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# Metabolomics of biomarker discovery in ovarian cancer: a systematic review of the current literature

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## Abstract

**Introduction—**Metabolomics is the emerging member of “omics” sciences advancing the understanding, diagnosis and treatment of many cancers, including ovarian cancer (OC).

**Objectives—**To systematically identify the metabolomic abnormalities in OC detection, and the dominant metabolic pathways associated with the observed alterations.

**Methods—**An electronic literature search was performed, up to and including January 15th 2016, for studies evaluating the metabolomic profile of patients with OC compared to controls. QUADOMICS tool was used to assess the quality of the twenty-three studies included in this systematic review.

**Results—**Biological samples utilized for metabolomic analysis include: serum/plasma (n = 13), urine (n = 4), cyst fluid (n = 3), tissue (n = 2) and ascitic fluid (n = 1). Metabolites related to cellular respiration, carbohydrate, lipid, protein and nucleotide metabolism were significantly altered in OC. Increased levels of tricarboxylic acid cycle intermediates and altered metabolites of the glycolytic pathway pointed to perturbations in cellular respiration. Alterations in lipid metabolism included enhanced fatty acid oxidation, abnormal levels of glycerolipids, sphingolipids and free fatty acids with common elevations of palmitate, oleate, and myristate. Increased levels of glutamine, glycine, cysteine and threonine were commonly reported while enhanced degradations of tryptophan, histidine and phenylalanine were found. N-acetylaspartate, a

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Compliance with ethical standards

**Conflict of Interest**

Onur Turkoglu, Amna Zeb, Stewart Graham, Thomas Szyperski, J Brian Szender, Kunle Odunsi and Ray Bahado-Singh declare that they have no conflict of interest.

**Ethical Approval**

This article does not contain any studies with human participants performed by any of the authors.

brain amino acid, was found elevated in primary and metastatic OC tissue and ovarian cyst fluid. Further, elevated levels of ketone bodies including 3-hydroxybutyrate were commonly reported. Increased levels of nucleotide metabolites and tocopherols were consistent through out the studies.

**Conclusion—**Metabolomics presents significant new opportunities for diagnostic biomarker development, elucidating previously unknown mechanisms of OC pathogenesis.

### Keywords

Ovarian cancer; Metabolomics; Metabolites; Systematic review; Biomarker

## 1 Introduction

Ovarian cancer (OC) is the leading cause of death from gynecologic malignancy in the United States. More than 80 % of patients present with advanced disease, with a five-year overall survival rate between 15 and 45 %. In contrast, patients with stage I disease, where the cancer is confined to the ovary, have a survival rate greater than 90 % (Siegel et al. 2014). The high survival rates for OC detected in early stages have fueled interest in developing novel screening and diagnostic strategies. Despite significant advances in the understanding of OC biology, no single screening modality currently exists that has demonstrated a survival benefit. Routine screening tests such as serum cancer antigen 125 (CA-125), ultrasound imaging, or combination of the two have been proposed to detect OC in early stages when therapeutic intervention is most effective; however, success has proved to be limited (Buys et al. 2011). In the recent United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKTOCS), 46,237 women, age 50 years or older underwent incidence screening by using annual serum CA-125 that was interpreted with the Risk of Ovarian Cancer Algorithm (ROCA) (Menon et al. 2015). While the ROCA algorithm had encouraging sensitivity (85.8 %) and specificity (99.8 %) for detecting ovarian cancer in low-risk postmenopausal women, only 41 % of screen-detected cancers were stage I or II disease (Menon et al. 2015), pointing to the continued need for novel early detection strategies.

The rapid development of systems biology and the ‘omics-cascade’, including genomics, transcriptomics, proteomics, and metabolomics, has forged a new era of research in understanding systems biology. Metabolomics is the newest member of this family and is based on the high-throughput identification and quantification of small molecule metabolites and their interactions within biological networks (German et al. 2005). The highly dynamic nature of the metabolites makes metabolomics the endpoint of the ‘omics’ cascade, and yields the closest profile to physiological phenotype (Fig. 1) (Dunn et al. 2011). Metabolomics’ still-evolving and dramatic technological developments are rapidly advancing our understanding of systems biology (Wishart 2007). Due to the vast chemical and physical diversity of metabolites, no single analytical method can currently measure the concentrations of all metabolites. Currently, the two dominant metabolomics platforms are nuclear magnetic resonance (NMR) and mass spectrometry (MS) (Weckwerth and Morgenthal 2005). NMR provides targeted, quantitative and structural information on each metabolite and can measure a wide range of metabolites with little to no sample preparation; however, low sensitivity and the higher limits of detection (lM concentrations) of

metabolites are some of the disadvantages of using this platform (Reo 2002). In contrast, MS provides untargeted semi-quantitative information with very high sensitivity, allowing for the analysis of low-abundance metabolites (nM-pM range). MS is conventionally coupled with either liquid chromatography (LC–MS), gas chromatography (GC–MS) or capillary electrophoresis (CE-MS) to enable successful separation between eluting metabolites. However, many of these MS techniques require extensive sample preparation and usually only measure specific subsets of compounds (Dettmer et al. 2007). Eventually, regardless of whether initial detection uses an NMR or MS platform, the identification and quantification of key metabolites is required.

The main principle of biomarker development in cancer metabolomics is the simultaneous, global and unbiased assessment of hundreds of endogenous metabolites by converting complex raw data into human-interpretable measurements. The first step includes pattern recognition in biofluids or tumor tissues that contain hundreds of signals from these endogenous metabolites. The spectrometric data is entered into advanced computational programs for multivariate data analysis and pattern recognition. The most widely used methods of multivariate analysis include principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), orthogonal partial least squares discriminant analysis (OPLS-DA) and regression analysis (Wishart 2009). Following the initial step of pattern recognition and group clustering, interpretation of the scores plot reveals information about the relationships between samples, trends, groupings and/or outliers (Spratlin et al. 2009). The second step is the use of compound-identification strategies that typically involve matching spectral features of the unknown compound(s) to curated spectral databases of reference compounds. To accomplish this step a database search is needed, for which the Human Metabolome Database, MassBank, KEGG and METLIN are often used (Wishart et al. 2013; Horai et al. 2010). These libraries are freely available online. The third and final step of analysis includes quantitation and accurate identification of biomarkers with respect to a particular characteristic of the cancer, such as prognosis or response to therapy (Spratlin et al. 2009). Although there are still existing challenges surrounding biomarker development (mainly metabolite identification), newly emerging techniques and technologies in computational metabolomics are making metabolite identification easier and more robust (Wishart 2011). The ability of metabolomics to measure high-throughput, system-wide phenotypes gives it significant potential power in the field of oncology to understand cancer metabolism and carcinogenesis while allowing biomarker discovery-based analysis (Vermeersch and Styczynski 2013). The metabolic alterations of cancers are now considered to be at the core of both tumorigenesis and phenotypic changes (Spratlin et al. 2009; Kwon et al. 2015). Correspondingly, metabolomics has showed great promise to date for advancing the understanding, diagnosis and treatment of many cancers including breast (Asiago et al. 2010; Jobard et al. 2014), brain (Locasale et al. 2012), prostrate (Zang et al. 2014), lung (Carrola et al. 2011), and ovarian (Odunsi et al. 2005). This systematic review aims to present the current status of the role of metabolomics in understanding the biology of OC with an eye on its future potential utility as a validated diagnostic tool.

# 2 Methodology

## 2.1 Search strategy

A literature search (title and abstract) of PubMed, Embase and the Web of Science electronic databases was conducted up to and including January 15th 2016 for metabolomics studies profiling ovarian cancer. After consulting a medical librarian, a search strategy based on a combination of Medical Subject Headings (MeSH) and free text words was formulated including the keywords “metabolomics”, “metabolite”, “metabolome”, “metabolic profiling”, “ovarian cancer”, “ovarian neoplasm”, and “metabolic biomarker” in any field. Two researchers (OT and AZ) independently assessed all articles based on the title, abstract or full article. Full texts of potentially relevant articles were retrieved. Further potentially relevant articles were identified manually through the searching of reference lists of relevant studies.

## 2.2 Eligibility criteria

Experimental studies investigating the metabolomic profile of biological samples from patients with ovarian cancer compared to an appropriate control group were included in our analysis. Inclusion criteria were: use of MS or NMR analytical platforms, English language, and an available abstract. Exclusion criteria were: animal studies, in vitro cell culture studies, studies analyzing the proteome rather than the metabolome and studies without a control group. Studies that reported the same patient population were also excluded. Articles for which there was disagreement regarding inclusion or exclusion were discussed and a consensus was reached.

## 2.3 Data extraction and analysis

Data on population characteristics, metabolomic platforms and study results were extracted by one investigator (OT) and verified by a second investigator (SG). Due to limited number of studies on ovarian cancer and metabolomics, the heterogeneity in analytical platforms, and the variation in multivariate analysis, a quantitative meta-analysis of the data was not appropriate.

## 2.4 Methodological quality assessment

The methodological quality of the selected studies was assessed using the QUADOMICS tool, an adaptation of QUADAS (quality assessment tool for diagnostic accuracy studies), which takes into account the particular challenges encountered when performing systematic reviews of ‘omics’-based techniques (Lumbreras et al. 2008). The quality of the studies was summarized by the percentage of applied criteria that scored positively. We did not use a threshold integer while assessing the quality of studies as has been previously reported (Huynh et al. 2014; Galazis et al. 2013). A cutoff assessing the quality of published studies has not been published by either QUADAS or QUADOMICS, as such a cutoff would not sufficiently discriminate between a study with a major methodological flaw that invalidates the results as compared to a study with minor methodological flaws (Whiting et al. 2005; Lumbreras et al. 2008; Parker et al. 2010).

# 3 Results

## 3.1 Study characteristics

Twenty-three studies met inclusion criteria and were eligible for systematic review. The selection flow diagram used is detailed in Fig. 2. Biological samples utilized for metabolomic analysis included serum/plasma in 13 studies, urine in 4 studies, cyst fluid in 3 studies, tissue in 2 studies, and ascitic fluid in one study. Validation of the results of serum profiling using tissue was conducted in two studies (Chen et al. 2011a; Hilvo et al. 2015). The analytical platforms used for metabolite detection included LC–MS in 12 studies, GC–MS in 6 studies, NMR in 5 studies, and direct analysis in real time–time of flight mass spectrometry (DART/TOF–MS) in one study. The methodologies, specimen types, study groups, metabolites and their regulation pattern (‘up-regulated’, ‘down-regulated’) are summarized in Table 1. Detailed regulation of metabolites according to related pathways is presented in the electronic supplementary materials (Supplementary Tables S1, S2, S3, S4).

## 3.2 Quality assessment of the studies

The quality assessment results in accordance with the QUADOMICS tool are shown in electronic supplementary Table S5. According to the quality assessment, 52 % of the studies were not able to avoid overfitting due to lack of an independent validation set. All the studies included in this systematic review were phase I. Thus, items questioning the availability of the clinical data and the representative nature of the spectrum of patients when a metabolomics platform is used in practice were not applicable for all the studies included. The majority of studies were not able to interpret results without knowledge of the diagnosis.

## 3.3 Detection rates of metabolites in early stage ovarian cancer

A systematic review of literature revealed four studies evaluating metabolomic biomarkers that are specifically associated with early stage OC (Chen et al. 2011a; Garcia et al. 2011; Ke et al. 2015; Zhang et al. 2013). Garcia et al. used a targeted NMR approach to evaluate the diagnostic value of serum metabolites in 120 early stage OC subjects (Stage I/II) compared to 132 controls. This study produced an excellent separation between cases and controls and was independently validated [50 epithelial ovarian cancer (EOC) vs 50 controls]. The independent validation group produced a receiver operating characteristic plot with a calculated area under the curve (AUC) of 0.949. The prediction model was also tested with renal cell carcinoma (RCC) and the models did not diagnose RCC incorrectly as EOC, indicating cancer-type specificity (Garcia et al. 2011). More recently, Ke et al. performed a large study comparing EOC cases (n = 140) to benign ovarian tumor (BOT) (n = 158) and uterine fibroid (UF) (n = 150) patients using ultra performance liquid chromatography mass spectrometry (UPLC–MS/MS). Positive and negative modes of electrospray ionization were used to acquire the data and the metabolic profiles yielded a high diagnostic accuracy, discriminating OC from BOT with an AUC of 0.910 and OC from UF with an AUC of 0.942. Fifty-three metabolites were identified to distinguish early stage EOC (Stage I/II) from BOT with an AUC value of 0.838. Additionally, metabolomic profiling of localized stage I disease showed excellent separation from advanced stages (Stage II, III, IV), with an AUC of 0.955. After the metabolite identification, lysophosphatidylethanolamines (LPEs),

lysophosphatidylcholines (LPCs), and CerP(d18:1/12:0) levels were found to be significantly upregulated in women with stage I-localized cancer compared to metastatic EOC, BOT, and UF (Ke et al. 2015). In another study, Zhang et al. reported the altered concentrations of 9 metabolites (imidazol-5-yl-pyruvate, N4-acetylcytidine, pseudouridine, succinic acid, (S)-reticuline, N-acetylneuraminic acid, 3-sialyl-N-acetyllactos-amine, β-nicotinamide mononucleotide, and 3-sialyllactose) that were significantly different ($p < 0.05$) in early stage EOC (stage I and II) when compared to advanced stages (III and IV) with an AUC value of 0.828 (Zhang et al. 2013). Moreover, a study by Chen et al. using an LC-MS platform reported 27-nor-5beta-cholestane-3,7,12,24,25 pentol glucuronide (CPG) as a potential marker of OC (Chen et al. 2011a). This metabolite was found to be significantly different in cases with stage I disease as compared to controls. An AUC value of 0.750 was reported with a sensitivity and specificity of 70 and 77 %, respectively. Additionally, to validate the metabolic origin of CPG, selective ion monitoring mass spectrometry was used to profile OC (n = 13) and BOT tissues. Higher levels of CPG were reported in cancer tissue as compared to benign lesions.

### 3.4 Altered metabolites in ovarian cancer

**3.4.1 Cellular respiration/carbohydrate metabolism—**Metabolomics studies of OC reported altered levels of metabolites that are related to glycolysis, the TCA cycle and anaerobic respiration (Supplementary Table S1). The majority of reported metabolites related to these pathways show contradictory results across the different studies. Lactate, a product of anaerobic glycolysis, was found to be elevated in malignant cyst fluid, primary and metastatic OC tissue (Fong et al. 2011; Kyriakides et al. 2016; Boss et al. 2000) while serum and urine profiling showed a reduction (Slupsky et al. 2010; Hilvo et al. 2015). Levels of lactate in tissue were down-regulated in OC when compared to borderline ovarian tumors (Denkert et al. 2006). Glucose was upregulated in serum profiled using NMR (Odunsi et al. 2005), while decreased levels were reported in urine (Slupsky et al. 2010) and malignant ascitic fluid when compared to ascitic fluid from cirrhosis cases (Denkert et al. 2006). Furthermore, Fong et al. and Kyriakides et al. reported no change in glucose levels in tissue and cyst fluid, respectively (Kyriakides et al. 2016; Fong et al. 2011). The TCA intermediates fumarate and malate were reported to be elevated in two metabolomic profiling studies of OC tissue using a MS platform (Denkert et al. 2006; Fong et al. 2011). Another TCA intermediate, succinate, showed contradictory results in two studies based on urinary metabolomic profiling (Zhang et al. 2013; Slupsky et al. 2010).

**3.4.2 Lipid metabolites—**Free fatty acids and metabolites related to fatty acid oxidation are frequently altered in OC patients (Supplementary Table S2). Fong et al. reported increased levels of carnitine, acetylcarnitine, butyrylcarnitine, and propionylcarnitine in patients with OC and metastatic OC (Fong et al. 2011). These findings were supported by Ke et al., who reported increased levels of the metabolites 3,5-tetradecadiencarnitine, dodecanoylcarnitine, cis-5-tetradecenoylcarnitine, elaidiccarnitine, tetradecanoylcarnitine, and trans-2-dodecenoylcarnitine in the plasma of OC patients (Ke et al. 2015). Alterations in metabolites related to glycerolipid metabolism were also a commonly reported finding in this systematic review; however, the majority of these metabolites show contradictory results across different studies. Lysophosphatidylcholine (LPC) levels were shown to be decreased

in the serum and urine of OC patients in two untargeted studies (Chen et al. 2011b; Zhang et al. 2013), while LPC (18:2) and several phosphatidylcholines (PC) were found to be increased (Guan et al. 2009). Ke et al. reported increased LPC and lysophosphatidylethanolamines (LPE) levels in stage I cancer compared to advanced stages of OC (Ke et al. 2015). Increased lysophosphatidic acid (LPA) levels were reported in urine and ascitic fluid in OC patients (Shender et al. 2014; Zhang et al. 2013). Ceramides and gangliosides were also increased in the serum and ascitic fluid of OC patients (Fan et al. 2012; Shender et al. 2014; Ke et al. 2015; Hilvo et al. 2015). Increased levels of long chain fatty acids were a commonly reported finding in serum and tissue of OC. Elevated palmitate, myristate and oleate concentrations were commonly reported in serum, ascitic fluid, and metastatic OC tissue (Guan et al. 2009; Fong et al. 2011; Shender et al. 2014; Hilvo et al. 2015). Further, a recent study by Buas et al. used lipidomics profiling and identified 17 metabolites including glycerolipids and glycerophospholipids that were decreased in OC cases relative to controls (Buas et al. 2016).

**3.4.3 Amino acid metabolites—**Amino acid metabolism is another novel pathway that has been commonly reported to be altered in OC in the studies included in this systematic review (Supplementary Table S3). Concentrations of valine and alanine were different across the studies included herein, where increased (Odunsi et al. 2005; Zhou et al. 2010; Boss et al. 2000; Kyriakides et al. 2016) and decreased (Garcia et al. 2011; Buas et al. 2016; Hilvo et al. 2015; Slupsky et al. 2010) levels were reported. Concentrations of the nitrogen donor amino acids glutamine and glutamate were increased in metastatic tissues, serum, cyst fluid and urine of OC cases (Denkert et al. 2006; Zhang et al. 2013; Fong et al. 2011; Hilvo et al. 2015; Boss et al. 2000). Denkert et al. and Zhou et al. both found increased levels of cysteine, glycine, and threonine using MS platforms (Denkert et al. 2006; Zhou et al. 2010). L-tryptophan metabolism alteration was shown in OC with decreased plasma levels of tryptophan and its metabolites 3-indolepropionic acid and 5-hydroxyindoleacetaldehyde (Ke et al. 2015; Zhang et al. 2012; Hilvo et al. 2015). The enhanced degradation of L-tryptophan was supported by increased urine levels of 3-indolelactic acid (Zhang et al. 2013), and increased serum and tissue concentrations of kynurenine (Zhang et al. 2013; Ke et al. 2015; Fong et al. 2011). Additionally, levels of the major metabolites of phenylalanine catabolism (phenylpyruvate, phenyllactate, and phenylacetate) were found to be increased in two studies using MS platform (Fong et al. 2011; Ke et al. 2015). Phenyllactate (PLA) levels were found to be increased 195.45-fold in EOC tissue (Fong et al. 2011). Consistent with the enhanced catabolism, serum levels of the phenylalanine were decreased (Hilvo et al. 2015). Hippuric acid, a product obtained following the formation of phenylalanine, showed decreased levels in urine studies (Chen et al. 2012; Slupsky et al. 2010). Further, the increased levels of phenylalanine-tyrosine metabolism products thyroxine and 3-(4-hydroxyphenyl) lactate were shown in plasma and tissue of OC patients (Ke et al. 2015; Fong et al. 2011; Hilvo et al. 2015). Another amino acid, L-histidine was decreased in urine and serum of EOC patients (Zhang et al. 2013; Ke et al. 2015). The product of L-histidine metabolism, imidazol-5-yl-pyruvate, was increased in OC patients, suggesting enhanced metabolism of this amino acid (Zhang et al. 2013). Further, a brain amino acid, N-acetylaspartate (NAA) and its' derivative N-acetylaspartatyl-glutatmate (NAAG) were elevated in primary and metastatic OC tissue and ovarian cyst fluid of serous type ovarian tumors (Boss et al. 2000;

Kolwijck et al. 2010; Kyriakides et al. 2016; Fong et al. 2011). Additionally, most recent study by Kyriakides et al. presented the unassigned resonances at ~2.03 ppm which is thought to belong to N-acetyl functional groups including NAA and NAAG (Kyriakides et al. 2016).

**3.4.4 Nucleotide metabolites—**The majority of nucleotide metabolite patterns reported supports an up-regulation in purine and pyrimidine metabolism (Supplementary Table S4). Pseudouridine was commonly found to be elevated in urine (Chen et al. 2012; Zhang et al. 2013) and serum (Ke et al. 2015) while hypoxanthine was found elevated in serum (Chen et al. 2011b), malignant cyst fluid (Kyriakides et al. 2016) and tissue (Denkert et al. 2006) of OC patients. Woo et al. reported elevated levels of 1-methyladenosine and 3-methyluridine in urine (Woo et al. 2009). In addition, Zhang et al. described elevated urinary concentrations of N4-acetylcytidine and urate-3-ribonucleoside in EOC (Zhang et al. 2013).

**3.4.5 Other significant metabolites—**Increased levels of ketone bodies were one of the common findings, where three studies reported increased levels of 3-hydroxybutyrate (Odunsi et al. 2005; Garcia et al. 2011; Hilvo et al. 2015) in the serum, whilst increased tissue concentrations were presented in two studies (Hilvo et al. 2015; Fong et al. 2011). Tissue levels of 3-hydroxybutyrate were increased 8.63-fold in metastatic OC compared to normal subjects (Fong et al. 2011). Acetoacetate, acetone (Garcia et al. 2011), 3,4-dihydroxybutyric and 2,4-dihydroxybutyric acid concentrations (Hilvo et al. 2015) were also elevated in a study profiling serum of patients with EOC. Additionally, increased concentrations of 2-hydroxybutyric acid was shown in primary and metastatic OC tissue (Fong et al. 2011). Furthermore, increases of α, δ and γ-tocopherol levels were reported in metastatic OC tissue (Fong et al. 2011). Ke et al. (2015) also described alterations in tocopherol metabolism, reporting decreased levels of the tocopherol metabolites, γ-CEHC and δ-CEHC in the plasma. Denkert et al. (2006) reported increased levels of α-tocopherol in OC tissues compared to borderline ovarian tumors (Supplementary Table S4). Ovarian cancer metabolomics also revealed altered histamine metabolites (Zhou et al. 2010; Fong et al. 2011). Zhou et al. (2010), demonstrated that a considerable proportion of the serum metabolites detected in OC cases were related to the histamine pathway, while Fong et al. (2011) reporting decreased levels of methylimidazoleacetate, a major metabolite of the histamine pathway (Supplementary Table S3).

## 4 Discussion

This systematic review provides a qualitative assessment of studies conducted on metabolomic profiling in ovarian cancer and a detailed overview of the pathological pathways in OC. The identified studies using untargeted and targeted approaches demonstrated variation in the relative abundance of metabolites in biological samples of patients versus controls with different diagnostic accuracies. Additionally, the results of several studies in this review were contradictory, which may be related to population differences, the diversity of specimen types, metabolomic analytical methods and/or sample sizes. Quality screening revealed that due to the nature of supervised analyses, the majority of the studies could not interpret metabolomics results without a priori knowledge of diagnosis, which may introduce a risk of bias.

In this review, we present the diagnostic value of metabolomics profiling in detection of OC. Currently, the routine diagnostic tools in clinical use are: (i) serum CA-125; (ii) ultrasound; and (iii) a combination of the two. CA-125 screening is widely available; however, the test has a very low positive predictive value (Jacobs and Bast 1989; Milojkovic et al. 2004). Further, screening using ultrasound is operator-dependent and has a low specificity ranging between 70 and 80 % in OC detection (Lachance et al. 2011). Although better results are obtained with the combination of CA-125 and ultrasound, sensitivity and specificity values are below the requirement for an effective screening method (Nossov et al. 2008). By comparison, metabolomic profiling of OC serum samples have reported promising results that may be able to ameliorate the lack of highly accurate biomarkers in ovarian malignancies (Table 1) (Chen et al. 2011a, b; Garcia et al. 2011; Guan et al. 2009; Ke et al. 2015; Silva et al. 2010; Zhang et al. 2012; Odunsi et al. 2005; Zhou et al. 2010; Buas et al. 2016; Hilvo et al. 2015; Cheng et al. 2015). Moreover, urinary metabolomic profiling has also identified significant metabolites able to distinguish patients with OC (Zhang et al. 2013; Slupsky et al. 2010; Chen et al. 2012). However, urinary metabolomic profiling was not able to achieve separation between ovarian and cervical cancer (Woo et al. 2009) while it showed a good separation between cancers of ovary and breast (Woo et al. 2009; Slupsky et al. 2010). These results indicate that further assessment of metabolomic profiling for its ability to discriminate different types of gynecological malignancies is required to better understand the specificity of these metabolites.

Most women with early stage OC are asymptomatic and three-quarters of the OC population have regional or distant metastasis at the time of diagnosis (Holschneider and Berek 2000). Thus, identifying early stage biomarkers could have a transformative impact on OC management, vastly improving survival rates. However to date; only a few studies were able to evaluate the metabolomic profiles of OC specifically diagnosed at early stages. This limitation is inherent to the low rates of early stage OCs at the time of presentation. Prediction models obtained in the study by Garcia et al., was able to achieve an AUC value of 0.949 in distinguishing stage I/II disease from controls (Garcia et al. 2011). Further, Chen et al. identified CPG in serum, which achieved an AUC value of 0.750 for the detection of stage I OC compared to benign ovarian neoplasms and healthy ovaries (Chen et al. 2011a). While existing data is very preliminary, it suggests a potential role for metabolomics in improving early stage OC detection especially when it is compared to detection rates of CA-125 in early stages (Menon et al. 2015). However, it is important to emphasize that all the studies in our systematic review were at the phase I stage in validating metabolomics as a diagnostic tool.

Metabolite perturbations and their relationships with major biological pathways were examined in this systematic review. These pathways include: cellular respiration, fatty acid β-oxidation, carbohydrate, amino acid, glycerolipid, nucleotide, and ketone metabolisms. There were significant alterations in metabolites of glycolysis, TCA cycle and anaerobic respiration pathways indicating significant perturbations of energy metabolism in OC. Altered energy metabolism as a hallmark of cancer was first identified almost a century ago when Warburg discovered that cancer cells primarily use anaerobic glycolysis to produce energy, even in the presence of oxygen (Warburg 1956). This phenomenon is called the Warburg effect and was demonstrated in OC metabolomics with findings of increased

glucose 6-phosphate and glucose 1-phosphate in malignant ascites and cancer tissue (Denkert et al. 2006; Fong et al. 2011; Shender et al. 2014). Further, the Warburg effect is known to cause an increase in lactate production and lower the pH of malignant tissue, which in turn impairs DNA repair mechanisms (Vander Heiden et al. 2009). However, increase in lactate concentration was not consistent through out the studies included in our systematic review, where elevation was shown in three studies evaluating malignant cyst fluid and tumor tissue (Fong et al. 2011; Boss et al. 2000; Kyriakides et al. 2016). Moreover, succinate dehydrogenase and fumarate hydratase are enzymes in the TCA cycle whose substrates have been designated as onco-metabolites that lead the overexpression of a transcription factor, hypoxia-inducible factor 1 (HIF-1). The loss of their enzymatic activities increases the succinate and fumarate levels, correspondingly (Pollard et al. 2005). Common to all studies reported herein, urinary and tissue concentrations of succinate and fumarate were increased in patients with EOC (Zhang et al. 2013; Denkert et al. 2006; Fong et al. 2011). These alterations in TCA intermediates and other metabolites related to glycolysis indicate high metabolic turnover rates and high demands on energy supply. Further, the increases of the TCA intermediates reported in several studies, in spite of the Warburg effect, can be explained by the process of anaplerosis (Denkert et al. 2006). This phenomenon refers to replenishment of intermediates through the generation of α-ketoglutarate from glutamate following its conversion from glutamine, generating macromolecules that are essential for cellular proliferation (DeBerardinis et al. 2007; Schulze and Harris 2012). This was shown in metabolomic profiling of OC tissues with elevated levels of glutamate/glutamine (Fong et al. 2011; Denkert et al. 2006).

Lipid metabolism also has an essential role in malignant proliferation, suggesting that adipocytes act as an energy source for cancer cells. Current evidence supporting this phenomenon includes increased levels of fatty acid β-oxidation in cancers such as prostate and kidney (Carracedo et al. 2013; Wu et al. 2014; Ganti et al. 2012). Increased fatty acid oxidation is associated with an over-expression of uncoupling proteins that can promote chemoresistance in cancer cells through mitochondrial “uncoupling,” helping cancer cells to survive (Derdak et al. 2008). In our systematic review, enhanced fatty acid β-oxidation was a commonly reported finding, manifesting as high levels of carnitine proteins in the serum and tissue of patients with OC (Fong et al. 2011; Ke et al. 2015). Enhanced oxidation of lipids results in elevated levels of acetyl-CoA, which normally enters the Krebs cycle. However, in cancer, due to increased turnover of TCA metabolites and their usage for gluconeogenesis, oxaloacetate level is depleted in the liver. This impedes the entry of acetyl-CoA into the Krebs cycle, and it is subsequently converted to the ketone bodies. In this review, increased levels of ketone bodies, especially 3-hydroxybutyrate, were found in serum (Garcia et al. 2011; Odunsi et al. 2005; Hilvo et al. 2015) and tissue of metastatic OC patients (Fong et al. 2011). Further, the recent study by Hilvo et al. presented elevated levels of 3-hydroxybutyrate in OC tissue with other hydroxybutyric acids including 3,4-dihydroxybutyric and 2,4-dihydroxybutyric acid. Although the common elevations of ketone bodies seem promising for a biomarker development, future validation is required for a better understanding of their diagnostic role (Hilvo et al. 2015).

Consistent with enhanced fatty acid oxidation, abnormal glycerolipid and sphingolipid metabolism and altered concentrations of long chain fatty acid derivatives were other

common findings of the studies reviewed (Fan et al. 2012; Shender et al. 2014; Buas et al. 2016; Hilvo et al. 2015). A recent study by Buas et al. presented lipidomics profiling of OC serum and identified decreased concentrations of 17 lipid metabolites including glycerolipids and glycerophospholipids (Buas et al. 2016). Further, elevated levels of LPC and LPA were previously reported as potential markers for OC (Sutphen et al. 2004). Although gylcerolipids including LPC and LPE were also commonly altered, contradictory results were reported across various studies. The aforementioned study by Ke et al. reported higher levels of LPCs and LPEs in early stages of OC compared to metastatic OC. This interesting finding was linked to phosholipase A2 and its catalytic activity which may be associated with OC initiation, while the down regulation of this pathway may correlate with metastasis and progression of EOC (Ke et al. 2015). Moreover, sphingolipids and fatty acids including palmitate, oleate, myristate and linoleate were commonly elevated in serum, urine, tissue and malignant ascitic fluid of patients with OC (Fan et al. 2012; Shender et al. 2014; Chen et al. 2012; Fong et al. 2011; Guan et al. 2009; Hilvo et al. 2015). Similar to LPCs and LPEs, sphingolipids and fatty acids have cellular signal transduction activity that contributes to genetic instability and cancer initiation (Coussens and Werb 2002). The altered lipid metabolism reported may be related to OC initiation and the contradictory relative abundance of glycerolipids and sphingolipids in the cancer versus control groups may be related to differences in the distribution of early and advanced stage cancers within each study.

Amino acid metabolism is another novel pathway that is commonly altered in cancer cells. Due to high cell cycle turnover, cancer cells exhibit increased uptake of glutamine, which plays a role as the amino group donor for many biosynthetic pathways. Elevated levels of glutamate and glutamine were reported in studies profiling metastatic tumor tissue, serum, cyst fluid and urine of OC cases (Denkert et al. 2006; Zhang et al. 2013; Fong et al. 2011; Hilvo et al. 2015). Further, amino acids such as alanine (Odunsi et al. 2005; Zhou et al. 2010; Boss et al. 2000; Buas et al. 2016; Kyriakides et al. 2016), glycine, threonine and cysteine (Denkert et al. 2006; Zhou et al. 2010), which are all regarded as important building blocks in protein biosynthesis, were also found to be elevated in several studies, indicating the rapid cell cycling and high turnover of cancer metabolism. Another commonly altered amino acid was L-tryptophan, whose degradation is one of the mechanisms by which tumors resist immune destruction. Enhanced degradation leading to tryptophan depletion causes T cell anergy and apoptosis via the GCN2 pathway, while increased levels of the tryptophan breakdown product kynurenine suppresses T cell differentiation (Platten et al. 2012). L-tryptophan degradation in OC has been previously reported (Inaba et al. 2009; Sperner-Unterweger et al. 2011) and is supported in metabolomics studies with decreased plasma levels of L-tryptophan and its metabolites (5-hydroxyindoleacetaldehyde, 3-Indolepropionic acid) (Ke et al. 2015; Zhang et al. 2012). Additionally, serum concentrations of kynurenine and urine concentrations of 3-indolelactic acid were found to be increased (Zhang et al. 2013; Ke et al. 2015). Moreover, major metabolites of phenylalanine catabolism (phenylpyruvate, PLA and phenylacetate) and phenylalanine-tyrosine metabolism were found to be increased in the serum and tissue of EOC patients (Fong et al. 2011; Ke et al. 2015; Hilvo et al. 2015) whilst one study reported decreased levels of phenylalanine in serum (Hilvo et al. 2015). The increase in phenylalanine-associated metabolites induces

oxidative stress. Phenylacetate has been shown to have an inhibitory effect on growth in OC cell lines (Melichar et al. 1998), whereas PLA can promote growth (Collier et al. 1980). Additionally, benzoate degradation is known to be a part of phenylalanine pathway, which is associated with altered hippurate levels (Chen et al. 2012; Ke et al. 2015; Slupsky et al. 2010). Decreased hippurate levels were found in the urine of OC patients (Chen et al. 2012; Slupsky et al. 2010). Despite all the significant alterations seen in the phenylalanine pathway, its role in OC is not yet fully understood. Furthermore, L-histidine, was shown to be decreased in urine and serum of EOC patients (Zhang et al. 2013; Ke et al. 2015). The increase in the product of L-histidine degradation, midazol-5-yl-pyruvate, also suggests enhanced metabolism of this amino acid (Fong et al. 2011). The enzyme histidine decarboxylase converts histidine to histamine. Over the past decade, considerable data suggest an important function of histamine in malignant cell proliferation (Falus 2003). Histamine serves as a receptor-dependent growth factor in malignant melanoma, breast and some gastrointestinal cancers (Medina et al. 1999). In this review, Zhou et al. reported a considerable proportion of histamine metabolites in OC serum (Zhou et al. 2010) while Fong et al. reported decreased urine levels of a histamine metabolite, methylimidazoleacetate (Fong et al. 2011). Interestingly, NAA, a free amino acid that exists in brain at very high concentrations was found in OC tissue (Fong et al. 2011) and in several studies profiling ovarian cyst fluid (Boss et al. 2000; Kolwijck et al. 2010; Kyriakides et al. 2016). NAA, a precursor of NAAG, plays a role in osmotic activity, lipid and myelin synthesis in glia cells (Baslow 2002, 2003). Although the role of NAA and NAAG in ovarian cyst fluid is still unknown, elevated levels were reported in metabolomic profiling of serous subtype of ovarian tumors (Boss et al. 2000; Kolwijck et al. 2010; Kyriakides et al. 2016). Additionally, Kolwijck et al. presented high levels of NAA in hydrosalpingeal cyst fluid, which resembles the fallopian tube epithelium lining similar to serous EOC. High levels of NAA in cyst fluid of serous cystadenoma and hydrosalpinx support the evidence that NAA might be due to specific histological serous subtype that is independent of the malignant differentiation (Kolwijck et al. 2010).

Consistent with the metabolites related to high cell turnover, nucleotide metabolism was found to be enhanced in OC metabolomics. Elevated levels of uracil, 5-methyluridine, pseudouridine and hypoxanthine were reported in tissue, cyst fluid and urine of OC patients (Denkert et al. 2006; Woo et al. 2009; Chen et al. 2012; Zhang et al. 2013; Kyriakides et al. 2016). It is known that *de novo* synthesis of purine and pyrimidine nucleotides is required for sustained tumor growth; however, more evidence is required to fully the understand the specificity of the nucleotide pathway in OC pathogenesis.

Another commonly reported altered metabolic pathway in OC was tocopherol metabolism. Four main isoforms of tocopherols (α, β, δ, and γ), accounting for approximately 90 % of vitamin E, are known to have antioxidant activity and possess a protective effect against many cancers (Odin 1997). The enhanced accumulation of tocopherols in cancer is attributed to have an immunosuppressive effect, which may provide cancer cells a survival mechanism. Elevated levels of α, δ, and γ tocopherols were reported in metastatic OC tissue (Fong et al. 2011). In contrast, high levels of tocopherols were not shown in primary EOC tissues, explained by the fact that this metabolic alteration develops during the metastatic stage. Ke et al. reported decreased plasma levels of the tocopherol metabolites γ-CEHC and

δ-CEHC in OC patients which is linked to a reduction in vitamin E metabolism, increasing the tocopherol levels correspondingly (Ke et al. 2015).

There are several limitations of this analysis. Several studies reviewed herein report contradictory findings in the regulation of identified metabolites and pathways. These opposing results may be due to the differences in specimen types, patient selection and/or analytical platforms. Moreover, in several studies there was a lack of a discovery and a cross validation group, which may have led to potential over-fitting in the interpretation of results. The biomarker development in OC metabolomics has not progressed beyond Phase 1 pre-clinical exploratory studies. Further, the studies published are largely limited to individual cancer centers so their applicability to the general population must be questioned at this stage.

## 5 Concluding remarks

Overall, metabolomics has revealed multiple dysregulated metabolites that related to the differences in metabolic pathways between OC and control samples, and potentially may result in multiple clinically useful biomarkers. Despite the promising preliminary results a consensus group of biomarkers for OC has not yet emerged. Such a group of biomarkers is a necessary prerequisite for larger scale studies of OC detection. The standardization of metabolomics platforms including separating techniques is crucial to minimize variability due to equipment and approaches to metabolite identification and quantitation. Subsequently, larger studies addressing a more diverse population need to be designed and executed. Beyond the question of screening biomarkers, our review provided insights into the biology of OC development. Apart from the obvious scientific interest, such knowledge will form the basis for new therapeutic interventions that can interrupt these neoplastic pathways. Rigorous adherence to these approaches will set the stage for metabolomics to be validated both as a diagnostic tool and as the basis for a new generation of therapeutic agents for OC.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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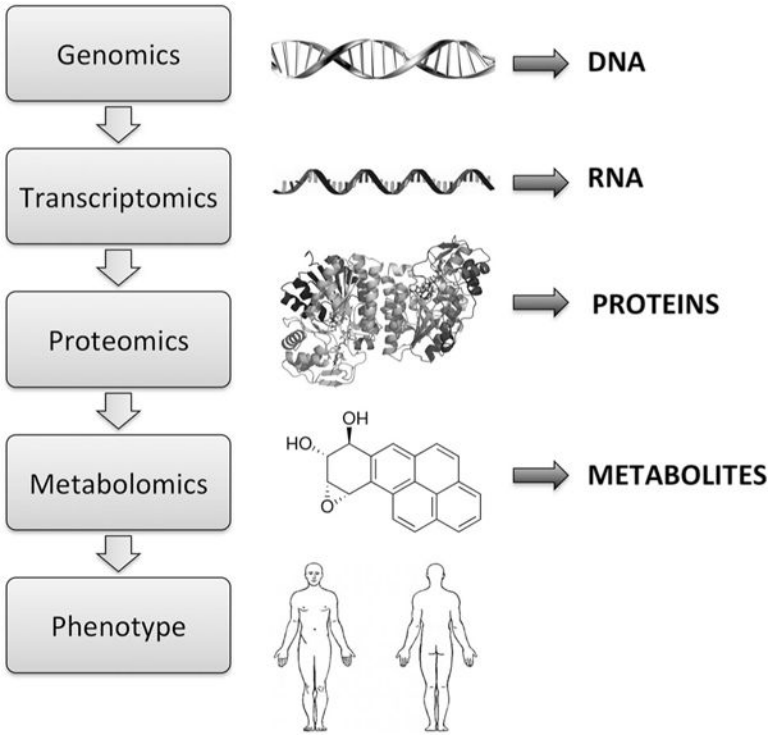


**Fig. 1.**
"Omics" cascade

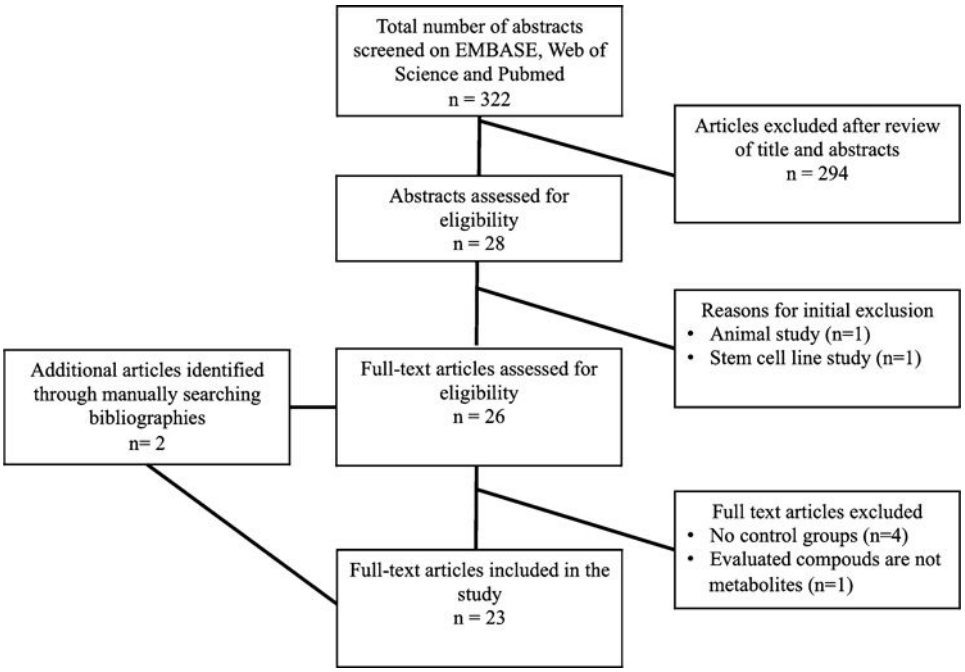


**Fig. 2.**
Systematic search and selection strategy

**Table 1**

Current literature in metabolomics of ovarian cancer detection

| Ref | Specimen | Cases/controls | Platform | Up-regulated metabolites | Down-regulated metabolites |
|---|---|---|---|---|---|
| Odunsi et al. (2005) | Serum | EOC (n = 38), BOT (n = 12), controls (n = 53) | $^{1}$H-NMR | Alanine; valine; glucose; 3-hydroxybutyrate | N/A |
| Garcia et al. (2011) | Serum | EOC (stage I/II, n = 120), controls (n = 132), RCC (n = 30) | $^{1}$H-NMR | Acetoacetate; acetone; 3-hydroxybutyrate | Alanine, the choline moiety of phospholipids; creatine/creatinine; 'LDL1' representing CH3(CH2)n of lipid mainly in LDL; CH2CH2CH2CO of lipid mainly in VLDL; = CHCH2CH2 of unsaturated lipid; valine; 'VLDL1' representing CH3CH2CH2C = of lipid mainly in VLDL |
| Zhou et al. (2010) | Serum | Serous papillary OC (n = 44), BOT (n = 50) | DART/TOF–MS | Canonical pathways of alanine; serine; cysteine; threonine; glycine; histamine pathway members | N/A |
| Ke et al. (2015) | Serum | EOC (n = 140), BOT (n = 158), UF (n = 150) | UPLC-MS | 3, 5-tetradecadiencarnitine; cis-5-tetradecenoylcarnitine; elaidic carnitine; dodecanoylcarnitine; kynurenine; tetradecanoylcarnitine; trans-2-hexadecenoylcarnitine; Phe Phe; L-thyroxine L-(-)-3-phenyllactic acid; hydroxyphenyllactic acid; pseudouridine *Stage I vs MOC* LPC (14:0); LPC (18:2); LPC (18:3); LPE (0:0/18:1(9Z)); LPE (0:0/16:0); LPE (20:2(11Z,14Z)/0:0) | L-Tryptophan; 3-indolepropionic acid; 5-hydroxyindoleacetaldehyde; 3b-hydroxy-5-cholenoic acid; glycoursodeoxycholic acid; deoxycholic acid; androsterone glucuronide; prasterone sulfate; hippuric acid; piperine; 2-piperidinone; γ-CEHC; δ-CEHC; L-histidine |
| Zhang et al. (2012) | Plasma | EOC (n = 80), BOT (n = 90) | UPLC/QTOF–MS | N/A | L-Tryptophan; LPC(18:3); LPC(14:0); 2-piperidinone |
| Fan et al. (2012) | Plasma | EOC (n = 80), controls (n = 93) | UPLC/QTOF-MS | Ganglioside; lysophospholipids; ceramides; phytosphingosine; N-formylkynurenine | Demethylphylloquinone |
| Guan et al. (2009) | Serum | EOC (n = 37), controls (n = 35) | LC/TOF-MS | 12-Hydroxy-8E;10E-heptadecadienoic acid; palmitic acid; stearic acid; GlnHisAla; DHEA sulfate; PC(P-16:0/0:0); PC(10:0/4:0); PE(9:0/10:0); LPC(18:2); PC(14:0/20:1(11Z)); PE-NMe(18:1(19E)/18:1(9E)); PC(14:0/22:4 (7Z,10Z,13Z,16Z)); PC(14:0/22:1(13Z)) | N/A |
| Chen et al. (2011a) | Serum | EOC (n = 235), BOT (n = 135), controls (n = 218) | LC–MS | 27-nor-5beta-cholestane-3,7,12,24,25 pentol glucuronide (CPG) | N/A |
| Silva et al. (2010) | Serum | EOC (n = 67), borderline (n = 4), BOT (n = 14), controls (n = 60) | HPLC-HR-MS | Ion 472 | N/A |
| Chen et al. (2011b) | Serum | EOC (n = 21), recurrent EOC (n = 36), non-recurrent EOC (n = 25), controls (n = 24) | LC–MS | Hypoxanthine; guanidinosuccinic acid; cortisol; lyso PE(22:6); lyso PE(22:6) fragment | LPC (18:2) |
| Hilvo et al. (2015) | Serum | EOC (n = 158), controls (n = 100) | GC/TOF–MS | 3,4-Dihydroxybutyric acid; 3-hydroxybutyric acid; 2-hydroxybutyric acid; acetoacetic acid; | Tryptophan; alanine; methionine; threonine; proline; serine; phenylalanine; tyrosine; valine; 2- |

| Ref | Specimen | Cases/controls | Platform | Up-regulated metabolites | Down-regulated metabolites |
|---|---|---|---|---|---|
| | | | | 2,4-Dihydroxybutyric acid; glycine; glutamic acid; glutamine; 4-hydroxyphenyllactic acid; maltose; turanose; melibiose; mannonic acid; xylitol; arabinose; glucopyranose; mannopyranose; xylose; linolenic acid (C18:3); Palmitelaidic acid (C16:1); oleic Acid (C18:1); myristic acid (C14:0); myristoleic acid (C14:1); palmitic acid (C16:0); lauric acid (C12:0); adipic acid; Myo-Inositol; ethanolamine; glycerol; 3-hydroxyvaleric acid; erythritol; maleic acid; 3-hydroxyisovaleric acid; 3-hydroxycaproic acid | oxo-3-methylpentanoic acid; indole-3-acetic acid; 2-oxoisovaleric acid; 2-hydroxy-3-methylvaleric acid; lactic acid; malic acid; glycerol-3-phosphate; 2,3,4-trihydroxybutyric acid; ketoleucine; cholesterol; 2-aminobutyric acid; glyceric acid |
| Cheng et al. (2015) | Serum | OC (n = 21), BOT (n = 17), controls (n = 20) | LC–MS | *OC vs controls* arabitol; maltose; maltotriose; raffinose; mannitol; glucose<br>*OC vs BOT* arabitol; maltose; maltotriose; raffinose; mannitol; glucose; erythritol | *OC vs controls* inosine; ribose *OC vs BOT* inosine; ribose |
| Buas et al. (2016) | Plasma | OC (n = 50), controls (n = 50) | LC–MS | N/A | Alanine; C52H79 NO5S; PS(O-18:0/0:0); 18:3 Cholesteryl ester + 22.7; PG(P-20:0/12:0); TG(16:0/16:1(9Z)/16:1(9Z))[iso3] + 23.5; TG(16:1(9Z)/16:1(9Z)/16:1(9Z)) + 22.7; PS(O-18:0/16:1(9Z)); TG(17:2(9Z,12Z)/17:2(9Z, 12Z)/20:5(5Z,8Z,11Z,14Z,17Z)); TG(16:1(9Z)/ 17:2(9Z,12Z)/17:2(9Z,12Z))[iso3] + 22.2; PE(18:1(9Z)/20:3(8Z,11Z,14Z)); TG(16:1(9Z)/ 17:1(9Z)/17:2(9Z,12Z))[iso6]; C57H102N2OS; TG(16:0/16:0/16:1(9Z)) [iso3]; C29 H47 N9 O2; PS(O-20:0/0:0); PE(18:1(9Z)/20:3(8Z,11Z,14Z)); TG(16:1(9Z)/17:0/17:2(9Z,12Z))[iso6] |
| Woo et al. (2009) | Urine | OC (n = 9), controls (n = 22), BC (n = 10), CC (n = 12) | GC–MS | 1-Methyladenosine; 3-methyluridine; 4-androstene-3,17-dione | N/A |
| Zhang et al. (2013) | Urine | EOC (n = 40), BOT (n = 62), controls (n = 54) | UPLC-QTOF/MS | Imidazol-5-yl-pyruvate; N4-acetylcytidine; urate-3-ribonucleoside; pseudouridine; N-acetylneuraminate 9-phosphate; 3-dehydroquinic acid; 3-indolelactic acid; succinic acid; LPA (P-16:0e/0:0); (S)-reticuline; N-acetylneuraminic acid; taurine; N-acetylgalactosamine 4-sulfate; 3-sialyl-N-acetyllactosamine; β-nicotinamide mononucleotide; 3′-sialyllactose; prolylhydroxyproline; selenocystathionine | L-Histidine; N-acetylglutamine; prasterone sulfate; glycodeoxycholate |
| Slupsky et al. (2010) | Urine | EOC (n = 50), BC (n = 48), controls (n = 62) | NMR | N/A | Creatine; acetate; succinate; levoglucosan; lactate; pyroglutamate; formate; isoleucine; sucrose; methanol trigonelline; leucine; asparagine; urea; glucose; ethanolamine; dimethylamine; 4-hydroxyphenylacetate; creatinine; alanine; valine; hippurate; 1-methylnicotinamide; unknown singlet at 3.79 ppm; uracil; trans-aconitate; singlets at 2.60 ppm/3.94 ppm/4.34 ppm/2.36 ppm |
| Chen et al. (2012) | Urine | EOC (n = 22), BOT (n = 29), controls (n = 25) | HILIC-RPLC-MS | Pseudouridine; phytosphingosine | Hippuric acid; homovanillic acid sulfate |
| Shender et al. (2014) | Ascites | OC (n = 10), cirrhosis (n = 5) | GC–MS | Glucose-1-phosphate; glycero-3-phosphate; amide of linoleic acid; amide of oleic acid; amide of pentadecanoic acid; amide of stearic acid; arachidic acid; arachidonic acid; behenic acid; capric acid; ceramide; cholesta-4,6-dien-3-ol; cholesteryl acetate; lauric acid; | 2-Hydroxybutanoate; glycolate; furanose; glucose; fructose; sorbitol; galacturonic acid; 2-hydroxyisovalerate; 2,4-dihydroxybutanoate; pyranose1853; xylitol |

| Ref | Specimen | Cases/controls | Platform | Up-regulated metabolites | Down-regulated metabolites |
|---|---|---|---|---|---|
| | | | | docosahexaenoic acid; eicosadienoic acid; eicosenoic acid; lignoceric acid; LPA; 16:0 monoacylglycerol; methyl phosphate; myristic acid; nOH14:0 itoleic acid; stearic acid; cholesterol linoleic acid; palmitic acid; oleic acid; vaccenic acid | |
| Kyriakides et al. (2016) | Ovarian cyst fluid | EOC (n = 10), borderline (n = 5), BOT (n = 8) | $^{1}$H-NMR | *EOC vs BOT* lysine; alanine; valine; leucine; hypoxanthine; N-acetyl groups (~2.03 ppm)<br>*EOC/Borderline vs BOT* lactate; choline | *EOC vs BOT* citrate |
| Kolwijck et al. (2010) | Ovarian cyst fluid | EOC (n = 25), borderline (n = 8), BOT (n = 36) | GC–MS | NAA | |
| Boss et al. (2000) | Ovarian cyst fluid | EOC (n = 12), BOT (n = 28) | $^{1}$H-NMR | Isoleucine; valine; threonine; choline; lactate; lysine; alanine; methionine; glutamine; singlets at 2.25 ppm/2.77 ppm | NAA; 5-oxoproline (elevated in serous cystadenoma subtype) |
| Fong et al. (2011) | Tissue | EOC (n = 11), MOC (n = 7), controls (n = 12) | GC–MS/LC–MS | *EOC* phenyllactate; phenylacetate; phenylpyruvate; 3-(4-hydroxyphenyl) lactate; 4-hydroxyphenylpyruvate; thymine; kynurenine; 3-methyl-2-oxovalerate; N-formylmethionine; trans-4-hydroxyproline; Indole-3-acetic acid; N-acetylputrescine; N-acetylthreonine; erythronate; N-acetylglycine; uracil; cytidine; xanthine; nonadecanoate (19:0); adenosine 5′-monophosphate (AMP); 2-hydroxybutyrate; 2-linoleoylglycerophosphoethanolamine<br>*MOC* Glutamine; N-acetylglutamine; pro-hydroy-pro; 2-methylbutyroylcarnitine; ophthalmate; cis-vaccenate (18:1n7); N-acetylglucosamine 6-P; 4-hydroxybutyrate; gamma-glutamylvaline; mannose-6-phosphate; glucose-6-phosphate; fructose-6-phosphate; 1-octadecanol; glycerol; 1-oleoylglycerol; squalene; 3-hydroxybutyrate; glucarate; alpha/delta/gamma-tocopherol<br>*EOC/MOC* N-acetylaspartate (NAA); fucose; lactate; N-acetyl-aspartylglutamate (NAAG); pyroglutamine; betaine; 2-aminobutyrate; N-acetylglycine; malate; glycylleucine; cytidine-3′-monophosphate (3′-CMP); fumarate; cysteinylglycine | *MOC* phenol sulfate; taurine; 2-palmitoylglycerophosphoethanolamine; 2-arachidonoylglycerophosphoethanolamine; 2-docosahexaenoylglycerophosphoethanolamine; 2-arachidonoylglycerophosphocholine; 6-phosphogluconate *EOC/MOC* 1-Methylimidazoleacetate; 1-arachidonoylglycerophosphoethanolamine; creatinine; cytidine 5′-monophosphate (5′-CMP) |
| Denkert et al. (2006) | Tissue | OC (n = 66), borderline (n = 9) | GC/TOF–MS | Glycerolphosphate-α; uracil; hypoxanthine; malic acid; pyrazine-2,5-bishydroxy; inositol-2-phosphate; proline; phosphoric acid; glutamic acid; glycine; threonine; g-aminobutyric acid; asparagine α-tocopherol; glycine minor; 2 glucose-1-phosphate degr; fumaric acid; butyric acid 2-hydroxy; creatine; cysteine; glutamine | Nonadecanoic acid; heptadecanoic acid; stearic acid; benzoic acid; lactic acid |

*EOC* epithelial ovarian cancer, *UF* uterine fibroid, *BOT* benign ovarian tumor, *BC* breast cancer, *CC* cervical cancer, *RCC* renal cell cancer, *MOC* metastatic ovarian cancer, *HPLC* high-performance liquid chromatography, *HRMS* high-resolution mass spectrometry, *GC/TOF–MS* gas chromatography/time of flight-mass spectrometry, *SVM* support vector machines, *SVM_NL* support vector machines non-linear, *HILIC* hydrophilic interaction chromatography, *RPLC* reversed-phase liquid chromatography, *N/A* not available