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Review

Metabolomic profiling of oesophago-gastric cancer: A systematic review

N. Abbassi-Ghadi [a], S. Kumar [a], J. Huang [a], R. Goldin [b], Z. Takats [a], G.B. Hanna [a,*]

[a] Department of Surgery and Cancer, Imperial College London, 10th Floor, QEQM Wing, St Mary's Hospital, London W2 1NY, UK
[b] Centre for Pathology, Imperial College London, 4th Floor, Clarence Wing, St Mary's Hospital, London W2 1NY, UK

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Abstract ***Aims:*** This review aims to identify metabolomic biomarkers of oesophago-gastric (OG) cancer in human biological samples, and to discuss the dominant metabolic pathways associated with the observed changes.
Methods: A systematic review of the literature, up to and including 9th November 2012, was conducted for experimental studies investigating the metabolomic profile of human biological samples from patients with OG cancer compared to a control group. Inclusion criteria for analytical platforms were mass spectrometry or nuclear magnetic resonance spectroscopy. The QUADAS-2 tool was used to assess the quality of the included studies.
Results: Twenty studies met the inclusion criteria and samples utilised for metabolomic analysis included tissue ($n = 11$), serum ($n = 8$), urine ($n = 1$) and gastric content ($n = 1$). Several metabolites of glycolysis, the tricarboxylic acid cycle, anaerobic respiration and protein/lipid metabolism were found to be significantly different between cancer and control samples. Lactate and fumurate were the most commonly recognised biomarkers of OG cancer related to cellular respiration. Valine, glutamine and glutamate were the most commonly identified amino acid biomarkers. Products of lipid metabolism including saturated and un-saturated free fatty acids, ketones and aldehydes and triacylglycerides were also identified as biomarkers of OG cancer. Unclear risk of bias for patient selection was reported for the majority of studies due to the lack of clarity regarding patient recruitment.
Conclusion: The application of metabolomics for biomarker detection in OG cancer presents new opportunities for the purposes of screening and therapeutic monitoring. Future studies should provide clear details of patient selection and develop metabolite assays suitable for progress beyond phase 1 pre-clinical exploratory studies.

1. Introduction

Oesophago-gastric (OG) cancer affects approximately 1.5 million people a year worldwide and accounts for

* *Corresponding author:* Address: Head of Division of Surgery, Department of Surgery and Cancer, 10th Floor, QEQM, St Mary's Hospital, Praed St., London W2 1NY, UK. Tel.: +44 2078862124; fax: +44 2078866309.
E-mail address: g.hanna@imperial.ac.uk (G.B. Hanna).

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15% of cancer related deaths.[1] In Western countries, the incidence is increasing, in particular adenocarcinomas of the distal oesophagus and gastro-oesophageal junction.[2–4] Prognosis remains poor with 5-year survival rates of 34% for localised disease and 17% for all stages combined.[5]

Treatment options and overall survival depend on both the stage of the disease and a patient's general health. The early symptoms of OG cancer are usually insidious and the majority of patients present late to their doctor with incurable disease. Surgical resection of the tumour remains the mainstay of curative treatment. In the United Kingdom (UK), only 38% percent of newly diagnosed patients are suitable for treatment with curative intent.[6] Identification of these cancers at an earlier stage could potentially improve the survival outcomes of OG cancer. There are currently no screening tests in mainstream clinical practice, despite evidence that treatment of early OG cancer has more favourable outcomes.[7–9] Oesophago-gastroduodenoscopy (OGD) and biopsy remain the gold standard procedure for the diagnosis of OG cancer. However, this is an invasive procedure and is too costly to be employed in a screening capacity. Radiological tests such as barium oesophograms and serum biomarkers are available; however their use has been limited due to poor sensitivity and specificity.

Metabolomics is defined as a quantitative description of all endogenous low-molecular-weight components (<1 kDa) in a biological sample, such as tissue, urine or plasma.[10] It is an evolving field and it has the potential to be an effective tool for the early diagnosis of OG cancer, through identification of one or more diagnostic biomarkers. The composition of these endogenous compounds is affected by the upstream influence of the proteome and genome as well as environmental factors, lifestyle factors, medication and underlying disease. The exact number of different metabolites in humans is currently unknown. Recent estimates by the human metabolite database (HDMB) suggest up to 5000 compounds, with reference to bio-fluid or tissue concentration data existing in the current literature.[11]

The majority of analytical platforms for metabolomic profiling are based on spectroscopic techniques. Mass spectrometry (often combined with chromatographic separation) and nuclear magnetic resonance (NMR) spectroscopy are the two most commonly employed analytical platforms; both techniques allow extensive and rapid analysis of small molecule metabolites,[12] resulting in multi-parameter datasets containing quantitative information on a range of metabolites. Due to the complexity and volume of the resultant spectral data, computer-based pre-processing and multivariate modelling techniques have been developed to facilitate the analysis and interpretation of the data.

Metabolomics studies have primarily focussed on the identification of cancer-specific metabolites. Examples include analysis of serum for the diagnosis of colorectal cancer,[13] exhaled breath for lung cancer[14] and urine for prostate cancer.[15] The aim of these studies has been to identify a panel of metabolites that may provide a metabolic fingerprint of malignancy. In combination, their sensitivity and specificity of predicting cancer has greater statistical power than single metabolites.

This systematic review aims to identify metabolomic biomarkers of OG cancer, found in human biological samples, and to assess the quality of the published data. The dominant metabolic pathways of malignant transformation, associated with the observed changes, will also be discussed.

2. Methods

2.1. Search strategy

A literature search (title and abstract) of Ovid Medline(R) (1948–2012), Embase (1974–2012), Web of Science and PubMed electronic databases was conducted up to and including 9th November 2012 for studies of metabolomic profiling of oesophago-gastric cancer. The search was conducted using the MeSH terms: *mass spectrometry, nuclear magnetic resonance spectroscopy, metabolomic, metabonomic, metabolic profiling* in multiple combinations (AND) with *gastric cancer* and *oesophageal/esophageal cancer*.

Two reviewers (N.A. and S.K.) independently screened titles and abstracts of studies identified through the electronic search. Full texts of potentially relevant articles were retrieved. Further potentially relevant articles were identified through the searching of reference lists of relevant studies. Experimental studies investigating the metabolomic profile of human biological samples from patients with gastric or oesophageal cancers, compared to an appropriate control group were included in our analysis. Inclusion criteria for analytical platforms were mass spectrometry (MS) or NMR. Exclusion criteria were: studies analysing the proteome rather than the metabolome, *in vitro* cell line studies, animal studies and studies without a control group. Studies that reported the same patient population were also excluded, except for the most recent or complete publication.

Two reviewers (N.A. and S.K.) independently extracted data from selected studies including primary author; year of publication; number and types of specimen; analytical platform and significantly different metabolites in the cancer and control groups. Compounds presented with varying chemical nomenclature in different articles are described by their common name in this review. The primary outcome measure was the

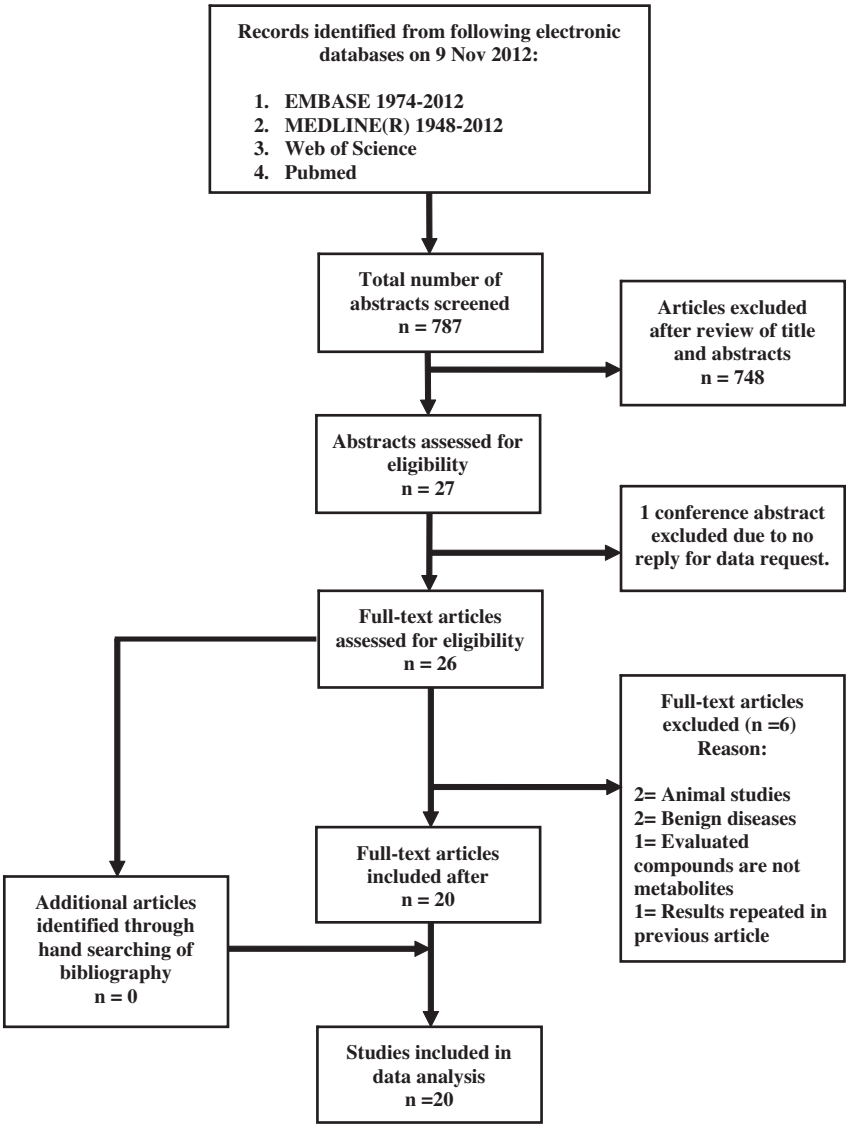

Fig. 1. Systematic search and selection strategy.

identification of metabolites found to have different abundances between cancer and control samples at a statistically significant level.

2.2. Study quality assessment

The QUADAS-2 tool[16] was used to assess the quality of the included studies. It consists of four key domains covering patient selection, index test, reference standard and flow of patients through the study. The reference standard is defined as the best available method to establish the presence or absence of the target condition.[17] In this review, the reference standard is considered to be histological examination. Each domain is assessed in terms of the risk of bias and the first three are also assessed in terms of concerns regarding applicability. To help reach a judgement on the risk of bias, signalling questions are included, full details of which are provided in Supplementary file 1.

3. Results

Twenty studies met the inclusion criteria and were eligible for systematic review. A kappa score of 0.85 confirmed excellent agreement between assessors. Search results are presented in Fig. 1.

3.1. Study characteristics

There were 12 studies of gastric cancer, six of oesophageal cancer and the remaining two had a mixed cohort (Table 1). Biological samples utilised for Metabolomic analysis included tissue ($n = 11$), serum ($n = 8$), urine ($n = 1$) and gastric content ($n = 1$). The analytical platforms used for metabolite detection included Gas Chromatography–Mass Spectrometry (GC–MS) ($n = 10$), High Resolution-Magic Angle Spinning-NMR ($n = 3$), Capillary Electrophoresis–Mass Spectrometry ($n = 1$), NMR ($n = 3$), Liquid Chromatography–Mass

Table 1
Summary of included studies.

Author and year of publication	Analytical platform	Cancer site	Sample type	Cancer type	NCa	Control	Nc	Metabolites of cancer compared to control		Other findings	Refs.
								Up-regulated	Down-regulated		
Mun CW (2004)	^{1}H NMR	Gastric	Tissue	GC	13	NGT	22	Choline Lactate	Lipids		18
Tugnioli V (2006)	HR-MAS-NMR	Gastric	Tissue	GC-AC HP +ve	5	NGT HP −ve	11	Triacylglycerides* Choline and Gly			19
Ligor T (2007)	SPME–GCMS	Gastric	Tissue Breath	GC	3 3	NGT	13 10	Carbon disulphide* Nil	Nil	No difference in VOCs of exhaled breath of patients with cancer versus control	20
Buszewski B (2008)	SPME–GCMS	Gastric	Tissue	GC	5	NGT	5	Propanol* Carbon disulphide		Butane & carbon disulphide present in the headspace of HP culture	21
Hirayama A (2009)	CE–MS	Gastric	Tissue	GC	12	NGT		Lactate, Glucose, fructose, Fumurate Malate, Glyceraldehyde-3P and All common AA apart from Gln & Asp	Citrate		22
Cai Z (2010)	GC–MS	Gastric	Tissue	GC	65	NGT	65	Pyruvic acid, Lactic acid Fructose, Glyceraldehyde and Isocitric acid	Fumaric acid	Confirmation of findings with western blot proteomic analysis	23
Wu H (2010)	GC–MS	Gastric	Tissue	GC	18	NGT	18	Val, Ile, Ser, Gln Heptanedioic acid, Propanoic acid, Butenoic acid, Galactofuranoside, Phenanthrenol, butanetriol, Acetamide Oxazolethione and Naphtalene	Phosphoserine L-Altrose L-Mannofuranose and D-Ribofuranose	Higher levels of L-Cysteine, hypoxanthine, L-Tryosine and lower levels of phenanthrenol and butanoic acid in late versus early stage cancer	24
Song H (2011)	GC–MS	Gastric	Tissue	GC	30	NGT	30	Fumaric acid, Valeric acid, α-Ketoglutaric acid, Benzenepropanoic acid, 1-Phenanthrene, Squalene, Carboxylic acid, Xylonic acid and Octadecanoic acid	3-Hydroxybutanoic acid 9-Hexadecanoic acid Hexadecanoic acid Cis-vaccenic acid Arachidonic acid and 9-Octadecenamide	Failed to differentiate pathological stage using multivariate analysis	25
Calabrese C (2012)	HR-MAS-MRS	Gastric	Tissue	GC-AC	5	NGT AAG HP	12 5 5	Choline Gly, Ala and Triacylglycerides		Increased presence of lipid bodies found in the cytoplasm of cancer cell with TEM/SEM imaging	26

(*continued on next page*)

Table 1 (*continued*)

Author and year of publication	Analytical platform	Cancer site	Sample type	Cancer type	NCa	Control	Nc	Metabolites of cancer compared to control		Other findings	Refs.
								Up-regulated	Down-regulated		
Yu L (2011)	GC–MS	Gastric	Serum	GC-AC	9	GSG	19	Urate, Ornithine, Pyroglutamate, Azelaic acid 11-Eicosenoic acid Glu, Asn and y-tocopherol 2-Hydroxybutyrate	Creatinine Threonate	Surgical removal of GC tissue restored the levels of some metabolites back to those found in CSG	27
Ikeda A	GC–MS	Gastric	Serum	GC-AC	11	HV	12	3-Hydroxypropionic acid and 3-Hydroxyisobutyric acid,	Octanoic acid, Phosphoric acid Pyruvic acid		28
		Oesophageal		EAC	15			Lactic acid, Glycolic acid, Malonic acid, Fumaric acid, Ser, Gln and Asp	Pyruvic acid		
Song H (2012)	GC–MS	Gastric	Serum	GC	30	HV	30	Val Sarcosine and Hexadecanenitrile	Gln, Hexanedioic acid, 9,12 Octadecadienoic acid, 9-Octadecenoic acid, Trans-13-octadecenoic acid, Nonahexacontanoic acid, Cholesterol, fumaric acid, mesyl-arabinose Benzeneacetonitrile, 2-Amino-4-hydroxy-pteridinone and 1,2,4,-Benzenetricarboxylic acid	Failed to differentiate pathological stage using multivariate analysis	29
Aa J (2012)	GC–MS	Gastric	Serum	CSG	20	CSG	17	Citrate, Succinate, Fumurate, Malate Glu, β-hydroxybutyrate Hexadecenoic acid, Docosahexaenoic acid and hepatanoic acid	Glucose	Surgical removal of GC tissue restored the levels of some metabolites back to normal	30
Wu H (2009)	GC–MS	Oesophageal	Tissue	EAC 2	20	NOT	20	Val, Ile, Tyr, Asn, andAla	L-Altrose		31
				ESC 18				Arabinofuranoside, Tetradecanoic acid, Hexadecanoic acid, Naphthalene, 1-Butanamine, Aminoquinolone, Myo-inositol, Phosphoric acid and Pyrimidine nucleoside	D-Galactofuranoside Arabinose and Bisethane		
Yakoub D (20100	HR-MAS-NMR	Oesophageal	Tissue	EAC	35	NOT	35	Phospho-choline, Myo-inositol, Glu,		Stepwise up-regulation of metabolites from cancer to adjacent tissue to healthy distant tissue	32
						NOTH	52	Inosine, Adenosine and uridine containing compounds			

(*continued on next page*)

Table 1 (*continued*)

Author and year of publication	Analytical platform	Cancer site	Sample type	Cancer type	NCa	Control	Nc	Metabolites of cancer compared to control		Other findings	Refs.
								Up-regulated	Down-regulated		
Djukovic D (2010)	HPLC–MS	Oesophageal	Serum	EAC				1-Methyladenosine, N2,N2-dimethylguanosine, N2-methylguanosine and Cytidine	Uridine		33
Zhang J (2011)	NMR	Oesophageal	Serum	EAC	68	HV	34	Citrate, lactate, a-glucose		Up-regulation of metabolites shown in BE-HGD-EAC sequence	34
						BE	5	Gln, Lys, B-hydroxybutyrate and			
						HGD	11	Creatinine			
Zhang J (2012)	LC–MS	Oesophageal	Serum	EAC	67	HV	34	Lactic acid	Val, Leu, Ile, Met, Tyr and Trp		35
						BE	3	Carnitine and	Myristic acid, Linolenic acid and		
								Margaric acid	Linoleic acid		
						HGD	9				
Kumar S (2012)	SIFT–MS	Gastric and Oesophageal	GastricContent	Cancer	19	HV	11	Acetaldehyde, Acetone and Acetic acid	Formaldehyde		36
						ID	9	Hexanoic acid, Hydrogen sulphide, Hydrogen cyanide, Methanol and Methyl phenol			
Hasim A (2012)	^{1}H NMR	Oesophageal	Urine	SCC	108	HV	40	Mannitol, Glucose, Pyruvate, Glu, Tyr c-Propalanine, Phenylalanine, Acetate and Allantoin	N-acetylcysteine, Val Dihydrothymine, Hippurate, Methylguanidine, Citric acid and 1-methylnicotin-amide,	Metabolites in plasma positively correlated to lymph node metastatic rate	37
			Serum					Dimethylamine a-Glucose b-Glucose and Citric acid	Leu, Ala, Ile, Val, Glycoprotein, Lactate, Acetone, Acetate, Choline Isobutyrate, Unsaturated lipids VLDL and LDL		

^{1}H NMR, Proton Nuclear Magnetic Resonance; HR-MAS-NMR, High Resolution-Magic Angle Spinning-Nuclear Magnetic Resonance Spectroscopy; SPME, Solid Phase Micro-extraction; GC–MS, Gas Chromatography–Mass Spectrometry; CE–MS, Capillary Electrophoresis–Mass Spectrometry; HPLC–MS, High Performance Liquid Chromatography–Mass Spectrometry; LC–MS, Liquid Chromatography–Mass Spectrometry; NCa, number of cancer patients; Nc, number of control patients; GC, gastric cancer; EAC, oesophageal adenocarcinoma; ESC, oesophageal squamous cell carcinoma; NGT, normal gastric tissue matched to the same patient; AAG, autoimmune atrophic gastritis; CSG, chronic superficial gastritis; NOT, normal oesophageal tissue from the same patients; NOTH, normal oesophageal tissue from healthy patients; HV, healthy volunteers; BE, Barrett's oesophagus; HGD, high grade dysplasia; ID, inflammatory disorders; HP, *Helicobacter pylori*; VOCs, volatile organic compounds; Val, valine; His, histidine; Phe, phenylalanine; Thr, threonine; Leu, leucine; Met, methionine; Trp, tryptophan; Ile, isoleucine; Lys, lysine; Tyr, tyrosine; Gln, glutamine; Glu, glutamate; Ser, serine; Asn, asparagine; Gly, glycine; Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartic acid; Cys, cysteine; Pro, proline; TEM, tunnel electron microscopy; SEM, surface electron microscopy.

* Results not statistically significant.

Spectrometry ($n = 2$) and Selected Ion Flow Tube–Mass Spectrometry ($n = 1$).

3.2. Quality assessment of studies

The outcomes of the QUADAS-2 study quality assessment are shown in Fig. 2. Further details regarding individual studies are presented in Supplementary file 1. Unclear risk of bias for patient selection was reported for the majority of studies due to the lack of clarity regarding patient recruitment. It is unclear whether consecutive patient data is included or whether highly selected samples were chosen. As a consequence, 35% of studies have an unclear/high risk of applicability with respect to the target population defined by the review.

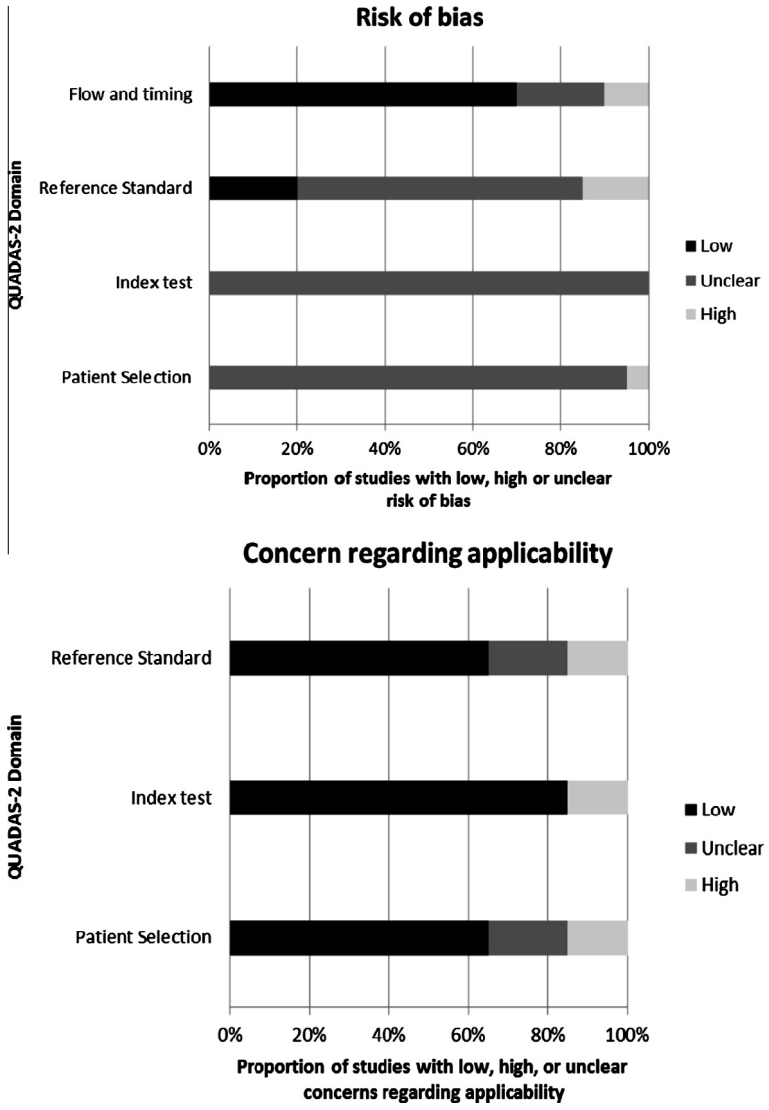

Fig. 2. Outcome of QUADAS-2 study quality assessment.

The risk of bias for the index test and reference standard was unclear for the majority of studies due to lack of clarity regarding blinding of assessors. Thirty-five percent of studies had an unclear/high risk of applicability with regard to the reference standard, mostly due to lack of information regarding histological confirmation of control samples.

3.3. Metabolites of cellular respiration

The metabolite intermediates of glycolysis, the tricarboxylic acid (TCA) cycle and anaerobic respiration that were found to be significantly different between cancer and control samples are shown in Table 2. The majority of metabolites that have been identified show contradictory results in different studies, in terms of relative abundance in the cancer versus control groups. Lactate and fumurate were the most commonly recognised biomarkers.

3.4. Amino acid metabolites

Essential and non-essential amino acids that were found to be significantly different between cancer and control samples are shown in Table 3. Valine, glutamine and glutamate were the most commonly identified amino acid biomarkers in descending order of frequency. The metabolite glutamine is the most consistent biomarker showing up-regulation in the serum, urine and tumour tissues of patients with OG cancer. There is a general trend of up-regulation of amino acids across the majority of studies, however in a study by Zhang et al.,[35] they observed contradictory findings in serum samples from patients with oesophageal adenocarcinoma analysed by LC–MS.

Several other metabolites related to amino acid metabolism have been identified in relation to OG cancer. Sulphur containing compounds, thought to be generated from the incomplete metabolism of methionine in

Table 2
Relative abundance of metabolite intermediates of glycolysis, the tricarboxylic acid (TCA) cycle and anaerobic respiration, in biological samples of patients with oesophageal/gastric cancer in comparison to normal controls. All metabolite comparisons are determined at a statically significant level ($P < 0.05$).

Author	Type of sample	Analytical platform	Glycolysis				Anaerobic respiration	The TCA cycle					
			Glucose	Fructose	Glyceraldehyde	Pyruvic acid	Lactic acid/ Lactate	Citrate	Isocitric acid	α-Ketoglutaric acid/ α-ketoglutarate	Succinate	Fumaric acid/ Fumurate	Malate
Gastric													
Hirayama A (2009)	Tissue	CE–MS	↑	↑	↑		↑	↓				↑	↑
Cai Z (2010)	Tissue	GC–MS		↑	↑	↑	↑		↑			↓	
Song H (2011)	Tissue	GC–MS								↑		↑	
Ikeda A (Part B) (2011)	Serum	GC–MS				↓							
Song H (2012)	Serum	GC–MS										↓	
Aa J (2012)	Serum	GC–MS	↓					↑			↑	↑	↑
Oesophageal													
Zhang J (2011)	Serum	NMR	↑				↑	↑					
Ikeda A (2011)	Serum	GC–MS				↓	↑					↑	
Zhang J (2012)	Serum	LC–MS					↑						
Hasim A (2012)	Urine	^{1}H NMR	↑			↑		↓					
	Serum		↑				↓	↑					

CE–MS, Capillary Electrophoresis–Mass Spectrometry; GC–MS, Gas Chromatography–Mass Spectrometry; NMR, Nuclear Magnetic Resonance Spectroscopy; LC–MS, Liquid Chromatography–Mass Spectrometry; ^{1}H NMR, ^{1}H Nuclear Magnetic Resonance.

Table 3
Relative abundance of essential and non-essential amino acids in biological samples of patients with oesophageal/gastric cancer, in comparison to normal controls. All comparisons are determined at a statically significant level ($P < 0.05$).

Author	Sample type	Analytical platform	Essential amino acid									Non-essential amino acid											
			Val	His	Phe	Thr	Leu	Met	Trp	Ile	Lys	Tyr	Gln	Glu	Ser	Asn	Gly	Ala	Arg	Asn	Asp	Cys	Pro
Gastric																							
Tugnioli V (2006)	Serum	HR-MAS-NMR															↑						
Hiarayama A (2009)	Tissue	CE–MS	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑		↑	↑	↑	↑	↑	↑	↑		↑	↑
Wu H (2010)	Tissue	GC–MS	↑							↑			↑		↑								
Calabrese C (2012)	Tissue	HR-MAS-MRS															↑	↑					
Yu L (2011)	Serum	GC–MS												↑		↑							
Song H (2012)	Serum	GC–MS	↑										↓										
Aa J (2012)	Serum	GC–MS												↑									
Oesophageal																							
Wu H (2009)	Tissue	GC–MS	↑							↑		↑			↑	↑		↑					
Yakoub D (2010)	Tissue	HR-MAS-NMR												↑									
Zhang J (2011)	Serum	NMR									↑		↑										
Ikeda A (2011)	Serum	GC–MS											↑	↑	↑						↑		
Zhang J (2012)	Serum	LC–MS	↓				↓	↓	↓	↓		↓											
Hasim A (2012)	Urine	^{1}H NMR	↓		↑									↑									
	Serum		↓				↓			↓								↓					

CE–MS, Capillary Electrophoresis–Mass Spectrometry; GC–MS, Gas Chromatography–Mass Spectrometry; NMR, Nuclear Magnetic Resonance Spectroscopy; LC–MS, Liquid Chromatography–Mass Spectrometry; ^{1}H NMR, ^{1}H Nuclear Magnetic Resonance; HR-MAS-NMR, High Resolution-Magic Angle Spinning-Nuclear Magnetic Resonance Spectroscopy; Val, valine; His, histidine; Phe, phenylalanine; Thr, threonine; Leu, leucine; Met, methionine; Trp, tryptophan; Ile, isoleucine; Lys, lysine; Tyr, tyrosine; Gln, glutamine; Glu, glutamate; Ser, serine; Asn, asparagine; Gly, glycine; Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartic acid; Cys, cysteine; Pro, proline.

the transamination pathway or by bacterial metabolism has been found to be up-regulated.[20] Two studies (from the same research group) investigating the volatile organic compounds (VOCs) in the headspace of tissue found a significant increase in the presence of carbon disulphide in cancer versus normal tissue. Buszewski et al., also demonstrated the presence of carbon disulphide in the headspace of *Helicobacter pylori* cultures.[21]

3.5. Lipid metabolites

The saturated and un-saturated free fatty acids (FFA) that were found to be significantly different between cancer and control samples are shown in Table 4. Metabolites of FFA oxidation, such as aldehydes and ketones have also been identified as potential biomarkers. Of the three endogenous ketones,[38] acetone and β-hydroxybutyrate have been described as potential biomarkers of oesophago-gastric cancer. β-Hydroxybutyrate has been shown to be up-regulated in the serum of patients with gastric cancer using GC–MS[27,30] and in the serum of patients with oesophageal cancer using NMR.[35] The median concentration of acetone was found to be higher in the headspace of gastric content from patients with OG cancer versus controls.[36] However, it was found in lower quantities in the serum of

Table 4
Relative abundance of saturated and un-saturated free fatty acids in biological samples of patients with oesophageal/gastric cancer, in comparison to normal controls. All comparisons are determined at a statically significant level ($P < 0.05$).

Author	Sample type	Analytical platform	Un-saturated fatty acid								Saturated fatty acid			
			Palmitoleic acid	Cervonic acid	Linoleic acid	Oleic acid	Linolenic acid	Vaccenic acid	Gondoic acid	Arachidonic acid	Myristic acid	Enanthic acid	Margaric acid	Caprylic acid
Gastric														
Mun CW (2004)	Tissue	^{1}H NMR	Decrease in lipids											
Tugnioli V (2006)	Tissue	HR-MAS-NMR	Increase in tryiacyglycerides											
Song H (2011)	Tissue	GC–MS	↓			↑		↓		↓				
Calabrese C (2012)	Tissue	HR-MAS-MRS	Increase in tryiacyglycerides											
Ikeda A (2011)	Serum	GC–MS												↑
Yu L (2011)	Serum	GC–MS							↑					
Song H (2012)	Serum	GC–MS			↓	↓								
Aa J (2012)	Serum	GC–MS	↑	↑								↑		
Oesophageal														
Wu H (2009)	Tissue	GC–MS	↑								↑			
Zhang J (2012)	Serum	LC–MS			↓		↓				↓		↑	
Hasim A (2012)	Urine Serum	^{1}H NMR	Decrease in unsaturated lipids, VLDL and LDL											

CE–MS, Capillary Electrophoresis–Mass Spectrometry; GC–MS, Gas Chromatography–Mass Spectrometry; LC–MS, Liquid Chromatography–Mass Spectrometry; ^{1}H NMR, ^{1}H Nuclear Magnetic Resonance; HR-MAS-NMR, High Resolution-Magic Angle Spinning-Nuclear Magnetic Resonance Spectroscopy; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein.

oesophageal cancer patients when compared to healthy controls.[37] The study by Kumar et al. also identified aldehydes as potential biomarkers for cancer in the headspace of gastric content. This study demonstrated an increase in acetaldehyde in cancer patients compared with controls.[36]

Lipid metabolites of the cell membrane including: myo-inositol up-regulated in three studies using HR-MAS-NMR[19,32] and GC–MS[31]; choline and phosphocholine up-regulated in three studies of OG cancer tissue using HR-MAS-NMR[18,26,32] and down-regulated in the serum of oesophageal cancer using ^{1}H NMR,[37] and squalene up-regulated in the gastric cancer tissue using GC–MS,[25] have also been identified as potential biomarkers.

3.6. Nucleotide metabolites

Studies of oesophageal cancer tissue have shown up-regulation of pyrimidine nucleotides using GC–MS,[31] and adenine and uridine containing compounds using HR-MAS-NMR.[32] A study of the serum of patients with oesophageal cancer demonstrates up-regulation of guanosine and cytidine containing compounds in comparison to healthy controls.[37]

4. Discussion

This systematic review demonstrates variation in the relative abundance of the metabolites of glycolysis, lactic acid fermentation, *de novo* lipid and amino acid synthesis in biological samples of patients with OG cancer versus controls. The results of several studies are contradictory; this variation may be due to sample selection and preparation, type of biological medium investigated or analytical technique. The metabolite glutamine is the most consistent biomarker, showing up-regulation in the serum, urine and tumour tissues of patients with OG cancer.[22,27,28,30,32,37]

Weinberg and Hanahan described six essential alterations in cell physiology which collectively dictate malignant transformation.[39] These characteristics were identified as the hallmarks of cancer and since 2000 reprogramming of energy metabolism has been added to the list.[40,41] The increased rate of cell proliferation in cancer requires metabolic pathways to be redesigned to satisfy large demands for adenosine triphosphate (ATP), nicotinamide adenine dinucleotide phosphate (NADPH), nicotinamide adenine dinucleotide (NADH) and carbon skeletons.[42] The emerging field of metabolomics has aided in the identification of metabolite intermediates involved in these pathways, which have been identified as potential biomarkers in biological samples from cancer patients (Fig. 3).

The metabolic intermediates of glycolysis including glucose, fructose, glyceraldehyde and pyruvic acid are shown to be up-regulated in several studies of gastric cancer tissue.[22,23] This may be due to a phenomenon known as the Warburg effect,[43,44] which states that even in the presence of oxygen, cancer cells reprogram their glucose metabolism to produce energy largely by glycolysis rather than oxidative phosphorylation via the tricarboxylic acid (TCA) cycle.[45] The poor efficiency of generating ATP by glycolysis is rationalised by the advantages of diverting glycolytic intermediates into various biosynthetic pathways, which are essential for the synthesis of necessary macromolecules (i.e. amino acids, nucleosides and lipids required for assembling new cells).[45,46] The increased production of pyruvate by the Warburg effect and subsequent lactic acid fermentation propagated by the enzyme lactate dehydrogenase, may also explain the increased levels of lactate/lactic acid found in the tissues of gastric cancer,[22,23] as well as in the serum of patients with oesophageal cancer.[28,34,35]

The metabolite intermediates of the TCA cycle have been identified as biomarkers in the tissues of gastric cancer,[22,23,25] and in the serum of gastric and oesophageal cancer.[28–30,34,35,37] Fumurate was the most frequently identified biomarker, but showed inconsistencies in relative abundance, with two studies observing down-regulation in cancer. The increased abundance of Krebs cycle metabolites, despite cancer cell preference of glycolysis to oxidative phosphorylation, is dependent on the process of anaplerosis. It refers to replenishment of metabolites in the cycle through the generation of α-ketoglutarate from the amino acid glutamate after its conversion from glutamine.[47] The aneplerotic flux generates macromolecules for cellular proliferation and is more specific than a high glycolytic flux, which can be induced by other factors such as hypoxia.[48,49] This review confirms that in the majority of studies, there is an increased presence of glutamine and glutamate, suggesting an important role of this pathway in OG cancer metabolism.

In addition to higher levels of glutamine and glutamate, our review demonstrates the increase of other amino acid metabolites in the serum and tissues of oesophago-gastric cancer. The availability of amino acids in the tumour environment is important for cell proliferation and as an energy substrate. The source of these amino acids has not been determined; however it is likely to be a combination of systemic protein catabolism,[50] tumour environment degradation of extracellular matrix and *de novo* biosynthesis.[22] Contradictory findings of low levels of amino acids in the serum of oesophageal cancer patients in one study were attributed to the over-utilisation of amino acids in the tumour tissue.[35]

Lipids and triglycerides have been shown to be present in increased levels in gastric cancer tissue by two studies using HR-MAR-NMR.[19,26] One study confirmed these findings by demonstrating increased

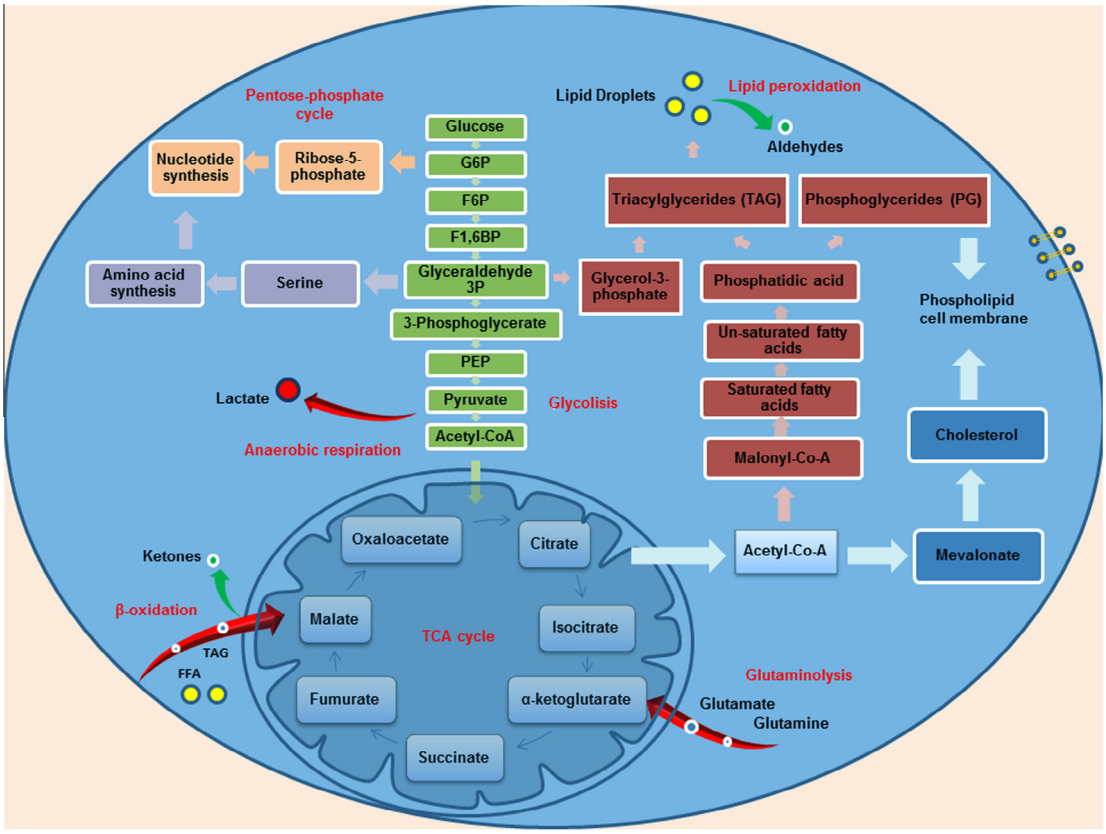

Fig. 3. Metabolic pathways associated with oesophago-gastric (OG) cancer.

abundance of cytosolic lipid droplets with scanning electron microscopy.[26] Studies using GC–MS and LC–MS have demonstrated a mixed outcome of up-regulation and down-regulation, of saturated and unsaturated FFA in both tissue and serum. Low levels of FFA or lipids have been attributed to increased consumption by tumours due to their anabolic metabolism. However, much of the scientific evidence demonstrates the increased presence of fatty acids in the tumour environment as a consequence of metabolic reprogramming in the cancer disease state.[51,52] There may be increased lipid availability from the bloodstream as a consequence of systemic lipolysis secondary to cancer cachexia or *de novo* fatty acid synthesis leading to FFA accumulation.[53] The role of lipid synthesis in cancer is not fully understood; however it is likely that *de novo* lipogenesis contributes to the formation of structural lipids for cell membrane synthesis, generation of energy by β-oxidation and cell signalling molecules in the cancer cell.[51,54,55]

Fatty acid β-oxidation has been confirmed as a dominant pathway for energy generation in numerous malignancies including prostate and pancreatic cancer.[56,57] Ketones and aldehydes, which are metabolic products of β-oxidation, are shown to be increased in biological samples of patients with OG cancer.[27,30,34,36] Up-regulation of acylcarnitines, which are metabolic intermediates of β-oxidation involved in the transfer of FFA across the mitochondrial membrane,[58] has also been confirmed in metabolomic studies of kidney,[59] liver[60] and colorectal cancer.[61] Converse findings have been confirmed in a recent metabolic profiling study of the plasma of patients with oesophageal squamous cell cancer using LC–MS.[62]

Local inflammation and oxidative stress associated with the cancer disease state have been shown to influence the production of eicosanoids from the lipid cell membrane and FFAs.[63] The increased abundance of eicosanoids have been confirmed in cell line,[64] animal[65] and human studies[66,67] of OG cancer. Oxidative stress also promotes lipid peroxidation leading to the production of aldehydes,[68] which has been found in the headspace of gastric content for patients with OG cancer.[36] Lipid metabolic profiles have great potential in detecting OG cancer; however a limitation of current research is that only small molecular endogenous FFAs and intermediate metabolites have been investigated. Therefore, the roles of macromolecular lipids warrant further investigation.

The changes in the metabolic pathways that have been described may explain some of the observed differences in metabolites found in cancer and control samples. However, several of the hypotheses of cancer cell metabolism do not always hold true in human samples and contradictory findings are evident. The differences in study findings may be due to the type of biological

sample analysed, its preparation and metabolite derivatisation, but consideration must also be given to the analytical platform employed. Variable detection limits and sensitivities of instruments may also influence the detection of metabolites that are present in a sample.

The majority of studies were unclear as to the nature of their patient selection and therefore we do not know if positive results are due to highly selected test subjects. Therefore, there can only be limited application of these results to real patient groups. A further weakness of this systematic review is the potential for publication bias and the lack of data representing no difference between compared groups. Due to lack of reported statistical data, we were not able to account for this phenomenon in the full cohort of included studies.

The application of metabolomics in cancer biomarker detection and development has largely not advanced beyond Phase 1 pre-clinical exploratory studies. Future studies have an obligation to provide transparent details of patient selection with attention being paid to variability in patient demographics, histology and treatment modalities (e.g. chemotherapy). Control subjects should be matched to cancer case subjects for factors that may potentially influence the identified biomarkers including age, sex, race, medication and lifestyle factors. Discovery of a biomarker or group of biomarkers with adequate sensitivity and specificity should be cross-validated across several analytical platforms and with different statistical methods. The development of an appropriate clinical assay suitable for screening purposes will then allow advancement of metabolomic biomarker discovery into phase 2 clinical studies and beyond.

Conflict of interest statement

None declared.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejca.2013.07.004.

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