



How previous treatment changes the metabolomic profile in patients with metastatic breast cancer

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Abstract

Purpose Metabolites are in the spotlight of attention as promising novel breast cancer biomarkers. However, no study has been conducted concerning changes in the metabolomics profile of metastatic breast cancer patients according to previous therapy.

Methods We performed a retrospective, single-center, nonrandomized, partially blinded, treatment-based study. Metastatic breast cancer (MBC) patients were enrolled between 03/2010 and 09/2016 at the beginning of a new systemic therapy. The endogenous metabolites in the plasma samples were analyzed using the AbsoluteIDQ[®] p180 Kit (Biocrates Life Sciences AG, Innsbruck) a targeted, quality and quantitative-controlled metabolomics approach. The statistical analysis was performed using R package, version 3.3.1. ANOVA was used to statistically assess age differences within groups. Furthermore, we analyzed the CTC status of the patients using the CellSearch[™] assay.

Results We included 178 patients in our study. Upon dividing the study population according to therapy before study inclusion, we found the following: 4 patients had received no therapy, 165 chemotherapy, and 135 anti-hormonal therapy, 30 with anti-Her2 therapy and 38 had received treatment with bevacizumab. Two metabolites were found to be significantly different, depending on the further therapy of the patients: methionine and serine. Whereas methionine levels were higher in the blood of patients who received an anti-Her2-therapy, serine was lower in patients with endocrine therapy only.

Conclusion We identified two metabolites for which concentrations differed significantly depending on previous therapies, which could help to choose the next therapy in patients who have already received numerous different treatments.

Keywords Metastatic breast cancer · Metabolomics · Serine · Methionine · Circulating tumor cells

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Abbreviations

ANOVA	Analysis of variance
CA15-3	Cancer-antigen 15-3
CEA	Carcinoembryonic antigen
CT	Computed tomography
CTC	Circulating tumor cells
DCIS	Ductal carcinoma in situ
EDTA	Ethylenediaminetetraacetic acid
ER	Estrogen receptor
ET	Endocrinological therapy
FDR	False discovery rate
FIA-MS/MS	Flow injection analysis tandem mass spectrometry
GGR	Glutamate-to-glutamine ratio
Her 2	Human epidermal growth factor receptor 2
IDH 2	Isocitrate dehydrogenase 2
LC-MS/MS	Liquid chromatography tandem mass spectrometry

MBC	Metastatic breast cancer
MTA	5 Deoxy 5methylthioadenosin
NCT	National Center for Tumor Diseases
PR	Progesterone receptor
SAH	S-Adenosyl homocysteine
SAM	S-Adenosylmethionine
TNBC	Triple-negative breast cancer

Introduction

Breast cancer

Breast cancer (BC) is the most common female cancer all over the world. Whereas over 90% of patients diagnosed with primary cancer survive the first 5 years, only 15% of patients with advanced BC live more than 5 years [1]. These facts underline the significance of improving therapy monitoring, especially in patients with metastatic BC (MBC), to avoid ineffective therapies. Metastatic patients usually receive numerous different kinds of therapies so that sometimes it is difficult to find a new treatment when disease is progressing. With all the new therapeutic options that are available, a physician is left alone with non-tested and non-approved combinations to choose the next therapeutic regimen [2]. The established methods for monitoring therapy include procedural imaging [e.g., magnetic resonance imaging (MRI) and computed tomography (ct)], and blood tests, such as CA15-3 and CEA. Blood tests seem to be very attractive but they are not sensitive and specific enough [3]. Among the new methods for monitoring therapy and predicting overall and progression-free survival, is measuring the circulating tumor cells (CTC) providing deeper prognostic information than state-of-the-art imaging [4] and may help to identify patients with a high therapy pressure due to bad prognosis without an aggressive therapy [5]. Nevertheless, CTC are not used in the clinical routine until today. All in all, new and better markers are needed for predicting therapy response and therapeutic monitoring.

Metabolomics

Metabolomics, measuring low molecular weight molecules, represents a recent development attempting to comprehensively and quantitatively, representing a lot of intermediary metabolism pathways by covering systematically the key metabolites [6]. Metabolomics depict functional information and bridge this information gap [7]. Differences in metabolites found in fluid or tissues provide the closest connection to the various phenotypic responses; changes might both provide new diagnostic information and help develop therapeutic options for malignant diseases. Metabolites can be detected in the tissue, blood,

urine and saliva of patients and are therefore very easy to retrieve. Recent analysis of the function of tumor suppressors and oncogenes discovered that many of them play an important role in cell metabolism [8]. In particular, changes in the metabolic systems of cancer cells in comparison to healthy include the induction of the cell membrane and the use of glucose through non-oxidative pathways [9]. In patients with prostate cancer, androgen deprivation therapy changed the metabolomics profile: steroid levels dropped, bile acids increased, and biomarkers of lipid metabolism and insulin resistance decreased [10]. In BC, significant changes in the metabolomics profile could be also detected between healthy controls and early stage cancer patients [11].

Methionine

Methionine is an essential amino acid and cannot be synthesized by humans. Its three main functions are glutathione formation via cysteine, methyl group donation for example at DNA modification via methylation and polyamine synthesis [12] which always starts with methionine on the N-terminus. Methionine can be converted to S-adenosylmethionine (SAM) by linking with ATP through methionine adenosyltransferase, an important methyl group donor in most organisms. After giving the methyl group, S-adenosyl homocysteine (SAH) is formed, which is ultimately converted to homocysteine and from which methionine is recovered. Tissues with a high turnover rate require more methionine to produce proteins they need to grow. Furthermore, methionine being leading targets is converted to methionine sulfoxide by the influence of reactive oxygen species [13].

Serine

Serine is a proteinogenic nonessential amino acid playing an important role in activating and inactivating several enzymes, especially peptidases. Serine can either be synthesized from glucose as a component of the glycolysis-diverting pathway [14] or imported from the extracellular environment. In detail, glucose-6-phosphate is formed to 3-phosphoglycerate and via phosphoglycerate dehydrogenase into the serine precursor 3-phosphohydroxypyruvate. 3-Phosphohydroxypyruvate is converted into serine via transamination and phosphate ester hydrolysis reactions. This biosynthesis is said to play an important role for many cancers due to its link to nucleotide biosynthesis [15]. And its pathway with one-carbon is upregulated metastatic subclones of the BC cell line MDA-MB-231 driving faster proliferation [16].

Circulating tumor cells

Using the CellSearch™ system (Veridex), CTCs can be detected in about 50% of patients with MBC [17]. A positive CTC status (≥ 5 CTCs/7.5 ml peripheral blood) correlates with shorter PFS and OS [18–20]. Furthermore, serial CTC enumeration is an effective test for predicting treatment outcome in MBC and a versatile addendum to classic diagnostic tools for therapy tailoring [21].

Metabolomics in BC

There is explicit evidence that currently established clinical diagnostic tools for BC can be complemented by metabolomics to, including the possibility to detect BC in early stages [22], relapse [23] and to predict treatment response, OS and PFS by analyzing blood and tissue samples.

To identify new therapeutic targets, deeper knowledge of the molecular changes depending on subtypes is essential to monitor treatment responses [24]. Recent studies found the estrogen receptor (ER) status of the tumor influencing the glutamate-to-glutamine ratio (GGR). That ratio can also be used to predict PFS and OS: a higher level is associated with longer OS [25]. Budczies et al. also found that the GGR significantly correlated with tumor grade and the ER status.

Triple-negative breast cancer (TNBC) is reported as a very metabolic disease [26] with elevated energy metabolism pathways that may directly impact the aggressive nature of this cancer [27].

Objective

The objective of this study was to gain a better understanding of the changes in metabolism in MBC according to the previous treatment. Furthermore, the CTC status of the patients was analyzed at study inclusion.

Methods

Patients

MBC patients were consecutively included in the study between 2010 (March) and 2016 (September) at the start of a new line of systemic therapy selected by the attending physician independent from the current study. Their blood was analyzed to determine the metabolomics profile. Previous treatments were clustered into chemotherapy, endocrinological therapy (ET), targeted therapy against human epidermal growth factor receptor 2 (Her2), and therapy with bevacizumab and the combination of these different therapy strategies. Subsequently, the metabolomics profile according to the previous treatment regimen was compared.

Blood sample processing

Blood samples (30 ml) were collected in standard 10-ml tubes containing ethylene-diamine-tetra-acetic acid and a cellular preservative before patients received surgery or chemotherapy. Before being analyzed, the samples were centrifuged 20 min at 2700 rpm (1300×g) at 10 °C. The blood pellets were put in small cryo tubes (1 ml) and shock frozen in liquid nitrogen in a –80 °C freezer. The plasma was transferred into 2 ml tubes and again centrifuged 10 min at 1200 rpm (12,000×g) and the supernatant was put in small cryo tubes and shock frozen.

Targeted metabolome analysis using LC–MS/MS

The plasma with the endogenous metabolites was analyzed with a targeted quality, and quantitative-controlled metabolomics approach using the AbsoluteIDQ® p180 Kit (Biocrates Life Sciences AG, Innsbruck), as published previously [28, 29]. With this kit up to 188 endogenous metabolites can be quantified, including 21 biogenic amines, 21 amino acids, 90 glycerophospholipids, 40 acylcarnitines, 15 sphingolipids, and hexoses. Liquid chromatography tandem mass spectrometry (LC–MS/MS) was applied to detect amino acids and biogenic amines, whereas using flow injection analysis tandem mass spectrometry (FIA–MS/MS) we could quantify acylcarnitines, sphingolipids, glycerophospholipids, and hexoses. Sample preparation was performed according to the user manual of the kit. Samples were randomized, including multiple quality control samples in the measurement sequence. The complete analytical process (sample registration, work list generation, and data processing) was carried out using the Biocrates MetIDQ™ software, version Boron 2693 (Biocrates Life Sciences AG, Innsbruck), which is an integral part of the AbsoluteIDQ® p180 Kit. Metabolite concentrations were calculated using the MetIDQ™ software and reported in $\mu\text{mol/l}$.

CTC detection

7.5 ml peripheral whole blood was drawn in a CellSave tube (Janssen Diagnostics, LLC, Raritan, NJ, USA) for CTC enumeration. Blood samples were kept at room temperature for ≤ 96 h until analysis using the CellSearch™ assay (CellSearch™ Epithelial Cell Kit/CellSpotter™ Analyzer, Janssen Diagnostics, LLC, Raritan, NJ, USA). Sample analysis and processing were done exactly according to the manufacturer's instructions [5].

Study design

The study was performed as a retrospective, single-center, nonrandomized, treatment-based, partially blinded study.

Treating physicians and patients were blinded to metabolomics status. The selection of the next system therapy was not based on the metabolic profile. All technical staff and investigators performing or reviewing the metabolomics were blinded to patient (treatment) history. The study was conducted at the National Center for Tumor Diseases (NCT), Heidelberg, Germany, and the Department of Obstetrics and Gynecology, University of Heidelberg, Heidelberg, Germany.

Statistical analysis

An analysis of variance (ANOVA) was done to statistically assess age differences within groups. To statistically assess the differences in metabolite concentrations between CTC groups and healthy controls, a *t* test was conducted with equal variances to consider different sample sizes. Since comparisons for each metabolite were made, a multiple testing correction procedure by Benjamini and Hochberg was done to calculate the false discovery rate (FDR) as presented in Tables 3 and 4. To consider significant differences of contributing factors between groups, Tukey's range test (Tukey 1949), Kruskal–Wallis rank-sum test, and the Pearson's Chi-squared test were executed and results are presented in Table 1. A *p* value (FDR) of ≤ 0.05 was regarded as significantly different (*).

Results

Patient clinical characteristics

178 patients with metastatic breast cancer were included in this study (Table 1). The median age was 59 years and ranged from 31 to 89 years. Half of the study population suffered from bone metastasis, 33% had liver metastasis, and 35% metastasis of the lung. When first diagnosed with breast cancer, 30% had a T1 tumor stage, 46% a T2 stage, and 9% T3 stage. One patient only had ductal carcinoma in situ (DCIS) and with unknown initial tumor stage of 3% of the patients. Most of the tumors were intermediate grade (46%), 37% were high-grade, and 4% low-grade tumors. The tumor grade is unknown for 13%. Most of the patients (75%) had an ER-positive tumor, 22 were ER negative, and 3% are unknown. Concerning the PR status 65% were positive, 30% were negative, and of 5% indefinite. Most of the patients had a Her2 negative tumor (75%); only 14% were Her2 positive, and 12% are unknown. Of the patients 62% were CTC negative and 36% positive; for 2% of the patients, the CTC status was unknown. Most of the patients (84%) had received surgery before being included in the study and 62% received radiation.

When the study population was divided according to therapy before study inclusion (see Table 2), it was found that 4 patients had received no therapy before being included in the study, 165 had received any chemotherapy, and 13 no chemotherapy at all. Seventeen patients had chemotherapy only and no other therapy. In all, 135 patients had received any kind of anti-hormonal therapy, and 43 did not have such therapy. Eight of our patients had systemetically anti-hormonal therapy only. In all, 30 MBC patients were treated with anti-Her2 therapy, and 148 were not. Finally, 38 patients in our study received therapy with bevacizumab, and 140 did not have such a therapy.

Differences in the concentrations of metabolites

There is a significant difference in the metabolomics profile between patients who received anti-Her2 therapy and those who did not receive such a therapy. The patients who received therapy had higher concentrations of methionine in their blood than the patients who did not receive anti-Her2 therapy (see Table 3). We also found a significant difference in the patients who were treated with endocrine therapy only: concentrations of serine were lower than in the other patients (see Table 4). The concentration differences depending on the previous therapy for serine and methionine are shown in Fig. 1.

Neither other significant changes were found in the metabolomics profile for the patients who received chemotherapy or bevacizumab, nor were any significant differences discovered in the combination of two or three therapeutic options.

Discussion

It is already known that the metabolomics levels depend on internal factors, such as gender, age, race and (cancerous) disease, but also on externals for example nutrition and therapies, e.g., medicinal but also radiation [30, 31]. In breast cancer patients, metabolomics concentrations are additionally dependent on tumor type (ER, PR, and Her2) [32] and the extent and site of metastasis [33, 34].

Methionine

A statistically significant difference was found in the concentration of methionine, which plays a rate-limiting role in tumorigenesis [35], associated with anti-Her2 therapy. The patients who had received this treatment had a higher concentration of methionine in their blood than the patients who did not receive anti-Her2 therapy. Willmann et al. previously reported changes in the methionine cycle at breast cancer cell lines. There significantly different levels

Table 1 Patient characteristics

Parameter	Statistics	Anti-Her2 group	Exclusively ET Group	Study population	<i>p</i> value
Age	Median	53	65.5	59	*1.63E–02
	Range	36–72	52–78	31–89	
Bone metastasis					
No	<i>N</i> (%)	18 (60)	6 (75)	89 (50)	1.41E–01
Yes	<i>N</i> (%)	12 (40)	2 (25)	89 (50)	
Liver metastasis					
No	<i>N</i> (%)	21 (70)	7 (87.5)	119 (66.9)	3.89E–01
Yes	<i>N</i> (%)	9 (30)	1 (12.5)	59 (33.1)	
Lung metastasis					
No	<i>N</i> (%)	19 (63.3)	5 (62.5)	116 (65.2)	9.57E–01
Yes	<i>N</i> (%)	11 (36.7)	3 (37.5)	62 (34.8)	
T					
T1	<i>N</i> (%)	10 (33.3)	2 (25)	54 (30.3)	8.83E–01
T2	<i>N</i> (%)	13 (43.3)	3 (37.5)	82 (46.1)	
T3	<i>N</i> (%)	3 (10)	0 (0)	16 (9)	
T4	<i>N</i> (%)	3 (10)	2 (25)	19 (10.7)	
Tis	<i>N</i> (%)	0 (0)	0 (0)	1 (0.6)	
NA	<i>N</i> (%)	1 (3.3)	1 (12.5)	6 (3.4)	
Grading					
G1	<i>N</i> (%)	1 (3.3)	1 (12.5)	7 (3.9)	1.84E–01
G2	<i>N</i> (%)	10 (33.3)	6 (75)	82 (46.1)	
G3	<i>N</i> (%)	13 (43.3)	0 (0)	66 (37.1)	
NA	<i>N</i> (%)	6 (20)	1 (12.5)	23 (12.9)	
Estrogen receptor (ER)					
Positive	<i>N</i> (%)	15 (50)	8 (100)	134 (75.3)	*8.37E–04
Negative	<i>N</i> (%)	15 (50)	0 (0)	39 (21.9)	
NA	<i>N</i> (%)	0 (0)	0 (0)	5 (2.8)	
Progesterone receptor (PR)					
Positive	<i>N</i> (%)	13 (43.3)	6 (75)	116 (65.2)	*1.08E–02
Negative	<i>N</i> (%)	17 (56.7)	2 (25)	54 (30.3)	
NA	<i>N</i> (%)	0 (0)	0 (0)	8 (4.5)	
Her2					
Negative	<i>N</i> (%)	8 (26.7)	8 (100)	133 (74.7)	*2.63E–16
Positive	<i>N</i> (%)	19 (63.3)	0 (0)	24 (13.5)	
NA	<i>N</i> (%)	3 (10)	0 (0)	21 (11.8)	
CTC					
Negative	<i>N</i> (%)	25 (83.3)	5 (62.5)	110 (61.8)	*1.40E–05
Positive	<i>N</i> (%)	5 (16.7)	1 (12.5)	64 (36)	
NA	<i>N</i> (%)	0 (0)	2 (25)	4 (2.2)	
Surgery					
No	<i>N</i> (%)	7 (23.3)	3 (37.5)	28 (15.7)	8.05E–02
Yes	<i>N</i> (%)	23 (76.7)	5 (62.5)	150 (84.3)	
Radiation					
No	<i>N</i> (%)	16 (53.3)	6 (75)	67 (37.6)	*7.80E–03
Yes	<i>N</i> (%)	14 (46.7)	2 (25)	111 (62.4)	

Anti-Her2 Group are the patients who received anti-Her2 therapy before being included in the study: exclusively ET group are the patients who did not receive any other therapy except for endocrine therapy

Table 2 Patient numbers according to the previous therapy

Therapy	Yes	No	Exclusively
Any	174 (97.8%)	4 (2.2%)	–
Chemotherapy	165 (92.7%)	13 (7.3%)	17 (9.5%)
Endocrine therapy	135 (75.8%)	43 (24.2%)	8 (4.5%)
Anti-Her 2	30 (16.9%)	148 (83.1%)	0
Bevacizumab	38 (21.3%)	140 (78.7%)	0

Table 3 Metabolomics according to therapy against Anti-Her2

Metabolite	pVal	FDR	Fold Change	log2FC
Met	0.000225	*0.033471	1.14504	0.195398
Met-SO	0.00418	0.311396	1.183643	0.243233
C5	0.029313	0.796629	−1.71614	−0.77917
GDCA	0.053344	0.796629	1.613991	0.690632
SM C18:0	0.057353	0.796629	−1.10374	−0.1424
Ser	0.061827	0.796629	1.102561	0.140859
PC aa C42:0	0.0629	0.796629	1.128321	0.174178
Tyr	0.068192	0.796629	1.069945	0.097537
His	0.068498	0.796629	1.047076	0.066366
Gln	0.075358	0.796629	1.056773	0.079666

*FDR ≤ 0.05 (regarded as significantly different)

pVal *p* value; *FDR* false discovery rate; *Log2FC* log2 fold change; *Met* methionine; *Met-SO* methionine sulfoxide; *C5* valerylcarnitine; *GDCA* glycodeoxycholic acid; *SM C18:0* sphingolipid octadecanoylcarnitine, *Ser* serine; *PC aa C42:0* glycerolipid dotetracontaylcarnitine *Tyr* tyrosine; *His* histidine; *Gln* glutamine

Table 4 Differences in the metabolomics for patients with only endocrine therapy

Metabolite	pVal	FDR	Fold change	log2FC
Ser	9.92E-05	*0.014775	−1.37295755	−0.45729
TCDCA	0.002563	0.129588	−8.16052906	−3.02866
TUDCA	0.002609	0.129588	−4.17712275	−2.06251
Spermine	0.005563	0.207221	1.16611114	0.221705
TCA	0.039891	0.984027	−20.4249691	−4.35226
PC ae C36:4	0.058837	0.984027	1.21862174	0.28525
PC aa C28:1	0.062185	0.984027	1.11377595	0.155459
SM C18:0	0.12694	0.984027	1.22327802	0.290752
lysoPC a C16:1	0.133064	0.984027	−1.31319242	−0.39308
PC ae C38:5	0.133169	0.984027	1.19877895	0.261566

* FDR ≤ 0.05 (regarded as significantly different)

pVal *p* value; *FDR* false discovery rate; *Log2FC* log2 fold change; *Ser* serine; *TCDCA* taurochenodeoxycholic acid; *TUDCA* tauroursodeoxycholic acid; *TCA* taurocholic acid; *PC ae C36:6* glycerolipid hexatriacontylcarnitine; *PC ae 28:1* glycerolipid octacosylcarnitine; *SM C18:0* sphingolipid octadecanoylcarnitine, *lysoPC a C16:1* lysophosphatidylcholine hexadecenoylcarnitine; *PC ae C38:5* glycerolipid octatriacontylcarnitine

of SAM, SAH, MTA (5 deoxy 5 methylthioadenosine), MTA-SO (sulfoxide), and 3-(3-Amino-carboxypropyl)-uridine were identified in breast cancer cells compared to normal breast tissue [36]. An elevated level of SAM was detected in the Her2-positive cell line. Thus, the elevated methionine levels found might have been caused by the Her2-positive tumor and not the therapy. SAH was elevated in TNBC and hormone receptor-negative and Her2-positive breast cancer but not in hormone receptor-positive and Her2-positive breast cancer. MTA and MTA-SO levels were lower in all BC cell lines, probably because they were involved in the recycling process of methionine.

Serine

Significantly lower concentrations of serine were observed in patients who received endocrine therapy only. Similar data were published by Kim et al., who reported higher serine levels for patients with TNBC but low serine concentrations in the blood of patients with luminal A cancer [37] who normally are treated with endocrine therapy only. Related to pre-therapies, serine accumulation has been described at PARP inhibition [38]. Furthermore, Possemato et al. found increased serine synthesis flux for patients with estrogen receptor-negative breast cancer, which is also associated with poor 5-year survival [39]. Thus, changes in metabolite concentrations can also be useful for predicting overall survival for breast cancer patients. Additionally, changes in serine concentration may be caused by different tumor types, which are treated differently, and therefore changes in the metabolite concentrations may be related to the tumor type and not the therapies.

As for challenges in interpreting these results, some subgroups are small and the heterogeneity of the study population and their primary tumor is high, which was not correlated in the description of the metabolomics profile.

Conclusion

There are significant differences in the metabolomics profile of MBC patients according to their previous therapy. Patients who received anti-Her2 therapy had a higher concentration of methionine in their blood than the patients who did not have such a therapy. Furthermore, serine concentrations were lower in patients who were treated with endocrine therapy only. These findings and further metabolomics studies may help to find new therapeutic strategies for the individual patient and perhaps to monitor treatment by determining changes of metabolites during therapy.

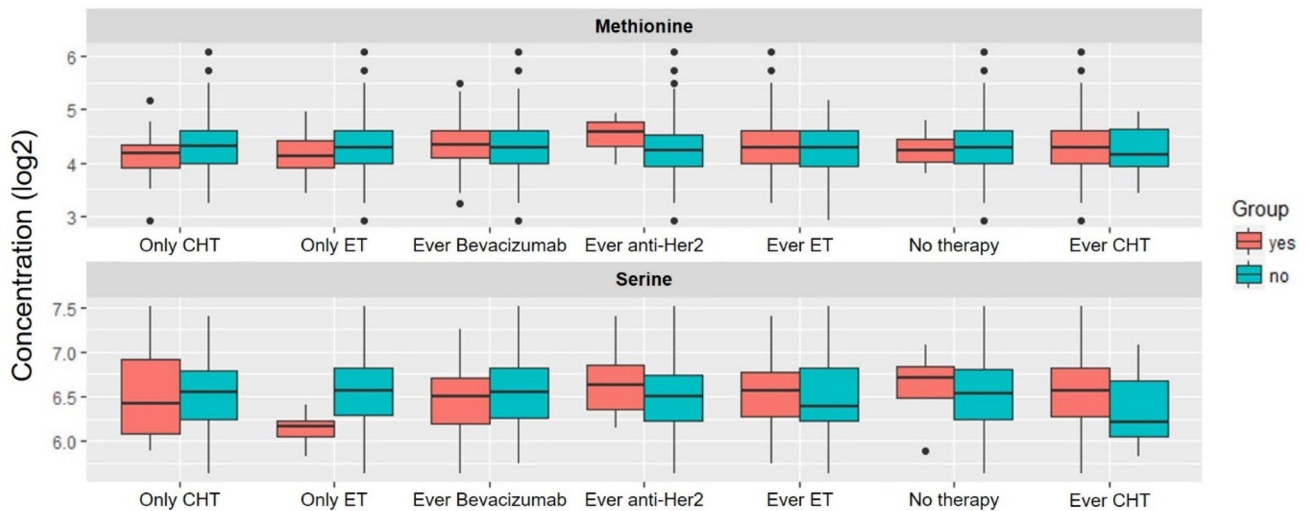


Fig. 1 Plasma levels of methionine (upper panel) and serine (lower panel) to different previous therapies. *CHT* chemotherapy, *ET* endocrine therapy

Outlook

In future, metabolomics might help to improve diagnosis, therapeutic monitoring, and aid in finding new therapy targets and drugs such as AG-221, an inhibitor of mutated iso-citrate dehydrogenase 2 (IDH2) for patients with acute myeloid leukemia [26].

Metabolomics offer the possibility to improve diagnosis, disease control, and monitoring breast cancer therapy with high sensitivity and specificity in a low invasive way [3]. They may help to detect relapse and progression and to discover breast cancer early in patients when other methods are not sensitive enough. Metabolomics may also support to improve treatment strategies [40].

Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by JN, SS, MW and BB. The first draft of the manuscript was written by JN and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The authors confirm that the data supporting the findings of this study are available within the article.

Code availability R software, version 3.3.1.

Declarations

Ethics approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Ethics approval was obtained from the Ethics Committee of the Medical Faculty of the University of Heidelberg (S-052/2010).

Consent to participate Informed consent was obtained from all individual participants included in the study.

Consent for publication Patients signed informed consent regarding publishing their data.

Conflict of interest The authors declare that they have no conflict of interest.

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References

1. Cancer Research UK (2015) Breast cancer survival statistics.
2. Hartkopf AD et al (2018) Treatment landscape of advanced breast cancer patients with hormone receptor positive HER2

- negative tumors—data from the German PRAEGNANT breast cancer registry. *Breast* 37:42–51
3. Zhang AH et al (2013) Metabolomics in noninvasive breast cancer. *Clin Chim Acta* 424:3–7
 4. Budd GT et al (2006) Circulating tumor cells versus imaging—predicting overall survival in metastatic breast cancer. *Clin Cancer Res* 12(21):6403–6409
 5. Deutsch TM et al (2020) Cut-off analysis of CTC change under systemic therapy for defining early therapy response in metastatic breast cancer. *Cancers (Basel)* 12(4):1055
 6. Zhang A et al (2012) Modern analytical techniques in metabolomics analysis. *Analyst* 137(2):293–300
 7. Kewal KJ (2009) Textbook of personalized medicine. Springer, New York
 8. Boroughs LK, DeBerardinis RJ (2015) Metabolic pathways promoting cancer cell survival and growth. *Nat Cell Biol* 17(4):351–359
 9. Tenori L et al (2012) Exploration of serum metabolomic profiles and outcomes in women with metastatic breast cancer: a pilot study. *Mol Oncol* 6(4):437–444
 10. Saylor PJ, Karoly ED, Smith MR (2012) Prospective study of changes in the metabolomic profiles of men during their first three months of androgen deprivation therapy for prostate cancer. *Clin Cancer Res* 18(13):3677–3685
 11. Yuan B et al (2019) A plasma metabolite panel as biomarkers for early primary breast cancer detection. *Int J Cancer* 144(11):2833–2842
 12. Wanders D, Hobson K, Ji X (2020) Methionine restriction and cancer biology. *Nutrients* 12(3):684
 13. Moskovitz J (2005) Methionine sulfoxide reductases: ubiquitous enzymes involved in antioxidant defense, protein regulation, and prevention of aging-associated diseases. *Biochim Biophys Acta* 1703(2):213–219
 14. Amelio I et al (2014) Serine and glycine metabolism in cancer. *Trends Biochem Sci* 39(4):191–198
 15. Locasale JW (2013) Serine, glycine and one-carbon units: cancer metabolism in full circle. *Nat Rev Cancer* 13(8):572–583
 16. Li AM et al (2020) Metabolic profiling reveals a dependency of human metastatic breast cancer on mitochondrial serine and one-carbon unit metabolism. *Mol Cancer Res* 18(4):599–611
 17. Giordano A et al (2012) Circulating tumor cells in immunohistochemical subtypes of metastatic breast cancer: lack of prediction in HER2-positive disease treated with targeted therapy. *Ann Oncol* 23(5):1144–1150
 18. Cristofanilli M et al (2004) Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *N Engl J Med* 351(8):781–791
 19. Smerage JB, Hayes DF (2008) The prognostic implications of circulating tumor cells in patients with breast cancer. *Cancer Invest* 26(2):109–114
 20. Wallwiener M et al (2013) The prognostic impact of circulating tumor cells in subtypes of metastatic breast cancer. *Breast Cancer Res Treat* 137(2):503–510
 21. Wallwiener M et al (2014) Serial enumeration of circulating tumor cells predicts treatment response and prognosis in metastatic breast cancer: a prospective study in 393 patients. *BMC Cancer* 14(1):512
 22. Huang S et al (2016) Novel personalized pathway-based metabolomics models reveal key metabolic pathways for breast cancer diagnosis. *Genome Med* 8(1):34
 23. Tenori L et al (2015) Serum metabolomic profiles evaluated after surgery may identify patients with oestrogen receptor negative early breast cancer at increased risk of disease recurrence. Results from a retrospective study. *Mol Oncol* 9(1):128–139
 24. Cui M, Wang Q, Chen G (2016) Serum metabolomics analysis reveals changes in signaling lipids in breast cancer patients. *Biomed Chromatogr* 30(1):42–47
 25. Budczies J et al (2015) Glutamate enrichment as new diagnostic opportunity in breast cancer. *Int J Cancer* 136(7):1619–1628
 26. Mullard A (2016) Cancer metabolism pipeline breaks new ground. *Nat Rev Drug Discov* 15(11):735–737
 27. Kanaan YM et al (2014) Metabolic profile of triple-negative breast cancer in African-American women reveals potential biomarkers of aggressive disease. *Cancer Genomics Proteomics* 11(6):279–294
 28. Koal T et al (2015) Sphingomyelin SM(d18:1/18:0) is significantly enhanced in cerebrospinal fluid samples dichotomized by pathological amyloid-β42, tau, and phospho-tau-181 levels. *J Alzheimers Dis* 44(4):1193–1201
 29. Klavins K et al (2015) The ratio of phosphatidylcholines to lysophosphatidylcholines in plasma differentiates healthy controls from patients with Alzheimer’s disease and mild cognitive impairment. *Alzheimers Dement (Amst)* 1(3):295–302
 30. McCartney A et al (2018) Metabolomics in breast cancer: a decade in review. *Cancer Treat Rev* 67:88–96
 31. Arenas M et al (2018) Metabolite normalization with local radiotherapy following breast tumor resection. *PLoS ONE* 13(11):e0207474
 32. Cao MD et al (2014) Metabolic characterization of triple negative breast cancer. *BMC Cancer* 14(1):941
 33. Kim HY et al (2016) Comparative metabolic and lipidomic profiling of human breast cancer cells with different metastatic potentials. *Oncotarget* 7(41):67111–67128
 34. Dupuy F et al (2015) PDK1-Dependent metabolic reprogramming dictates metastatic potential in breast cancer. *Cell Metab* 22(4):577–589
 35. Wang J et al (2017) Application of liquid biopsy in precision medicine: opportunities and challenges. *Front Med* 11(4):522–527
 36. Willmann L et al (2015) Exometabolom analysis of breast cancer cell lines: metabolic signature. *Sci Rep* 5:13374
 37. Kim SK, Jung WH, Koo JS (2014) Differential expression of enzymes associated with serine/glycine metabolism in different breast cancer subtypes. *PLoS ONE* 9(6):e101004
 38. Prokhorova E et al (2021) Serine-linked PARP1 auto-modification controls PARP inhibitor response. *Nat Commun* 12(1):4055
 39. Possemato R et al (2011) Functional genomics reveal that the serine synthesis pathway is essential in breast cancer. *Nature* 476(7360):346–350
 40. Zhang A et al (2012) Urine metabolomics. *Clin Chim Acta* 414:65–69

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