Pharmaco-metabonomic profiling as a predictor of toxicity in patients with inoperable colorectal cancer treated with capecitabine

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STATEMENT OF TRANSLATIONAL RELEVANCE

In patients undergoing chemotherapy systemic toxicity remains the major limitation to adequate dosing. The ability to predict adverse events prior to drug administration, and to provide individualized treatment, is likely to have a significant impact on clinical outcomes and quality of life, particularly in the palliative setting. 'Pharmaco-metabonomics' - using pre-treatment profiles of endogenous metabolite levels to predict the metabolic fate and toxicity of drugs - potentially offers phenotypic information not captured by genetic profiling. A patient's response to treatment relies on a complex array of genetic and environmental factors and this approach has the ability to identify in a non-invasive manner a downstream profile that describes the 'current state' biological system, beyond the potential state defined by the genome. In the future models based on the combination of both pharmaco-genetic and pharmaco-metabonomic information using up- and down-stream data can help delineate the optimum therapeutic pathway for the individual patient.

Abstract

Purpose: Endogenous metabolic profiles have been shown to predict the fate and toxicity of drugs such as acetaminophen in healthy individuals. However, the clinical utility of metabonomics in oncology remains to be defined. We aimed to evaluate the effect of pre-treatment serum metabolic profiles generated by ^{1}H NMR spectroscopy on toxicity in patients with inoperable colorectal cancer receiving single agent capecitabine.

Experimental Design: Serum was collected from 54 patients with a diagnosis of locally advanced or metastatic colorectal cancer prior to treatment with single agent capecitabine. ^{1}H NMR spectroscopy was used to generate metabolic profile data for each patient. Toxicities were graded according to National Cancer Institute Common Toxicity Criteria version 2.0.

Results: Higher levels of LDL-derived lipids, including polyunsaturated fatty acids and choline phospholipids predicted for higher-grade toxicity over the treatment period. Statistical analyses revealed a 'pharmaco-metabonomic' lipid profile that correlated with severity of toxicity.

Conclusions: This study suggests that metabolic profiles can delineate sub-populations susceptible to adverse events and have a potential role in the assessment of treatment viability for cancer patients prior to commencing chemotherapy.

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Introduction

Metabolic profiling (metabonomics/metabolomics) is a flexible approach that can be used to investigate in a systematic manner the metabolic composition of cells, tissues and biofluids (1-4). It has recently been demonstrated that pre-treatment biofluid metabolic profiles can be used to predict the metabolic fate and toxicity of drugs *in vivo*, specifically for acetaminophen exposure in rodents (5), an observation subsequently shown to translate to man (6, 7). This strategy, termed 'pharmaco-metabonomics', potentially offers phenotypic information not captured by genetic profiling that can be used to predict pharmacology. In the study by Winnike et al. (7) a combination of both the early drug metabolite profile and observed changes in common urinary endogenous metabolites were able to identify a subpopulation of individuals who experienced ALT elevation in response to 4g/day acetaminophen, several days before the phenotype was apparent by conventional clinical chemistry.

While this experiment shows in principle how pharmaco-metabonomics could help to reduce adverse events in susceptible individuals, the trial was conducted in otherwise healthy volunteers with no clinical requirement for treatment. In patients undergoing chemotherapy systemic toxicity remains the major limitation to adequate dosing. The ability to predict adverse events prior to drug administration, and to provide individualized treatment, is likely to have a significant impact on clinical outcomes and quality of life, particularly in the palliative setting. While extensively used to characterise the tumour metabolome (8-10) and for the discovery of diagnostic biomarkers in body fluids (11, 12), there are relatively few examples of metabolic profiling being used to derive prognostic or predictive biofluid biomarkers in oncology (13). In a previous study we used an NMR-based approach to define a serum metabolic signature predictive of weight gain secondary to chemotherapy in patients with breast cancer,

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demonstrating that this platform can potentially identify phenotypes related to poorer outcomes (14). However, the utility of an NMR-based approach as a prognostic or predictive marker of clinical outcome remains to be evaluated.

Capecitabine is an oral prodrug of 5-fluorouracil (5-FU) which was designed to minimize gastrointestinal toxicity whilst maintaining anti-tumour activity. The pharmacologically inactive capecitabine is absorbed from the gastrointestinal tract and undergoes a three-step activation process to 5-FU within the tumour (15). During the first step, capecitabine is converted to 5′-deoxy-5-fluorocytidine by carboxylesterases primarily in the liver (15-18). This is then converted to 5′-Deoxy-5-fluorouridine (5′-DFUR) by cytidine deaminase within the liver and tumour tissue. In the final step, 5-FU is ultimately formed from 5′-DFUR by thymidine phosphorylase, an enzyme that is predominant in tumour tissues. This process results in improved bioavailability of 5-FU by reducing the catabolism of 5-FU in the liver; and leads to higher intra-tumoural 5-FU delivery. The intermediate, 5′-DFUR, is toxic in itself causing diarrhoea through formation of 5-FU as a result of metabolism by thymidine phosphorylase present in the small intestinal mucosa. Capecitabine has been shown to have equivalent efficacy in the management of colorectal cancer in both the metastatic and adjuvant setting (19-23). The dose-limiting side-effects are diarrhoea, stomatitis and palmar-plantar erythema. The aim of this study was to determine whether a pre-treatment serum metabolic profile could predict toxicity from capecitabine in patients with advanced colorectal cancer.

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Materials and Methods

Patients. The study was conducted as part of a previously published trial assessing the tolerability of fixed dose capecitabine (24). Consenting patients with locally advanced or metastatic colorectal inoperable cancer with measurable or evaluable disease who had adequate organ function and performance status were enrolled from three centers in Australia between January 2002 and August 2003. Each patient received single agent capecitabine 2000mg twice daily. The efficacy data of fixed-dose capecitabine have been published elsewhere (24).

Evaluation of patients. Complete history was recorded, full physical examination performed, and blood samples collected at baseline. Baseline computed tomography imaging of the chest, abdomen and pelvis were obtained within 3 weeks of treatment commencement. Patients were reviewed weekly during cycle 1 and then every 3 weeks for safety assessment. All safety evaluations were graded according to the National Cancer Institute Common Toxicity Criteria version 2.0. Hand-foot syndrome was classified as grade 1 (numbness, dysesthesia, painless swelling, or erythema not disrupting normal activities), grade 2 (painful erythema with swelling or affecting daily living activities), or grade 3 (moist desquamation, ulceration, blistering, severe pain, or any symptoms leading to an inability to work or to perform daily living activities (25).

Sample preparation. Serum samples (stored at -80°C and transported on dry ice) were defrosted at room temperature and randomized. For each sample a 200 μL aliquot of serum was mixed with 400 μL saline (0.9% NaCl in 10% D_2O) followed by centrifugation at 16000 g for 5 minutes. 550 μL of this solution was pipetted into a 5 mm NMR tube. Samples in NMR tubes were frozen at -40°C until NMR analysis. Serum preparation was done with samples on ice.

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^{1}H NMR Spectroscopy. All ^{1}H NMR spectra were acquired using a Bruker DRX600C spectrometer (Bruker Biospin, Rheinstetten, Germany), operating at a temperature of 300K, at a ^{1}H NMR frequency of 600.13MHz using automatic sample delivery into a 5mm TXI NMR probe. Gradient shimming was performed prior to acquisition of spectra. ^{1}H NMR spectra of the samples were acquired using the Carr-Purcell-Meiboom-Gill (CPMG) and 1D presaturation pulse sequences and were the sum of 256 free induction decays (FIDs) collected into 32k data-points with a spectral width of 12019.230 Hz.

Data treatment and statistical analysis. Exponential line broadening (1Hz), Fourier Transformation and manual spectral phasing and linear baseline correction were conducted using XWIN NMR 3.5 software (Bruker). Data were imported and manipulated in Matlab (Mathworks) using in-house software 'NMRProc' and 'Metaspectra' written and compiled by Dr. T.M.D. Ebbels, Dr H.C.Keun, Mr. J.T. Pearce, and Dr O. Cloarec. Two spectra were excluded due to poor spectral quality. The remaining spectra were calibrated to glucose at 5.23ppm using an automated calibration script (26), normalized (27) (by the median fold change to the median spectrum) and 'binned' in Matlab (this was done by selective 'peak-picking'; the data was visually assessed and 89 metabolite resonances identified). Varying bin widths were allocated based upon metabolite resonances and regions. Binned data was exported to SIMCA (Umetrics) for multivariate analysis. For relative quantification of the individual metabolites, signals were integrated with local linear baseline correction applied.

Statistical analyses were undertaken using SPSS Statistics version 17.0 (SPSS Inc.) and Microsoft Excel. Differences between metabolite integral regions were assessed by T-test (assuming unequal variance and corrected for multiple testing, q<0.05). Correlation between metabolite regions and severity of toxicity was assessed using Kendall's tau (rank-based, 2

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tailed). The criteria for metabolite selection was correspondence between visual identification of average differences in resonance intensity and spectral position of integral regions with the significance level as assessed by T-test and Kendall's tau. The relationship between metabolite signals and clinical data (BMI, weight at baseline, age, and sex) was assessed using correlation analysis (Kendall's tau).

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Results

Patients and treatment outcome. NMR spectra of sufficient quality could not be obtained from two samples; therefore, metabonomic-outcome analyses were conducted on 52 patients. The demographic and clinical characteristics of the patients are summarized in Table 1. The majority of patients had liver function tests within normal range at the time of diagnosis; mean albumin 39g/L (range 29 – 46), mean bilirubin 7μmol/L (range 4 – 147), mean AST 23U/L (range 9 – 125), mean ALT 20U/L (range 10 – 101) and mean ALP 116U/L (range 53 – 484).   Patients received 2g capecitabine twice daily as a single agent for a median duration of 3 months (range 1 - 7 months). The median number of cycles received was 4.9 (range 1-8 cycles). As previously published, the response rate to therapy was 28% (95% CI: 15.7 - 40.3) (24) .

Clinically significant toxicities are reported in Table 2. Overall, capecitabine was well tolerated with no grade 4 non-hematologic or grade 3/4 hematologic adverse events recorded. There were no adverse event–related deaths during the study. The most common treatment-related adverse events were diarrhoea, hand-foot syndrome, and fatigue.   Toxicity led to the cessation of treatment in eight patients (15%) and of these, five patients ceased treatment after cycle 1. The most frequent adverse event leading to discontinuation was grade 3 diarrhoea. These patients were included in the final analysis.

Relationship between ^{1}H NMR metabolic profile and toxicity

We hypothesised that there is a relationship between features in the ^{1}H NMR metabolic profile of sera taken from patients pre-treatment, and subsequent toxicity as a result of capecitabine exposure. Figure 1 is aliphatic region of the mean CPMG spectrum of the sera collected from

patients who experienced no toxicity overlaid with the mean spectrum of sera from those who experienced severe toxicity (grade 3) over the total treatment period. The CPMG experiment attenuates signals from macromolecules, in particular serum proteins, allowing a better focus on metabolic features. Visual inspection indicated that there were major differences on average between the spectra, including resonances from lipid fatty acid chains, glutamate, glutamine, polyunsaturated fatty acids (PUFA), and choline phospholipids.

Following these initial visual indications, the CPMG spectrum for each patient was integrated at 89 targeted spectral regions encompassing resolved resonances apparent by visual inspection. As an initial test of the hypothesis, a partial least squares-discrimination analysis (PLS-DA) was performed using the integrated ^{1}H NMR spectral regions from patients experiencing grade 0 and grade 3 toxicity. Figure 2A shows the scores plot for the resulting model, indicating separation between the two groups and when the data for grades 1 and 2 were predicted into the model the trend was for these intermediate toxicity groups to cluster between the two extremes. This suggested that there were some features in ^{1}H NMR spectral profile dependent on the severity of the experienced toxicity. However the cross-validation predictivity of the model approached but did not reach significance by permutation analysis (Q^2=0.42, p=0.098), limiting the further use of the model to identify the discriminatory features. The PLS-DA loadings coefficients (Figure 2B) indicated that the resonances from lipid fatty acid chains, glutamate, polyunsaturated fatty acids (PUFA), and choline phospholipids were also influencing the multivariate model, consistent with our initial observations.

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To define more precisely which spectral features were significantly associated with toxicity from capecitabine, we assessed the mean difference in intensity of each metabolite resonance (89 resonances, the same as used for the multivariate analysis) between the grade 0 and grade 3 groups. Table 3 shows the ^{1}H NMR resonances that were identified as being significantly different between the two patient groups. These included fatty acid chains, polyunsaturated fatty acids, choline phospholipid, valine, adipic acid, tyrosine and one unassigned resonance. We then applied the method of Benjamini and Holchberg (28) to evaluate the false discovery rate (FDR) and P-value corrected for multiple testing (q). Four spectral regions were still considered significant (q<0.05) after multiple testing correction, corresponding to the following moieties; -CH3 (δ 0.8-0.86); $(-CH_2-)_n$ (δ 1.21-1.24); =CH-CH$_2$-CH= (δ 2.72-2.78), and N(CH$_3$)$_3$ (δ 3.2-3.2). The levels of these resonances were all higher in the patients who experienced severe toxicity compared to no toxicity. These moieties were assigned to LDL-like lipid particles (δ0.8-0.86 & 1.21-1.24), including polyunsaturated fatty acids (δ2.72-2.78), and choline phospholipids (δ3.2-3.22). The assignments were made on the basis of previous literature (1, 2, 29), describing variation in the frequency of lipoprotein resonances as a result of susceptibility anisotropy (example 2D NMR data is also available as supplementary data). The annotation of the ^{1}H NMR spectrum of human plasma and serum has been comprehensively described in many publications which show that the metabolites detectable by NMR in serum are highly consistent and that significant variation in chemical shift due to inter-sample variation in pH, temperature or ionic strength is also minimal in this biological matrix.

We also compared the CPMG spectra to those acquired using a standard pre-saturation sequence and observed a similar or lower magnitude of differences between the toxicity groups (Figure 3).

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As the CPMG suppresses high-molecular weight species such lipoprotein particles, this suggested that the CH_2 and CH_3 moieties were from smaller, higher density particles such LDL, rather than very low density lipoprotein (VLDL). Finally, the fact that choline phospholipid-based and polyunsaturated fatty acid-based resonances were observed this also supports the assignment of the lipid-based resonances to LDL, as phosphatidylcholine is the main phospholipid in LDL, and the most common fatty acyl chain in LDL is from a polyunsaturated fatty acid. Despite this there is substantial evidence to suggest that NMR lipid profiles are sensitive to subtle differences in lipoprotein particle size and composition that is not reflected by conventional estimates of lipoprotein particle distribution (30, 31) and hence we define our lipid profile primarily by resonance frequency rather than lipoprotein species.

Having identified a pharmaco-metabonomic lipid profile discriminating between the most extreme toxicity groups (grade 0 and grade 3), we observed that those patients experiencing intermediate levels of toxicity (grade 1 and 2) also appeared on average to possess an intermediate spectral profile in terms of the same selected lipid resonances, and that there was a visual indication of a progression with severity of toxicity (Figure 3; Figure 4). To assess more objectively the progressive nature of the relationship between lipid profile and toxicity grade, a non-parametric correlation analysis was conducted on the resonance intensities using Kendall's tau statistic. Significant correlations ($p<0.05$) were observed for four lipid resonances in our pharmaco-metabonomic profile (Table 2; tau 0.227-0.286) and also to a lesser extent for resonances from glutamate (δ 2.31-2.37) and unsaturated lipids/triglycerides (δ5.26-5.36). These results support the hypothesis that a more quantitative relationship exists between the pre-treatment metabolic profile and subsequent toxicity from capecitabine.

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Finally, to test if the lipid resonances in the pharmaco-metabonomic profile were providing information beyond basic anthropometric parameters, we calculated all possible non-parametric correlations between each of the four selected resonances and age, gender, BMI, and baseline body weight. Weight at baseline, but not BMI, age or gender, was inversely correlated to toxicity grade (tau=-0.233), consistent with previous reports of a significant relationship between low lean body mass and increased 5-FU-induced adverse events (32). BMI and baseline weight were negatively correlated to both lipid CH_3 and $N(CH_3)_3$ resonances (-0.232 to -0.305), but not to $(-CH_2-)_n$ or $=CH-CH_2-CH=$ resonances, and the correlation between the lipid resonances was variable (0.193-0.538). Collectively these observations indicate that the lipid profile provides information beyond basic baseline patient characteristics on the risk and possible severity of toxicity.

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Discussion

In the last few years the ^{1}H NMR-based metabolic profiling approach has shown potential in the prediction of response to treatment (pharmaco-metabonomics) using the fate of paracetamol in rats and man as an example of the method in principle (6, 7, 33). These studies highlight the predictive potential of metabonomics-based personalised health care in a clinical setting. The current challenge is to assess how well this pharmaco-metabonomic approach translates to the clinic. A patient's response to a given chemotherapeutic treatment relies on a complex array of factors that are broadly encompassed by both the genome and environment. Unlike pharmaco-genomics which focuses on the genetic/enzymatic factors in disease and drug metabolism, pharmaco-metabonomics interrogates the metabolism of a particular biofluid or tissue from a patient, providing a profile that is derived from combined genetic and environmental influences (34). The advantage of this approach is the ability to identify a downstream profile that provides a picture of the biological system in its 'current state', as opposed to a potential state (as may be indicated by a genetic factor).

Previous research using metabolic profiling of biofluids in the oncology setting has focused on identifying panels of metabolites that show potential for aiding current diagnostic methodology (11). To our knowledge this is the first study to report the predictive capacity of metabonomics to allow identification of toxicity severity secondary to chemotherapy using pre-treatment serum samples from patients with colorectal cancer. In this study we showed an association with lipid-based resonances and increased toxicity experienced by patients. This study highlights the possible role of this technique in individualizing chemotherapy regimens to avoid intolerable side-effects, thereby improving treatment outcomes.

Whilst provocative, the mechanism by which this association occurs remains to be elucidated and a number of hypotheses may account for our findings. One hypothesis may be that of inflammation. It is well established that raised levels of inflammatory markers are associated with elevated serum lipids, in particularly LDLs (35-37). Furthermore, the presence of inflammation has been shown in a number of studies to be a predictor of clinical outcome in malignancy (38-41). A number of *in vivo* studies have illustrated alteration in hepatic expression of nuclear receptors, LXR, FXR, PPAR, CAR and PXR, involved in lipid handling in the presence of inflammation (42). It is therefore plausible that the NMR signature observed in this study is a result of alteration in hepatic metabolism due to the presence of circulating pro-inflammatory cytokines. Previous work by a number of investigators suggest that the presence of extra-hepatic malignancy can impact negatively on hepatic drug metabolism; the mechanism by which this occurs remains to be elucidated but is postulated to be the presence of inflammation (43)(44, 45). Furthermore, previous reports utilizing the lipid lowering agent, omega-3 fatty acids, as an adjunct to chemotherapy, has shown these to be beneficial in reducing toxicity from chemotherapy in colorectal cancer patients, and this has been attributed to the anti-inflammatory effect of these interventions, rather than the lipid lowering effects of the fatty acids (46). This again lends support to the hypothesis that abnormal lipid profiles may reflect inflammation secondary to malignancy. Serum lipids as a predictor of either toxicity or response have not been studied in oncology as lipids are not routinely measured in patients with malignancy and this remains an open area of investigation.

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A further hypothesis for our findings is the impact of lipids on the protein binding of capecitabine itself and its metabolites. Protein binding strongly influences a drug's distribution and/or clearance, and a number of studies have illustrated altered protein binding in patients with type II diabetes and hyperlipidaemia (47-49). Lipids are known to interact directly with proteins to alter their capacity for drug binding via competitive or allosteric modulation. Capecitabine is relatively hydrophobic in comparison to its metabolites, and it is possible that patients with raised lipids may have a greater circulating pool of capecitabine and therefore may experience greater drug exposure. Future studies should incorporate first dose PKs of capecitabine and its metabolites to further investigate this hypothesis, as it is possible that the association between pre-treatment serum lipid profiles and the level of toxicity experienced in this patient cohort may represent subtle differences in lipid-based metabolism that result in altered clearance in capecitabine, or its metabolites, leading to the differences in toxicity reported.

Previous meta-analysis identified poor performance status, age greater than 70 and female gender as predictors of toxicity to single agent 5-FU (50)(51). However, there have been no studies in patients with metastatic colorectal cancer investigating BMI or base weight as predictors of toxicity. A number of papers have shown that in the adjuvant setting, a BMI of greater than 30 is predictive of worse toxicity (52, 53). However, there are a number of limitations relating to BMI mainly that it is entirely based on a Caucasian population and may not relate to other ethnicities, which contributed to our study population. Furthermore, BMI does not consider the physiological state of the patient which is important particularly in patients with malignancy. Other anthropometric measures have been suggested to be more accurate in determining body composition and a study by Prado and colleagues found an association

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between low lean body mass, as measured by CT, and worse toxicity with 5-FU, lending support to our findings (32). Therefore, whilst our findings of weight and toxicity are interesting they raise the need further investigation with more detailed anthropometric measures in a larger patient population.

We chose to study the metabonomic profile of patients prior to receiving capecitabine with the primary aim to identify a metabonomic profile predictive of toxicity. Whilst these results are provocative they need to be validated in larger confirmatory studies. The results of this study will act as a training set that will be validated in a larger patient group to confirm the utility of metabonomics as a predictor of toxicity prior to patients receiving chemotherapy. From this planned future study it will be possible to more accurately define specific cut-off values for spectral species above which patients presenting to the clinic would be likely to experience toxicity. Chemotherapy doses could then be prospectively altered. These studies will be difficult to conduct with single agent capecitabine as combination therapy now remains the mainstay of treatment in colorectal cancer, and furthermore individual drugs themselves may impact on the metabonomic profiles. For example there have been several reported incidences of patients treated with capecitabine developing hypertriglyceridemia which would influence post-therapy profiles obtained (54-58). How these drug effects are treated remains to be considered. In future studies it would also be of interest to investigate impact of chemotherapy on the metabonomic profile by considering pre- and post-treatment NMR spectra, and correlating any change with clinical outcome.

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Chemotherapy-induced toxicities have direct impact on cancer treatment outcomes including response rates and survival (59). Moreover, adverse effects not only limit the ability of the oncologist to effectively deliver treatment, but they also have a significant negative impact on the patient's quality of life (60-62). It can be argued that a significant level of toxicity is acceptable if the ultimate goal of treatment is cure, however severe treatment related toxicity is unacceptable where the objective of treatment is symptom palliation (63, 64). The use, therefore, of serum metabonomics as a non-invasive tool for the prediction of toxicity could greatly benefit this patient population and these data require further investigation prospectively in a large clinical trial. The next step in the process of defining a predictive set of markers is to move to a much larger patient cohort to generate predictive models with training and validation sets. In all such predictive studies there remains the challenge of balancing findings identified in populations, e.g. geographical differences in folate intake, versus those derived from the study of inter-individual differences. Our eventual goal is to produce models based on the combination of both pharmaco-genetic and pharmaco-metabonomic information using up- and down-stream data to help delineate the optimum therapeutic pathway for the individual patient(65)(66).

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Acknowledgements

Lynn Maslen, Maciej Kaliszczak, Eric Aboagye

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Figure Legends

Figure 1   Metabonomic analysis reveals a metabolic signature associated with capecitabine toxicity. Overlay of mean ^{1}H NMR CPMG spectra from the patient groups who experienced no toxicity (grade 0, solid line) and those that experienced severe toxicity (grade 3, dashed line).

Figure 2   Partial least squares-discriminant analysis (PLS-DA) models of grade 0 (no toxicity, n=3, circles) and grade 3 (severe, n=9, squares) toxicity. (A) Scores plot from the 2 component PLS-DA model, showing the grade 1 and 2 data (mild + moderate) predicted using the model. (B) The PLS-DA loadings coefficients most influential on the model. The magnitude of the coefficients corresponds to the importance of a particular resonance in explaining the toxicity status. R^2X, R_2Y, and Q^2 for the model are 0.417, 0.917 and 0.42, respectively. Data were UV scaled. Permutation analysis p=0.098 (supplementary figure 1)

Figure 3   Comparison of the mean 1D presaturation and CPMG spectra for each toxicity grade for (A) fatty acid chain, and (B) choline phospholipid spectral regions.

Figure 4   The metabolites that differ significantly between patients that consequently experience no toxicity and those that experience a severe response show intermediate levels in the patients that experience mild and moderate toxicity (grade 1 and 2). In all cases p<0.05 for both Kendall's tau across all toxicity grades, and a T test comparing no toxicity (grade 0) and severe groups (grade 3)

Table 1 Demographic and clinical characteristics (n=52)

Characteristic	No. of patients (%)
Male	34 (65)
Median age, yrs (range)	79 (42 - 86)
Median bodyweight, kg (range)	70 (40 – 117)
Performance status	
0	18 (35)
1	32 (62)
2	2 (4)
Primary tumour site	
Colon	38 (73)
Rectal	14 (27)
No. of metastatic sites	
1	31 (60)
2	15 (29)
≥3	6 (11)
Prior adjuvant chemotherapy	12 (23)
Prior chemotherapy for metastatic disease	2 (4)
Prior pelvic radiotherapy	6 (12)

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Table 2   **G**rade 2 and 3 adverse events experienced by patients over the study period. All safety evaluations were graded according to the National Cancer Institute Common Toxicity Criteria version 2.0 (n=52).

	% of patients	
Toxicity[§]	**Grade 2**	**Grade 3**
Fatigue	25	2
Hand-foot syndrome	11	11
Stomatitis	15	0
Diarrhea	25	9
Nausea and vomiting	9	2
Anaemia	13	0
Bilirubin	4	0

Table 3   Spectral regions from pre-treatment serum associated with the level of toxicity experienced in patients receiving fixed-dose capecitabine

^{1}H shift (ppm)	multiplicity	molecule	assignment	% change from grade 0 toxicity			Grade 0 toxicity versus grade 3 t-test p value (q-value)	correlation with grades 0-4 Kendall's tau (p-value)
				Grade 1 (n=9)	Grade 2 (n=26)	Grade 3 (n=9)		
0.8-0.86	m	lipid, fatty acid chain	$-CH_3$	47.3	49.3	70.1	0.0012 (0.027) **	0.271 (0.018*)
1.21-1.235	m	lipid, fatty acid chain	$(-CH_2-)_n$	35.6	38.4	53.9	<0.001 (0.006) ***	0.265 (0.021*)
2.720-2.780	m	PUFA	$-CH-CH_2-CH=$	66.2	67.6	117.8	<0.001 (0.021) ***	0.227 (0.047*)
3.2-3.22	m	choline phospholipid	$-N(CH_3)_3$	45.0	53.5	71.6	0.001 (0.03) **	0.286 (0.013*)
1.235-1.295	m	lipid, fatty acid chain	$(CH_2CH_2CH_2)_n$	44.5	75.9	103.0	<0.01 (0.14)	0.227 (<0.05*)
0.99	d	valine	$-CH_3$	20.1	19.9	23.2	<0.01 (0.14)	0.113 (0.323)
7.2-7.3	m	unassigned	-	-23.8	-21.7	-36.0	0.011 (0.14)	-0.224 (0.051)
1.57	m	adipic acid	CH_2CH_2CO	851.7	590.5	263.8	0.014 (0.16)	0.132 (0.250)
6.88	d	tyrosine	H3, H5	-23.4	-18.5	-32.1	<0.05 (>0.20)	-0.157 (0.169)
2	m	lipid, fatty acid chain	$CH_2C=C$	8.4	14.3	23.5	<0.05 (>0.20)	0.169 (0.140)

Spectral regions were screened using a T test comparing the patients that experienced no toxicity versus severe toxicity (2 tailed, assuming unequal variance and corrected for multiple testing, q<0.05). These regions were then tested for correlation across all four

toxicity grades using Kendall's tau (rank-based correlation, 2 tailed). Tests were considered significant at the following levels: ***p≤0.001, **p≤0.01, *p≤0.05 after correction for multiple testing; PUFA, polyunsaturated fatty acid
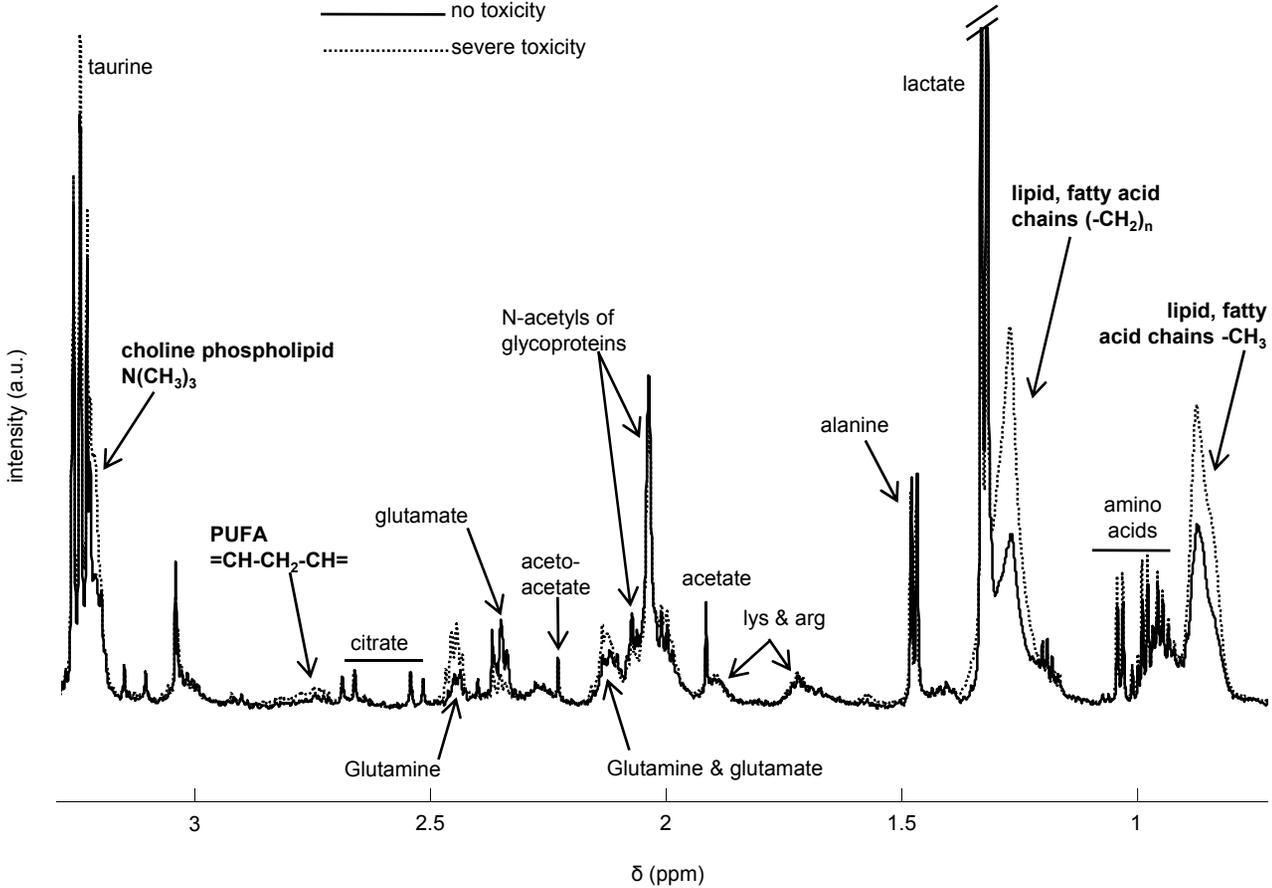
Figure 1

Figure 2

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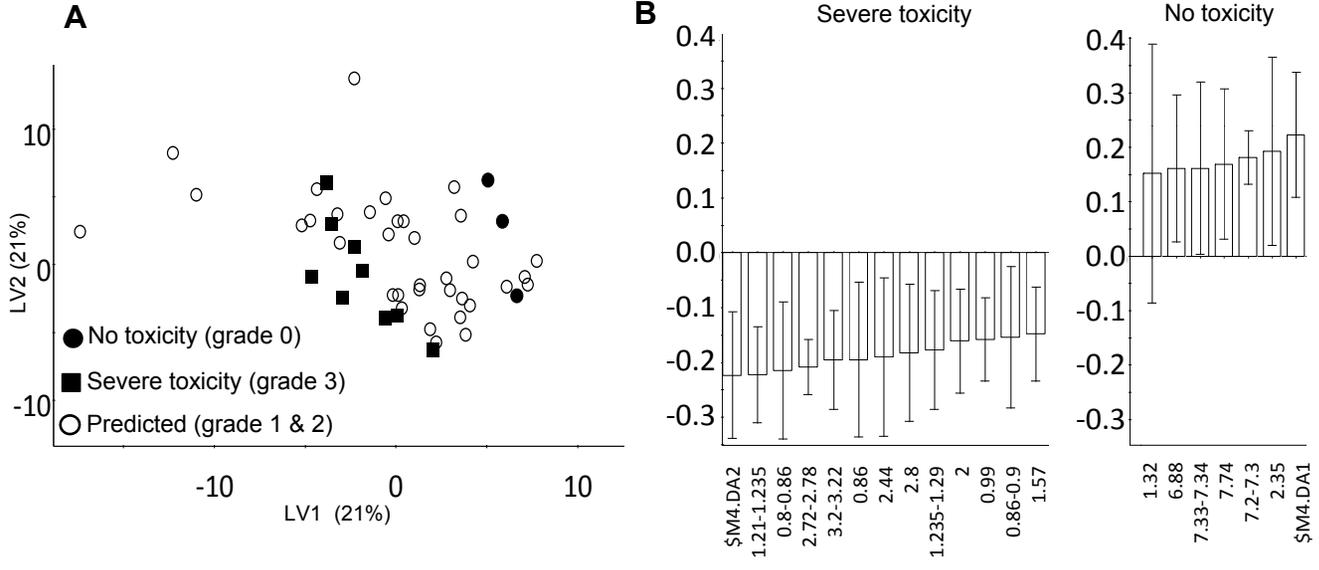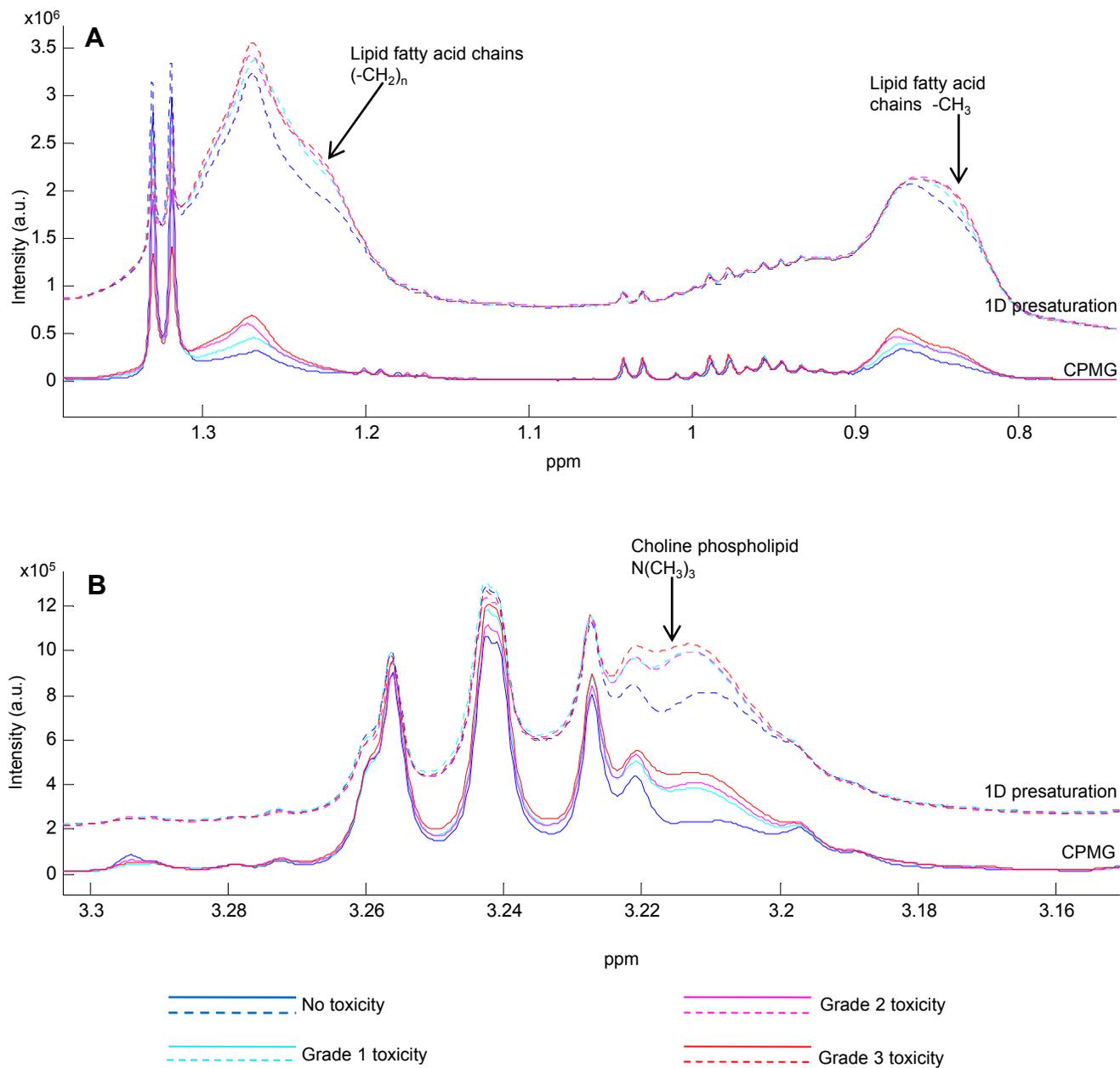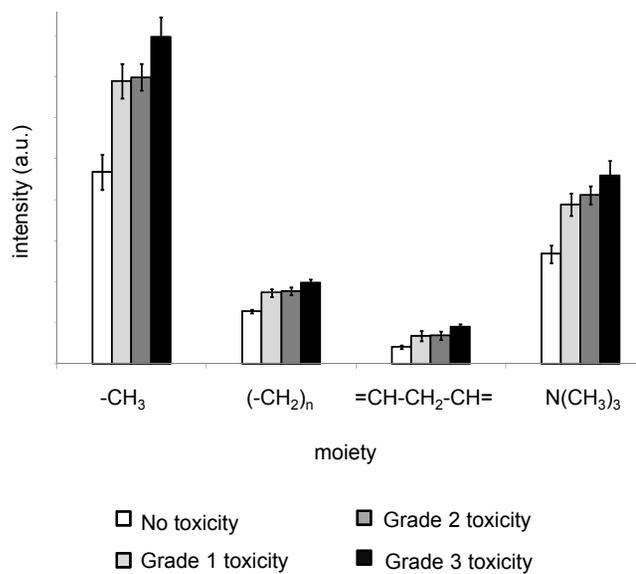
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Pharmaco-metabonomic profiling as a predictor of toxicity in patients with inoperable colorectal cancer treated with capecitabine

Alexandra Backshall, Rohini Sharma, Stephen Clarke, et al.

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