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SYSTEMATIC REVIEW

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Metabolomics in endometrial cancer diagnosis: A systematic review

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Abstract

Introduction: Endometrial cancer (EC) is the most common gynecological malignancy in the developed world. The prognosis of EC strongly depends on tumor stage, hence the importance of improving diagnosis. Metabolomics has recently appeared as a promising test for a non-invasive diagnosis of several diseases. Nevertheless, no metabolic marker has been approved for use in the routine practice. We aimed to provide an overview of metabolomics findings in the diagnosis of EC.

Material and methods: A systematic review was performed by searching eight electronic databases from their inception to October 2019 for studies assessing metabolomics in EC diagnosis. Extracted data included characteristics of patients and EC, serum concentration of metabolites in women with and without EC and its association with EC diagnosis, tumor behavior and pathological characteristics.

Results: Six studies with 732 women (356 cases and 376 controls) were included. Several metabolites were found able to predict the presence of EC, tumor behavior (progression and recurrence) and pathological characteristics (histotype, myometrial invasion and lymph vascular space invasion).

Conclusions: Metabolomics might be suitable for a non-invasive diagnosis and screening of EC, offering the possibility to predict tumor behavior and pathological characteristics. Further studies are necessary to validate these results.

KEYWORDS

early detection, endometrial cancer, metabolomics, screening, tailored: precision, carcinoma, oncology

1 | INTRODUCTION

Endometrial cancer (EC) is the most prevalent gynecologic cancer in the developed world. In the last decades, there has been an increase in the incidence, mainly due to an increased prevalence of risk factors such as obesity.[1-3] It is estimated that the incidence of EC will increase by 55% from 2010 to 2030.[4]

The prognosis of EC strongly depends from tumor stage: the 5-year survival decreases from 85% at International Federation of Gynecology and Obstetrics (FIGO) stage I to 25% at FIGO stage IV.[5] Therefore, improving diagnosis appears crucial.[6]

A step FORWARD has been made in the field of biomarker discovery with the introduction of the "-omics" technologies, including genomics, transcriptomics, proteomics and metabolomics.[7,8] Metabolomics is

Abbreviations: EC, endometrial cancer; FIGO, International Federation of Gynecology and Obstetrics.

the systematic identification and quantification of metabolic products to provide information on cellular activities and phenotypic changes linked to biological function.[9,10] Metabolomics ensures a reading of the physiological state of the system, representing the end point of cellular processes.[11] This approach is becoming a powerful tool for biomarker discovery and has proven to be useful in the study of many metabolic diseases including cancer. In this regard, oncometabolites have highlighted the involvement of novel and unexpected cellular pathways in several studies.[12,13] Components of such a pathway may serve as diagnostic or prognostic biomarkers or may be therapeutically targeted for tailored treatment.[12] To date, interesting results have been achieved for diagnosis in breast, ovarian, cervical, hepatic, pulmonary, pancreatic, prostatic, renal, cerebral and endometrial carcinomas.[14-18] Nevertheless, no metabolic marker has been approved for use in the routine practice, and the potential role of metabolomics in cancer diagnosis is still unclear to date.

The aim of this systematic review was to provide an overview of metabolomics findings in the diagnosis of EC.

Key message

In endometrial cancer patients, metabolomics might be useful for a non-invasive diagnosis, screening and prediction of tumor behavior, and pathological characteristics.

2 | MATERIAL AND METHODS

2.1 | Study protocol

The study was designed a priori following a protocol recommended for systematic review.[19] Two authors independently performed all review stages, that is, electronic search, study selection, risk of bias within studies assessment, data extraction and analysis. Disagreements were solved by discussion with a third author.

The Preferred Reporting Item for Systematic Reviews and Meta-analyses (PRISMA) statement was followed for study reporting.[19]

2.2 | Search strategy and study selection

Eight electronic databases—Web of Sciences, Cochrane Library, Scopus, ClinicalTrial.gov, Google Scholar, EMBASE, OVID and MEDLINE—were searched from their inception to October 2019. A combination of the following text words was used for several searches: "metabolomics", "metabolit*", "endometr*", "tumor", "tumour", "cancer", "carcinoma", "uterus"; "uterine"; "diagnosis".

All peer-reviewed studies assessing metabolomics in the diagnosis of EC were included in the systematic review. Case reports and reviews were defined a priori as exclusion criteria. Title, abstract and references of each relevant article were screened. The full text of each eligible article was assessed.

2.3 | Risk of bias within studies assessment

The methodological index for non-randomized studies (MINORS) was used for risk of bias within study assessments.[20] Five applicable domains related to risk of bias were evaluated in each included study:

- aim (ie, if the aim was clearly stated);
- inclusion of consecutive patients (ie, if all eligible patients were included in the study during the study period);
- prospective collection of data (ie, if data were collected following an a priori defined protocol);
- endpoints appropriate to the aim (ie, if criteria used to assess outcomes were clearly stated);
- unbiased assessment of the study endpoints (ie, if the study endpoints were evaluated without bias).

Authors judged each domain of each included study as "low risk", "unclear risk" or "high risk" of bias, whether reported data were "reported and adequate", "not reported" or "reported but inadequate", respectively.

2.4 | Data extraction and analysis

Data extraction from the included studies was performed without modification of original data. Extracted data included characteristics of patients and EC, serum concentration of metabolites in women with and without EC, and its association with diagnosis and characteristics of EC (histotype, myometrial invasion, lymphovascular invasion, progression at advanced FIGO stages and recurrence after surgery).

REVIEW MANAGER 5.3, 2014 (The Nordic Cochrane Center - Cochrane Collaboration, Copenhagen, Denmark) was used to analyze data.

3 | RESULTS

3.1 | Study selection

In total, 3223 articles were identified by searching eight electronic databases. A total of 49 articles remained after duplicate removal. Thirty-three articles remained after title screening. In total, 15 articles were evaluated for eligibility after abstract screening. Lastly, six studies were included in the systematic review.[13,21-25] Details of the whole process of study selection are reported in Figure S1.

3.2 | Risk of bias within studies assessment

Overall quality of the included studies was high, given that all domains related to risk of bias of each included study were judged as

"low risk" of bias, with the exception of the "Inclusion of consecutive patients" domain. In that domain, all included studies were categorized as "unclear risk" of bias because they did not report whether all eligible patients were included, not allowing a selection bias to be excluded. Results of risk of bias within study assessments are graphically reported in Figure 1.

3.3 | Characteristics of included studies and study population

A total of 732 women, 356 (48.6%) cases with EC and 376 (51.4%) controls, were included in this systematic review. All the included studies were prospective (two of them were pilot studies) (Table 1).

Patient age ranged from 54 ± 8.0 to 67.5 ± 9.4 years in the cases, and from 59.2 ± 12.7 to 63.2 ± 9.4 years in the controls. Body mass index was around 36.9 ± 17.3 kg/m^2 in the cases, and from 27.5 ± 7.2 to 28.8 ± 6.8 kg/m^2 in the controls. Smokers ranged from 17% to 22% in the cases, and from 22% to 29% in the controls. Diabetes mellitus ranged from 10.2% to 23.2% in the cases, and from 3.3% to 15.4 in the controls. Blood hypertension ranged from 23.2% to 57.4% in the cases, and from 33.3% to 35.4% in the controls. Three studies included only postmenopausal women,[13,21,22] and two studies did not report the menopausal status;[23,25] the remaining study also included premenopausal women (Table 2).

ECs were histological grade 1-2 in 73.3% and grade 3 in 25.7%, endometrioid in 80.3%, at FIGO stage I in 58.5%, stage II in 28.7%, stage III in 11.2%, and stage IV in 1.3% (Table 3).

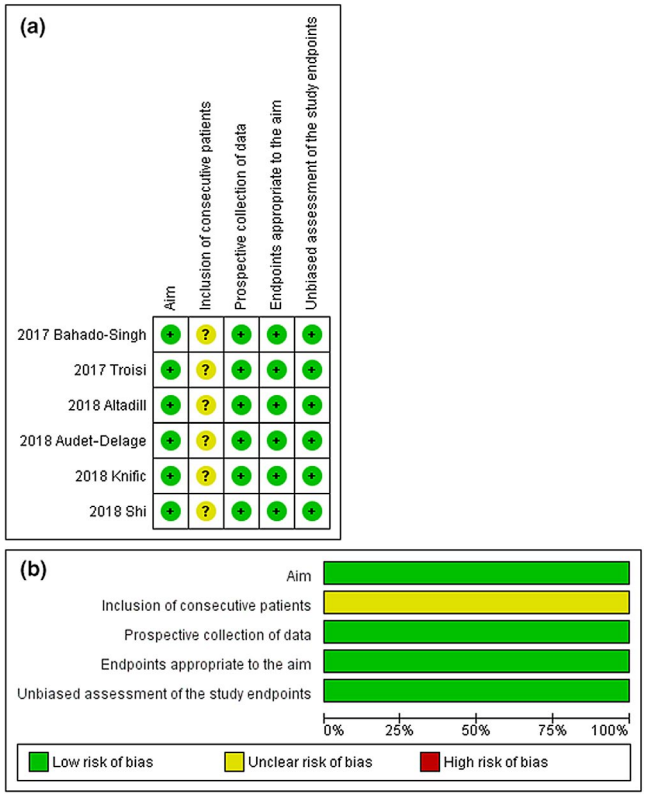

FIGURE 1 (a) Assessment of risk of bias. Summary of risk of bias for each study. Plus sign: low risk of bias; minus sign: high risk of bias; question mark: unclear risk of bias. (b) Risk of bias graph for each risk of bias item presented as percentages across all included studies [Color figure can be viewed at wileyonlinelibrary.com]

3.4 | Metabolites

The most relevant metabolites for diagnosis of EC were:

- tetradecadienoylcarnitine,[23]
- phosphatidylcholine with acyl-alkyl residue sum C38:1,[23]
- 3-hydroxybutyric acid,[23]
- hexadecanoylcarnitine/phosphatidylcholine with acyl-alkyl residue sum C40:1,[24]
- proline/tyrosine,[24]
- phosphatidylcholine with diacyl residue sum C42:0/ phosphatidylcholine with acyl-alkyl residue sum C44:5,[24]
- DL-phenylalanine,[25]
- indoleacetic acid,[25]
- phosphocholine,[25]
- lyso-platelet-activating factor-16;[25]

for diagnosis, screening and prediction of tumor histotype:

- lactic acid,[22]
- progesterone,[22]
- homocysteine,[22]
- 3-hydroxybuthyrate,[22]
- linoleic acid,[22]
- stearic acid,[22]
- myristic acid,[22]
- threonine,[22]
- valine;[22]

for prediction of myometrial invasion:

- hydroxysphingomyelins C14:1/hydroxysphingomyelins C24:1,[24]
- phosphatidylcholine with diacyl residue sum C40:2/ phosphatidylcholine with diacyl residue sum C42:6;[24]

for prediction of lymphovascular invasion:

- phosphatidylcholine with diacyl residue sum C34:4/phosphatidylcholine with acyl-alkyl residue sum C38:3,[24]
- hexadecadienyl carnitine/ phosphatidylcholine with diacyl residue sum C38:1;[24]

for diagnosis and prediction of cancer progression:

- picolinic acid (also showing antitumoral activity),[21]
- vaccenic acid,[21]

TABLE 1 Characteristics of the included studies

Study[Ref]	Country	Design	Study period	Sample size	Distribution of patients n (%)	
					Cases group	Control group
Bahado-Singh 2018[23]	Department of Obstetrics and Gynecology, William Beaumont Health, Royal Oak, MI 48073, USA	Prospective case-control study	–	116	56 (48.3)	60 (51.7)
Troisi 2017[22]	Department of Medicine, Surgery and Dentistry "Scuola Medico Salernitana", University of Salerno, Baronissi, Italy	Prospective case-control pilot study	May 2012-November 2016	288	118 (41)	170 (59)
Knific 2018[24]	Department of Obstetrics and Gynecology, University Medical Center Ljubljana, Ljubljana, SLOVENIA	Prospective case-control study	June 2012-December 2014	126	61 (48.4)	65 (51.6)
Shi 2018[25]	Department of Obstetrics and Gynecology, Guangzhou Women and Children's Medical Center, Guangzhou, China	Prospective case-control study	January 2013-December 2013	92	46 (50)	46 (50)
Altadill 2018[21]	Biomedical Research Group in Gynecology, Vall Hebron Research Institute (VHIR), Universitat Autonoma de Barcelona, Ciberonc, Barcelona, Spain	Prospective case-control study	–	56	39 (69.6)	17 (30.4)
Audet-Delage 2018[13]	Center Hospitalier Universitarie de Quebec Research Center, Faculty of Pharmacy, Laval University, Quebec, QC, Canada	Prospective case-control pilot study	2002-2014	54	36 (66.6)	18 (33.4)
Total	–	–	–	732	356 (48.6)	376 (51.4)

- arachidonic acid (peroxide-free),[21]
- phosphatidic acid,[21]
- phosphatidylglycerol,[21]
- inosine,[21]
- palmitic amide,[21]
- gleamide,[21]
- stearamide,[21]
- 13Z-docosenamide,[21]
- glutamate/phenylalanine/arginine/tryptophan,[21]
- linoleic acid,[21]
- 5,8,11-eicosatrienoic acid,[21]
- UDP-*N*-acetyl-D-galactosamine,[21]
- 1-palmitoyl-2-linoleoyl,[21]
- phosphatidylserine,[21]
- phosphatidylethanolamines,[21]
- phosphatidylinositols,[21]
- glycerophosphocholines;[21]

for diagnosis and prediction of recurrence after surgery:

- linoleic acid and myristic acid,[13]
- intermediates from the branched chain amino acid pathway,[13]
- polyamines,[13]
- spermine,[13]
- acylcholines,[13]
- acylcarnitines,[13]
- monoacylglycerols,[13]
- bradykinin,[13]
- sulfated androgens,[13]
- heme,[13]
- bile acids,[13]
- sphingolipids,[13]
- ceramides[13] (Table 4).

All assessed metabolites with diagnostic accuracy data are shown in detail in Table S1.

4 | DISCUSSION

4.1 | Main findings and interpretation

This study showed that several metabolites might be useful for diagnosis, screening and prediction of tumor histotype, myometrial invasion, lymph vascular invasion and cancer progression in patients with EC.

Metabolomics is the study of chemical processes that involve the products of cellular metabolism.[26] Metabolites are detectable on biological samples and may highlight the correct functioning of cellular metabolism. This is leading to the discovery of novel diagnostic, prognostic and therapeutic biomarkers for several diseases, making metabolomics one of the most promising "omics" technologies.[27] Furthermore, metabolomics offers the possibility to assess

TABLE 2 Characteristics of women in the included studies

	Age (Mean ± SD or median), [years+]		BMI (Mean + SD or median) [kg/m^2]		Smoking status [%]						Postmenopausal women [%]		Diabetes [%]		Hypertension [%]		Hormonal replacement therapy or tamoxifene in the past [%]	
					Cases			Controls										
Study[Ref]	Cases	Controls	Cases	Controls	No	Yes	Other	No	Yes	Other	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Bahado-Singh 2018[23]	59.1 ± 12.8	59.2 ± 12.7	36.9 ± 17.3	28.8 ± 6.8	–	–	–	–	–	–	–	–	23.2	3.3	23.2	33.3	T 1.8	T 5.0
Troisi 2017[22]	68[a] 66[b] 63[c] 68[d]	60[e] 65[f]	28.3[a] 28.9[b] 27.8[c] 27.1[d]	27.8[e] 27.1[f]	–	–	–	–	–	–	100	100	10.2[a] 13.3[b] 10.0[c] 10.0[d]	15.0[e] 14.0[f]	–	–	–	–
Knific 2018[24]	65.1 ± 8.7	63.2 ± 9.4	32.1 ± 7.3	28.3 ± 4.7	78.7	21.3	0	67.7	29.2	3.1	94.4	81.5	16.4	15.4	57.4	35.4	HRT 8.2	HRT 13.9
Shi 2018[25]	54 ± 8.0	57 ± 10	26.9 ± 5.1	25.8 ± 3.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Altadill 2018[21]	–	–	–	–	–	–	–	–	–	–	100	100	–	–	–	–	0	0
Audet-Delage 2018[13]	67.5 ± 9.42[g] 66.3 ± 8.3[h]	58.9 ± 10	28.0 ± 6.4[g] 28.4 ± 7[h]	27.5 ± 7.2	72[g] 61[h]	17[g] 22[h]	11[g] 17[h]	67	22	11	100	100	–	–	–	–	HRT 33[g] 39[h]	HRT 22

Abbreviations: HRT, Hormonal replacement therapy; T, tamoxifen.

[a]Patients with endometrial cancer of recruitment I (patients with endometrial cancer compared with a control group of healthy women).

[b]Patients with endometrial cancer of recruitment II (patients with endometrial cancer, ovarian cancer, benign endometrial disease compared with a control group of healthy women).

[c]Ovarian cancer of recruitment II.

[d]Benign endometrial disease of recruitment II.

[e]Healthy women of recruitment I.

[f]Healthy women of recruitment II.

[g]Patients with recurrent endometrial cancer.

[h]Patients with not-recurrent endometrial cancer.

TABLE 3 Endometrial cancer characteristics

Study	FIGO grade n (%)				Histopathological type n (%)			FIGO stage n (%)					Lymphovascular invasion n (%)			Miometrial invasion n (%)			Presence of metastases n (%)		
	G1	G2	G3	Missing	Endometrioid	Non endometrioid	Missing	I	II	III	IV	Missing	No	Yes	Missing	<50%	>50%	Missing	No	Yes	Missing
Bahado-Singh 2018[23]	–	–	–	–	–	–	–	45 (80.3)	1 (1.7)	8 (14.5)	2 (3.5)	0	–	–	–	–	–	–	–	–	–
Troisi 2017[22]	6 (5.1)	75 (63.5)	37 (31.4)	0	90 (76.3)	28 (23.7)	0	42 (35.6)	60 (50.8)	10 (8.4)	0	0	–	–	–	–	–	–	–	–	–
Knific 2018[24]	36 (59.0)	12 (19.7)	13 (21.3)	0	54 (88.6)	6 (9.8)	1 (1.6)	51 (83.6)	0	7 (11.5)	2 (3.3)	1 (1.6)	51 (83.6)	9 (14.8)	1 (1.6)	44 (73.3)	16 (26.2)	1 (1.6)	50 (82.0)	10 (16.4)	1 (1.6)
Shi 2018[25]	20 (43.4)	13 (28.3)	13 (28.3)	0	–	–	–	27 (58.7)	19 (41.3)	0	0	0	–	–	–	–	–	–	–	–	–
Altadill 2018[21]	36 (92.3)		0	3 (7.7)	36 (92.3)	0	3 (7.7)	19 (48.8)	10 (25.6)	10 (25.6)	0	0	-	–	–	–	–	–	–	–	–
Audet-Delage 2018[13]	6 (16.7)	16 (44.4)	14 (38.9)	0	24 (66.7)	12 (33.3)	0	–	–	–	–	–				17 (47.3)	19 (52.7)	0	29 (80.6)	7 (19.4)	0
Total	220 (73.3)		77 (25.7)	3 (1.0)	204 (80.3)	46 (18.1)	4 (1.6)	184 (58.5)	90 (28.7)	35 (11.2)	4 (1.3)	1 (0.3)	51 (83.6)	9 (14.8)	1 (1.6)	61 (63.0)	35 (36.0)	1 (1.0)	79 (81.5)	17 (17.5)	1 (1.0)

–, not reported.

clinically relevant biomarkers through non-invasive sampling (eg, blood sample).[22] Oncology appears to be one of the most applicable fields for metabolomics, given the reprogramming in the cellular metabolism associated with cancer processes.[28] In fact, the cancer growth requires an increased synthesis of phospholipids, proteins and nucleic acid compared with normal tissues.[28] It has long since been known that cancer cells tend to favor glycolysis rather than oxidative phosphorylation even in aerobic conditions (the so called "Warburg effect").[29] Other alterations of carbon metabolism involve the citric acid cycle and pentose phosphate pathway.[29] More recently, a role of amino acids in maintaining cancer cell viability and growth was described.[29] Beyond their obvious biosynthetic action, amino acids also serve as energy sources and in maintaining redox balance of cancer cells. Moreover, amino acid-derived metabolites support epigenetic and immune regulation related to cancer arising, growth and diffusion.[29] In relation to this, transporters and transaminases involved in amino acid uptake and biosynthesis have recently been proposed as potential targets for therapeutic intervention.[30]

With particular regard to EC, differences in serum concentration of many metabolites between healthy and EC women were found in several studies, with the potential to improve diagnosis.[13,21-25] These differences are substantiated by the biological evidence of a well-known or supposed role in carcinogenesis for each metabolite.

Progesterone showed lower concentration in EC patients than in healthy ones, consistent with the anti-estrogenic action and estrogen-sensitivity of ECs.[28]

Among metabolites with increased concentration in EC patients, homocysteine has shown a crucial role in the stability of DNA, and its high levels correlate with the risk of malignant neoplasms of epithelial cells.[31,32] 3-Hydroxybutyrate is synthesized from acetyl-coA by the liver and used as an energy source when blood glucose levels are low; this may be the basis of its correlation with cancer stage.[33] Linoleic acid has a controversial role in carcinogenesis: on one hand, it promotes mammary tumors in rodents[34,35] and its high dietary income correlates with a higher risk of cancer; on the other hand, several studies have been reported a linoleic acid-related reduction in human cancer risk.[36] Indoleacetic acid has shown that it can interfere with the cellular proliferation, migration, invasion and autophagy by interfering with the metabolism of tryptophan; this might result in anergy and apoptosis of effector T-cells.[37] Instead, phenylalanine inhibits proliferation without effect on apoptosis or autophagy.[38,39] Phosphocholine has shown inhibitory activity on invasion and migration, stimulating activity on proliferation and autophagy, and protective activity against tumor necrosis factor alpha-induced apoptosis.[40] Despite being nonsignificant, the higher concentration of lactic acid in EC cases is in accordance with the higher speed of anaerobic glycolysis in cancer cellular metabolism; the resulting low pH suppresses T-cells and promotes angiogenesis through the increase of interleukin 8, favoring cancer growth.[41-44] Lyso-platelet-activating factor-16 is an induced lipid mediator which promotes inflammation and has been shown to be associated with skin cancer.[45]

TABLE 4 Most relevant metabolites

Study/year[Ref]	Metabolites or their association	Possible use in endometrial cancer management	Analytical platform	Selection criteria
Bahado-Singh 2017[23]	Tetradecadienoylcarnitine Phosphatidylcholine with acyl-alkyl residue sum C38:1 3-Hydroxybutyric acid	Diagnosis (higher concentration in endometrial cancer than controls)	NMR (untargeted) + DI-MS/MS and LC-MS/MS	PLS-DA + LASSO
	Tetradecadienoylcarnitine Phosphatidylcholine with acyl-alkyl residue sum C40:1 (combined with BMI)			
Troisi 2017[22]	Lactic acid[a] Progesterone Homocysteine 3-Hydroxybuthyrate Linoleic acid Stearic acid Myristic acid Threonine Valine	Diagnosis, screening and prediction of tumor histotype (higher concentration in endometrial cancer than controls)	GC-MS (untargeted)	PLS-DA VIP Score
Knific 2018[24]	Hexadecanoylcarnitine/ Phosphatidylcholine with acyl-alkyl residue sum C40:1 Proline/tyrosine[a] Phosphatidylcholine with diacyl residue sum C42:0/ Phosphatidylcholine with acyl-alkyl residue sum C44:5	Diagnosis (lower concentration in endometrial cancer than controls of phospholipids and amino acids – higher concentration of acetylcarnitine in endometrial cancer than controls)	LC-MS/MS (targeted)	Odd ratio
	Hydroxysphingomyelins C14:1/ Hydroxysphingomyelins C24:1 Phosphatidylcholine with diacyl residue sum C40:2/ Phosphatidylcholine with diacyl residue sum C42:6	Prediction of myometrial invasion (lower concentration in endometrial cancer than controls of phospholipids – higher concentration of sphingolipids in endometrial cancer than controls)		
	Phosphatidylcholine with diacyl residue sum C34:4/ Phosphatidylcholine with acyl-alkyl residue sum C38:3 Hexadecadienyl carnitine/ Phosphatidylcholine with diacyl residue sum C38:1	Prediction of lymphovascular invasion (lower concentration in endometrial cancer than controls of phospholipids – higher concentration of acetylcarnitine in endometrial cancer than controls)		

(Continues)

TABLE 4 (Continued)

Study/year[Ref]	Metabolites or their association	Possible use in endometrial cancer management	Analytical platform	Selection criteria
Shi 2018[25]	DL-Phenylalanine Indoleacetic acid Phosphocholine Lyso-platelet-activating factor-16	Diagnosis (higher concentration in endometrial cancer than controls)	UPLC-Q/ToF-MS (untargeted)	PLS-DA and OPLS-DA VIP-score and *t* test
Altadill 2018[21]	Picolinic acid	Diagnosis and prediction of cancer progression -Antitumoral activity (lower concentration in endometrial cancer than controls)	UPLC-Q/ToF-MS (untargeted)	Student *t* test
	Vaccenic acid Arachidonic acid (peroxide free) Phosphatidic acid Phosphatidylglycerol Inosine Palmitic amide Oleamide Stearamide 13Z-Docosenamide Glutamate/phenylalanine/arginine/tryptophan	Diagnosis and prediction of cancer progression: (lower concentration in endometrial cancer than controls)		
	Linoleic acid 5,8,11-eicosatrienoic acid UDP-*N*-acetyl-D-galactosamine 1-Palmitoyl-2-linoleoyl Phosphatidylserine Phosphatidylethanolamines Phosphatidylinositols Glycerophosphocholines	Diagnosis and prediction of cancer progression: (higher concentration in endometrial cancer than controls)		

(Continues)

TABLE 4 (Continued)

Study/year[Ref]	Metabolites or their association	Possible use in endometrial cancer management	Analytical platform	Selection criteria
Audet-Delage 2018[13]	Linoleic acid (C18:2) and myristic acid (C14:0)	Diagnosis and prediction of recurrence after surgery (lower concentration in endometrial cancer than controls)	UPLC-Q-ORBITRAP (targeted)	Student *t* test
	Intermediates from the branched chain amino acid pathway, such as isovalerylcarnitine/2-methylbutyrylcarnitine	Diagnosis and prediction of recurrence after surgery (higher concentration in endometrial cancer than controls)		
	Polyamines			
	Spermine			
	Acylcholines, acylcarnitines, and monoacylglycerols			
	Bradykinin			
	Sulfated androgens			
	Heme			
	Bile acids			
	Sphingolipids and ceramides			

Abbreviations: DI, direct injection; GC-MS, gas chromatography-mass spectrometry; LASSO, least absolute shrinkage and selection operator technique; LC, liquid chromatography; MS, mass spectrometry; NMR, nuclear magnetic resonance; OPLS-DA, orthogonal partial least squares discriminant analysis; PLS-DA, partial least squares discriminant analysis; UPLC-Q-ORBITRAP, ultra-high performance liquid chromatography coupled with quadrupole and orbitrap mass spectrometers; UPLC-Q-TOF/MS, ultra-high performance liquid chromatography quadrupole time-of-flight mass spectrometry; VIP, variable importance in projection score.

[a]*P* value not significant.

Among metabolites with decreased concentration in EC patients, stearic acid has shown an in vitro inhibition capacity of tumor cell growth.[46] Decrease in inosine concentration might be related to an imbalance in the isoleucine/alanine ratio, the most common RNA modification by RNA-specific adenosine deaminase enzymes; such enzymes appear upregulated in several tumors, including EC.[47]

Amino acids showed overall lower levels in EC patients probably due to a hypermetabolic activity of the tumor cells which is accompanied by increased gluconeogenesis and protein catabolism.[48] However, this difference was not significant and would have to be further investigated. Modifications in intermediates from the branched chain amino acid pathway, such as isovalerylcarnitine/2-methylbutyrylcarnitine, were observed in cases, as well as the increased levels of polyamines and bradykinin (associated with cancer progression), spermine and heme.[49-53]

Low levels of phospholipids found in women with EC could be related to an improvement in cell membrane synthesis, and oncogenic changes in the activity of lipid transporters and catabolic enzymes. In accordance, high levels of phospholipase-A2 and lysophospholipase-D were found in cases.[54] A decrease in free fatty acid (eg, linoleic and myristic acid) levels and an increase in the concentration of conjugated fatty acids (eg, acylcholine, acylcarnitine and monoacylglycerols) were also found in EC patients.[55] Acetylcarnitine has a crucial role in the transport of fatty acids through the mitochondrial membrane for beta oxidation; therefore, high levels are related to disturbed beta oxidation with increased energy consumption and lipolysis.[56,57] The increase in monoacylglycerols could also be explained by the reduced expression of the monoacylglycerol lipase, which deals with their metabolism.[58] Overall, this unbalance might favor an estrogenic environment related to the pathogenesis of many ECs.[59]

Lastly, the possible ability of bile acids to predict recurrence after surgical treatment might be explained with the fact that they increase the sensitivity of the myometrium to hormones, show inflammatory functions, and contribute to cholesterol homeostasis that drives EC progression.[60]

As regards this, previous finding seems to support the promising results of metabolomics in diagnosis and prediction of pathological features of EC. In fact, diagnosis and characterization of EC are currently based on histologic examination and may be subjective and only slightly reproducible, even by experienced pathologists.[61-65] Therefore, great efforts have been made to find diagnostic, prognostic and predictive markers to improve the definition of uterine neoplastic lesions.[66-73] In this field, strengths of metabolomics may lie in the reproducibility of the results and in mini-invasiveness.[74,75] Furthermore, given that the prognostic stratification of EC will be revised in the light of recent molecular findings,[76-78] metabolomics might also find a role in the risk assessment of EC, as it is cheaper than sequencing analyses.[74,76] Until the association of metabolites with The Cancer Genome Atlas (TCGA) novel prognostic group of EC is assessed, metabolomics might still be useful as a prognostic tool. In fact, several metabolites seem to predict currently used prognostic factors, such as tumor histotype, myometrial invasion, lymph vascular invasion and even cancer progression. Since such factors appear to have a prognostic value independently of the TCGA molecular subgroups of EC, to predict them preoperatively through metabolomics might drive patient management (eg, in the decision whether or not to perform lymph node dissection in FIGO Stage I).[76,79]

However, further studies are necessary to validate metabolite panels and metabolomics platforms. In fact, several metabolomics platforms have been used, adopting both targeted and untargeted approaches (Table 4). The most used one appears to be liquid chromatography coupled with quadrupole, time of flight and orbitrap mass spectrometers. Although all platforms have shown promising results, prospective validation studies on larger samples are necessary. This is a crucial step to evaluate the real applicability of metabolomics in this field. Validation will pose new technical challenges due to the need for long-term reproducibility assurance and costs sustainability. Among metabolomics platforms, gas chromatography-mass spectrometry could be the best candidate to overcome these issues, as it is the cheapest in terms of both equipment and cost-per-analysis. Moreover, it shows the highest chromatographic resolution, which could reduce the need for expensive high-resolution mass spectrometers.

To our knowledge, this study is the first systematic review of metabolomics in EC diagnosis. A limitation of this study may be the impossibility of performing sub-analyses based on patient diseases (eg, diabetes mellitus, hypertension, benign gynecologic diseases and malignancy other than EC) because data were not extractable from the included studies (ie, the included studies did not perform metabolite comparisons stratified by patient diseases).

5 | CONCLUSION

Metabolomics might be useful for non-invasive diagnosis, screening and prediction of tumor histotype, myometrial invasion, lymphovascular invasion and cancer progression in patients with EC. Further studies are necessary to validate relevant metabolite panels and metabolomics platforms.

CONFLICT OF INTEREST

JT, MG and FZ applied for a Patent entitled 'Method for the diagnosis of endometrial cancer' (International Application No. PCT/EP2016/053726). The other authors have stated explicitly that they have no conflicts of interest in connection with this article.

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SUPPORTING INFORMATION

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