software, G Smith: None declared, D Prytherch Shareholder with: Spouse holds shares in the Learning Clinic software development company, R Aspinall: None declared.

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PWE-290 ACTIVE ALCOHOL CONSUMPTION INDUCES FUNCTIONAL IMMUNE PARESIS BUT PARADOXICALLY PROMOTES ENDOTOXIN TOLERANCE IN THOSE WITH ADVANCED ALCOHOL-RELATED CIRRHOSIS

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Introduction Patients with alcohol-related cirrhosis (ARC) are particularly prone to infection which is frequently a precipitant of organ failure and death. Neutrophil dysfunction has been reported in patients with ARC. Sepsis and associated endotoxemia occur in approximately 40% of hospitalised patients. Neutrophil TLRs are able to sense pathogens and induce inflammatory responses but whether active alcohol drinking is protective or detrimental in this context remains unknown. The aim of this study was to characterise the neutrophil phenotype, TLR2/4/9 expression and plasma cytokine profile in patients with ARC who were abstinent (n=13) compared to those who were drinking (n=16), split by MELD score15 compared to healthy controls (n=12).

Methods Neutrophils isolated from patients with ARC were studied ex vivo at baseline, and following 2-h stimulation with lipopolysaccharide (LPS) and the bacterial degradation protein fMLP. Neutrophil phenotype and TLR expression were determined using anti-CD16 (PE); -CD11b (APC-Cy7); -TLR2 (Alexa Fluor 488); -TLR4 (biotin conjugated PE-Cy7 Streptavidin) and -TLR9 (APC) by flow cytometry. Intracellular cytokine production pre- and post-stimulation will be measured by CBA.

Results The severity of cirrhosis (MELD15) or abstinent/active drinking status did not significantly impact on resting CD16, CD11b, TLR2 and 9 expression. TLR 2/9 expression on exposure to LPS and fMLP showed downregulation in those who were active alcohol-drinkers compared to the baseline. Conversely TLR4 expression was upregulated in the abstinent group with MELD>15; an effect significantly abrogated by the effect of active drinking (p=0.004). Active alcohol consumption did not however change TLR4 expression in those with MELD.

Conclusion Active alcohol consumption was shown to downregulate functional TLR2/9 expression resulting in immunoparesis with potential susceptibility to Gram-positive infection. However, active alcohol consumption was shown to abrogate the increased TLR4 responses to endotoxin stimulation seen in the abstinent ARC cohort with MELD >15. This implies that alcohol paradoxically promotes endotoxin tolerance and may act as a potential protective mechanism against Gram-negative insults in those with advanced cirrhosis.

Competing interests None declared.

PWE-291 MAPK SIGNALLING REGULATES THE DEVELOPMENT OF A CHOLANGIOCELLULAR PHENOTYPE FROM HCC IN POST-TACE LIVER TRANSPLANTS

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Introduction During normal liver wound-healing, tissue injury causes inflammation and cell death, which in turn orchestrates compensatory cell proliferation and regeneration.[1] If resolution of the injury cannot be obtained the resulting unchecked healing process can lead to cancer. Similar injury-induced repair mechanisms occur in response to chemotherapeutic treatments.[1] We recently found that the use of pre-transplantation TACE for HCC promotes the development of a mixed cholangiocellular phenotype that is associated with the expression of CD133, a marker of hepatic progenitor cells (HPC). This phenomenon is associated with higher post-transplantation tumour recurrence.[2] The molecular bases for the development of the cholangiocellular phenotype have not been investigated before. In this study we identified key oncogenic effectors involved with a *"switch"* from HCC to cholangiocellular phenotype resulting from liver injury induced by TACE treatment.

Methods Ten cases of post-TACE (doxorubicin/lipiodol) treated HCC examined after transplantation at King's College Hospital were selected for the study. HCC patients not treated by TACE before transplantation were used as control group. Formalin-fixed section from main tumour mass embedded in paraffin blocks were used to perform immunohistochemistry in order to delineate which component of the MAPK signalling pathway is associated with the hepatocholangiocellular phenotype and expression of HPC markers (CD133 and CK19).

Results Among the post-TACE liver explants, approximately 50% of the neoplastic cells resembling bile ductules (cholangiocellular differentiation) showed nuclear expression of a specific phospho-activated component of the MAPK cascade. Furthermore, half of the cells positive for activated-MAPK showed proliferative activity, as determined by Ki67 staining. Expression of activated-MAPK was not detected in the non-TACE treated group of liver transplant.

Conclusion The development of the mixed hepato-cholangiocellular phenotype in HCC patients after TACE-inflicted liver injury relates to activation of a specific MAPK signalling pathway that promotes proliferation of HPC. Attenuation of this signalling pathway could be used to prevent the *"uncontrolled"* proliferation and differentiation of progenitor cells before liver transplantation in HCC patients, and thus improving the tumour recurrence rate.

Competing interests None declared.

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PWE-292 NAFLD: BELOW THE RADAR EVEN ON SURFACING IN SECONDARY CARE!

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Introduction Non-alcoholic fatty liver disease (NAFLD) is becoming a common cause for secondary care referral. While simple steatosis does not lead to liver related morbidity, non-alcoholic steatohepatitis (NASH) may lead to cirrhosis and hepatocellular carcinoma. Currently, non-invasive imaging including ultrasonography (USS) may reveal fatty infiltration. Many patients attend secondary care for USS, in whom NAFLD may be identified incidentally. While the true prevalence and burden of NAFLD in the community remains unclear, patients attending for USS in whom NAFLD is identified could provide the opportunity to reduce disease burden by monitoring for any complications. The aims of this study were to assess

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whether patients reported to have fatty liver on USS, irrespective of clinical indication, were adequately assessed by testing of liver function tests (LFTs) and if abnormal, subsequently referred to a specialist clinic.

Methods A single centre, retrospective analysis of all patients who underwent USS over a 5-month period (January–May 2011) at Chase Farm Hospital was performed. Patients who had LFTs within 8 weeks of USS were said to have had their LFTs checked appropriately. Data were obtained from radiology reports via PACS/EPR reporting systems.

Results 258 patients were investigated over the audit period. 69 (26.7%) patients (42 male, 27 female), median age 58 years (25–91 years) were reported to have fatty liver on ultrasound. 52 (75.3%) of these patients had their LFTs checked of which 37 (71.2%) were abnormal. 12 (17.3%) patients with fatty liver on ultrasound were formally seen in a specialist clinic. Over half of patients (27, 51.9%) with fatty liver and abnormal LFTs were never seen in a specialist clinic.

Conclusion A quarter of patients with USS diagnosis of fatty liver did not have their LFTs checked potentially missing an opportunity to monitor for complications of NAFLD. While only the tip of the iceberg of NAFLD patients are referred to secondary care, a large portion of the iceberg goes unnoticed even on surfacing. An increased awareness of NAFLD needs to relayed to all healthcare professionals including radiologists and general practitioners coming into contact with this "iceberg."

Competing interests None declared.

PWE-293 LOW GLYCAEMIC INDEX DIETARY INTERVENTION FOR PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE IN THE GENERAL POPULATION—A RANDOMISED CONTROLLED TRIAL

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Introduction Low glycaemic index diet improves insulin sensitivity and adiposity. Its effect on non-alcoholic fatty liver disease (NAFLD) is unclear.

Methods This was a single-blind, randomised controlled trial of NAFLD patients aged 18–70 years who were recruited through population screening. All patients were instructed to exercise around 90 min/week, and were randomised to participate in a low glycaemic index dietary intervention programme or receive usual care. The dietary intervention programme was led by dietitians and consisted of education on food components, interpretation of food labels, food exchanges, healthy eating out techniques and cooking methods. The primary endpoint was the proportion of patients with resolution of NAFLD at month 12, defined as intrahepatic triglyceride content (IHTG) <5% by proton-magnetic resonance spectroscopy.

Results At the time of analysis, 104 patients (51 in the intervention group and 53 in the control group) had completed month 12 assessment. The adherence to the intervention programme was excellent, with attendance over 80%. Resolution of NAFLD occurred in 35 (69%) patients in the intervention group and 11 (21%) in the control group ($p<0.001$). IHTG decreased from 11.7±6.2% to 4.8±4.6% in the intervention group but remained static from 11.7±6.9% to 9.7±6.7% in the control group (mean difference in IHTG change 4.9%; 95% CI 2.6% to 7.1%). Reduced body mass index (BMI) was observed in the intervention group first at month 3 and maintained through month 12. At month 12, BMI decreased by 9.2±7.8% from baseline in the intervention group, compared to 1.1±5.5% in the control group ($p<0.001$). By multivariate analysis, dietary intervention (OR 4.2; 95% CI 1.1 to 15.4), baseline IHTG (OR 0.82; 95% CI 0.72 to 0.93) and percentage change in BMI (OR 0.78; 95% CI 0.68 to 0.89) were independent factors associated with NAFLD resolution.

Conclusion Low glycaemic index dietary intervention is effective in reducing liver fat in NAFLD patients in the general population. (This study was supported in part by the Nutritional Research Foundation of the UK and the Centre for Nutritional Studies, Faculty of Medicine, The Chinese University of Hong Kong.

Clinical trial registration number ClinicalTrials.gov number, NCT00868933.

Competing interests None declared.

PWE-294 MICROPARTICLE DEPENDENT PROCOAGULANT ACTIVITY AND THROMBIN GENERATION IS INCREASED IN PATIENTS WITH CIRRHOSIS INDUCED COAGULOPATHY

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Introduction Recent data suggests stable cirrhotics may have a hypercoagulable phenotype. Microparticles (MPs) are submicron plasma particles formed by the exocytic budding of cell membranes and play an important role in haemostasis due to phosphatidylserine (PS) surface expression which provides a phospholipid surface for assembly of coagulation enzymes and/or the expression of tissue factor (TF), the primary initiator of coagulation. To determine whether MPs may contribute to this hypercoagulable phenotype, we assessed microparticle associated functional procoagulant and phenotypic characteristics in cirrhotics.

Methods 72 consecutive cirrhotics and 30 healthy volunteers were recruited. Platelet free plasma (PFP) was prepared by two centrifugations and MP-free plasma (MP-FP) by filtration of PFP through a 200 nm microparticle filtration unit. Microparticle (MP) associated procoagulant activity (PCA) was measured using the STA Procoag PPL (phospholipid) assay (Stago Diagnostics) and MP associated thrombin generation (TG) measured using the calibrated automated thrombogram (CAT). For the CAT assay TG was initiated by adding $CaCl_2$ and 1 pM tissue factor, but no phospholipid (PRP reagent), therefore TG was dependent upon phospholipid present in the sample. Flow cytometry (LSRII) was used to determine MP size, number and cellular origin using marker specific antibodies.

Results PFP from cirrhotics generated significantly more thrombin than healthy volunteers reflected in the ETP (1374.3 vs 1142.6 nM/min, $p=0.04$), the peak (101.0 vs 66.5 nM, $p=0.001$) and a shorter time to peak (13.0 vs 14.2 min, $p=0.03$). Similarly, MP associated PCA was significantly increased in cirrhotics (65.9±13.2 s), compared to healthy volunteers (74.6±13.9 s, $p=0.005$). Following filtration of MPs >200 nM in size, there was a large reduction in ETP and peak in both cirrhotics and healthy volunteers, with prolongation of both the time to peak and PPL time. There was significant inverse correlation between the PPL assay and parameters of the TG test [ETP ($r=-0.57$, $p<0.001$), Peak ($r=-0.43$, $p<0.001$)]. Cirrhotic patients had high levels of Annexin V binding PS positive MPs compared to controls (1412 vs 279 per u/l, $p<0.05$).

Conclusion Microparticle dependent procoagulant activity and thrombin generating capacity is increased in plasma from cirrhotics. High levels of annexin-V positive procoagulant MPs are a likely key and previously undescribed mechanism contributing to the hypercoaguable phenotype observed in cirrhotics.

Competing interests None declared.

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