Urinary Metabotyping of Hepatocellular Carcinoma in a UK Cohort Using Proton Nuclear Magnetic Resonance Spectroscopy

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Background: Discriminatory metabolic profiles have been described in urinary ¹H nuclear magnetic resonance (NMR) spectroscopy studies of African patients with hepatocellular carcinoma (HCC). This study aimed to assess similarities in a UK cohort, where there is a greater etiological diversity. **Methods:** Urine from cirrhosis and HCC patients was analyzed using a 600 MHz ¹H NMR system. Multivariate analysis and median group MR spectra comparison identified metabolite alterations between groups. Metabolite identification was achieved through literature reference and statistical total correlation spectroscopy. Diagnostic accuracy was compared to serum alpha-fetoprotein (AFP). **Results:** Of the 52 patients recruited, 13 samples from HCC and 25 from cirrhosis patients were selected. At 200 IU mL⁻¹, diagnostic sensitivity of AFP was 27%. Multivariate analysis of urinary spectra generated diagnostic models with a sensitivity/specificity of 53.6%/96%. p-Cresol sulfate ($P = 0.04$), creatinine ($P = 0.03$), citrate ($P = 0.21$) and hippurate ($P = 0.52$) were reduced in the HCC patients. Carnitine ($P = 0.31$) and formate ($P = 0.44$) were elevated. **Conclusion:** Diagnostic sensitivity was lower than previous African studies, but still outperformed serum AFP. Reduced creatinine, citrate and hippurate and elevated carnitine are comparable with the African studies. p-Cresol sulfate alteration is a novel finding and may indicate an altered sulfonation capacity of the liver in patients with HCC. (J CLIN EXP HEPATOL 2016;6:186–194)

Hepatocellular carcinoma (HCC) is the commonest primary liver cancer and the second global cause of cancer mortality. Although the heaviest disease burden is in sub-Saharan Africa and Eastern Asia,¹ recent evidence suggests HCC incidence is rising in

previously low-risk countries such as the United Kingdom.²

The 5-year mortality for HCC in the developed world is poor.³ The Surveillance Epidemiology and End Results (SEER) database from the United States documents a 9.9% HCC 5-year survival, compared to over 60% for all tumors.⁴ The current HCC surveillance guidelines in the developed world recommend 6-monthly hepatic ultrasonography (US) in patients with cirrhosis, but requirement for serum alpha-fetoprotein (AFP) measurement has been dropped, owing to poor sensitivity and specificity of this test.^{5,6} However, a retrospective SEER-database based study between 1994 and 2001 reported 6-monthly regular surveillance rates of only 17% in patients over 65 years with cirrhosis.⁷ This highlights problems concerning patient attendance under surveillance, in particular those who live greater distances from medical centers.

“Metabonomics”, the study of metabolic responses to physiological, drug and disease stimuli, may be utilized to identify profiles of biomarkers that characterize HCC. The

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Abbreviations: ¹H NMR: proton nuclear magnetic resonance; AFP: alpha-fetoprotein; ALT: alanine transaminase; BCLC: Barcelona Clinic Liver Cancer; BMI: body mass index; HBV: hepatitis B virus; HCC: hepatocellular carcinoma; HCV: hepatitis C virus; HIV: human immunodeficiency virus; INR: International Normalized Ratio; NASH: non-alcoholic steatohepatitis; PCA: principal component analysis; PLS-DA: partial least squares discriminant analysis; SEER: surveillance Epidemiology and End Results; STOCSSY: statistical total correlation spectroscopy; TSP: trimethyl-silyl phosphate; US: ultrasonography
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most common metabonomic technique is proton nuclear magnetic resonance (^1H NMR) spectroscopy.⁸ Previous ^1H NMR studies performed in Nigerian, Egyptian and Gambian patients have identified a number of altered metabolites in the urine, implicating changes in hepatic function according to Warburg's hypothesis of altered metabolism.^{9–11} Applying this theory, a simple, non-invasive test that identifies tumor profiles from the urine would be invaluable for cirrhosis patients, potentially allowing surveillance in the community by local general practitioners or even patients themselves.

The etiological variation of HCC patients in the UK is greater than African patients, including viral hepatitis, alcohol and non-alcoholic steatohepatitis (NASH). The collection of samples in the UK also allowed detailed patient and disease demographics to be collated.

The main aims of this study were to identify tumor-specific urinary metabolite changes in patients with HCC of varying etiology and to compare findings to previous African studies with larger tumors, caused solely by viral hepatitis.

MATERIALS AND METHODS

Patient Selection

Patients were recruited at six hospital sites around the United Kingdom: London, Manchester, Newcastle, Nottingham, Plymouth and Southampton. All subjects gave written, informed consent, in accordance with local Research Ethics Committee approval (LREC reference no. 06/Q041/10).

HCC was diagnosed with two confirmatory imaging modalities and cirrhosis with histological and/or radiological confirmation. Tumor staging was according to the Barcelona Clinic Liver Cancer (BCLC) staging system,⁵ based upon tumor size and multiplicity, patient performance status, presence or absence portal vein invasion, presence or absence of portal hypertension, and Child–Pugh scores of liver function.^{12,13} Patients with cirrhosis were graded according to the Child–Pugh score. Participant height, weight, medical, drug, smoking, and alcohol intake history were recorded. A 24-h dietary history was also recorded, paying attention to foodstuffs known to produce prominent nuclear magnetic resonance (NMR) signals, such as vanilla (vanillin) and vinegar (acetic acid). Exclusion criteria included those patients not meeting the diagnostic criteria cited above for HCC and cirrhosis, those patients with HCC who had undergone curative resection or transplant, patients co-infected with human immunodeficiency virus (HIV) and those samples identified as outliers on principal component analysis.

Sample Collection

15 mL of random, non-fasted urine were collected into preservative-free universal containers and placed

immediately on ice and centrifuged within 2 h at 4 °C, 1000 rpm for 10 min. The supernatant was then transferred as 2 mL aliquots into 2 mL microvial tubes and stored at –80 °C until analysis.

Sample Preparation

Urine samples were thawed at room temperature and 400 μL were transferred to 1.5 mL microvial tube to which 200 μL of phosphate buffer solution (pH 7.4), containing 1 mM trimethyl silyl phosphate (TSP), sodium azide 3 mM (as a bacterial biocidal agent to inhibit bacterial growth and contamination) and D_2O 20%. Samples were centrifuged for 5 min at 13,000 rpm and 550 μL of supernatant were transferred to 5 mm NMR tubes (Norell, Landisville, NJ, USA).

Proton Nuclear Magnetic Resonance Spectroscopy

Samples were run in random non-grouped order under automation on a Bruker DRX-600 NMR system operating at 600.44 Hz ^1H frequency (Bruker Biospin, Rheinstetten, Germany). The system was tuned, matched and frequency locked to deuterated hydrogen as the nucleus of interest. A representative sample was utilized to set shim gradients to ensure a homogenous magnetic field across the sample, a 90° pulse length and water suppression offset parameters.¹⁴ Spectra were acquired at 300 K using a one-dimensional (1-D) noesypr1d pulse sequence with water presaturation during relaxation delay (RD) and mixing time (t_m) using the following pulse program: -RD-90°- t_m -90°- t_m -90°-acquire; where RD = 2.0 s and t_m = 0.1 s. For each sample, 128 FIDs were collected into 32,000 data points with a spectral width of 20 ppm. A line broadening function of 0.3 Hz was applied prior to Fourier transformation. Spectra were manually phased, baseline corrected and referenced to TSP at 0 ppm using TOPSPIN v2.0 (Bruker Biospin, Rheinstetten, Germany). Spectral peaks were assigned with reference to the literature.^{15–17}

Spectral Processing

Proton NMR spectra were exported to MATLAB R2010 (MathWorks, Natick, MA, USA) and to avoid influence on analyses from water suppression aberration, the water region from 4.5 ppm to 6 ppm was excluded. Spectra were aligned to negate the effect of pH dependent variation in metabolite resonances which may occur despite sample buffering. To remove the effect of differential urine concentrations, data were normalized using median fold-change normalization. Median spectra for both clinical groups were generated to allow direct visual comparison of average spectra and allow the selection of regions that were visually divergent, in addition to those identified by multivariate analysis, for peak integration.

Statistical Total Correlation Spectroscopy (STOCSY)

Using in-house software, developed at Imperial College London, the spectral database was interrogated for correlated peaks across the whole spectrum. Peaks that co-varied with high correlation were identified by input of one peak region of interest. Correlation factors varied from 0 to 1. This method was utilized to identify unknown peaks to extract the total spectrum for an individual metabolite.

Multivariate and Univariate Statistical Analyses

Full resolution data were used for analyses. Data matrices were generated in MATLAB containing ppm variables as columns and sample identities as rows. After water exclusion, this amounted to a matrix of 28,238 variables and 43 observations. This matrix was exported to SIMCAP-12 (Umetrics, Umea, Sweden) for multivariate analysis and variables from 0.2 to 10.0 ppm were used for analysis, thus removing any influence from variation in TSP concentration between samples. Data were mean-centered and principal component analysis (PCA) was performed first to model variation and identify outliers. Pareto-scaled data and unit variance data were also generated, but both tended to model noise rather than signal. Therefore, only mean-centered data were used for further analysis. After outliers were identified and excluded, partial least squares discriminant analysis (PLS-DA) was performed to identify the discriminant strength of the model and to generate a loadings plot from which metabolites could be identified which contributed to differences between groups. In SIMCAP-12, PLS-DA models were generated through sevenfold cross validation. In this method, every 7th sample is excluded (1st, 7th, 14th, 21st and so on), a model generated from the remaining samples and the excluded “training set” predicted back into the model. This was repeated for all the samples (grouping the 2nd, 9th, 16th and 3rd, 10th, 17th and so on) until all the samples have been excluded

once. The results were averaged to produce a model that was externally cross-validated.

Spectral peaks that were most contributory to PLS-DA models and those peaks that appeared divergent on comparison of median spectra were selected for peak integration. Comparison of integrals was performed using GraphPad Prism (La Jolla, CA, USA) using Mann–Whitney non-parametric tests of significance, as normal Gaussian distribution could not be assumed (*P*-values of <0.05 were considered significant).

RESULTS

Subject Recruitment, Exclusion and Demographics

A total of 51 patients were recruited: 19 with HCC and 32 with cirrhosis. From the HCC cohort, two patients were excluded as they had undergone curative liver transplantation or hepatic resection prior to sample collection; one patient was excluded as she/he was co-infected with HIV and further three samples were excluded on PCA analysis (see below). The total number of HCC samples after exclusions was therefore 13. From the cirrhosis cohort, four patients were excluded owing to insufficient radiological or biopsy evidence of cirrhosis status and two samples were excluded following PCA, leaving a total of 25. Median ages were similar in both groups: 61 years for HCC and 58 years for cirrhosis patients. There were proportionally more males in the HCC group (85% versus 56%) but this was not statistically significant (*P* = 0.26). Body mass indices were comparable, 25.9 kg/m² in the HCC group and 27.3 kg/m² in the cirrhosis group, again the difference not reaching significance (*P* = 0.23) (Table 1). All patients studied were ethnically Caucasian and born in the United Kingdom or in Europe.

There were greater numbers of Child's-Pugh grade A patients in the cirrhosis group (74% versus 40%), but

Table 1 UK Patient Demographics.

Characteristic	HCC	Cirrhosis	P-value
N	13	25	
Median age (range) (years)	61 (29–82)	58 (28–79)	0.55 ^a
Male <i>n</i> (%)	11 (85%)	14 (56%)	0.26 ^b
Median body mass index (kg/m ²)	25.9	27.3	0.23 ^a
Child–Pugh A	4 (40%)	17 (74%)	
Child–Pugh B	3 (30%)	4 (17%)	
Child–Pugh C	1 (10%)	2 (9%)	
Non-cirrhotic	2 (20%)	0	
Unknown due to insufficient data	3	2	

^aMann–Whitney test for non-parametric data.

^bFisher's exact test for categorical data.

Table 2 Barcelona Clinic Liver Cancer Stages of Hepatocellular Carcinoma.

Stage	Number
A	3 (30%)
B	0
C	5 (50%)
D	2 (20%)
Unknown due to insufficient data	3 (30%)
Total	13

Serum biochemical analysis.

cumulatively, grade A + B patients accounted for the majority of patients in both groups: 70% in the HCC cohort and 91% in the cirrhosis cohort. The liver disease etiologies of both HCC and cirrhosis patients were hepatitis C virus (HCV) (6;6), hepatitis B virus (HBV) (3;3), alcohol (2;9), NASH (0;2), hemochromatosis (1;1), HCV + alcohol (1;2), HBV + alcohol (0;0), NASH + alcohol (0;1), idiopathic (0;1), respectively.

Disease stages of HCC are displayed in Table 2. Three tumors were BCLC stage A, five grade C and two grade D. The tumor sizes ranged from a single 1 cm lesion (BCLC stage A) to a multifocal tumor with a combined diameter of 8 cm (BCLC stage D). Three tumors could not be staged owing to insufficient data.

AFP, biochemical and liver function test results are detailed in the [supplementary results \(supplementary Tables 1 and 2\)](#). Median AFP levels were significantly higher in the HCC group (18.1 IU mL^{-1} versus 4.6 IU mL^{-1}). Using an AFP cut-off level of 20 IU mL^{-1} , sensitivity and specificity were 45% and 95%, respectively and at 200 IU mL^{-1} , were 27% and 100%, confirming the poor sensitivity of AFP for HCC in this cohort.

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The median serum biochemistry profiles of serum creatinine, alanine transaminase, bilirubin and International Normalized Ratio were similar, with the exception of albumin which was lower in the HCC group, compared to the patients with cirrhosis (30 g L^{-1} and 35 g L^{-1} , respectively).

Multivariate Statistical Analysis

The initial PCA scatter demonstrated two HCC and one cirrhosis outlying samples, positioned outside the Hotelling's T^2 limit (see [supplementary results: supplementary Figure 2](#)). Examination of the NMR spectra of these samples identified significant glycosuria in one HCC sample,

which affected further analysis. The cirrhosis and remaining HCC outlying samples displayed dominant resonances at 1.18 ppm (triplet) and 3.65 ppm (quadruplet), which corresponded to ethanol resonances (see [supplementary results: supplementary Figure 3](#)).

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Following exclusion of these three datasets, PCA identified further significantly outlying samples (see [supplementary results: supplementary Figure 4](#)). Two outliers in this analysis revealed heavy influence from glucose metabolites. One of these patients, although not formally diagnosed as diabetic, had a high capillary glucose level (18.2 mmol L^{-1}) at the time of sampling. The other had no documented diagnosis of diabetes. These samples were excluded and a third PCA scatter plot was generated with R^2 and Q^2 values of 0.51 and 0.31, respectively (see [supplementary results: supplementary Figure 5](#)).

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To identify discriminatory metabolites between the two groups, PLS-DA was performed (see [supplementary results: supplementary Figure 6](#)). In a two component model, model fitting was poor ($R^2 = 0.48$) with negative Q^2 values, suggesting that, as a predictive tool, the model would be poorly predictive. The misclassification matrix from the PLS-DA predicted 7 as HCC and 6 as cirrhosis in the HCC group, and predicted 1 as HCC and 24 as cirrhosis in the cirrhosis group, resulting in a diagnostic sensitivity of HCC of 53.6% and specificity of 96%. The most influential variables, from PLS-DA loadings plot and visual comparison of median spectra are summarized in Table 3.

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Table 3 Discriminatory Metabolites Comparison Between Subject Cohorts.

Discriminatory metabolite	HCC median (arbitrary units)	Cirrhosis median (arbitrary units)	P-values
↑ Carnitine (3.24 ppm)	1.16×10^7	9.37×10^6	0.31
↑ Formate (8.5 ppm)	6.36×10^6	3.13×10^6	0.44
↓ Citrate doublet (2.56 ppm)	4.65×10^7	7.33×10^7	0.21
↓ Citrate doublet (2.68 ppm)	5.39×10^7	7.20×10^7	0.25
↓ Hippurate (3.97 ppm)	4.25×10^7	4.68×10^7	0.52
↓ Hippurate (7.56 ppm)	3.94×10^6	4.45×10^6	0.69
↓ p-cresol sulfate (2.35 ppm)	1.15×10^7	2.61×10^7	0.06*
↓ p-cresol sulfate (7.21 ppm)	6.68×10^6	1.32×10^7	0.04*
↓ Creatinine methyl (3.05 ppm)	4.50×10^8	5.01×10^8	0.26
↓ Creatinine methylene (4.06 ppm)	2.83×10^8	3.62×10^8	0.03*

*P-values calculated using Mann–Whitney tests of significance.

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Univariate Statistical Analysis

Influential metabolite peak integrals are displayed in Table 3 and Figure 1. Metabolites elevated in the urine of patients with HCC were carnitine (3.24 ppm, $P = 0.31$) and formate (8.5 ppm, $P = 0.44$), although neither result reaching significance. Urinary metabolites reduced in patients with HCC included citrate double doublet (2.56 ppm and 2.68 ppm, $P = 0.21$ and $P = 0.25$, respectively) and both the aliphatic and aromatic resonances of hippurate (3.97 ppm, $P = 0.52$ and 7.56 ppm, $P = 0.69$), neither of these results reaching significance. Peaks at 2.35 ppm ($P = 0.06$) and 7.21 ppm ($P = 0.04$) were significantly reduced in patients with HCC and were identified as arising from the same metabolite when a STOCSY analysis was performed. This metabolite was identified as p-cresol sulfate from the literature,¹⁵ the resonance at 2.35 representing the methyl signal and a pseudo-doublet at 7.21 ppm, the aromatic signal. A comparison of the median spectral integrals of the 7.21 ppm region is displayed in Figure 2. Finally, urinary creatinine was found to be reduced in patients with HCC, at both the creatinine methyl singlet at 3.05 ppm ($P = 0.26$) and significantly, at the methylene singlet at 4.06 ppm ($P = 0.03$) (Figure 2).

DISCUSSION

This is the first reported study to identify HCC-specific urinary ^1H NMR markers in a closely-matched group of patients with HCC of differing etiology. Similar to the studies of Gambian, Nigerian and Egyptian patient cohorts, reduced urinary creatinine, citrate and hippurate

and elevated carnitine levels were found in patients with HCC in our study, in comparison to those with cirrhosis, albeit not to levels of statistical significance.^{9–11} The study presented here also identified significant reductions in urinary p-cresol sulfate, a metabolite which was not discriminatory in previous studies.

Although this is a small study in relative terms, study patients were well matched, the majority being Child–Pugh stage A and B. In these patients, serum AFP, the most widely used marker for HCC, displayed poor diagnostic sensitivity (27%) at a cut-off of 200 IU mL^{−1}, while urinary multivariate analysis, despite displaying poor fitting and predictive parameters, produced a superior sensitivity (53.6%). Diagnostic specificity was similar for both tests (100% for AFP and 96% for multivariate models). These results do not compare as well as the published West African, Nigerian and Egyptian urinary ^1H NMR studies, which displayed multivariate model sensitivities and specificities approaching >90%.^{9–11} The reasons for poorer discrimination in this UK cohort are probably multifactorial. First, the cohorts for this study were small, but they were intentionally selected to be well matched, both for demographic and physical status and for etiology of liver disease. No healthy control group was included as the metabolic profiles of healthy patients have been shown to be very different to those of diseased groups, which may be due to global metabolic and hepatic function rather than tumor-specific changes. Furthermore, the stage of the tumors tended to be more advanced in the Nigerian and Egyptian cohorts, compared to the UK cohort. It is therefore possible that the close matching of cirrhosis and HCC patients, with ≥70% in both groups being Child–Pugh stage A or B, resulted in higher similarity of spectra. Certainly, BMI was similar in both groups, so it is unlikely that cancer cachexia, which may have been a prominent factor in causing altered spectral profiles in the Nigerian and Egyptian groups, had a great influence on discriminatory profiles in this study. Nevertheless, PLS-DA and

Figure 1 Median value scatter plots of discriminatory variables. *P*-values calculated using Mann-Whitney tests of significance.

Legend: p-cresol sulfate (the top two plots) is represented by a methyl resonance at 2.35 ppm and a pseudo-doublet aromatic resonance at 7.21 ppm. Citrate (the second two plots) is represented by a double doublet at 2.56 ppm and 2.68 ppm. Hippurate (the last two plots) comprises aliphatic and aromatic resonances at 3.97 ppm and 7.56 ppm.

Figure 2 (a) Comparison of median spectra of creatinine (4.06 ppm). (b) Comparison of median spectra of p-cresol sulfate (7.21 ppm).

median MR spectral comparison identified a number of spectral regions elevated or reduced in the HCC compared to the cirrhosis cohorts.

The metabolic alterations that we observed in this study may be partially explained by the Warburg phenomenon, where rapidly proliferating cancer cells with increased energy requirements preferentially switch to glycolysis with

little oxidative phosphorylation with potentially a series of measurable alternative energy intermediates in the plasma and urine.¹⁸

Therefore of note, urinary citrate levels (2.56 ppm and 2.68 ppm, $P = 0.21$ and $P = 0.25$, respectively) were reduced in the HCC cohort, although not to a level of significance. This finding is similar to that of the previous Egyptian

studies¹⁰ and may represent a repression of oxidative phosphorylation in the mitochondria, as predicted by the Warburg hypothesis.^{18,19} In addition, a previous study in Sprague-Dawley rats, by Bollard and colleagues, observed reduced urinary citrate after partial hepatectomy, which they proposed to be a marker of heightened energy demand and cellular stress, as would be the case in patients with HCC.²⁰

Urinary creatinine was reduced in patients with HCC, the methylene (CH₂) singlet at 4.06 ppm significantly reduced ($P = 0.03$), while the methyl singlet (CH₃) at 3.05 ppm, non-significantly reduced ($P = 0.26$). Similar findings were present in both previous West African and Egyptian cohorts of patients.^{9–11} Chen and colleagues, using HILIC and RPLC MS, also observed reduced urinary creatinine levels in HCC subjects.²¹ Creatinine is a breakdown product of creatine phosphate in muscle and is eliminated from the body by glomerular filtration and partial tubular excretion. Renal impairment will cause a rise in serum creatinine and a concomitant rise in urine creatinine. Serum creatinine levels were similar in both groups, so is unlikely to have contributed to urinary differences. Lower total body creatinine may be due to reduced muscle mass. Although the BMI of each group was non-significantly different (HCC = 25.9 kg/m² and cirrhosis = 27.25 kg/m², $P = 0.23$) it may be that the slight difference in weight is due to reduced muscle mass in HCC patients, which is reflected in lower urinary creatinine levels. A study of urinary creatinine in a US population observed that lower muscle mass was a major contributing factor to lower urinary creatinine levels.²²

Formate (8.5 ppm, $P = 0.44$) was non-significantly elevated in the HCC group and was not identified as a discriminatory metabolite in West African or Egyptian groups in the past. Formate is produced from the folate cycle in hepatic embryonic cells. Similar to the observable rise in other embryonic glycoproteins, such as AFP, elevated formate is the consequence of hepatocyte tumorigenesis.²³

Urinary hippurate levels (3.97 ppm, $P = 0.52$ and 7.56 ppm, $P = 0.69$), an aromatic acyl glycine, that is formed by conjugation of benzoate with glycine in liver and kidney mitochondria,²⁴ was non-significantly reduced in the urine of patients with HCC. Hippurate was similarly reduced in the urine of the Egyptian cohort of HCC patients. The gut microbiota play a key role in hippurate homeostasis through metabolizing aromatic compounds to form benzoate and modulating hippurate urinary excretion.^{25,26} It is possible that the decreased hepatic function, as evidenced by higher Child–Pugh scores in patients with HCC translates into less efficient benzoate conjugation and subsequently lower urinary hippurate excretion levels. In this way, hippurate may be seen as a surrogate marker of hepatic function. Alternatively, Williams and colleagues described that alteration in urinary hippurate in Crohn's disease was due to the alteration in the gut microbiota.²⁷ This may be suggestive of an interaction between

microbiotal modulation and HCC tumorigenesis. Alterations in the urinary level of this metabolite have also been reported in inflammatory bowel disease²⁸ and pneumonia,²⁹ questioning its specificity for a particular disease.

Urinary p-cresol sulfate was significantly reduced in the HCC cohort, represented by a pseudo-doublet at 7.21 ppm ($P = 0.04$). This corresponded to one of the aromatic resonances of the molecule, a further pseudo-doublet being found at 7.29 ppm. The methyl group, on the benzene ring, resonated at 2.35 ppm, and comparative median values approached significance ($P = 0.06$). This finding was not evident in the Nigerian and Egyptian groups. p-Cresol is a product of colonic bacterial metabolism of tyrosine. Certain bacteria, such as *Clostridium difficile*, are particularly high producers. Sulfonation to p-cresol sulfate occurs in the liver. Clayton and colleagues in 2008, observed that healthy human subjects with high pre-test urinary p-cresol sulfate levels sulfonated acetaminophen (paracetamol) to the less toxic acetaminophen glucuronide to a lesser extent than those with lower pre-test p-cresol sulfate levels.¹⁵ This was proposed to be due to the competitive inhibition of p-cresol sulfonation in the presence of acetaminophen (paracetamol). This study was performed in healthy patients, so it is not possible to surmise whether this would translate in higher likelihood of drug-induced liver toxicity, although sulfonation of hepatically-metabolized drugs is a common occurrence and it is possible that p-cresol sulfate is a surrogate marker of liver function, lower levels reflecting reduced sulfonation in those patients with HCC and worse liver function. Furthermore, there is accumulating evidence toward the role of microbiota in hepatic disease progression, the so called “microbiota–liver axis”. Chassaing and colleagues have pointed out that microbiota may be significant sources of inflammatory agents that contribute to both gastrointestinal and hepatic diseases.³⁰ The changes in p-cresol, therefore, may be an indication of a primary dysfunction of host microbiota, leading to less tyrosine metabolism, or a responsive change in the gut microbial environment, secondary to liver disease.

CONCLUSIONS

This is the first urinary ¹H NMR spectroscopy metabolic profiling study of HCC to be performed in a UK population. Overall, the findings provide further insight into the metabolic pathogenesis of liver disease and HCC and corroborate some of the findings highlighted in earlier studies in Nigerian, Egyptian and Gambian cohorts.^{9,10} Similarities include reduced urinary creatinine, citrate and hippurate and raised carnitine, albeit not to significant level. This study has also identified p-cresol sulfate as a discriminatory urinary metabolite in this UK cohort, significantly reduced in patients with HCC compared to those with cirrhosis. The explanation for this is not clear but may be due to altered liver function or to a change in the

microbiome-host interaction. Larger cohort numbers are required for validation of these data. Study of the host microbiome is also warranted.

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CONFLICTS OF INTEREST

The authors have none to declare.

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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.jceh.2016.03.003>.

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