Method We identified consecutive 271 patients who had undergone HCC surveillance between January 2007 and January 2009. We divided the patients into high risk (hepatitis B, hepatitis C, men with alcoholic liver disease or PBC and haemochromatosis) and low risk groups. We collected their demographic data, surveillance results, treatment details and outcome.

Results Mean duration of follow-up was 22 months (range 1 to 32). 156/272 (57%) patients belonged to high risk group. Ten patients (4%) ceased surveillance as they underwent liver transplantation with indication other than HCC. Eight patients (3%) died during surveillance, unrelated to HCC and one patient was found to have pancreatic carcinoma on ultrasound done for HCC surveillance. 18 (6%) were found to have HCC, of whom (72%) were in the high risk group. Number of HCC present varied from 1 (72%), 2 (22%) and 3 HCC in just 1 (6%). Two patients (11%) were detected by abnormal AFP alone with further imaging confirming the diagnosis. Both of these were unsuitable for curative treatment. Majority (44%) were picked up on surveillance USS demonstrating abnormality then confirmed with further imaging. Fifty-six percent of patients with HCC underwent attempted curative treatment (liver transplantation 28%, resection 6% and radiofrequency ablation 22%); 23% received controlling treatment (TACE 6%, ethanol injection 17%) and 22% were referred for palliation.

Conclusion Our results show that a large proportion of HCC were detected in high risk group and 56% of these patients underwent curative treatment for HCC. A significant proportion (23%) also underwent other definitive treatment. There was a significant burden of HCC in the group conventionally classified as low risk group for HCC (18% of total HCC we identified in our series). 22% of our total identified HCC cases were not suitable for treatment other than palliation despite being on recommended surveillance and this may be improved by modifying surveillance interval or better definition of the high risk group. With this study we recommend present surveillance guidelines to be followed in all patients with cirrhosis, however, there may be scope for further improvement in outcome for cirrhosis patients developing HCC by modification of surveillance protocol in selected patients and also by modification of risk groups.

P27 NEUTROPHIL DYSFUNCTION: THE MISSING LINK BETWEEN AMMONIA, INFECTION AND HEPATIC ENCEPHALOPATHY?

doi:10.1136/gut.2010.223362.53

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Introduction Neutrophil phagocytic dysfunction is associated with increased risk of infection and mortality in patients with cirrhosis. The p38-mitogen-activated protein kinase (MAPK) signalling pathway is a critical step in neutrophil activation and is modulated by chemokines, ammonia and endotoxaemia.

Aim This longitudinal study aims to characterise the relationship between neutrophil function and volume, plasma cytokine profile and ammonia, in order to assess neutrophil function as a biomarker of susceptibility to infection and contributor to the development of hepatic encephalopathy (HE) in cirrhosis.

Method Neutrophils were isolated at baseline, following the development of HE, and post-LT, from a cohort of 75 patients with cirrhosis and controls during an 18-month follow-up period. Phagocytosis was analysed by flow cytometry using FITC-labelled *E. coli* and oxidative burst (OB) was determined by the percentage of neutrophils producing reactive oxygen species (ROS) at rest and after stimulation with opsonised *E. coli*. Neutrophil volume was measured using flow cytometry and transmission electron microscopy. Clinical data, blood biochemistry, arterial ammonia and microbial cultures were collected prospectively. Analysis of stimulated neutrophil intracellular cytokine production, plasma cytokine profile and neutrophil basal levels of phosphorylated P38-MAPK were performed.

Results At baseline patients had a median age of 54 (44–62), 31% were female, 43% were on antibiotics. Median MELD score was 17 (12–22). During follow-up nine patients developed overt grade 2–4 HE, 12 patients underwent LT and nine died. Neutrophil phagocytic capacity was significantly impaired in patients with advanced cirrhosis ($p=0.001$) and was associated with a 20% increase in neutrophil volume. In those who underwent uncomplicated LT, neutrophil phagocytic capacity improved by 15% within 72 h. Phagocytic impairment correlated with increasing plasma concentrations of CRP*, ammonia*, IgG**, proinflammatory cytokines TNF-*, IL-6* and the anti-inflammatory cytokine IL-10* (*$p<0.05$, **$p<0.1$). Resting neutrophil production of ROS as a measure of neutrophil activation was significantly increased in patients with cirrhosis with further elevation following the development of HE.

Conclusion Phagocytic dysfunction is universal in patients with cirrhosis and is related in part to the development of ammonia-induced neutrophil swelling, which is reversible following LT. Increasing levels of endotoxin and/or ammonia leading to neutrophil activation via the p38-MAPK pathway and resultant generation of ROS may prove the link between neutrophils, ammonia and infection in the development of HE in cirrhosis.

P28 OPTIMISING THE ANALYSIS OF THE ELECTROENCEPHALOGRAM IN HEPATIC ENCEPHALOPATHY

doi:10.1136/gut.2010.223362.54

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Introduction Hepatic encephalopathy (HE) is the commonest complication of cirrhosis. It has a detrimental effect on quality of life and on survival. Nevertheless, there is no recognised gold standard for its diagnosis. The only objective diagnostic technique, which does not require patient co-operation, is the electroencephalogram (EEG). The current assessment of the EEG is based on measures of the mean dominant frequency (MDF) and the percentage θ (θ%) power from spectral analysis. However, this represents a fairly simplistic approach to analysis of complex shifting wave forms.

Aim To optimise the analysis of the EEG to provide more reliable criteria for the diagnosis of hepatic encephalopathy and the grading of its severity.

Method The patient population comprised 169 individuals (108 men, 61 women; mean (range) age, 55 (26–80) yr) with biopsy-proven cirrhosis, classified on the basis of clinical (Conn *et al*, 1977) and psychometric performance (PHES; Weissenborn *et al*, 2001), as neuropsychiatrically unimpaired (n=85), or as having minimal (n=21) or overt (n=63) HE. The reference population comprised 48 healthy individuals (25 men, 23 women; mean (range) age, 39 (22–58) yr). Standard 21-lead, eyes-closed EEG recordings were obtained in all subjects. EEGs were visually inspected and spectral analysis undertaken on a standard P3–P4 derivation (Amodio *et al*, 1999) and a SEDACA component (Montagnese *et al*, 2007). In addition, a measure of the stability of the posterior rhythm, F-mean, was obtained using a novel computational technique. A subset of 74 patients underwent a mean of 3 (2–9) repeat assessments over time. Performance of the variables was assessed using ROC analysis.

Results Four variables had good performance characteristics.

All four variables correlated significantly with the PHES scores ($p<0.05$). Significant changes in F-Mean ($p<0.05$), α-P3P4 and

SEDACA ($p<0.05$) were observed over time in relation to changes in neuropsychiatric status.

Conclusion Two additional variables have been identified which have good performance characteristics for the diagnosis of any degree of HE. These new variables, in combination with the currently used EEG measures, can be used to optimise the diagnosis of HE and the grading of its severity.

Abstract P28 Table 1 Results

Variable	Threshold	Sensitivity	Specificity	PPV	NPV
MDF	9.2 Hz	74.7	58.7	53.2	78.7
θ %	46.8	76.9	69.8	61.2	83.0
α %	20.0	71.8	71.4	60.9	80.4
F-Mean	10.2 Hz	75.6	47.7	47.7	75.6

P29 DO DISTURBANCES IN CEREBRAL OSCILLATORY NETWORKS EXPLAIN SLEEP AND NEUROPSYCHIATRIC ABNORMALITIES IN PATIENTS WITH CIRRHOSIS?

doi:10.1136/gut.2010.223362.55

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Introduction Patients with cirrhosis have significantly disturbed sleep–wake behaviour. However, the cause of these disturbances is uncertain and their relationship to hepatic encephalopathy (HE) is debated. Sleep is regulated by circadian and homeostatic processes; circadian abnormalities, while present in these patients, do not correlate with the observed disturbances in sleep–wake behaviour (Montagnese *et al*, 2010). Homeostatic sleep mechanisms are difficult to access; the possibility that homeostatic control is disturbed in these patients has not been systematically studied. Sleep-spindles, which are a feature of the sleep electroencephalogram (EEG), are generated by thalamo-cortical oscillatory networks and are a surrogate marker for homeostatic sleep processes. The same oscillatory networks have been implicated in the pathogenesis of HE.

Aim To examine homeostatic sleep mechanisms in patients with cirrhosis and to determine the relationship between sleep abnormalities and HE.

Method 39 patients with cirrhosis (24 men; 15 woman; mean (range) age 60 (37–85) years) were classified using clinical, psychometric and electrophysiological variables as neuropsychiatrically unimpaired (n=20), or as having minimal (n=6) or overt (n=13) HE. The reference population comprised 50 healthy individuals (26 men; 24 woman; age 55 (41–65) years. Sleep–wake behaviour was characterised using validated questionnaires. Sleep deprived EEGs were obtained in 14 patients (unimpaired (n=5), minimal (n=3) and overt (n=6) HE). The reference population comprised 26 healthy individuals (15 men; 11 woman; age 49 (39–59) years). The EEG was band-pass filtered (12.75–15.0 Hz) and the envelop of the sleep spindles obtained using the Hilbert transform. A threshold was identified which allowed the maximal rate of spindle occurrence to be defined. Variables were compared between patients and controls and in patients by degree of neuropsychiatric impairment.

Results Patients with overt HE were significantly more likely to report night-time sleep disturbances and day-time napping than the control subject and unimpaired patients ($p<0.05$).

The rate of sleep-spindle occurrence was significantly greater in the EEGs of patients with overt HE than in control subjects and unimpaired patients (59.2 ± 3.8 vs 52.4 ± 2.8, $p<0.001$ and 53.9 ± 1.5, $p<0.005$).

Conclusion Sleep–wake disturbances were prevalent in the patients with cirrhosis and increased with the degree of neuropsychiatric impairment. Significant abnormalities were observed in the sleep EEGs in patients with overt HE. Thus, abnormalities in cerebral oscillatory networks may underlie both the sleep disturbances and the neuropsychiatric abnormalities observed in patient with cirrhosis

Abstract P29 Table 1 Results

Variable	Healthy controls (n=50)	Unimpaired (n=20)	Minimal HE (n=6)	Overt HE (n=13)
Night sleep disturbance	54%	37%	67%	80%
Day-time sleepiness	14%	5%	17%	70%

P30 CYTOPLASMIC EXPRESSION OF TOLL-LIKE RECEPTOR-9 IS ASSOCIATED WITH INCREASED CELLULAR PROLIFERATION IN HEPATOCELLULAR CARCINOMA

doi:10.1136/gut.2010.223362.56

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Introduction Effective treatment of Hepatocellular carcinoma (HCC) still remains an unmet need. Inflammation plays a crucial role in the pathogenesis of HCC by causing repeated cell damage and creating a microenviroment rich in cytokines that can enhance cell replication, angiogenesis and invasion into the surrounding structures. Toll-like receptors (TLRs) are important pathogen recognition receptors. Stimulation of TLRs is followed by cascade of transcriptional and translational reactions resulting in activation of NF-kB which subsequently serves as a common- link between the chronic inflammation and tumour development.

Aim The aim of this study was to characterise the expression of TLR-9 along the HCC tumour genesis pathway including the normal liver, viral hepatitis, cirrhosis and HCC and the results were collated with cell proliferation as determined by Ki-67 staining.

Method We used a human tissue array platforms (Vbiolab, Cambridge, UK) which includes 102 cores of liver tissue including nine normal livers, 26 hepatitis (B, C and Non-b, Non-C hepatitis), 25 cirrhosis and 42 HCC. Immunohistochemistry was performed for the expression of TLR-9 and Ki-67. The scoring was performed in a blinded fashion by two individual pathologists. For the quantitation of Ki-67 expression, we counted the positively stained nuclei among 1000 hepatocytes in the highest expression area using a standardised grid.

Results TLR-9 expression was noticed as membranous staining in 2/9 cases in normal liver. 12/26 cases of hepatitis and 13/25 of cirrhosis. Weak cytoplasmic staining was noticed in 4/26 cases of hepatitis and one case in cirrhosis whereas the staining was predominantly cytoplasmic in HCC; weak (+1) in 17/42 cases and moderate to strong (+2 and+3) in 17/42 cases, whilst in eight cases it was negative. In 13/42 cases, it was found in both cytoplasm and cell membrane. There was a close correlation between the proliferative index (Ki-67 staining) and TLR-9 staining; $r=0.8$, $p<0.001$. The proliferative index was <100, 100–200, >200 in TLR9 negative, weak and moderate-to-strong cases, respectively.

Conclusion The results of our study show, for the first time, a strong correlation between the amount of cellular proliferation and TLR-9 expression in HCC. Also we found the shifting of TLR9 expression from the membranous type in hepatitis and cirrhosis into cytoplasmic in HCC. Our data suggest that TLR-9 might have a pivotal role in cellular proliferation in HCC and merits further studies to explore the possibility of exploiting it as a potential target for future therapy.