proline metabolism, glycerophospholipid metabolism, phenylalanine, tyrosine, tryptophan biosynthesis	Arginine, tyrosine, PE (24:0), PE (22:0), PC (18:0): elevated levels in BC Proline, kynurenic acid, 3-hydroxybutyryl carnitine, myoinositol, TG (20:1), TG (22:0), PS (24:0), PS (22:0), lysoPE (14:0), lysoPE (20:1), lysoPE (24:0), PG (18:1): decreased levels in BC
Xu, *et al*. [10]	2023	China	143	86	UPLC-QTRAP-MS	Plasma	*N*-acetylpyrrolidine, phenethylamine, L-isoleucine, 6-aminocaproic acid, dL-leucine, 4-hydroxybenzoic Acid, 2,4-dihydroxypteridine, 4-hydroxytryptamine, kynurenic acid, L-tryptophan, pantothenate, Tyr-Asn, carnitine C10:0, sorbitol 6-phosphate	Fourteen-metabolite panel: high reproducibility, diagnostic model with an AUC of 0.0.792
Mao, *et al*. [11]	2022	China	20	30	UPLC-TOF-MS	Serum	L-Arginine, arachidonic acid	Elevated levels in HER2-positive BC
Huang, *et al*. [12]	2022	China	135 (discovery set), 34 (validation set)	125 (discovery set), 31 (validation set)	NPELDI-MS + machine learning	Serum	Seven metabolites, including L-glyceric acid, nicotinamide, His, uracil, thymine, 3,4-dihydroxybenzylamine, dehydrophenylalanine	Seven metabolites panel: high reproducibility, diagnostic model with an AUC of 0.948
Gong, *et al*. [13]	2024	China	53 (BC) 23 (TNBC), 30 (Non -TNBC)	57	UHPLC-MS	Serum	Four metabolites, including *N*-acetyl-L-tryptophan, 2-arachidonoylglycerol, pipecolic acid, oxoglutaric acid	Four metabolites panel: high diagnostic performance with an AUC of 0.995
Park, *et al*. [14]	2019	Korea	40 (discovery set), 30 (validation set)	30 (discovery set), 16 (validation set)	LC-MS	Plasma	L-Octanoylcarnitine, 5-oxoproline, hypoxanthine, docosahexaenoic acid (DHA)	5-Oxoproline and hypoxanthine levels elevated in BC L-Octanoylcarnitine and DHA levels decreased in BC
Onesti, *et al*. [15]	2019	Belgium	202	146	MS/MS (TQ5500)	Plasma	Kynurenine, tryptophan, kynurenine:tryptophan ratio	Kynurenine, tryptophan, kynurenine:tryptophan ratio decreased in BC
Jasbi, *et al*. [16]	2019	USA	102	99	LC-MS/MS	Plasma	Six-metabolite panel, including proline, myoinositol, 2-hydroxybenzoic acid, gentisic acid, hypoxanthine, acetylglycine	Six-metabolite panel: high diagnostic performance with an AUC of 0.89

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Table 1. Continued 1

Author, reference	Year	Country	Cancer cases (N)	Control cases (N)	Analytical method	Sample type	Biomarkers	Key findings
Zhang, *et al*. [17]	2024	China	27	30	UHPLC-HRMS	Serum	Arginine, proline, nicotinate, nicotinamide, caffeine, arachidonic acid	Significant alterations in metabolic pathways, potential biomarkers for BC detection
Yuan, *et al*. [21]	2019	Germany	80 (training set), 109 (validation set)	100 (training set), 50 (validation set)	UHPLC-MS/MS and FIA-MS/MS	Plasma	Glutamate, ornithine, threonine, tryptophan, methionine sulfoxide, C2 (acetylcarnitine), C3 (propionylcarnitine)	Seven-metabolite panel: high diagnostic performance with AUCs of 0.87 and 0.80 in training and validation sets, respectively.
Wang, *et al*. [28]	2018	China	34 (training set), 30 (validation set)	Benign: 38 (training set), 30 (validation set) Control: 44 (training set), 30 (validation set)	LC-MS for amino acids; GC-MS for organic acids	Serum	Taurine, glutamic acid, ethylmalonic acid	Taurine and glutamic acid levels elevated in BC; ethylmalonic acid levels decreased in BC
Suman, *et al*. [30]	2018	India	72	50	^{1}H NMR spectroscopy	Plasma	Hydroxybutyrate, lysine, glutamate, glucose, NAG, lactate	Hydroxybutyrate, lysine, glutamate, glucose, and lactate levels elevated in BC; NAG level decreased in BC
Budczies, *et al*. [31]	2015	Germany	270	97	GC-TOFMS	Tissue	Glutamate:glutamine ratio	Breast cancer tissues exhibited higher glutamate levels and an increased glutamate: glutamine ratio compared to normal breast tissues.
Mrowiec, *et al*. [32]	2024	Poland and Norway	112	207	HRMS	Serum	Twenty-three metabolites, including several amino acids (alanine, aspartate, glutamine, histidine, phenylalanine, leucine/isoleucine), lysophosphatidylcholines, and diglycerides	Twenty-three-metabolite panel (multicancer signature): high diagnostic performance with an AUC>0.95
An, *et al*. [33]	2022	China	75 (training set), 32 (test set)	Benign: 30 (training set), 30 (test set) Healthy: 20 (training set), 29 (test set)	UPLC-MS	Plasma	Forty-seven metabolites, including sphingomyelins, glutamate, cysteine; Proteins like GOT1, LDHB, GSS, GPX3	Forty-seven-metabolite panel: High accuracy in BC prediction (AUC=1). high predictive power in the test cohort between BC vs. HC (AUC=0.794) and benign vs. HC (AUC=0.879)
Kozar, *et al*. [39]	2021	Slovenia and Spain	39	21	Targeted metabolomics using HPLC-MS	Serum	Acylcarnitines, 9,12-linoleic acid	Acylcarnitine and 9,12-linoleic acid levels decreased in BC
Cala, *et al*. [40]	2018	Colombia	31	29	LC-MS and GC-MS	Urine	Dimethylheptanoylcarnitine, succinic acid	Combination of dimethylheptanoylcarnitine, and succinic acid: high diagnostic performance with an AUC of 0.915

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Table 1. Continued 2

Author, reference	Year	Country	Cancer cases (N)	Control cases (N)	Analytical method	Sample type	Biomarkers	Key findings
Wei, *et al*. [41]	2021	United States	124	86	Untargeted LC-QTOF-MS and targeted LC-QQQ-MS	Plasma	Six metabolites: ethyl (R)-3-hydroxyhexanoate, caprylic acid, hypoxanthine, m/z 358.0018, 354.0053, and 356.0037	Six-metabolite panel: diagnostic performance with an AUROC of 0.938, with 90% sensitivity and 90% specificity
Jiang, *et al*. [44]	2017	China	37	41	UPLC-QTOF-MS	Plasma	Six differentiating lipids: PC (20:2/20:5), PC (22:0/24 :1), TG (12:0/14 :1), DG (18:1/18 :2), PE (15:0/19 :1), N-palmitoyl proline	PC (20:2/20:5), PC (22:0/24:1), TG (12:0/14:1), DG (18:1/18:2), PE (15:0/19:1), N-palmitoyl proline levels decreased in BC
Buentzel, *et al*. [45]	2021	Germany	78	30	Targeted and untargeted mass spectrometry	Blood-derived microvesicles	Eight significant metabolites: lysoPC a C26:0, PC aa C38:5, PC ae C32:2, PC ae C34:2, PC ae C38:3, PC ae C40:2, PC ae C40:6, SM (OH) C16:1	Eight-metabolite panel: diagnostic performance with an AUC of 0.78 Higher levels of lysoPCaC26:0 and PCaaC38:5 correlated with shorter overall survival
Wang, *et al*. [50]	2014	China	39	45	GC-MS	Exhaled breath	5,6-Trimethyloctane, 1,4-dimethoxy-2,3-butanediol, cyclohexanone	5,6-Trimethyloctane, 1,4-dimethoxy-2,3-butanediol, cyclohexanone levels elevated in BC patients compared to controls and patients with benign breast disease

Abbreviations: TNBC, triple-negative breast cancer; LC-MS, liquid chromatography-mass spectrometry; DHA, docosahexaenoic acid; NAG, N-acetyl glycoprotein; HPLC-MS, high-performance liquid chromatography-mass spectrometry; LC-QTOF-MS, liquid chromatography quadrupole time-of-flight mass spectrometry; LC, liquid chromatography; LC-MS/MS; liquid chromatography coupled with tandem mass spectrometry; PE, phosphatidylethanolamine; PC, phosphatidylcholine; BC, breast cancer; TG, triacylglycerol; PS, phosphatidylserine; PG, phosphatidylglycerol; AUC, area under the curve; UPLC-QTRAP-MS, ultraperformance liquid chromatography-triple quadrupole-linear ion trap mass spectrometry; UPLC-TOF-MS, ultrahigh-performance liquid time-of-flight mass spectrometry; NPELDI-MS, nanoparticle-enhanced laser desorption mass spectrometry; UHPLC-MS; ultrahigh-performance liquid chromatography-mass spectrometry; UHPLC-MS/MS, ultrahigh-performance liquid chromatography-tandem mass spectrometry; FIA-MS/MS, flow injection analysis tandem mass spectrometry; GC-TOFMS, gas chromatography time-of-flight mass spectrometry; HRMS, high-resolution mass spectrometry; UPLC-MS, ultraperformance liquid chromatography-mass spectrometry; LDHB, L-lactate dehydrogenase B chain; GSS, glutathione synthetase; GPX3, glutathione peroxidase 3; HC, healthy control; GC-MS, gas chromatography-mass spectrometry; LC-QQQ-MS, liquid chromatography-triple quadrupole mass spectrometry; AUROC; area under the ROC curve; UPLC-QTOF-MS, ultrahigh-performance liquid chromatography quadrupole time-of-flight tandem mass spectrometry; HPLC, high-performance liquid chromatography; DG, diacylglycerol; SM, sphingomyelin.

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and supplying the energy necessary for cancer cell growth and survival [28, 29]. Park, *et al*. [14] reported that the level of 5-oxoproline, also known as pyroglutamic acid, is significantly increased in breast cancer. Glutamate levels are also elevated in the serum and plasma [28, 30] and cancer tissues [31] of patients with breast cancer, whereas serum and plasma glutamine levels are decreased [30, 32]. However, An, *et al*. reported that plasma glutamate levels were decreased in patients with breast cancer [33]. The authors attributed this decrease to tumor cells absorbing glutamate from the bloodstream to enhance tumorigenesis. These findings suggest that glutamate may be a potential biomarker for breast cancer diagnosis. However, further research is necessary to validate these findings.

Nicotinic acid and nicotinamide metabolism are associated with the rapid turnover of nicotinamide adenine dinucleotide (NAD^+) in cancer cells. Cancer cells utilize the salvage pathway to maintain high NAD^+ levels, promoting cancer cell synthesis [34]. Several studies have suggested the potential of nicotinic acid and nicotinamide as biomarkers for breast cancer diagnosis. Huang, *et al*. [12] reported that serum levels of nicotinic acid and nicotinamide were significantly higher in patients with breast cancer than in individuals with benign breast disease or healthy controls. However, Zhang, *et al*. [17] reported a significant decrease in serum nicotinamide levels in breast cancer. This conflicting result can be explained by variations in metabolic states and cancer subtypes. Some studies have reported decreased nicotinamide levels due to the increased NAD+ demand of cancer cells, whereas others attribute elevated nicotinamide levels to inflammation and stress responses. These discrepancies are likely caused by variations in study populations and conditions [34].

Lipid metabolic biomarkers

Lipid metabolism is associated with cell growth, proliferation, and differentiation, and certain changes in lipid metabolism are related to cancer development and progression [35]. Studies have focused on changes in lipid metabolism and their use as early diagnostic biomarkers in breast cancer.

Carnitine is related to lipid metabolism in breast cancer. Acylcarnitine helps regulate the balance between carbohydrate and lipid metabolism in cells [36–38]. The level of L-carnitine, essential for transporting fatty acids into mitochondria for fatty acid oxidation, is significantly decreased in patients with breast cancer, as are plasma, serum, and urine levels of acylcarnitine derivatives, such as L-octanoylcarnitine, dimethylheptanoylcarnitine, and 3-hydroxybutyrylcarnitine [9, 14, 39, 40]. These changes indicate alterations in lipid metabolism that may be linked to cancer development and progression.

Cala, *et al*. [40] demonstrated that using a combination of dimethylheptanoylcarnitine and succinic acid in urine achieved a sensitivity of 93% and specificity of 86%. For dimethylheptanoylcarnitine alone, the sensitivity was 71%, and the specificity was 76%. This highlights the synergistic or complementary effects of metabolic biomarkers. Plasma levels of ethyl (R)-3-hydroxyhexanoate and caprylic acid are decreased in patients with breast cancer. A combination of these metabolites has been proposed as a potential diagnostic panel for breast cancer detection [41].

Arachidonic acid is a membrane polyunsaturated fatty acid vital in cell signaling and inflammatory responses [42]. The arachidonic acid pathway is a potential target for overcoming drug resistance in breast cancer [43, 44]. Mao, *et al*. [11] reported elevated serum arachidonic acid levels in patients with HER-2-positive breast cancer, suggesting its potential as a diagnostic biomarker. Gong, *et al*. [13] reported that the monoacylglycerol derivative 2-arachidonoylglycerol is decreased in the serum of patients with breast cancer.

Changes in other polyunsaturated fatty acids in breast cancer include decreased levels of docosahexaenoic acid in plasma and linoleic acid in serum, suggesting their potential as diagnostic biomarkers [14, 39]. In early-stage breast cancer, the levels of phosphatidylcholine (PC) (20:2/20:5), PC (22:0/24:1), glycerol, and glycerol lipids such as triacylglycerol (12:0/14:1) and diacylglycerol (18:1/18:2) are increased [33, 44], whereas phosphatidylethanolamine (15:0/19:1) and *N*-palmitoyl proline levels are decreased [44]. Serum levels of diglycerides, triglycerides, and lysophosphatidylcholines are decreased in patients with breast cancer [32].

A combination of PC ae C40:6, lysoPC a C26:0, PC aa C38:5, PC ae C34:2, PC ae C32:2, PC ae C38:3, and sphingomyelin (OH) C16:1 in blood plasma-derived microvesicles from patients with breast cancer showed diagnostic potential, with an AUC of 0.78 (95% CI, 0.690–0.876) [45].

Carbohydrate metabolic biomarkers

Several carbohydrate metabolites have been suggested as potential diagnostic biomarkers. Plasma and serum myoinositol levels are decreased in patients with breast cancer [9, 16]. Myoinositol is involved in various physiological and pathological processes, including intracellular signaling, cell cycle control, and apoptosis. In the context of cancer, it inhibits invasiveness and mobility [45]. Plasma glucose and lactate levels are increased in patients with breast cancer [30, 33]. Lactate contributes to

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breast cancer development and aggressiveness. Its accumulation is linked to the Warburg effect, with cancer cells preferentially using glycolysis for energy production, even in oxygen-rich conditions. This metabolic shift results in excess lactate production, contributing to an acidic tumor microenvironment that promotes invasion, immune evasion, and metastasis. Elevated lactate levels are associated with poor prognosis and increased tumor aggressiveness in breast cancer [47, 48].

Other metabolic biomarkers

Regarding nucleic acid metabolism, increased plasma hypoxanthine and decreased serum uracil levels have been reported [12, 14, 41]. Hypoxanthine is related to purine metabolism. Cancer cells utilize the purine salvage pathway for energy synthesis, leading to increased hypoxanthine levels as the purine biosynthesis pathway is overridden [49]. Jasbie, *et al*. [16] demonstrated the potential of using a panel including proline, myoinositol, 2-hydroxybenzoic acid, gentisic acid, hypoxanthine, and 2,3-dihydroxybenzoic acid for breast cancer diagnosis.

Wang, *et al*. [50] found increased levels of cyclohexanone, 1,4-dimethoxy-2,3-butanediol, and 2,5,6-trimethyloctane in the exhaled breath of patients with breast cancer, suggesting that these compounds may serve as specific volatile markers for breast cancer diagnosis. Ketones, alcohols, and alkanes are the subject of ongoing research to understand the biological mechanisms underlying their production. Their presence may be related to oxidative stress and estrogen metabolism in breast cancer [51].

RISK FACTOR BIOMARKERS FOR BREAST CANCER

Breast cancer risk is influenced by multiple factors, including genetic, environmental, hormonal, and lifestyle elements. Identifying the roles of metabolites would help improve the prediction or reduce the risk of breast cancer development and contribute to the establishment of preventive strategies and early risk assessment tools by integrating metabolic insights with other contributing factors. Population-based case–cohort studies are essential for this purpose. Examining large, diverse groups of individuals helps to establish associations between specific metabolite profiles and breast cancer risk. Table 2 summarizes population-based case studies on risk factor biomarkers for breast cancer.

Amino acid metabolic biomarkers

Several amino acid metabolites have been linked with the risk of developing breast cancer. Higher plasma levels of valine, lysine, arginine, and glutamine are associated with an elevated risk of breast cancer in young women [52]. The mTOR pathway, a key pathway in cell proliferation, is activated by valine and glutamine and further promoted by citrate produced via the glutamine pathway, which supports fatty acid synthesis for membrane formation [52]. Findings from another study in the same cohort revealed that a reduced plasma level of *O*-succinyl homoserine and increased plasma levels of valine/norvaline, glutamine/isoglutamine, 5-aminovaleric acid, phenylalanine, tryptophan, and γ-glutamyl-threonine are associated with an increased risk of breast cancer [53].

The relationship between arginine and breast cancer remains inconclusive. A large case–control study (1,624 patients with breast cancer and 1,624 matched controls) revealed that plasma levels of arginine and asparagine are inversely associated with breast cancer risk, particularly in women not using hormone therapy [54]. Human and animal studies have shown that arginine depletion reduces antitumor immune responses in breast cancer, indicating a link between arginine and immunity. Higher plasma arginine levels have been linked to lower estradiol and IGF-1 levels, suggesting the role of arginine in breast cancer development [22, 55, 56]. These ambiguous findings imply that the role of arginine in breast cancer may be context-dependent and influenced by factors such as hormonal status, metabolic conditions, and, possibly, the overall amino acid balance of the individual. More research is needed to clarify the exact nature of the association between arginine and breast cancer risk, as well as the mechanisms involved.

Yoo, *et al*. [57] found that serum levels of valine, leucine, and isoleucine were increased in breast cancer and identified leucine as an independent factor influencing breast cancer incidence in a Korean cohort.

Lipid metabolic biomarkers

Plasma phosphatidylcholine levels are inversely related to breast cancer risk, whereas acylcarnitine C2 levels are positively associated with disease risk [26]. Lecuyer, *et al*. [52] reported that lower plasma levels of lipoproteins, unsaturated lipids, and other lipid-related metabolites, such as acetone and glycerol-derived compounds, were linked to an elevated breast cancer risk, potentially due to altered lipid synthesis and energy homeostasis. A large case–control study (1,531 patients with breast cancer and 1,531 matched controls) highlighted that plasma triacylglycerols with few double bonds were positively associated, whereas those with three or more double bonds were inversely associ-

Table 2. Studies on risk factor biomarkers for breast cancer

Author, reference	Year	Country	Cancer cases (N)	Control cases (N)	Analytical method	Sample type	Biomarkers	Key findings
Lecuyer, *et al*. [52]	2018	France	206	396	^{1}H NMR spectroscopy Nested case–control study	Plasma	Valine, lysine, arginine, glutamine, creatine, creatinine, glucose, lipoproteins, lipids, glycoproteins, acetone, glycerol-derived compounds, unsaturated lipids	Higher plasma levels of valine, lysine, and arginine, and lower levels of lipids and glycoproteins were associated with increased BC risk
Lecuyer, *et al*. [53]	2019	France	211	211	LC-MS Nested case–control study	Plasma	*O*-succinyl-homoserine, valine/norvaline, glutamine/isoglutamine, 5-aminovaleric acid, phenylalanine, tryptophan, γ-glutamyl-threonine, pregnene-triol sulfate, ATBC	Lower plasma levels of O-succinylhomoserine and higher plasma levels of valine/norvaline, glutamine/isoglutamine, 5-aminovaleric acid, phenylalanine, tryptophan, γ-glutamyl-threonine, ATBC, and pregnene-triol sulfate were associated with an increased risk of BC
His, *et al*. [54]	2019	10 European countries	1,624	1,624	MS using Absolute IDQ p180 platform Nested case–control study	Plasma	Arginine, asparagine, phosphatidylcholines, acylcarnitine C2	Levels of arginine, asparagine, phosphatidylcholines (ae C36:3, aa C36:3, ae C34:2 ae C36:2, ae C38:2) were inversely associated with breast cancer risk, whereas the acylcarnitine C2 level was positively associated with BC risk
Yoo, *et al*. [57]	2018	South Korea	84	88	UPLC-MS Prospective cohort study	Serum	Leucine, arachidonic acid, prostaglandin J2 (PGJ2), prostaglandin E2 (PGE2), γ-linolenic acid (GLA)	Leucine, AA, PGJ2, PGE2, and GLA were identified as independent variables affecting BC incidence
Brantley, *et al*. [58]	2022	USA	1,531	1,531	LC/MS-MS Nested case–control study	Plasma	Triacylglycerols	TAGs with three double bonds or more were inversely associated with BC at the proximate time point
Houghton, *et al*. [60]	2019	USA	610	1,207	HPLC with fluorescence detection Nested case–control	Plasma	B12, folate	Plasma vitamin B-12 level was positively associated with increased risk of BC, and plasma folate level was positively associated with risk of invasive BC
Playdon, *et al*. [61]	2017	USA	621	621	LC/MS-MS and GC-MS Nested case–control	Serum	Caprate (10:0), γ-carboxyethyl hydrochroman, vitamin E (γ-tocopherol) derivative, 4-androsten-3β,17β-diol-monosulfate, androgen Seven androgens, α-hydroxyisovalerate, 2-hydroxyoctanoate	Associations with dietary fats, alcohol, and tocopherol derivatives; caprate and androgen metabolite levels linked with ER(+) cancer risk
Kim, *et al*. [62]	2013	USA	307	300	LC/MS-MS Nested case–control	Urine	PGE2 metabolite (PGE-M)	Positive association between urinary PGE-M and BC risk in women not using non-steroidal anti-inflammatory drugs
Cui, *et al*. [63]	2014	China	504	1,082	LC/MS-MS Nested case–control	Urine	PGE2 metabolite (PGE-M)	Increased urinary PGE-M associated with breast cancer risk in normal-weight postmenopausal women

Abbreviations: LC-MS, liquid chromatography-mass spectrometry; ATBC, acetyl tributyl citrate; ER, estrogen receptor; BC, breast cancer; IDQ, integrated data quantification; UPLC-MS, ultrahigh-performance liquid chromatography-mass spectrometry; PGJ2; prostaglandin J_2; GLA, γ-linolenic acid; AA, arachidonic acid; LC/MS-MS, liquid chromatography-tandem mass spectrometry; TAG, triacylglycerol; GC-MS; gas chromatography-mass spectrometry; PGE-M: prostaglandin E metabolite.

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ated with breast cancer risk [58].

Carbohydrate metabolic biomarkers

Carbohydrate metabolism is key in cancer cell energy production and proliferation. Elevated levels of glucose in fasting plasma samples have been associated with increased breast cancer risk, emphasizing the role of glycolysis and insulin sensitivity in breast carcinogenesis [59].

Other metabolic biomarkers

Studies have highlighted the role of vitamins and cofactors in breast cancer risk. Houghton, *et al*. [60] found that plasma vitamin B12 levels were positively associated with an increased risk of overall breast cancer, and plasma folate levels were positively associated with the risk of invasive breast cancer in premenopausal women. Playdon, *et al*. linked serum vitamin E metabolite and certain dietary fat-related metabolite levels to breast cancer risk, emphasizing the influence of diet on estrogen receptor (ER)+ breast cancer development [61]. α-tocopherol levels were inversely associated with ER+ breast cancer, possibly reflecting a healthy lifestyle, and correlated with dietary supplement use. In contrast, the levels of δ-tocopherol and γ-carboxyethyl hydroxychroman, a conjugated form of γ-tocopherol, were positively associated with ER+ breast cancer, which may suggest a direct link between certain forms of vitamin E and breast cancer or may reflect the influence of specific dietary fat sources [61].

Urinary prostaglandin metabolites, such as prostaglandin E2 (PGE2), have been associated with an elevated risk in postmenopausal women with low non-steroidal anti-inflammatory drug use or normal weight [62, 63].

PROGNOSTIC BIOMARKERS FOR BREAST CANCER

Prognostic factors for breast cancer, which are key indicators for assessing patient survival and the likelihood of recurrence, including tumor size, lymph node metastasis, presence of distant metastasis, tumor histological grade, hormone receptor status, HER-2 expression, and Ki-67 level [64]. However, all these factors can only be confirmed through histological examination, and biological characteristics can vary even within tumors [65], emphasizing the need for prognostic biomarkers to overcome these limitations. The potential for developing metabolic biomarkers is actively researched. Table 3 summarizes studies on prognostic biomarkers for breast cancer.

Amino acid metabolic biomarkers

The amino acid metabolites glutamate and glycine have been linked with breast cancer prognosis [30, 66, 67]. Suman, *et al*. reported that in patients with late-stage breast cancer, glutamate levels were increased, whereas glycine levels were decreased as compared to those in patients with early-stage breast cancer [30]. In contrast, high levels of glycine in breast cancer tissues are associated with low survival rates and poor prognoses [66, 67]. Glycine promotes heme biosynthesis in the mitochondria, thereby maintaining oxidative phosphorylation [68, 69]. Therefore, increased glycine metabolism provides more energy necessary for cancer cell survival and growth, which are associated with cancer development and progression [70, 71]. The different study results may be attributed to differences in the study samples, and further research is necessary to validate these findings. Nevertheless, these findings suggest the potential of these metabolites as prognostic indicators.

Lipid metabolic biomarkers

Research on the prognosis of breast cancer in relation to lipid metabolism has shown that increased levels of PC aa C38:5 and lysoPC a C26:30 in the blood microvesicle metabolome of patients with breast cancer are associated with poor survival rates [44]. Increased levels of sphingomyelin in the breast cancer tissues of patients with triple-negative breast cancer are correlated with improved disease-free survival [72]. The levels of phospholipids, such as sphingosine-1-phosphate, have been linked to cancer proliferation, migration, and angiogenesis and are elevated in breast cancer versus normal tissues [73–75].

Carbohydrate metabolic biomarkers

Lactate is a carbohydrate metabolite associated with breast cancer prognosis related to the process of cellular anaerobic glycolysis. Plasma lactate levels are higher in patients with advanced breast cancer (stages III–IV) than in those with early-stage breast cancer (stages 0–II) [30]. Increased lactate levels in breast cancer tissues are associated with poor 5-yr survival rates [66]. Lactate has been suggested as a diagnostic biomarker for breast cancer [76]. Hypoxia is common in solid tumors and frequently occurs in aggressive or metastatic tumors [77]. Therefore, lactate may have significant value as a biomarker for predicting breast cancer prognosis. Further, β-glucose levels in breast cancer tissues are negatively correlated with the MIB-1 proliferative index, but were not directly associated with prognosis, indicating the need for further research to establish β-glucose as a prognostic biomarker for breast cancer [67].

Table 3. Studies on prognostic biomarkers for breast cancer

Author, reference	Year	Country	Cancer cases (N)	Control cases (N)	Analytical method	Sample type	Biomarkers	Key findings
Suman, *et al*. [30]	2018	India	72	50	^{1}H NMR spectroscopy	Plasma	Lactate, glutamate, lysine, NAG, β-glucose, 2-hydroxybutyrate, lipid, α glucose, formate, glutamine, glycine	Lactate, glutamate, lysine, NAG, β-glucose, 2-hydroxybutyrate, lipid, α glucose levels elevated in EBC vs. healthy individuals. Formate and glutamine levels decreased in EBC vs. healthy individuals. Glutamate levels elevated in LBC vs. EBC Glycine level decreased in LBC vs. EBC
Buentzel, *et al*. [45]	2021	Germany	78	30	MS	Blood-derived microvesicles	Eight significant metabolites: lysoPC a C26:0, PC aa C38:5, PC ae C32:2, PC ae C34:2, PC ae C38:3, PC ae C40:2, PC ae C40:6, SM (OH) C16:1	Eight-metabolite panel: diagnostic performance with an AUC of 0.78 Higher levels of lysoPCaC26:0 and PCaaC38:5 correlated with shorter overall survival
Giskeødegård, *et al*. [66]	2012	Norway	98	None	HR-MAS-MRS	Tissue	Lactate, glycine	Elevated glycine and lactate levels were associated with lower survival rates
Sitter, *et al*. [67]	2010	Norway	29	None	HR-MAS-MRS	Tissue	Glycine, β-glucose	Decreased glycine level was associated with good prognosis, β-glucose negatively correlated with the MIB-1 proliferative index
Purwaha, *et al*. [72]	2018	USA	70 (TNBC)	None	HR-LC-MS	Tissue	Sphingomyelins	Elevated sphingomyelin levels were associated with better disease-free survival
Terunuma, *et al*. [78]	2014	USA	67	65	MS	Breast cancer tissue and cell lines	2-Hydroxyglutarate (2HG)	Elevated 2HG level was associated with MYC pathway activation and poor prognosis
Jaskulski, *et al*. [81]	2018	Germany	1,743	None	TR-FIA	Serum and plasma	Enterolactone	Elevated enterolactone level was associated with lower mortality
Jaskulski, *et al*. [82]	2020	Germany	2,105	None	UPLC-MS/MS (ultraperformance liquid chromatography-tandem mass spectrometry), TR-FIA	Serum and plasma	Enterolactone, genistein, resveratrol, luteolin	Elevated enterolactone level was associated with improved survival. Elevated genistein, resveratrol, and luteolin levels were associated with poor prognosis

Abbreviations: NAG, *N*-acetyl glycoprotein; HR-MAS-MRS, high-resolution magic-angle spinning magnetic resonance spectroscopy; TNBC, triple-negative breast cancer; HR-LC-MS, high-resolution liquid chromatography-mass spectrometry; TR-FIA, time-resolved fluoroimmunoassay; EBC, early breast cancer; LBC, late breast cancer; PC, phosphatidylcholine; SM, sphingomyelin; AUC, area under the curve; MIB-1, mindbomb homolog 1; MYC, myelocytomatosis; UPLC-MS/MS, ultrahigh-performance liquid chromatography-tandem mass spectrometry.

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Other metabolic biomarkers

2-Hydroxyglutarate (2-HG), a glutaric acid derivative related to cellular energy metabolism, is associated with breast cancer prognosis. Increased levels of 2-HG in tissues of patients with breast cancer are associated with poor survival rates. 2-HG levels are significantly elevated in tissues of patients with breast cancer with activated *MYC*, an oncogene [78]. 2-HG is related to hypoxia, acidic pH, and immune cell fate [79].

Lignans, a major source of phytoestrogens found in oilseeds, have been associated with good prognosis in postmenopausal patients with breast cancer [80]. Jaskulski, *et al*. [81, 82] measured the levels of enterolactone, the major lignan metabolite in serum/plasma, and its association with prognosis (disease-free and 5-yr survival) in postmenopausal patients with breast cancer and found an inverse correlation between enterolactone levels and prognosis, highlighting enterolactone as a potential prognostic biomarker in postmenopausal patients with breast cancer. Plasma *N*-acetyl glycoprotein levels are elevated in patients with stage III–IV breast cancer, suggests its potential as a prognostic biomarker [30].

SUBTYPE BIOMARKERS FOR BREAST CANCER

Breast cancer subtypes are determined based on hormonal (estrogen, progesterone) receptor expression and HER-2 expression and inform patient treatment and prognosis [64]. Therefore, subtype identification, generally based on tissue biopsy, is essential in breast cancer treatment. When performing a tissue biopsy is challenging, e.g., in patients with metastatic breast cancer, treatment is often based on the primary tumor subtype. In some cases, the subtypes of the primary and metastatic tumors differ, making effective treatment difficult [83]. Biomarkers that can predict breast cancer subtypes would greatly aid in providing personalized treatment for patients with breast cancer. Table 4 summarizes studies on subtype biomarkers for breast cancer.

Amino acid metabolic biomarkers

The plasma kynurenine:tryptophan ratio and tryptophan levels can serve as diagnostic biomarkers for breast cancer, especially in distinguishing subtypes. Reduced tryptophan levels and increased kynurenine:tryptophan ratio in plasma are associated with negative ER and progesterone receptor (PR) status [15]. Tryptophan 2,3-dioxygenase was upregulated in an NF-κB-dependent manner in a hormone receptor-negative breast cancer cell line, indicating a relationship between tryptophan catabolism and hormone receptor status [84].

Table 4. Studies on subtype biomarkers for breast cancer

Author, reference	Year	Country	Cancer cases (N)	Control cases (N)	Analytical method	Sample type	Biomarkers	Key findings
Gong, *et al*. [13]	2024	China	53 (BC) 23 (TNBC) 30 (non-TNBC)	57 (non-BC)	UHPLC-MS	Serum	Two metabolites, including *N*-acetyl-d-tryptophan, 2-arachidonoylglycerol	Two-metabolite panel for distinguishing TNBC from non-TNBC with an AUC of 0.965
Park, *et al*. [14]	2019	Korea	40 (discovery set) 30 (validation set)	30 (discovery set) 16 (validation set)	LC-MS	Plasma	L-Octanoylcarnitine	L-Octanoylcarnitine level elevated in HR(+) BC
Onesti, *et al*. [15]	2019	Belgium	202	146	MS/MS (TQ5500)	Plasma	Tryptophan, kynurenine:tryptophan ratio	Tryptophan level decreased in HR(−) BC Kynurenine/tryptophan ratio elevated in HR(−) BC
Fan, *et al*. [85]	2016	China	51 (training set) 45 (test set)	79	LC-MS, GC-MS	Plasma	Carnitine, lysophosphatidylcholine (20:4), proline, alanine, lysophosphatidylcholine (16:1), glycochenodeoxycholic acid, valine, 2-octenedioic acid	Eight-metabolite panel for classification of subtype with AUCs of 0.925 and 0.893 in the training and test sets, respectively
Jin, *et al*. [86]	2023	China	79 (training set) 50 (validation set)	35 (training set) 25 (validation set)	^{1}H-NMR	Serum	Six-biomarker panel, including d-dimer, CA15-3, CEA, L5CH, glutamine, ornithine	Six-biomarker panel for distinguishing TNBC from non-TNBC with AUCs of 0.892 and 0.905 in the training and validation sets, respectively

Abbreviations: TNBC, triple-negative breast cancer; LC-MS, liquid chromatography-mass spectrometry; UHPLC-MS, ultra-hih-performance liquid chromatography-mass spectrometry; NMR, nuclear magnetic resonance; HR, hormone receptor; CEA, carcinoembryonic antigen; AUC, area under the curve; GC-MS, gas chromatography-mass spectrometry.

Plasma levels of proline, alanine, and valine are significantly elevated in ER-positive breast cancer, suggesting their potential for predicting breast cancer subtypes [85]. Jin, *et al*. [86] reported that serum glutamine and ornithine levels differ between triple-negative breast cancer (TNBC) and non-TNBC. They demonstrated that a panel of glutamine, ornithine, d-dimer, CA15-3, CEA, and L5CH had high diagnostic accuracy in distinguishing TNBC from non-TNBC (training set: N=12; AUC, 0.892; 95% CI, 0.778–0.967; validation set: N=60; AUC, 0.905; 95% CI: 0.754–0.987). TNBC cells are highly dependent on glutamine for rapid proliferation and utilize glutaminolysis to generate intermediate metabolites essential for the tricarboxylic acid cycle and antioxidant defense. This dependency is more pronounced in TNBC than in hormone receptor- or HER2-positive breast cancers [87]. Glutamine is a precursor to ornithine in the glutamate pathway. In the context of cancer, including TNBC, the elevated demand for glutamine ensures a supply of intermediates such as glutamate and ornithine [88, 89].

N-Acetyltransferase (NAT) has been proposed as a metabolite that differentiates TNBC from non-TNBC and as a biomarker for breast cancer diagnosis. Notably, serum NAT levels are significantly reduced in patients with TNBC [13].

Lipid metabolic biomarkers

Several lipid-related metabolites have been suggested as potential biomarkers for distinguishing breast cancer subtypes, diagnosis, and prognosis. 2-Arachidonoylglycerol levels are significantly increased in patients with TNBC. A combination of 2-arachidonoylglycerol and NAT could help differentiate TNBC from non-TNBC, with an AUC of 0.965 [13].

Plasma LysoPC (16:1) levels were decreased in ER(+) tumors, whereas the levels of lysoPC (20:4) and carnitine, which is related to fatty acid transport for mitochondrial β-oxidation, were increased in HER-2(+) tumors. 2-Octenedioic acid levels were decreased in ER(+) tumors and increased in HER-2(+) tumors. A combination of these metabolites with amino acid-related metabolites, such as proline, alanine, valine, and other metabolites such as glycochenodeoxycholic acid (GDCA), which is related to bile acid biosynthesis, has been used to predict breast cancer subtypes (training set [N=51], AUC, 0.925; 95% CI, 0.867–0.983; validation set [N=45]; AUC, 0.893; 95% CI, 0.847–0.939) [85].

Plasma octanoylcarnitine levels were increased in ER(+) or PR(+) tumors but showed no correlation with HER-2 expression [14], suggesting the need for further research. Wang, *et al*. performed single-cell metabolic analysis of cell lines and reported that PC has the potential as a biomarker for subtyping; however, this was an *in vitro* study [90].

Other metabolic biomarkers

GDCA levels are increased in the plasma of patients with ER(+) tumors. A panel of GDCA combined with other metabolites has been used to predict breast cancer subtypes [113]. GDCA promoted cell growth in ER(+) MCF-7 tumor cells [91].

TREATMENT RESPONSE-PREDICTING BIOMARKERS FOR BREAST CANCER

Biomarkers for predicting drug responses are essential for establishing efficient breast cancer treatment strategies tailored to individual patients and avoiding unnecessary treatments, as well as for early evaluation of drug responses and predicting recurrence and metastasis. Studies are ongoing to identify metabolic biomarkers for these purposes. Table 5 summarizes studies on treatment response-predicting biomarkers for breast cancer.

Amino acid metabolic biomarkers

Studies aimed at finding metabolic biomarkers to predict drug responses in breast cancer have been conducted in patients who received neoadjuvant chemotherapy (NAC). Zidi, *et al*. [92] predicted NAC responsiveness using fecal metabolites from patients who received NAC. Amino acids such as methionine, valine, alanine, isoleucine, and glutamate were significantly increased in the stool of the chemotherapy-sensitive group after NAC but not in the chemotherapy-insensitive group. In the chemotherapy-insensitive group, tyrosine levels were significantly increased after NAC. For 3-methylhistidine, a histidine derivative, levels in the chemotherapy-sensitive group were increased compared to before NAC but decreased compared to before NAC in the chemotherapy-insensitive group.

In a study aimed at developing biomarkers to predict NAC responses in patients who received anthracycline and taxane-based chemotherapy, a panel of serum cysteinyl-lysine and other lipid-related metabolites showed predictive ability, with an AUC of 0.957 [93]. Serum levels of threonine, isoleucine, and glutamine significantly differed between response and non-response groups in patients who received NAC [94]. Threonine and glutamine levels significantly decreased in the NAC response group, whereas isoleucine levels significantly increased. Leucine, valine, and proline, along with hormone receptor status, have been used to predict NAC response in breast cancer

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Table 5. Studies on treatment response-predicting biomarkers for breast cancer

Author, reference	Year	Country	Cancer cases (N)	Control cases (N)	Analytical method	Sample type	Biomarkers	Key findings
Mao, *et al*. [11]	2022	China	20	30	UPLC-TOF-MS	Serum	L-arginine	Elevated levels in trastuzumab-resistant BC
Zidi, *et al*. [92]	2021	Tunisia and France	8 patients 6 (good responders) 2 (non-responders)	0	^{1}H NMR spectroscopy	Stool	Methionine, valine, alanine, isoleucine, glutamate, tyrosine, succinate, fumarate, short-chain fatty acids (propionate, acetate, butyrate)	Levels of methionine, valine, alanine, isoleucine, and glutamate were increased in the stool of the chemotherapy-sensitive group succinate, fumarate, propionate, and acetate levels were increased in the stool of patients with a good response to chemotherapy
Lin, *et al*. [93]	2019	China	35 patients 19 (partial response) 16 (stable disease)	0	LC-MS	Serum	Nine metabolites, including prostaglandin C1, ricinoleic acid, oleic acid amide, ethyl docosahexaenoic, hulupapeptide, lysophosphatidylethanolamine 0:0/22:4, cysteinyl-lysine, methacholine, vitamin K2	Nine-metabolite panel: predictive of chemotherapy response with an AUC of 0.957
Wei, *et al*. [94]	2013	USA and Germany	28 patients 8 (complete response) 14 (partial response) 6 (no response)	0	NMR, LC-MS	Serum	Threonine, isoleucine, glutamine, linolenic acid	Threonine, glutamine, and linolenic acid levels decreased in response groups. Isoleucine levels increased in response groups
Cardoso, *et al*. [95]	2022	Brazil	80 patients 16 (sensitive) 64 (resistant)	0	^{1}H NMR spectroscopy	Serum	Leucine, formate, valine, proline	Interconnection of clinical variables (HR/Ki67/HER2) and serum metabolites can improve the prediction of the response to NACT
Silva, *et al*. [96]	2024	Brazil	75 patients Training set (sensitive: 12, resistant: 44) Validation set (sensitive: 4, resistant: 15)	0	LC-MS	plasma	Total 19 metabolites, including nine lipids (glycerophospholipids [N=7] and fatty acyls [N=2], amino acids (N=9), bile acids and derivatives (N=1)	Nineteen-biomarker panel for the prediction of the response to NACT showed 95.4% and 93.3% sensitivity, 94.6% and 94.7% accuracy, and 91.6% and 100.0% specificity in the training and validation sets, respectively
Tian, *et al*. [98]	2023	China	34 patients 34 (pretreatment samples) 26 (posttreatment samples)	17	^{1}H NMR spectroscopy	Serum	Asparagine, sarcosine	Asparagine and sarcosine levels elevated in treatment-resistant patients
Asten, *et al*. [103]	2015	Netherlands	Mouse model 23 (resistant) 26 (sensitive)	0	HRMAS ^{1}H NMR spectroscopy	Tissue	Choline metabolites	Choline metabolites were increased before docetaxel treatment in docetaxel-resistant tumors; shortly after docetaxel treatment, choline metabolites were increased in docetaxel-sensitive vs. -resistant tumors

Abbreviations: LC-MS, liquid chromatography-mass spectrometry; NMR, nuclear magnetic resonance; UPLC-TOF-MS, ultrahigh-performance liquid chromatography time-of-flight mass spectrometry; BC, breast cancer; AUC, area under the curve; NACT, neoadjuvant chemotherapy; HRMAS: high-resolution magic-angle spinning.

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[95]. Silva, *et al*. [96] have reported differences in seven plasma amino acid metabolites, including leucine, in relation to chemotherapy resistance. However, in contrast to the above findings, they reported that leucine and proline levels were increased in the chemotherapy resistance group. This discrepancy may be attributed to differences in patient cohorts, treatment regimens, and tumor biology. Further research is warranted to clarify these discrepancies.

L-Arginine is a potential biomarker for diagnosing HER-2(+) breast cancer and a candidate biomarker for predicting the response to trastuzumab, a crucial treatment for HER-2(+) breast cancer, as serum L-arginine levels were increased in patients with breast cancer with trastuzumab resistance [11]. This is likely related to the immune response-regulatory role of L-arginine [97].

Immunotherapy has gained attention as a novel treatment for TNBC. However, not all patients with TNBC respond effectively to immunotherapy, making treatment response prediction and patient selection for treatment important. Tian, *et al*. [98] studied metabolic biomarkers predicting treatment responses in patients receiving camrelizumab plus apatinib and eribulin and found that serum asparagine and sarcosine levels were increased in patients resistant to the treatment. Asparagine affects serine metabolism and is involved in protein and nucleotide synthesis, and therefore is associated with poor cancer prognosis [99, 100].

Lipid metabolic biomarkers

Drug resistance in breast cancer is linked to enhanced fatty acid synthesis and altered lipid composition, including sphingolipid and cholesterol dynamics in lipid rafts. Lipid metabolic pathways, including biosynthesis and catabolism, drive tumor progression and chemoresistance, with plasma lipoproteins implicated in oxidative stress-related pathology [101, 102].

Short-chain fatty acids (SCFAs) in breast cancer

Changes in SCFA levels have been investigated in the context of breast cancer treatment. Zidi, *et al*. [92] found changes in SCFA metabolite levels, including propionate, acetate, and butyrate, in the stool of patients who received NAC. Propionate and acetate levels were increased in the stool of patients responding positively to chemotherapy, whereas butyrate levels were decreased in the stool of poor responders.

Other lipid metabolites in breast cancer

In HER-2(+) breast cancer, arachidonic acid has been suggested to predict the response to trastuzumab when used with L-arginine; serum arachidonic acid levels were increased in trastuzumab-resistant patients and had predictive ability, with an AUC of 0.823 [11].

Docetaxel is one of the most commonly used chemotherapeutic agents for breast cancer, and response prediction is crucial and has been studied using a BRCA-1-mutant breast cancer mouse model. In docetaxel-resistant tumor tissues, choline metabolites such as glycerophosphocholine, phosphocholine, and choline were elevated before docetaxel treatment, whereas in docetaxel-sensitive tumor tissues, they increased shortly after docetaxel treatment, suggesting that choline metabolites have potential as biomarkers to predict the response to docetaxel [103]. Silva, *et al*. demonstrated the relationship between lipid metabolites, such as PCs, phosphatidylethanolamines (PEs), and fatty acyls (FAs), and chemoresistance in breast cancer. Notably, PCs, such as PC 20:3/18:1, PC 20:3/16:0, and PC 18:0/22:4, were upregulated in resistant plasma samples, whereas PEs, such as PE 20:4/22:0 and PE-NMe2 20:0/14:0 and FAs, such as DG 20:1/0:0/20:4 and FA 18:1;02 were downregulated [96].

Wei, *et al*. [94] predicted the response to chemotherapy in patients with breast cancer who received NAC. They identified threonine, isoleucine, and glutamine as candidate amino acid biomarkers using NMR. They also reported that serum linoleic acid levels were significantly reduced in patients who responded to chemotherapy when using liquid chromatography (LC-)MS, suggesting its potential as a biomarker [94].

Carbohydrate metabolic biomarkers

In a study of stool samples from patients who received NAC, changes were observed not only in amino acid and SCFA metabolites but also in carbohydrate metabolites, such as succinate and fumarate, the levels of which were elevated in chemotherapy responders [92]. Succinate and fumarate are oncometabolites and likely promote tumor growth and aggressiveness by accumulating within tumor cells [104, 105]. However, as the study included only eight patients, further research is necessary to validate these findings.

CONCLUSIONS

Recent metabolomics research in breast cancer has highlighted the potential of metabolite biomarkers for diagnosis, prognosis, subtype classification, and treatment response prediction. Key biomarkers, including amino acids, organic acids, lipids, and

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carbohydrates, reflect changes in energy metabolism, cell proliferation, and the tumor microenvironment. Metabolomics, i.e., the study of metabolic changes in cells, tissues, or the body, is a critical tool for early diagnosis, prognosis, and monitoring of treatment responses in breast cancer. This approach enhances diagnostic accuracy, facilitates personalized treatment strategies, enables disease progression monitoring, helps identify therapeutic targets, and reduces individual variability.

Several challenges hinder the clinical application of metabolite biomarkers. Metabolite profiles are influenced by diet, environmental factors, and lifestyle, highlighting the need for standardized protocols [106]. Additionally, most studies have been conducted in small cohorts of patients with early-stage breast cancer, emphasizing the necessity for large-scale clinical studies to validate biomarker efficacy across diverse populations [107].

Future research should focus on integrating advanced omics technologies to develop comprehensive biomarker panels. Cost-effective multiplex assays and point-of-care technologies must be designed to improve the accessibility of these panels across various healthcare settings. Artificial intelligence and machine learning tools can support the analysis of large datasets, facilitating real-time interpretation and decision-making for clinicians. Addressing these challenges will enhance the clinical utility of metabolite biomarkers and advance their integration into personalized healthcare.

In conclusion, metabolite biomarkers hold promise for breast cancer diagnosis, prognosis, subtype classification, and treatment response prediction. Nevertheless, further research and standardized methodologies are required to facilitate their clinical application. Advancements in this field are expected to contribute substantially to the progress of precision medicine.

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AUTHOR CONTRIBUTIONS

Conceptualization: Lee A and Lam CW; Methodology: Lee A and Lam CW; Investigation: Lee A and Lam CW; Visualization: Lee A and Lam CW; Supervision: Lam CW; Writing – original draft: Lee A; Writing – review & editing: Lam CW.

CONFLICTS OF INTEREST

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