glutamine levels ($r=0.78$, $p=0.002$). *Myo*-inositol concentration decreased significantly by $0.7 (\pm 0.7)$ mMol/l between scans and this correlated with the mean difference in ADC ($r=0.59$, $p<0.04$).

Conclusion These results show that hyperammonia can be derived from nitrogenous substrates in the colon and can *directly* drive changes in brain water distribution as a mechanism for cerebral oedema development. Since cerebral astrocytes contain glutamine synthetase, our MRS data suggests intracerebral formation of glutamine from ammonia. Developments in therapy for hepatic encephalopathy need to focus on amelioration of colonic ammonia formation.

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P12 MEASURING THE EFFECTIVENESS OF A MULTIDISCIPLINARY NASH CLINIC

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Introduction Non-alcoholic fatty liver disease (NAFLD) and specifically non-alcoholic steatohepatitis (NASH) are associated with both increased liver-related and cardiovascular morbidity and mortality. A multi-disciplinary, individualised approach targeting the complex pathogenesis of the disease has been employed in a tertiary/secondary care setting. The aims of this study were (1) to investigate the effectiveness of a multi-disciplinary NASH clinic by assessing the change in liver disease markers and risk factors for liver-related and cardiovascular mortality over time, and (2) to identify factors that influence patient response to treatment.

Method This was a retrospective survey of current clinical practice. NASH/NAFLD was defined histologically or NAFLD by echogenic liver on ultrasound with raised aminotransferase values in absence of a significant alcohol history or other hepatic co-morbidities. Interventions included: lifestyle advice; dietetic input; exercise classes; pharmacological therapy; bariatric surgery. Clinical and anthropometric data were collected including serum ALT, BMI, HBA1c, systolic blood pressure, total and HDL cholesterol values and analysed for the cohort overall and for patients who were obese, diabetic, hypertensive and dyslipidaemic respectively. Responders to treatment were defined as those with >10% decrease in ALT over the study period. Univariate and multivariate analysis were conducted to analyse baseline factors influencing patient response.

Results 145 patients were included with median follow-up of 12.5 months (range 3–44 months). Overall improvement was seen in ALT (-15% , $p=7 \times 10^{-6}$), BMI (-1.5% , $p=6 \times 10^{-6}$) and total cholesterol (-4.1% , $p=0.006$). BMI improved by >10% in 8%, by >7% in 16% and by >5% in 23% of patients. Patients categorised by abnormal baseline ALT, baseline obesity, baseline hypertension and baseline dyslipidaemia had improvements in ALT (-19% , $p=1 \times 10^{-7}$), BMI (-2.4% , $p=0.001$), systolic BP (-5.4% , $p=3 \times 10^{-4}$) and total cholesterol (-5.5% , $p=0.002$) respectively. Patients with type 2 diabetes made up a higher proportion of those who did not respond or who progressed compared to those who

responded on univariate analysis ($p=0.02$), but this was not significant on multivariate analysis. Moreover, patients with diabetes did not have a significant decrease in ALT (-8% , $p=0.06$).

Conclusion The management framework adopted by the multi-disciplinary NASH clinic is effective at reducing ALT overall. Cardiovascular risk factors were improved overall. Diabetic patients had a poor ALT response. These data support the use of a multidisciplinary NASH clinic, but long-term outcome data are awaited.

P13 THE PERFORMANCE VALIDITY OF BREATH SAMPLE ANALYSIS IN THE DIAGNOSIS OF HEPATIC ENCEPHALOPATHY IN PATIENTS WITH CIRRHOSIS

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Introduction Hepatic encephalopathy (HE) has a detrimental effect on patients' health-related quality of life and a significant negative impact on survival. Nevertheless, there is no diagnostic gold standard so the condition is often poorly diagnosed and managed.

Aim The aim of this study was to evaluate the performance validity of breath sample analysis for volatile organic compounds (VOCs) in the diagnosis of hepatic encephalopathy (HE) in patients with cirrhosis.

Method The patient population comprised 26 individuals (17 men, 9 women) of mean (range) age 60 (45 to 75) years, with biopsy-proven cirrhosis secondary to alcohol ($n=21$), or non-alcoholic steatosis, hepatitis C, autoimmune hepatitis, haemochromatosis or cryptogenic

Abstract P13 Figure 1 Classification scores for the model used to predict HE.

(n=1 each); none of the patients was currently misusing alcohol. Patients were classified using clinical, psychometric and electroencephalographic variables as either neuropsychiatrically unimpaired (n=10) or as having minimal (n=6) or overt (n=10) HE. Exhaled breath samples were collected, on the same day as the neuropsychiatric assessment, in a chemically inert 3L Tedlar bag (Adtech, UK). An evacuation device was used to evacuate 2L of gas from the bag into an empty glass tube which was packed with graphitised carbon adsorbents able to trap a wide range of VOCs (Sigma Aldrich, UK). Each tube was processed using a TurboMatrix automated thermal desorption unit (PerkinElmer, UK) attached to a Clarus 500 gas chromatograph mass spectrometer (GC-MS). In total 280 discrete peaks from the chromatographic output, excluding known experimental contaminants, were investigated for potential use as markers of HE.

Results A number of peaks were identified in the patients with cirrhosis which were absent or present in significantly different quantities in ten healthy controls. Discriminant analysis allowed the generation of two classification equations using standardised data from 12 peaks to build a predictive model for HE. This model correctly classified all patients from the original population (Abstract P13 figure 1).

Conclusion Analysis of VOCs in breath samples identifies patients with HE with a high degree of accuracy. Future work will validate the classification equations in a new group of patients, while identification of the individual compounds involved will provide insights into the pathogenesis of the syndrome and potential new therapeutic targets.

P14 PHENOTYPIC AND FUNCTIONAL PROFILE OF CD4^{POS}CD25^{HIGH} REGULATORY T CELLS IN AUTOIMMUNE HEPATITIS/SYSTEMIC LUPUS ERYTHEMATOSUS OVERLAP SYNDROME

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Introduction Autoimmune hepatitis (AIH) is a severe inflammatory liver disorder characterised by hypergammaglobulinaemia, seropositivity for autoantibodies and interface hepatitis on histology. Though the mechanisms leading to immune-tolerance breakdown have not been fully elucidated, a numerical and functional defect of circulating CD4^{POS}CD25^{HIGH} regulatory T-cells (T-regs) plays a key role in permitting effector lymphocytes to attack hepatocytes. We have identified a cohort of children with concomitant AIH and systemic lupus erythematosus (SLE), a multi-system autoimmune disease in the context of which most studies have reported numerical and functional T-reg impairment.

Aim To define the phenotypic and functional profile of T-regs in children with AIH and SLE (AIH/SLE).

Method 9 AIH/SLE patients (8 females; median age: 13.9 years), 16 AIH patients (13 females; median age: 13.3 years) and 9 healthy subjects (HS, 6 females; median age: 34 years) were studied. T-reg phenotype was determined by flow cytometry after cell incubation with anti-CD4, CD25 and CD127 monoclonal antibodies. Frequency of FOXP3^{POS} and IFN γ , IL-4, IL-17 and IL-9-producing cells was assessed by intracellular staining. T-reg suppressor function was evaluated as reduction of cell proliferation, measured by ³H-thymidine uptake, in co-culture experiments where CD4^{POS}CD25^{HIGH}CD127^{NEG}T-regs were added to CD25^{NEG} target cells.

Results The number of T-regs in AIH/SLE patients (7.06 $\pm$ 1.1) tended to be higher than in AIH (4.88 $\pm$ 1.1; p=0.1) and was similar to HS (7.03 $\pm$ 0.6). While the proportion of FOXP3^{POS} cells within T-regs did not differ, that of CD4^{POS}CD25^{HIGH}CD127^{POS} cells was higher in AIH/SLE (2.4 $\pm$ 0.99) than in AIH (0.46 $\pm$ 0.2; p=0.019) and

HS (0.63 $\pm$ 0.3; p=0.05). The frequency of IFN γ -producing cells within T-regs was higher in AIH/SLE (6.75 $\pm$ 1.7) than in AIH (4.8 $\pm$ 1.9; p=0.019) and HS (3.5 $\pm$ 0.82; p=0.05); conversely that of IL-4-producing cells within T-regs was lower in AIH/SLE (0 $\pm$ 0) than in AIH (0.86 $\pm$ 0.3; p=0.02) and HS (0.9 $\pm$ 0.47; p=0.06). The frequency of IL-17 and IL-9-producing cells was negligible and did not differ among the groups. Addition of CD127^{NEG}T-regs, while decreasing CD25^{NEG} cell proliferation by 31.6% in HS (p=0.08) and by 12.3% (p=0.056) in AIH, did not affect the proliferation of CD25^{NEG} cells isolated from AIH/SLE patients.

Conclusion Compared to AIH and HS, T-regs from AIH/SLE patients are more activated, are skewed towards a Th1 cytokine profile and are functionally defective. These data suggest that more severe alteration of T-reg phenotype and function may predispose to multiple autoimmune manifestations.

P15 MULTIPLE DEFECTS OF THE IMMUNOREGULATORY SYSTEM CONTRIBUTE TO THE DEVELOPMENT OF AUTOIMMUNE HEPATITIS

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Introduction Concordance for autoimmune hepatitis (AIH) is rare within families, though non-hepatic autoimmune disorders are frequent among first degree relatives (FDR) of AIH patients. While a defect in immunoregulation has been demonstrated in AIH patients, the mechanism preventing the development of AIH in FDR, who share genetic background, remains to be elucidated.

Aim To investigate multiple immunoregulatory systems in AIH patients and their FDR.

Method 44 children with AIH (33 AIH-1 and 11 AIH-2, median age 13.5 yrs, 23 females), 65 FDR from 34 families [23 fathers, 47 yrs (38–58); 28 mothers, 44 yrs (24–53) and 14 siblings, 7 females, 13 yrs (5–24)] and 42 healthy subjects [HS, 36 yrs (22–54), 37 females] were studied. Frequency of conventional CD4^{POS}CD25^{POS} regulatory T-cells (Tregs), CD4^{POS}CD25^{HIGH}CD127^{NEG} True Tregs, CD4^{POS}PD-1^{POS} and CD8^{POS}CD28^{NEG} T cells, CD3^{NEG}CD56^{POS} natural killer (NK) cells, CD3^{POS}TCRVa24^{POS}/TCRVb11^{POS} invariant NKT cells (iNKT) was defined by flowcytometry. Tregs and CD4^{POS}PD-1^{POS} T cells were purified from PBMCs using immunomagnetic beads. CD25^{NEG} and PD-1^{NEG} cells (responders) were co-cultured for 5 days with cells with regulatory potential and their proliferation was measured by ³H-thymidine incorporation.

Results Conventional Tregs were lower in patients (10.5% $\pm$ 1.1) than FDR (15.9% $\pm$ 1.1, p=0.001) and HS (14.7% $\pm$ 1.7, p=0.04), while 'True' Tregs were similar in all (6.0% $\pm$ 0.6, 6.3% $\pm$ 0.4 and 6.2% $\pm$ 0.5). CD4^{POS}PD-1^{POS} T cells were lower in patients (1.7% $\pm$ 0.2) and in FDR (1.9% $\pm$ 0.2) than in HS (3.0% $\pm$ 0.2, p<0.0001 for patients and p=0.0007 for FDR). NK cells were lower in patients (8.6% $\pm$ 1.2) than in FDR (15.8% $\pm$ 1.2, p=0.0004) and HS (12.3% $\pm$ 0.9, p=0.02). CD8^{POS}CD28^{NEG} T cells in patients tended to be lower (8.8% $\pm$ 1.46) than in FDR (12.43% $\pm$ 1.56, p=0.18) and HS (13.1% $\pm$ 1.82, p=0.11). The frequency of iNKT cells was similar in all groups. 'True' Tregs decreased CD25^{NEG} cell proliferation by 13.6% in patients, 28.8% (p=0.007) in FDR and 36.9% (p<0.0001) in HS, while CD4^{POS}PD-1^{POS} T cells decreased similarly PD-1^{NEG} cell proliferation in patients (25.4%), FDR (22.5%) and HS (26.8%).

Conclusion A numerical impairment of CD4^{POS}PD-1^{POS} T cells in patients and their FDR suggests that these defects are genetically determined and account for family clustering of autoimmune disorders. A numerical impairment of conventional Tregs and functional impairment of CD4^{POS}CD25^{HIGH}CD127^{NEG} 'True' Tregs, confined to patients with AIH, may be crucial to loss of liver tolerance.