Methods 27 healthy volunteers were randomly assigned to a vaccinated (n=14) or a control (n=13) group for Ty21a typhoid vaccine. Peripheral blood was collected from all volunteers prior to vaccination and 18 days following immunisation or recruitment. Mucosal samples (15 jumbo biopsies from duodenum (n=25) colon (n=18)) were collected from all volunteers at gastroscopy +/- sigmoidoscopy on day 18. Mononuclear cells were isolated from mucosal tissue by disruption and collagenase digestion, and from blood by blood centrifugation. Cells were stimulated with Ty21a or control antigens, and stained for surface phenotype and intracellular cytokine production. Antigen-specific IFN-γ, TNF-α, and IL-2 production was determined by flow cytometric analysis for CD8+/CD8- and CD3+/CD8- (CD4-) lymphocytes. Humoral IgA, IgM and IgG responses in blood were examined in relation to mucosal and peripheral cellular responses.

Results Oral immunisation with Ty21a significantly increased the proportion of antigen-specific cytokine-producing CD8-positive (p<0.05) and CD8-negative (p<0.05) lymphocytes within the duodenal mucosa, but no specific response was seen in colon. CD8-negative lymphocytes within the duodenal mucosa adopted a significantly more poly-functional phenotype following vaccination, expressing 2 or 3 cytokines simultaneously, while in contrast antigen-specific cytokine-producing CD8-positive lymphocytes in the duodenal mucosa were mono-functional expressing a single cytokine. In blood, the proportion of antigen-specific cytokine-producing CD8-positive lymphocytes was increased (p<0.05) following oral vaccination, but there was no significant increase in cytokine-producing CD4-positive lymphocytes. Differences in functionality of antigen-specific cytokine responses were less marked in peripheral blood lymphocytes following vaccination.

Conclusion These data show an antigen-specific response in human gut mucosal lymphocytes following oral vaccination, and directly demonstrate different immune functionality of CD8-positive compared to CD8-negative mucosal lymphocytes. These responses were more informative than surrogate measurements in peripheral blood lymphocytes. The absence of a detectable cognate response from the colon may indicate compartmentalisation of the gut mucosal response to the embryological mid-gut, where typhoid antigen is likely presented at immune inductive sites.

Competing interests None declared.

PWE-132 ENHANCED EXPRESSION OF SECRETORY PHOSPHOLIPASE A2 AND CRYPTIDINS IN SMALL INTESTINAL PANETH CELLS FOLLOWING TRICHINELLA SPIRALIS INFECTION

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Introduction T spiralis infection leads to a T cell-dependent enteropathy characterised by villus atrophy, crypt hyperplasia and an increase in Paneth and goblet cells. Paneth cells express a number of antimicrobial peptides and proteins. Our aim was to investigate changes in the expression of cationic antimicrobial peptides and proteins that are normally expressed by Paneth cells.

Methods Small intestinal epithelial cells were isolated from control mice and those infected with T spiralis. Concentrated cell extracts (in acetic acid) were studied by acid urea-polyacrylamide gel electrophoresis (AU-PAGE) and Western blot analysis. Samples with similar protein concentrations were used to assess antimicrobial activity against Escherichia coli, after 5 h of incubation at 37°C, using the following equation: antimicrobial activity = [(OD620 of control solution−OD620 of sample)/OD620 of control solution]×100.

Results The establishment of infection with the nematode was confirmed by the presence of worms in the small intestinal lumen, changes in mucosal architecture and increase in Paneth and goblet cell numbers. In contrast to controls, AU-PAGE analysis of Paneth cell-containing small intestinal epithelial cell extracts from T spiralis-infected mice showed two prominent bands. AU-PAGE-Western blot and amino acid sequence analyses identified one of these bands to be secretory phospholipase A2. Sequences for cryptidins were detected in the second prominent band. Acid extracts of epithelial cells isolated from T spiralis-infected mice showed significantly greater antimicrobial activity, compared to those from control mice [mean 54.7 (SEM 8.7) vs 7.3 (5.5); p=0.001].

Conclusion Following T spiralis infection, there was an increase in small intestinal epithelial expression of secretory phospholipase A2 and cryptidins. Enhanced production of these Paneth cell-derived peptides is likely to mediate greater antimicrobial activity against luminal bacteria in T spiralis-infected small intestine.

Competing interests None declared.

Hepatobiliary II

PWE-133 INCREASED LEVELS OF NEUTROPHIL GELATINASE ASSOCIATED LIPOCALIN (NGAL) IN THE PLASMA OF CHOLANGIOCARCINOMA PATIENTS

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Introduction We have previously demonstrated that the level of neutrophil gelatinase-associated lipocalin (NGAL) is increased in the bile of patients with pancreatobiliary malignancy. NGAL is expressed by activated neutrophils and many other cell types and is thought to have bacteriostatic, pro-proliferative and pro-metastatic functions. NGAL can be detected in the blood plasma. We hypothesised that the plasma NGAL level is elevated in patients with cholangiocarcinoma (CC) compared with patients with primary sclerosing cholangitis (PSC), and with healthy volunteers.

Methods Plasma samples were collected from 97 patients with confirmed CC, 62 patients with PSC and no CC and 82 healthy controls. Plasma NGAL quantification was performed in duplicate on plasma from each subject using a Quantikine ELISA kit (R&D Systems, Minneapolis, Minnesota, USA). CC and healthy control cohorts were compared using the Student t test and receiver operator characteristic (ROC) analysis. Differences between CC and PSC cohorts were then sought. Pearson’s correlation analysis was used to assess relationships between the levels of NGAL and other plasma markers.

Results Median NGAL concentrations (range) in CC, PSC and healthy controls were 92 ng/ml (14–644), 83 (48–171) and 64 (29–132) respectively. NGAL levels were significantly higher in plasma from CC patients compared with healthy controls (p<0.0001). The area under the ROC curve was 0.71 (95% CI 0.64 to 0.79 p<0.0001). NGAL levels were significantly higher in plasma samples from the CC cohort than those from the PSC cohort (p<0.01) with a ROC-AUC of 0.57 (95% CI 0.48 to 0.65 p<0.167). There was no relationship between NGAL levels and CRP (r²=0.14), white cell count (r²=0.09), bilirubin (r²=0.01), ALP (r²=0.02) or creatinine (r²=0.03). There was moderate correlation between NGAL and Ca19-9 concentrations (r²=0.38).

Conclusion NGAL is expressed at significantly higher concentrations in the plasma of patients with CC compared to plasma from healthy controls and from subjects with PSC. This finding appears to be independent of renal impairment, cholestasis or systemic inflammatory response, suggesting that NGAL may represent a novel plasma biomarker of CC.

Competing interests None declared.

PWE-135 RETROSPECTIVE AUDIT OF MANAGEMENT OF PATIENTS ADMITTED TO INTENSIVE CARE UNIT (ITU) WITH SEVERE ACUTE PANCREATITIS (SAP)

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Introduction Atlantaclassification stratifies acute pancreatitis (AP) into mild and severe. Severe acute pancreatitis (SAP) is best managed in HDU or ITU setting and associated with high mortality and morbidity despite best efforts at attaining early diagnosis and timely intervention.

Aim To compare management strategies and mortality of patients admitted to ITU with SAP against national standards and study the group who succumbed to their disease in detail in an attempt to define the circumstances that lead to this event and identify the most accurate prognostic indicators in this group of patients.

Methods Retrospective audit of management and outcome of consecutive patients admitted to ITU with SAP during the period of 2007–2010. The development of necrosis, infected necrosis (IN) or organ failure (OF) was recorded. Patients were classified into group I (No necrosis or OF), group II (sterile necrosis or transient OF), group III (IN or persistent OF) and group IV (infected necrosis and persistent OF). The four groups were compared regarding the clinical course, radiological/surgical intervention, any post-intervention complications, use of antibiotics/antifungal and nutritional support.

Results 51 patients were admitted to ITU with SAP (APACHE II ≥8, modified Glasgow score ≥5) during the period of 2007–2010. All cases fulfilled the Atlanta criteria of SAP. Median age: 66±17.5. The pancreatitis was alcohol induced in 12% and due to gallstones in 59% of patients; no cause was found in 25% of patients. Median ITU stay was 3.23 days. The overall mortality rate during the study period (5 years) was 38% (n=19) above national standard of 30%. All seven patients in group IV died, five of them underwent necrosectomy and one had CT guided drainage of infected acute fluid collection. The Abstract PWE-135 table 1 shows the total number of patients and respective mortality of SAP in all four groups. Forty-one patients (80%) received antibiotics and 35 patients (69%) had nutritional support but neither of them seems to have a significant impact on survival (p=0.6 and 0.06 respectively).

Outcome (death) correlated with organ dysfunction criteria (Atlanta criteria and APACHE II score).

Abstract PWE-135 Table 1 The mortality of SAP in the different groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Total number</th>
<th>Mortality</th>
<th>% of mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
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<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>II</td>
<td>2</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>III</td>
<td>30</td>
<td>12</td>
<td>40%</td>
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<tr>
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<td>7</td>
<td>100%</td>
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