BJO
Brazilian Journal of Oncology
REVIEW ARTICLES CLINICAL ONCOLOGY

METABOLOMIC APPROACHES APPLIED TO THE STUDY OF CERVICAL CANCER: A SYSTEMATIC REVIEW

Natalia Damasceno Almeida[1], Marcos Almeida Matos[1], Milena Bastos Brito[1], Amancio Jose de Souza[1,2]

ABSTRACT

Background: Cervical cancer (CC) is the fourth leading cause of cancer among women in the world. Metabolomics can provide a deeper understanding of the underlying metabolic alterations associated with its pathophysiology. **Objective:** To systematically analyze metabolomic approaches and findings used in the study of cervical cancer. **Selection Criteria:** Studies that included the use of metabolomics, obtained through biological samples, from patients diagnosed with CC. **Data collection and Analysis:** The review was conducted according to the PRISMA guidelines, and registered in PROSPERO. The terms of Medical Subject Headings (MeSH) and Health Sciences Descriptors (DeCS) corresponding to "Metabolomics" and "Cervical Cancer" were used as descriptors. Article quality was reviewed based on the QUADOMICS criteria. **Results:** A total of 17 articles were selected for systematic review. Study quality evaluation using QUADOMICS demonstrated heterogeneous results. The main changes in metabolite levels associated with cervical cancer were identified in alanine, creatine, valine, tyrosine, isoleucine, phosphatidylcholine, acetate, lactate and β-glucose. Some amino acid levels were reduced in patients with CC and changes in energy metabolism pathways were observed. Eight articles tested the diagnostic capacity of metabolomics, obtaining results for sensitivity > 90%, specificity between 73% and 99% and AUC between 0.78 and 0.99. **Conclusion:** The results suggest that patients with CC present alterations in energy metabolism, amino acids and glycerolphospholipids, pointing to a potential group of specific biomarkers.

Keywords: Metabolomics; Uterine Cervical Neoplasms; Metabolic Networks and Pathways; Biomarkers.

1. Bahiana School of Medicine and Public Health, Medicine - Salvador - Bahia - Brazil.
2. University of California Riverside, - - Riverside - California - United States.

Financial support: none to declare.
Conflicts of interest: The authors declare no conflict of interest relevant to this manuscript.
Correspondence author: Amancio Jose Souza.
E-mail: amancio@ucr.edu

Received on: January 3, 2022 | **Accepted on:** July 8, 2022 | **Published on:** September 29, 2022
DOI: https://doi.org/10.5935/2526-8732.20220328

INTRODUCTION

Cervical cancer (CC) is the fourth leading cause of cancer among women worldwide[1]. One of the possible causes of CC is the persistent infection with Human Papilloma Virus (HPV). HPV is one of the most common sexually transmitted infections in the world, being the main virus associated with the development of neoplasms[2-5].

In recent years the development of new analytical techniques in DNA sequencing and biomolecule measurements has expanded the ability to characterize phenotypic changes in pathological processes[6]. Metabolomics is capable of simultaneously analyzing multiple metabolic pathways in cells, tissues, and body fluids, connecting genetic processes to phenotypic expression, and listing specific metabolites involved in the pathophysiology of diseases[6,7].

Given the current scenario in which CC continues to be a serious health problem for women, metabolomics can be a useful tool in expanding the knowledge on this disease. This review aims to systematically analyze metabolomic approaches in the study of cervical cancer, describing the specific metabolites associated with the development of cervical cancer, identifying possible biomarkers and metabolomic profiles in the context of cervical neoplasia which can lead to innovative clinical approaches.

MATERIALS AND METHODS

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA)[8] guidelines and was registered in the International Prospective Register of Systematic Reviews (PROSPERO) database under number CRD42020215301.

A computerized bibliographic search was carried out in the electronic databases MEDLINE / PubMed, Latin American and Caribbean Literature in Health Sciences (LILACS), Scientific Electronic Library Online (SciELO), Virtual Health Library (VHL) and the databases of gray literature: Google Scholar and Open Gray. As descriptors, the terms in force in the Medical Subject Headings (MeSH) and Health Sciences Descriptors (DeCS) corresponding to "Metabolomics" and "Cervical Cancer" were used. The terms were combined with the Boolean operators "AND" and "OR" (Appendix S1). A manual search was also carried out on the reference list of the selected articles. All searches were carried out from 08/28/2020 to 05/11/2021.

The works were identified by title and abstract by two reviewers (N.D.A. and A.J.S.), who followed the inclusion and exclusion criteria. Studies without abstracts whose title suggested meeting the selection criteria were also selected for analysis. All divergences were resolved by consulting a third reviewer (M.A.M.), who finally defined which articles would be fully read. The selected works were read in full by three authors. Then, the studies were included in the systematic review upon agreement of the three reviewers.

Observational studies that included the use of metabolomics, obtained through biological samples, from patients diagnosed with CC were included in the search. Articles describing the metabolic profiles of tissues and samples and the potential use of metabolomics as a diagnostic and prognostic tool were included. There was no limitation on time or publication language. Studies whose interventions involved specific analysis on drug therapy, carried out on animals, case reports, letters, abstracts, conference proceedings, case studies, review or conglomerates were excluded.

The information extracted from studies included: author, date of publication, geographic origin, title, magazine, type of study, financing, among others. Data on the participants of each work, number of participants, sex, age, use of medications and comorbidities were recorded. The patients in the selected studies were divided into three groups: patients with cervical cancer, patients with intraepithelial lesions and the control group constituted by healthy women (not every selected article described all three categories). Finally, data from metabolomic analysis were collected, such as the method used, the analyzed sample (fluid or biological tissue) and the metabolites found in association with cancer.

Article quality was accessed using QUADOMICS[9], an adaptation of the Quality Assessment of Diagnostic Accuracy Assessment (QUADAS)[10] for studies using omic technologies.

RESULTS

Identification and selection of studies

A total of 124 references were identified in the initial search. 24 articles were selected for complete reading. Seven studies were further excluded for the following reasons: two for containing analysis exclusive for HPV, two for treating only high-grade intraepithelial lesions, two for using metabolomics for specific analysis and one due to the impossibility of separating the results from cervical cancer patients from other patients. Finally, 17 articles[11-27] were selected for systematic review (Figure S1).

General characteristics of the selected studies

All studies selected for systematic review were published in the English language, from 2004 to 2021 (Table 1). Nine were of the cross-sectional type and eight were prospective studies. Most articles were of Chinese origin (10) and the relationship between the origin and the number of studies is shown in Figure S2A.

The average age of patients with CC in the investigated articles ranged between 40 and 50 years. All 576 patients with CC were diagnosed through the association of clinical analysis and histological exams and a total of 529 patients made up the control group. Five articles presented HPV testing and of the 156 cervical cancer patients tested 129 (82.7%) were HPV +. Women with intraepithelial lesions totaled 505, 182 with LSIL (NIC I), 287 with HSIL (NIC II / III) and for 36 patients the lesion degree was not described.

Table 1 - Main features of selected articles.

Author, year	Origin	N (patients)			Age (average years old)	Sample type	Platform Analytics
		CC	CIN	Control			
Abdula *et al*. 2020[11]	China	21	20 CIN II/ III	11	45.2	Cervical tissue	HRMAS ^{1}H NMR
Rodrígues-Esquivel *et al*. 2018[12]	Mexico	15	-	15	Women over 20 years old	Volatile organic components	GC/MS
Ilhan *et al*. 2019[13]	USA	10	12 LSIL 27 HSIL	29	38+8	Cervical washes	UPLC/MS
Khan *et al*. 2019[14]	Korea	60	55 CIN I 42 CIN II/III	69	CC: 50 / Control: 48 / CIN I: 35 / CIN II / III: 39.5	Plasma	UPLC-QTOF/MS
Paraskevaidi *et al*. 2020[15]	England	5	30 CIN I 40 CIN II/III	49	CC: 47.6 / Control: 35.1 CIN I: 35 34.9 / CIN II: 35.3 / CIN III: 34.2	Cervical cells	LA-REIMS
Yang *et al*. 2017[16]	China	66	-	69	CC: 49.84 / Control: 54	Plasma	UPLC-QTOF/MS
Ye *et al*. 2015[17]	China	18	9	-	CC: 40 / CIN: 33	Serum	1H NMR
Zhou *et al*. 2019[18]	China	30 AT 30 PR 30 PB	-	-	BT: 52.20 / PP: 53.27 / GP: 52.40	Plasma	UPLC-QTOF/MS
De Silva *et al*. 2009[19]	England	23	5 CIN I 40 CIN II/III	5	CC + CIN: 37 / Control: 52	Cervical tissue	HRMAS 1H NMR
Hasim *et al*. 2012[20]	China	38	2 CIN I 36 CIN II/III	38	CC:45.6±0.3 / Control: 41.6 ± 0.3 / CIN: 39.6 ± 0.7	Plasma	1H NMR
Hasim *et al*. 2013[21]	China	22	10 CIN II 16 CIN III	35	CC: 52.7 / CIN: 46.3	Plasma	HPLC-MS
Shi *et al*. 2015[22]	China	5	-	6	CC: 35-43 Control: 25-28	Serum	GC-MS
Sitter *et al*. 2004[23]	Norway	8	-	8	50	Cervical tissue	HRMAS 1H NMR
Yin *et al*. 2015[24]	China	89	-	93	CC: 46.39±9.56 Control: 47.55 ±7.07	Plasma	UPLC-MS
Chen *et al*. 2021[25]	China	21	13 LSIL 22 HSIL	24	-	Cervical tissue	GC-MS
Nan *et al*. 2021[26]	Korea	60	55 CIN I 44 CIN II/III	66	CC: 50 / CIN I: 35 / CIN II / III: 39.5 Control: 48	Plasma	UPLC−QTOF−MS
Cheng *et al*. 2020[27]	China	25	27 CIN	20	CC: 48.48 ± 2.29 / CIN: 46.67 ± 1.64 / Control: 47.30 ± 2.71	Serum	UHPLC-QTOF/MS

Caption: 1H NMR = Proton nuclear magnetic resonance. HRMAS 1H NMR = High resolution magic angle rotating nuclear magnetic resonance. GC-MS = Gas chromatography coupled to mass spectrometry. UPLC-MS = Ultra-efficient liquid chromatography coupled with mass spectrometry. UPLC-QTOF / MS = Ultra high-performance liquid chromatography quadrupole time-of-flight mass spectrometry. LA-REIMS = Laser ablation coupled with rapid evaporative mass spectrometry. MALDI-TOF / MS = Matrix-assisted laser ionization and desorption coupled with mass spectrometry. CC = Cervical cancer. CIN = cervical intraepithelial neoplasia. Control= Healthy woman. LSIL= Low-grade intraepithelial lesion. HSIL = High-grade intraepithelial lesion. - = not reported.

Table 2 - Main results from selected articles.

Reference	Altered metabolites in the CC	P value	Sensitivity	Specificity	AUC	Conclusion
Khan *et al*. 2019[14]	CC x Control and CC x CIN: AMP, aspartate, glutamate, hypoxanthine, lactate, proline, pyroglutamate.	<0.01	-	-	CC x Control: 0.83* / CC X CIN: 0.78*	High level of the referred metabolites presents a greater risk of developing cervical dysplasia (OR 2.94-4.48).
Yang *et al*. 2017[16]	CC x Control: Bilirubin, lysoPC (17: 0), n-oleoyl threonine, 12-hydroxydodecanoic acid and tetracosahexaenoic acid.	<0.05	0.98	0.99	0.99*	The combination of 5 biomarkers establishing a promising method for the diagnosis and screening of cervical cancer.
Ye *et al*. 2015[17]	CC x CIN: Alanine, glutamine, carnitine, inositol, β-glucose and formate.	<0.05	-	-	-	These 6 metabolites can be identified as potential biomarkers and used to discriminate CC.
Zhou *et al*. 2019[18]	Phosphatidylcholine [PC (15: 0/16: 0)], phosphatidylglycerol [PG (12: 0/13: 0), lactosylceramide, D-Maltose and phthalic acid.	-	BT x PP: 0.94 BT x GP:0.92 GP x PP: 0.86	BT x PP: 0.87 BT x GP:0.89 GP x PP: 0.80	BT x PP: 0.97* BT x GP:0.97* GT vs PP: 0.91*	Five metabolites were identified as potential biomarkers for prognosis of patients with CC.
Hasim *et al*. 2012[20]	CC x control: Acetate, format, creatine, lactate, isoleucine, leucine, valine, alanine, glutamine, histidine and tyrosine. / CC X CIN: Acetate, acetone, format, glycoprotein, α-glucose and β-glucose.	<0.05	>0.90	>0.95	-	Differences in metabolomic signatures that can distinguish between CC, CIN and healthy controls.
Hasim *et al*. 2013[21]	CC x Control and CC X CIN: Arginine, threonine, aspartate, glutamate, asparagine, serine, glycine, histidine, taurine, tyrosine, valine, methionine, lysine, isoleucine, leucine and phenylalanine.	<0.04				Plasma free amino acid profiles may have the potential to be used to diagnose cancer and improve the understanding of it's mechanisms.
Shi *et al*. 2015[22]	CC x Control: Ethanedioic acid, phosphate, guloCIN acid, ethoxypropionic acid, pyruvic acid, l-valine, propanoic acid, 2-ketoiso-caprylic acid, isotridecyl alcohol, adenosine, l-oroline, dimethylpyruvic acid and tridecanol.	<0.05	-	-	-	The results suggested that metabolomics is capable of differentiating cervical cancer patients from healthy controls.
Yin *et al*. 2015[24]	CC x Control: PC (18: 2/20: 5), lysoPC (10: 0), lysoPC (18: 0) and PC (18: 1/15: 0)	-	0.93	0.91	0.972*	Four candidate biomarkers for diagnostic purposes, which when combined have high sensitivity, specificity and AUC.
Nam *et al*. 2021[26]	CC X control: Acylcarnitine, Diglyceride, LysoPE, PC, PE and free fatty acid./ CC X CIN: Acylcarnitine, ceramide, triglyceride, diglyceride, LysoPE, PC and free fatty acid.	<0.05	-	-	-	These results suggest that the lipid profile is a useful method for monitoring the progression to cervical cancer.

continue...

...continue

Table 2 - Main results from selected articles.

Reference	Altered metabolites in the CC	P value	Sensitivity	Specificity	AUC	Conclusion
Cheng *et al*. 2020[36]	PC 14:0/18:2, PE 15:1e/22:6, PE 16:1e/18:2, and PE 16:1e/20:5	-	0.920	0,915	0,961*	This panel was effective in distinguishing between CC and non-CC.
Abdula *et al*. 2020[11]	CC x CIN + Control: LDL, lactate, alanine, α-glucose, β-glucose, tyrosine and phenylalanine CC x Control: Creatine, acetate, scyllo-inositol, isoleucine and methylproline.	-	-	-	-	Reduction in the levels of α and β-glucose in CC and CIN group, increase in lactate and LDL and alteration in the expression of multiple amino acids in CC group compared with control group.
De Silva *et al*. 2009[19]	CC x Control: PE, phosphate, alanine, creatine CC x CIN: PC and choline.	<0.01	-	-	-	PE and phosphate were increased, and alanine and creatine reduced in patients with CC compared to control.
Sitter *et al*. 2004[23]	CC x Control: Lactate, methyl and methylene groups of lipids, compounds containing choline, creatine, taurine, alanine and β-glucose.	-	-	-	-	Larger sample studies are needed to assess the clinical value of this method in the CC.
Chen *et al*. 2021[25]	CC x Control Glucose, lactate, glycine, aspartate, alanine, tyrosine, phosphate, pyrimidine and arachidonate.	<0.05	-	-	-	The content of various amino acids, such as glycine, aspartate, alanine, tyrosine and serine, increased in CC.
Rodrígues-Esquivel *et al*. 2018[12]	CC x Control: Octane, 2,2,6-trimethyl-992 (alkane), decane, 2,6,10-trimethyl-1320 (alkane), octane, 2,2,6-trimethyl 1029 (alkane), undecane, 2,2- dimethyl-1828 (alkane), 2-methylpentyl 917 (Ester) and hexane, 2,2,4-trimethyl- (Alkane).	<0.05	0.93	0.93	0.86*	Specific VOCs are potential biomarkers and can function as a complementary tool for the diagnosis of the disease.
Ilhan *et al*. 2019[13]	CC x Control: 3-hydroxybutyrate, eicosenoate and oleate / vaccenate	-	-	-	>0.90**	Cervical dysplasia led to depletion of metabolites. These amino acid and nucleotide metabolites were able to distinguish the studied groups.
Paraskevaidi *et al*. 2021[5]	-	-	0.91	0.73	0.867 (95% CI 0.746-0.947)	LA-REIMS was able to classify 41 out of 45 patients with high-grade lesions and CC and 40 out of 55 healthy individuals.

Legend: AUC = Area under the curve. CC = Cervical cancer. CIN = cervical intraepithelial neoplasia. BT = before treatment / GP = good prognosis / PP = poor prognosis. LDL = low density lipoproteins. PC = phosphatidylcholine. PE = phosphatidylethanolamine. LysoPC = lysophosphatidylcholine. LA-REIMS = Laser ablation coupled with rapid evaporative mass spectrometry. VOCs = Volatile organic compounds. / - = not reported. * = AUC results obtained by combining the altered metabolites, the values for each specific metabolite can be found in the original article. ** = The AUC value was> 0.90 for each of the metabolites individually, the study did not present an analysis of the combined.

Sample types used for metabolomic analysis included blood serum/plasma (10), cervical tissue (4), cervical cells (1), volatile organic components (VOCs; 1) and cervical washes (1). Metabolomics studies were conducted using NMR (five articles), liquid chromatography coupled to mass spectrometry (LC-MS; eight articles), gas chromatography coupled to mass spectrometry (GC-MS; three articles) and laser ablation coupled to rapid evaporative mass spectrometry (LA-REIMS; 1 article) (Figure 2B and 2C).

Within the 17 selected studies, 16 reported some type of support or funding. Only Sitter et al. (2004) did not report any financial support.

Quality assessment of articles using QUADOMICS

From the 17 items recommended by QUADOMICS two were not used (items 2 and 14) since these are applicable to phase IV clinical studies which were not present in our sampled articles. Six articles reached 80% or more of the evaluated QUADOMICS criteria (12/15). Among these, Paraskevaidi et al. (2020) and Ying et al. (2015) obtained the highest score, reaching the proposed quality criteria for 13 items (87%; Table S1).

Metabolites Associated with Cervical Cancer

75 metabolite alterations were mentioned as relevant in the context of cervical cancer (Table 2). Of these, 24 were mentioned by two or more studies. Alanine was cited as relevant in six articles, lactate in five, β-glucose and creatine in four studies, these being the metabolites with the highest overall frequency among those listed (Table 3).

In 10 studies that used blood serum/plasma as a biological sample, 18 metabolites were found in common. Metabolites showing decrease in patients with CC were: valine, glutamine, histidine, isoleucine and leucine. In two articles lysophosphatidylcholines (lysoPC) and acetate were described as increased. B-glucose, alanine, lactate, aspartate, glutamate, formate, tyrosine and threonine were not consistentaly altered in the presented studies. Phosphatidylcholine was decreased in the three studies where it was detected, interestingly Zhou et al. in 2019 reported that this reduction occurred among patients with poor prognosis, whereas patients with good prognosis had an increase in this metabolite in their samples (Table 4).

Table 3 - Metabolites found in common in selected studies.

Metabolites	Studies	Sample	Disturbance	Statistical analysis
Alanine	Abdula *et al*. 2020	Cervical tissue	Increase	-
	Silva *et al*. 2008	Cervical tissue	Decrease	P=0.01
	Sitter *et al*. 2004	Cervical tissue	Increase	-
	Chen *et al*. 2021	Cervical tissue	Increase	P=0.003
	Ye *et al*. 2015	Plasma/Serum	Increase	P=0.024
	Hasim *et al*. 2012	Plasma/Serum	Decrease	P<0.05
Creatine	Abdula *et al*. 2020	Cervical tissue	Decrease	-
	Silva *et al*. 2008	Cervical tissue	Decrease	P= 0.008
	Sitter *et al*. 2004	Cervical tissue	Increase	-
	Hasim *et al*. 2012	Plasma/Serum	Decrease	P>0.05
Lactate	Abdula *et al*. 2020	Cervical tissue	Increase	-
	Sitter *et al*. 2004	Cervical tissue	-	-
	Chen *et al*. 2021	Cervical tissue	Increase	P=0.01
	Khan *et al*. 2019	Plasma/Serum	Increase	AUC= 0.74 / P<0.001
β-glucose	Hasim *et al*. 2012	Plasma/Serum	Decrease	P<0.05
	Abdula *et al*. 2020	Cervical tissue	Decrease	-
	Sitter *et al*. 2004	Cervical tissue	Decrease	-
	Ye *et al*. 2015	Plasma/Serum	Decrease	P=0.039
Valine	Hasim *et al*. 2012	Plasma/Serum	Increase	P>0.05
	Hasim *et al*. 2012	Plasma/Serum	Decrease	P<0.05
	Hasim *et al*. 2013	Plasma/Serum	Decrease	P=0.000
	Shi *et al*. 2015	Plasma/Serum	Decrease	P=0.001
Tyrosine	Abdula *et al*. 2020	Cervical tissue	Decrease	-
	Chen *et al*. 2021	Cervical tissue	Increase	P=0.009
	Hasim *et al*. 2012	Plasma/Serum	Decrease	P<0.05
	Hasim *et al*. 2013	Plasma/Serum	Decrease	P=0.027

continue...

...continue

Metabolites	Studies	Sample	Disturbance	Statistical analysis
Isoleucine	Abdula *et al*. 2020	Cervical tissue	Decrease	-
	Hasim *et al*. 2012	Plasma/Serum	Decrease	P<0.05
	Hasim *et al*. 2013	Plasma/Serum	Decrease	P=0.004
	Silva *et al*. 2008	Cervical tissue	Increase	P>0.05
PC	Nan *et al*. 2021	Plasma/Serum	Decrease	P<0.001
	Cheng *et al*. 2020	Plasma/Serum	-	AUC: 0.842
	Zhou *et al*. 2019	Plasma/Serum	Increase GP group Decrease PP group	P=0.00 / ATxPR -AUC:0.78 / ATxPB - AUC:0.78 / PB x PR - AUC:0.90
	Yin *et al*. 2015	Plasma/Serum	Decrease	AUC>0.79
Acetate	Abdula *et al*. 2020	Cervical tissue	Decrease	-
	Ye *et al*. 2015	Plasma/Serum	Increase	P<0.05
	Hasim *et al*. 2012	Plasma/Serum	Increase	P<0.05
α-glucose	Abdula *et al*. 2020	Cervical tissue	Decrease	-
	Hasim *et al*. 2012	Plasma/Serum	Increase	P>0.05
Aspartate	Chen *et al*. 2021	Cervical tissue	Increase	P<0.001
	Khan *et al*. 2019	Plasma/Serum	Increase	AUC= 0.76 / P<0.001
	Hasim *et al*. 2013	Plasma/Serum	Decrease	P=0.000
Glutamate	Khan *et al*. 2019	Plasma/Serum	Increase	AUC= 0.81 / P<0.001
	Hasim *et al*. 2013	Plasma/Serum	Decrease	P=0.007
Lyso PC	Yang *et al*. 2017	Plasma/Serum	Increase	AUC: 0.94
	Yin *et al*. 2015	Plasma/Serum	Increase	AUC>80
Threonine	Yang *et al*. 2017	Plasma/Serum	Increase	AUC: 0.85
	Hasim *et al*. 2013	Plasma/Serum	Decrease	P=0.007
Glutamine	Ye *et al*. 2015	Plasma/Serum	Decrease	P=0.030
	Hasim *et al*. 2012	Plasma/Serum	Decrease	P<0.05
Formate	Ye *et al*. 2015	Plasma/Serum	Decrease	P=0.012
	Hasim *et al*. 2012	Plasma/Serum	Increase	P<0.05
Choline	Silva *et al*. 2008	Cervical tissue	Increase	P>0.05
	Sitter *et al*. 2004	Cervical tissue	Increase	-
Phenylalanine	Abdula *et al*. 2020	Cervical tissue	Decrease	-
	Hasim *et al*. 2013	Plasma/Serum	Decrease	P=0.002
Histidine	Hasim *et al*. 2012	Plasma/Serum	Decrease	P<0.05
	Hasim *et al*. 2013	Plasma/Serum	Decrease	P=0.000
Phosphate	Silva *et al*. 2008	Cervical tissue	Increase	P=0.004
	Chen *et al*. 2021	Cervical tissue	Increase	P=0.017
	Shi *et al*. 2015	Plasma/Serum	Increase	P=0.02
Taurine	Sitter *et al*. 2004	Cervical tissue	Increase	-
	Hasim *et al*. 2013	Plasma/Serum	Decrease	P=0.047
Leucine	Hasim *et al*. 2012	Plasma/Serum	Decrease	P<0.05
	Hasim *et al*. 2013	Plasma/Serum	Decrease	P=0.003
Glycine	Chen *et al*. 2021	Cervical tissue	Increase	P=0.001
	Hasim *et al*. 2013	Plasma/Serum	Decrease	P=0.000
PE	Nan *et al*. 2021	Plasma/Serum	Decrease	P<0.05
	Cheng *et al*. 2020	Plasma/Serum	-	AUC>0.788

Caption: BT = before treatment / GP = good prognosis / PP = poor prognosis. / AUC = Area under the curve. / LysoPC = lysophosphatidylcholine. - = not reported

Nine of the reviewed articles performed statistical analysis on the accuracy of metabolimic techniques used in the diagnosis of CC (Table 3). Paraskevaidi et al. (2020) reported that the LA-REIMS of cell samples was able to classify 41 out of 45 patients with high-grade lesions and CC, presenting a sensitivity of 91%, specificity of 73% and AUC of 86%.

Only Zhou et al. (2019), used metabolomics as a prognostic analysis method in cervical cancer. This study, which analyzed blood plasma samples, demonstrated that phosphatidylcholine [PC (15: 0/16: 0)], phosphatidylglycerol [PG (12: 0/13: 0), lactosylceramide [LacCer (d18: 1/16: 0)], D-Maltose and phthalic acid can function as potential biomarkers when analyzed together to monitor the prognosis of patients with CC (Table 3).

Eleven studies analyzed patients with cervical intraepithelial lesions. Abdula et al. (2019) found that the levels of α-glucose and β-glucose were reduced in the tissue of patients with CIN when compared with patients with CC (Table 3). Ilhan et al. (2019) reported 71 metabolites which allowed the authors to distinguish between patients with cervical cancer and high-grade injuries with an AUC> 0.80. Ye at al. (2015) reported an increase in alanine and reduced levels of glutamine, carnitine, inositol, β-glucose and formate in cervical lavage samples from patients with cervical cancer compared to CIN. Hasim et al. (2012) found that changes in plasma levels of acetate, acetone, formate, glycoprotein, α-glucose and β-glucose can form a unique profile capable of differentiating CC from CIN. Chen et al. (2021) indicated that their results were able to distinguish precancerous cervical lesions from normal cervical epithelium and cervical cancer.

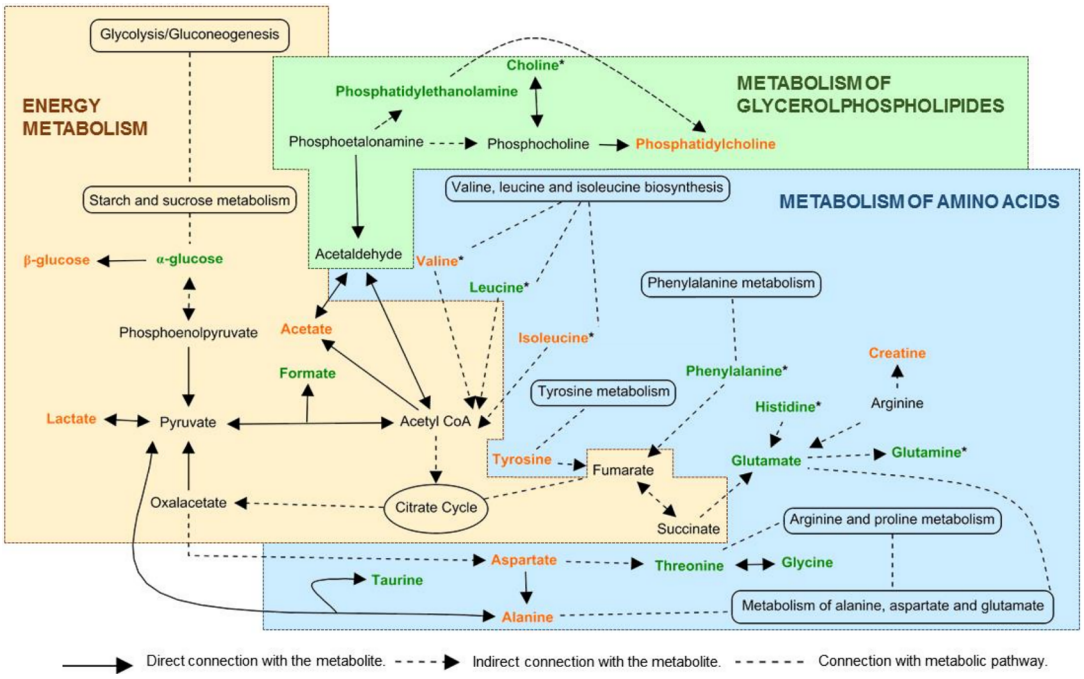
DISCUSSION

The age of patients with cervical cancer ranged between 40 and 50 years in the studies listed by this systematic review (Table 1). Such data is compatible with studies by the American Cancer Society that reports the average age at diagnosis of 50 years[28]. In addition, most studies used mass spectrometry (MS) as the basis for the metabolomic analysis (Figure S2B), the same was observed by Ahmed-Salim et al. (2020) in their systematic review on the use of metabolomics in the study of other types of cancer.

The main metabolites described as relevant by the articles studied in this review are amino acids and components related to carbohydrate metabolism (Table 2). Of the 24 compounds found in common, 14 were amino acids, which were largely reduced in samples from patients with CC (Table 3). These results are further supported by a previous systematic review by Ahmed-Salim et al. (2020) which also observed a decrease in amino acid levels in patients with ovarian cancer.

The decrease in amino acid levels in samples from patients with CC may be related to an increased demand for these metabolites by cancer cells, which need them for survival and growth[30]. These compounds are linked to several metabolic pathways, participating in anabolic and energetic processes by supplying raw materials such as carbon and nitrogen, in addition to playing an important role in cell signaling pathways[30]. Figure 1 illustrates the pathways in which the main metabolites described in this systematic review are involved, demonstrating the link between amino acids and energetic pathways. Glutamine is reported as decreased in the blood of patients with CC by the studies listed in this review.

Figure 1 – Connection between the metabolic pathways of the most cited compounds among the selected studies.



Legend: Metabolites in orange were cited as relevant by three or more articles, the metabolites in green were cited by at least two studies. Metabolites with (*) had a consistent disturbance in all articles in which they were mentioned. Metabolic pathway references extracted from Kanehisa (1996).

The decrease of this component in the plasma may be directly related to the activation of oncogenes and the inactivation of tumor suppressors, such as the Rb protein, which induce the use of glutamine and other important metabolites during the unregulated proliferation of neoplastic cells.

The most prominent altered metabolites within carbohydrate metabolism were β-glucose, α-glucose and lactate. β-glucose levels were decreased in most articles that reported it, in contrast, there was no consensus for α-glucose. Lactate was reported to be increased in three of the four studies that described lactate alteration (Table 3). Turkoglu et al. (2016) also found contradictory results regarding the regulation of carbohydrate related metabolites, in a systematic review of ovarian cancer biomarkers. Taken together these reports support the association between alterations in energy metabolism and cancer. However, the literature points to a lack of consistency in regards to the types and directional regulation of these cancer associated metabolites.

Glucose depletion and lactate increase maybe due to the Warburg effect, which suggests that an anaerobic glycolysis process can occur in tumors where glucose is converted into lactic acid even in the presence of oxygen[32]. As the tumor expands and the need for oxygen increases, the use of glycolysis becomes an advantageous option, to prevent the development of hypoxic conditions[33]. In addition, it is believed that this process creates a favorable catabolic state for tumor cells during rapid growth. The more undifferentiated the cancer cell, the greater the lactate production resulting from this process[32, 34]. The Warburg effect is further involved in cell proliferation and malignant progression of cancer through an intricate cell signaling pathway that promotes metabolic changes[32 35]. Thus, neoplasms in different stages may have different levels of energetic metabolites, which could possibly explain the contradictory findings in the listed studies.

Other metabolites such as phosphatidylcholine, phosphatidylethanolamine, lysophosphatidylcholine and choline were found to be significantly increased in the reviewed studies (Table 3). Wang et al. (2010) described a similar increase in choline levels in a systematic review focusing on colorectal cancer. Choline is an essential nutrient for the synthesis of the plasma membrane. Through the action of choline kinase (CHK), choline is phosphorylated into phosphocholine, a precursor to phosphatidylcholine (Figure 1). These compounds derived from choline contribute to cell proliferation and apoptosis processes, thus, changes in the metabolism of these components can affect cell growth and tumor survival[37, 38].

The diagnostic capacity of metabolomics was tested by nine studies in this review. Sensitivity was greater than 90%, specificity ranged from 73% to 99% and AUC varied from 0.78 to 0.99 for sets of metabolites. These results demonstrate that metabolomic approaches can be an accurate method for the diagnosis of cervical cancer. Ilhan et al. (2019), Khan et al. (2019), Hasim et al. (2012), Chen et al. (2021) and Cheng et al. (2020) also highlighted the ability of metabolomics to differentiate cancer patients from those with CIN (Table 3). Other systematic reviews have also pointed to metabolomics as a potential method to diagnose ovary[29]; colorectal[36] and endometrium cancers [39]. The use of metabolomics to identify specific types of cancer, by evaluating specific differences between metabolites, would be extremely useful for clinical practice. However, there are still no tools or research capable of establishing distinctions between different malignancies based solely on metabolic profiles [7].

The analysis carried out using QUADOMICS (Table S1) exposes a lack of detailed descriptions about patient selection and use of a standard diagnostic test for CC. Likewise, most studies were not blinded to patient diagnosis or did not make this information clear, potentially introducing bias. The heterogeneity of the results obtained using QUADOMICS was an important limitation, although most articles (70.5%) fulfilled at least 10 of the 15 items analyzed, others were rated poorly due to the lack of methodological and/or descriptive rigor in the study methodology. In addition, the different biological samples used, as well as the lack of cancer staging, were other limiting points within our analysis.

CONCLUSION

The reviewed CC metabolomic studies suggest blood as the main type of biological sample used and mass spectrometry as the preferred analytical platform. The average age of patients with cervical cancer ranged between 40-50 years and most studies added a comparative group of patients with cervical intraepithelial lesions to perform their analysis. Furthermore, we can conclude that the methodological quality of the identified articles was heterogeneous, with a gap of 8 QUADOMICS criteria being met between the studies with the highest scores compared to the lowest scores.

The main metabolites found to be associated with cervical cancer were alanine, creatine, valine, tyrosine, isoleucine, phosphatidylcholine, acetate, lactate and β-glucose. This suggest that a metabolomic profile associated with changes in energy pathways and their connections with amino acid depletion, as well as with changes in the metabolism of glycerophospholipids may be common to CC. In addition, the results described point to the effectiveness of metabolomics in the diagnosis of cervical cancer and in the differentiation of patients with intraepithelial lesions. The expansion and refinement of metabolomic approaches towards CC hold great potential for clinical applications.

CONTRIBUTION TO AUTHORSHIP

Data collection NDA, AJ de S. Data analysis: NDA, AJ de S, MAAM., MBB. Writing of the manuscript: NDA, AJ de S. Manuscript review: NDA, AJ de S, MAAM., MBB.

REFERENCES

1. World Health Organization. International Agency for Research on Cancer. Globocan. (World) in 2018, all cancers, females, all ages. World Heal Organ [internet] 2018 [Access in: 09/02/2020]. Available in: https://cutt.ly/ihjmtKW.
2. Instituto Nacional de Câncer (Brasil). Estimativa 2020. Incidência do Câncer no Brasil. Rio de Janeiro: INCA. [Internet]. 2020. [Access in: 09/02/2020]. Available in: https://www.inca.gov.br/sites/ufu.sti.inca.local/files/media/document/estimativa-2020-incidencia-de-cancer-no-brasil.pdf.
3. Instituto Nacional de Câncer (Brasil). Atlas da Mortalidade. [Internet]. 2020 [Access in: 09/02/2020]. Available in:https://mortalidade.inca.gov.br/MortalidadeWeb/.
4. Serrano B, Brotons M, Bosch FX, Bruni L. Epidemiology and burden of HPV-related disease. Best Pract Res Clin Obstet Gynaecol [Internet]. 2018 [Access in: 09/19/2020]; 47:14–26. Available in:https://doi.org/10.1016/j.bpobgyn.2017.08.006.
5. Petry KU. HPV and cervical cancer. Scand J Clin Lab Invest Suppl.[Internet].2014 [Access in: 09/02/2020]; 244:59-62. Available in:https://doi.org/10.3109/00365513.2014.936683.
6. Li B, He X, Jia W, Li H. Novel Applications of Metabolomics in Personalized Medicine: A Mini-Review. Molecules. [Internet]. 2017 [Access in: 09/02/2020]; 22(7):1–10. Available in:ttps://doi.org/10.3390/molecules22071173.
7. Kumar A, Misra BB. Challenges and Opportunities in Cancer Metabolomics. Proteomics. [Internet]. 2019 [Access in: 09/02/2020]; 19(21–22). Available in:https://doi.org/10.1002/pmic.201900042.
8. Galvão TF, Pansani, TSA, Harrad D. Principais itens para relatar Revisões sistemáticas e Meta-análises: A recomendação PRISMA. Epidemiol e Serviços Saúde. [Internet]. 2015 [Access in: 10/10/2020]; 24(2):335–42. Available in:https://doi.org/10.5123/S1679-49742015000200017,
9. Lumbreras B, Porta M, Márquez S, Pollán M, Parker LA, Hernández-Aguado I. QUADOMICS: An adaptation of the Quality Assessment of Diagnostic Accuracy Assessment (QUADAS) for the evaluation of the methodological quality of studies on the diagnostic accuracy of '-omics'-based technologies. Clin Biochem [Internet]. 2008 [Access in: 10/10/2020]; 41(16–17):1316–25. Available in:http://dx.doi.org/10.1016/j.clinbiochem.2008.06.018
10. Whiting, P., Rutjes, A. W., Reitsma, J. B., Bossuyt, P. M., & Kleijnen, J. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. BMC medical research methodology. [Internet]. 2003 [Access in: 10/9/2020]; 3, 25. Available in:https://doi.org/10.1186/1471-2288-3-25
11. Abudula, A., Rouzi, N., Xu, L., Yang, Y., & Hasimu, A.. Tissue-based metabolomics reveals potential biomarkers for cervical carcinoma and HPV infection. Bosnian journal of basic medical sciences. [Internet]. 2020 [Access in: 09/28/2020]; , 20(1), 78–87. Available in: https://doi.org/10.17305/bjbms.2019.4359.
12. Rodríguez-Esquivel, M., Rosales, J., Castro, R., Apresa-García, T., Garay, Ó., Romero-Morelos, P., Marrero-Rodríguez, D., Taniguchi-Ponciano, K., López-Romero, R., Guerrero-Flores, H., Morales, B., Mendoza-Rodríguez, M., Mosso-Lara, D., Núñez-Nolasco, I., Castro-Alba, P., Meza-Toledo, S. E., & Salcedo, M. Volatolome of the Female Genitourinary Area: Toward the Metabolome of Cervical Cancer. Archives of medical research. [Internet]. 2018 [Access in: 09/28/2020]; 49(1), 27–35. Available in:https://doi.org/10.1016/j.arcmed.2018.04.004.
13. Ilhan, Z. E., Łaniewski, P., Thomas, N., Roe, D. J., Chase, D. M., & Herbst-Kralovetz, M. M. Deciphering the complex interplay between microbiota, HPV, inflammation and cancer through cervicovaginal metabolic profiling. EBioMedicine. [Internet]. 2019 [Access in: 09/28/2020]; 44, 675–690. Available in: https://doi.org/10.1016/j.ebiom.2019.04.028.
14. Khan, I., Nam, M., Kwon, M., Seo, S. S., Jung, S., Han, J. S., Hwang, G. S., & Kim, M. K. LC/MS-Based Polar Metabolite Profiling Identified Unique Biomarker Signatures for Cervical Cancer and Cervical Intraepithelial Neoplasia Using Global and Targeted Metabolomics. Cancers. [Internet]. 2019 [Access in: 09/28/2020];11(4),511.Availablein:https://doi.org/10.3390/cancers11040511.
15. Paraskevaidi, M., Cameron, S., Whelan, E., Bowden, S., Tzafetas, M., Mitra, A., Semertzidou, A., Athanasiou, A., Bennett, P. R., MacIntyre, D. A., Takats, Z., & Kyrgiou, M. Laser-assisted rapid evaporative ionisation mass spectrometry (LA-REIMS) as a metabolomics platform in cervical cancer screening. EBioMedicine [Internet]. 2020 [Access in: 09/28/2020]; 60, 103017. Available in:https://doi.org/10.1016/j.ebiom.2020.103017.
16. Yang, K., Xia, B., Wang, W., Cheng, J., Yin, M., Xie, H., Li, J., Ma, L., Yang, C., Li, A., Fan, X., Dhillon, H. S., Hou, Y., Lou, G., & Li, K. A Comprehensive Analysis of Metabolomics and Transcriptomics in Cervical Cancer. Scientific reports. [Internet]. 2017 [Access in: 10/13/2020]; 7, 43353. Available in:https://doi.org/10.1038/srep43353.
17. Ye, N., Liu, C., & Shi, P. Metabolomics analysis of cervical cancer, cervical intraepithelial neoplasia and chronic cervicitis by 1H NMR spectroscopy. European journal of gynaecological oncology. [Internet]. 2015 [Access in: 10/13/2020]; 36(2), 174–180.Available in:https://pubmed.ncbi.nlm.nih.gov/26050356/.
18. Zhou, H., Li, Q., Wang, T., Liang, H., Wang, Y., Duan, Y., Song, M., Wang, Y., & Jin, H. Prognostic biomarkers of cervical squamous cell carcinoma identified via plasma metabolomics. Medicine. [Internet]. 2019 [Access in: 10/13/2020]; 98(26), e16192. Available in:https://doi.org/10.1097/MD.
19. De Silva, S. S., Payne, G. S., Thomas, V., Carter, P. G., Ind, T. E., & deSouza, N. M. Investigation of metabolite changes in the transition from pre-invasive to invasive cervical cancer measured using (1)H and (31) P magic angle spinning MRS of intact tissue. NMR in biomedicine. [Internet]. 2009 [Access in: 10/13/2020]; 22(2), 191–19841(16–17):1316–25. Available in:https://doi.org/10.1002/nbm.1302.
20. Hasim, A., Ali, M., Mamtimin, B., Ma, J. Q., Li, Q. Z., & Abudula, A. Metabonomic signature analysis of cervical carcinoma and precancerous lesions in women by (1) H NMR spectroscopy. Experimental and therapeutic medicine. [Internet]. 2012 [Access in: 10/10/2020];

3(6), 945–951. Available in:https://doi.org/10.3892/etm.2012.509

21. Hasim, A., Aili, A., Maimaiti, A., Mamtimin, B., Abudula, A., & Upur, H. Plasma-free amino acid profiling of cervical cancer and cervical intraepithelial neoplasia patients and its application for early detection. Molecular biology reports. [Internet]. 2013 [Access in: 10/13/2020]; 40(10), 5853–5859. Available in:https://doi.org/10.1007/s11033-013-2691-3.

22. Shi, P., Zhang, L., Ye, N. Serum Metabolomic Analysis of Cervical Cancer Patients by Gas Chromatography-Mass Spectrometry. Asian J. Chem. [Internet]. 2015 [Access in: 10/13/2020]; / 27(2) / pp 547-551. Available in:https://doi.org/10.14233/ajchem.2015.17027

23. Sitter, B., Bathen, T., Hagen, B., Arentz, C., Skjeldestad, F. E., & Gribbestad, I. S. Cervical cancer tissue characterized by high-resolution magic angle spinning MR spectroscopy. Magma (New York, N.Y.). [Internet]. 2004 [Access in: 10/13/2020]; 16(4), 174–181. Available in: https://doi.org/10.1007/s10334-003-0025-5

24. Yin, M. Z., Tan, S., Li, X., Hou, Y., Cao, G., Li, K., Kou, J., & Lou, G. (2016). Identification of phosphatidylcholine and lysophosphatidylcholine as novel biomarkers for cervical cancers in a prospective cohort study. Tumour biology: the journal of the International Society for Oncodevelopmental Biology and Medicine. [Internet]. 2016 [Access in: 10/13/2020]; 37(4), 5485–5492. Available in:https://doi.org/10.1007/s13277-015-4164-x.

25. Chen, X., Yi, C., Yang, M. J., Sun, X., Liu, X., Ma, H., Li, Y., Li, H., Wang, C., He, Y., Chen, G., Chen, S., Yu, L., & Yu, D. (2021). Metabolomics study reveals the potential evidence of metabolic reprogramming towards the Warburg effect in precancerous lesions. Journal of Cancer, 12(5), 1563-1574. https://doi.org/10.7150/jca.54252

26. Nam, M., Seo, S. S., Jung, S., Jang, S. Y., Lee, J., Kwon, M., Khan, I., Ryu, D. H., Kim, M. K., & Hwang, G. S. (2021). Comparable Plasma Lipid Changes in Patients with High-Grade Cervical Intraepithelial Neoplasia and Patients with Cervical Cancer. Journal of proteome research, 20(1), 740–750. https://doi.org/10.1021/acs.jproteome.0c00640

27. Cheng, F., Wen, Z., Feng, X., Wang, X., & Chen, Y. (2020). A serum lipidomic strategy revealed potential lipid biomarkers for early-stage cervical cancer. Life sciences, 260, 118489. https://doi.org/10.1016/j.lfs.2020.118489

28. Fontham, ETH, Wolf, AMD, Church, TR, et al. Cervical Cancer Screening for Individuals at Average Risk: 2020 Guideline Update from the American Cancer Society. CA Cancer J Clin. [Internet]. 2020 [Access in: 03/08/2021] Available in:https://doi.org/10.3322/caac.21628.

29. Ahmed-Salim, Y., Galazis, N., Bracewell-Milnes, T., Phelps, D. L., Jones, B. P., Chan, M., Munoz-Gonzales, M. D., Matsuzono, T., Smith, J. R., Yazbek, J., Krell, J., Ghaem-Maghami, S., & Saso, S. The application of metabolomics in ovarian cancer management: a systematic review. International journal of gynecological cancer : official journal of the International Gynecological Cancer SocietyAdvance online publication. [Internet]. 2020 [Access in: 02/23/2021] Available in:https://doi.org/10.1136/ijgc-2020-001862.

30. Li, Z., & Zhang, H. Reprogramming of glucose, fatty acid and amino acid metabolism for cancer progression. Cellular and molecular life sciences: CMLS, [Internet]. 2016 [Access in: 02/23/2021] 73(2), 377–392. Available in:https://doi.org/10.1007/s00018-015-2070-4.

31. Turkoglu, O., Zeb, A., Graham, S., Szyperski, T., Szender, J. B., Odunsi, K., & Bahado-Singh, R. Metabolomics of biomarker discovery in ovarian cancer: a systematic review of the current literature. Metabolomics: Official journal of the Metabolomic Society. [Internet]. 2016 [Access in: 02/27/2021] 12(4), 60. Available in:https://doi.org/10.1007/s11306-016-0990-0.

32. Kanehisa, M. Toward pathway engineering: a new database of genetic and molecular pathways. Science & Technology Japan, [Internet]. 1996 [Access in: 02/27/2021]. No. 59, pp. 34-38. Available in: https://www.kanehisa.jp/docs/archive/stj.pdf.

33. Liberti, M. V., & Locasale, J. W. The Warburg Effect: How Does it Benefit Cancer Cells?. Trends in biochemical sciences. [Internet]. 2016 [Access in: 02/27/2021] 41(3), 211–218. Available in:https://doi.org/10.1016/j.tibs.2015.12.001

34. Hsu, P. P., & Sabatini, D. M. Cancer cell metabolism: Warburg and beyond. Cell, [Internet]. 2008 [Access in: 03/14/2021] 134(5), 703–707. Available in:https://doi.org/10.1016/j.cell.2008.08.021

35. Vazquez, A., Liu, J., Zhou, Y., & Oltvai, Z. N. Catabolic efficiency of aerobic glycolysis: the Warburg effect revisited. BMC systems biology, [Internet]. 2010 [Access in: 03/23/2021] 4, 58. Available in:https://doi.org/10.1016/j.tibs.2015.12.001

36. Pascale, R. M., Calvisi, D. F., Simile, M. M., Feo, C. F., & Feo, F. The Warburg Effect 97 Years after Its Discovery. Cancers. [Internet]. 2020 [Access in: 02/27/2021] 12(10), 2819. Available in:https://doi.org/10.3390/cancers12102819

37. Wang, H., Tso, V. K., Slupsky, C. M., & Fedorak, R. N. Metabolomics and detection of colorectal cancer in humans: a systematic review. Future oncology (London, England). [Internet]. 2010 [Access in: 02/27/2021] 6(9), 1395–1406. Available in:https://doi.org/10.2217/fon.10.107.

38. Glunde, K., Penet, M. F., Jiang, L., Jacobs, M. A., & Bhujwalla, Z. M. Choline metabolism-based molecular diagnosis of cancer: an update. Expert review of molecular diagnostics. [Internet]. 2015 [Access in: 02/27/2021] 15(6), 735–747. Available in:https://doi.org/10.1586/14737159.2015.1039515

39. Ridgway N. D. The role of phosphatidylcholine and choline metabolites to cell proliferation and survival. Critical reviews in biochemistry and molecular biology. [Internet]. 2013 [Access in: 02/23/2021] 48(1), 20–38. Available in:https://doi.org/10.3109/10409238.2012.735643

40. Rižner T. L. Discovery of biomarkers for endometrial cancer: current status and prospects. Expert review of molecular diagnostics. [Internet]. 2016 [Access in: 03/02/2021] 16(12), 1315–1336. 1258302. Available in:https://doi.org/10.1080/14737159.2016.

APPENDIX S1

SEARCH STRATEGY IN THE ELECTRONIC DATABASE.

DATA BASE	SEARCH STRATEGY
PUBMED	"metabolome"[MeSH Terms] OR "metabolome"[All Fields] OR "metabolomes"[All Fields] OR "metabolomics"[MeSH Terms] OR "metabolomics"[All Fields] OR "metabolomic"[All Fields] AND "uterine cervical neoplasms"[MeSH Terms] OR ("uterine"[All Fields] AND "cervical"[All Fields] AND "neoplasms"[All Fields]) OR "uterine cervical neoplasms"[All Fields] OR ("cervical"[All Fields] AND "cancer"[All Fields]) OR "cervical cancer"[All Fields] OR ("cancer"[All Fields] AND "cervix"[All Fields]) OR "cancer of cervix"[All Fields] OR ("cervix"[All Fields] AND "neoplasms"[All Fields]) OR "cervix neoplasms"[All Fields] OR ("uterine"[All Fields] AND "cervix"[All Fields]) OR "uterine cervix"[All Fields]
LILACS, SCIELO AND BVS	(metabolomics cervical cancer) OR (metabolomics cancer of cervix) OR (metabolomics cervix neoplasms) OR (metabolomics uterine cervical neoplasms) OR (metabolomics of the uterine cervix) OR (metabolomics uterine cervix neoplasms)
GOOGLE SCHOLAR AND OPEN GRAY	Metabolomics cervical cancer Metabolomics cancer of cervix Metabolomics cervix neoplasms Metabolomics uterine cervical neoplasms Metabolomics of the uterine cervix Metabolomics uterine cervix neoplasms

Figure S1 – Flowchart of study selection.

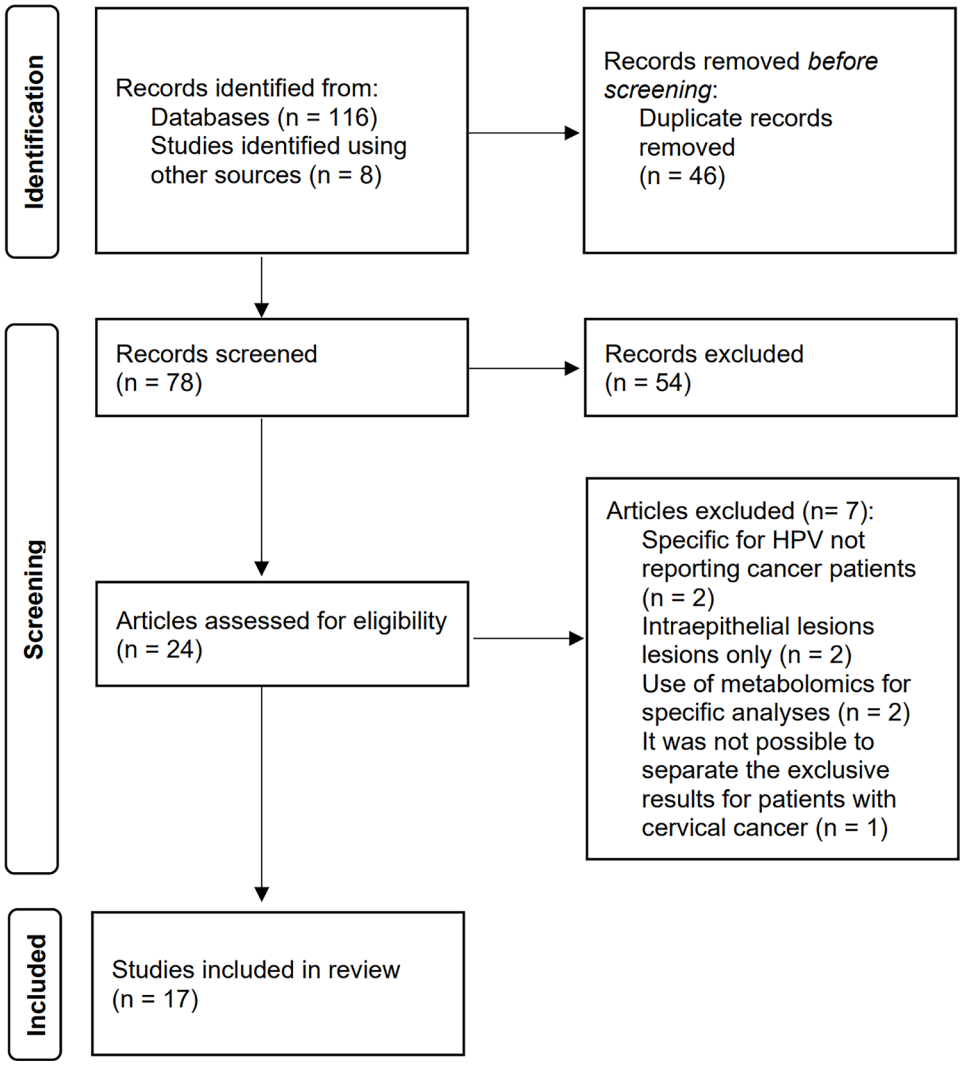
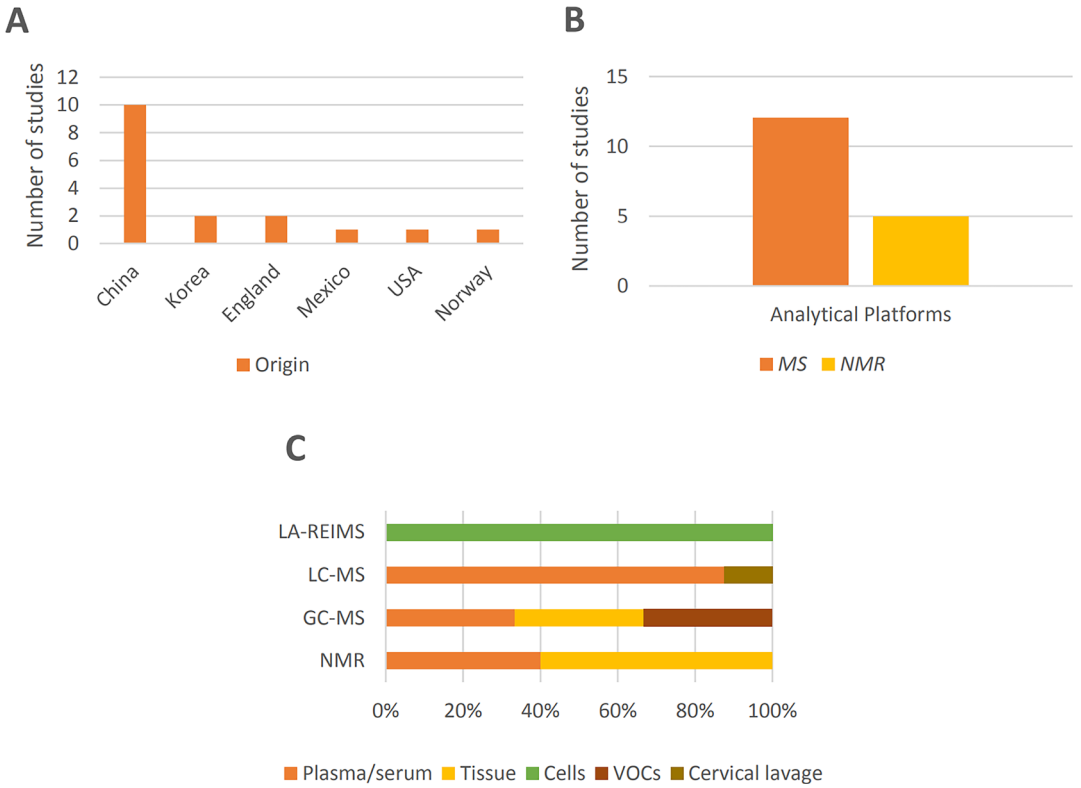


Figure S2 – General characteristics of the selected studies. A. Country of origin and number of studies. B. Analytical platforms used in CC metabolomic studies. C. biological sample type / analytical platform on CC metabolomics studies.



Caption: MS = Mass spectrometry. NMR = Nuclear magnetic resonance. LA-REIMS = Laser ablation coupled with rapid evaporative mass spectrometry. LC-MS = Liquid chromatography coupled to mass spectrometry. GC-MS = Gas chromatography coupled to mass spectrometry. VOCs = Volatile organic compounds

Table S1 - QUADOMICS evaluation of the studies included in the systematic review.

ITEM	Abdula *et al.* 2020[20]	Rodrígues-Esquivel *et al.* 2018[21]	Ilhan *et al.* 2019[22]	Khan *et al.* 2019[23]	Paraskevaidi *et al.* 2020[24]	Yang *et al.* 2017[25]	Ye *et al.* 2015[26]	Zhou *et al.* 2019[27]	Silva *et al.* 2008[28]	Hasim *et al.* 2012[29]	Hasim *et al.* 2013[30]	Shi *et al.* 2015[31]	Sitter *et al.* 2004[32]	Yin *et al.* 2015[33]	Chen *et al.* 2021[34]	Nan *et al.* 2021[35]	Cheng *et al.* 2020[36]
1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
3	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
4.1	N	Y	Y	Y	Y	Y	N	Y	N	Y	Y	N	N	Y	N	Y	Y
4.2	N	Y	Y	Y	Y	Y	N	Y	N	N	Y	N	Y	Y	N	Y	Y
5	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y
6	?	?	?	?	Y	Y	Y	Y	Y	Y	Y	?	Y	Y	?	Y	Y
7	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
8	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
9	?	Y	Y	?	Y	Y	?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
10	N	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y
11	N	Y	N	Y	Y	N	N	N	Y	N	N	N	Y	Y	N	N	N
12	?	Y	N	?	Y	N	N	N	N	N	N	N	N	N	N	N	N
13	Y	Y	Y	Y	?	Y	Y	Y	?	Y	Y	Y	Y	Y	Y	Y	Y
14	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
15	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
16	N	N	N	N	Y	Y	N	Y	N	N	N	N	N	Y	N	N	Y
TOTAL	5	12	10	10	13	12	7	12	8	10	11	8	10	13	8	11	12

Legend: Y = criteria met / N = criteria not met / ? = Unclear, N / A = not applicable