Introduction Rotavirus is the most common cause of severe diarrhoea among infants and young children. Rotavirus is usually an easily managed disease of childhood, but worldwide nearly 500,000 children under 5 years of age still die from rotavirus infection each year and almost two million more become severely ill. Rotavirus A (responsible for about 90% of infections) is typically diagnosed by finding the virus in the child’s stool by enzyme immunoassay. This study was undertaken to expand our knowledge of VOCs from the stool of children and assess whether rotavirus causes easily measurable changes in the gut chemistry of infected young children.

Methods The volatile organic compounds (VOCs) from the stool of 53 children from Malawi (26 non-infected children with an unspecified GI problem and 27 rotavirus children diagnosed with the virus) were analysed using Headspace Trap-GC/MS. The faecal samples were placed in headspace vials and were heated from frozen to 90°C. The VOCs were preconcentrated and focused prior to GC/MS analysis. Those VOCs were identified by comparing their mass spectra with those contained in the NIST/EPA/NIH Mass Spectral Database.

Results A total of 186 different compounds have so far been identified. Of the 53 stool samples ethanol was found in 69% and 63% samples respectively between the non and infected classes, which contrasts with previous work where ethanol was found in all healthy adult and all healthy neonate stool. This could be due to the more dilute stool due to diarrhoea. Carbon disulphide has previously been found to be ubiquitous in healthy adult stool, again the contrasts with previous work where ethanol was found in all samples respectively between the non-infected classes, which was much less at 27% and 22% respectively. In contrast there were very little differences in frequency (ca. 92%) and abundance of ethanol in both in the rotavirus group. There were very little differences in the frequency (ca. 92%) and abundance of ethanol in both sample classes, curiously ethanol has been previously shown to be significantly present by comparing their mass spectra with those contained in the NIST/EPA/NIH Mass Spectral Database.

Conclusion Very little work has been published on volatile compounds from stool in particular from the stool of children, this work adds to this knowledge. Some compounds such as ethanol were found in approx. equal amounts in the diarrhoea of infected and non-infected stool, however in general there was a greater frequency and abundance of VOCs in the rota infected samples, particularly of aldehydes and 2,3-butanedione.

Competing interests: None declared.

Abstract PWE-169 Figure 1 Combined peak area (arbitrary units) of selected carbonyl compounds (namely; 2,3-butanedione/propanal/2-heptenal, (Z)-octanal/nonanal combined) present in faecal samples analysed.

PWE-170 PROTON PUMP INHIBITORS AND CLOPIDOGREL COMBINATION: IS THERE ANY RISK?

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Introduction Dual anti-platelet therapy (DAT) with aspirin and clopidogrel is prescribed up to 1 year following Acute Coronary Syndrome (ACS). Proton Pump Inhibitors (PPIs) are often used in selective patients to reduce the risk of gastrointestinal (GI) bleeding. Some studies have suggested that there is a possible interaction between PPIs and clopidogrel resulting in an adverse cardiac event rate. Main objective of this study was to determine if prescription of PPIs in ACS patients on dual anti-platelet therapy results in increased cardiac events compared to patients not receiving PPIs.

Methods A retrospective observational, comparative study of 200 admissions with ST elevation myocardial infarctions (STEMIs) and non-ST elevation myocardial infarction (nSTEMIs) over a period of 1 year. Patients were divided in two groups: PPI group (Patients on DAT and PPIs) and Non-PPI group (Patients on DAT only).

Results Mean age was 67 (24–96), 63% male and 35% female, 75% Caucasian and 25% Asians. 27 patients were excluded (24 patients not receiving DAT, 3 patients on H2 antagonist). For PPI group (54% on lansoprazole, 35% on pantoprazole and 11% on omeprazole), baseline characteristics were (Patient number—89, 27-STEMIs, 62-nSTEMIs, mean age—69) whereas the corresponding values for non-PPI group were (Patient number—84, 58-STEMIs, 46-nSTEMIs, mean age—63). Mean GRACE score at admission (predictor of death or MI in 6 months) was 39% for PPI group whereas the corresponding value for the non-PPI group was 27% (p=0.075). 26 patients were re-admitted with ACS, 20 in PPI group and six in non-PPI group (OR 3.76, 95% CI 1.43 to 9.92, z statistic 2.68, p=0.007). For PPI group (54% on lansoprazole, 35% on pantoprazole and 11% on omeprazole), baseline characteristics were (Patient number—89, 27-STEMIs, 62-nSTEMIs, mean age—69) whereas the corresponding values for non-PPI group were (Patient number—84, 58-STEMIs, 46-nSTEMIs, mean age—63). Mean GRACE score at admission (predictor of death or MI in 6 months) was 39% for PPI group whereas the corresponding value for the non-PPI group was 27% (p=0.075). 26 patients were re-admitted with ACS, 20 in PPI group and six in non-PPI group (OR 3.76, 95% CI 1.43 to 9.92, z statistic 2.68, p=0.007). Five deaths due to ACS were observed, four in PPI group and one in non-PPI group (RR 3.77, 95% CI 0.43 to 35.09, p=0.23).

Conclusion This study suggests that there is an association between PPIs and clopidogrel resulting in increased readmission with acute coronary syndromes but not in increased mortality. Larger scale studies are required to confirm or refute these observations.

Competing interests: None declared.

PWE-171 STRUCTURE-FUNCTION ANALYSIS OF POLYMORPHIC VACA TOXIN VARIANTS FROM HELICOBACTER PYLORI USING A RECOMBINANT APPROACH

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Introduction The Gram negative bacterium Helicobacter pylori persistently colonises the stomachs of around half the world’s population. It causes chronic asymptomatic gastritis, and leads to peptic ulceration and gastric cancer in a small proportion of cases. One of the key factors influencing the development of more severe pathology and disease is the expression of virulence factors by the colonising strain. The vaculating cytotoxin, VacA, is a major determinant of H pylori pathogenicity and is highly polymorphic. Strains producing more active forms of VacA (I1 types) are more strongly associated with gastric cancer than strains producing less toxicogenic VacA (I2 types).1 The I-region polymorphism is characterised by 10 amino acid substitutions and a serine-asparagine-glutamine (SNQ) tri-peptide insertion in I2. We aimed to further characterise the structure and function of this important region.
Methods The SNQ tri-peptide from i2 was engineered into *H pylori* strain 60190 (VacA s1/l1/m1) to make strain 60190/SNQ. The vacuolating activities of 60190/SNQ and wild type strains were compared by incubating AGS cells with broth culture supernatants, and counting vacuolated cells in random fields by microscopy. To allow further structure-function characterisation of i-region variants, we modified a recombinant VacA system2 by expressing VacA p33 and p55 subunits in *Escherichia coli* and purifying both under denaturing conditions by metal affinity chromatography, then refolding by stepwise dialysis. Recombinant VacA was tested for vacuolating activity and previously described effects on Jurkat T-cells. Results Introduction of the i2-specific SNQ insertion into i1 VacA in the *H pylori* 60190 strain resulted in a 1.8-fold decrease in native toxin vacuolating activity on AGS cells lines (*pH pylori*—they induced extensive vacuolation of gastric epithelial cell lines at concentrations down to 0.2 μg/ml. The recombinant toxin also inhibited proliferation of, and IL-2 production by Jurkat cells. Engineering the SNQ insertion into recombinant i1 VacA resulted in modest reductions in the stability of the toxin.

Conclusion A naturally-occurring three amino acid insertion in the VacA i-region renders the toxin less active in vitro. We speculate that this small insertion is a major contributor to the reduced association with cancer of strains with type i2 VacA. Recombinant VacA toxin is highly active on both epithelial and T cells and can be genetically modified to explore VacA structure-function relationships. We aim to use this system to further characterise the structure and function of i-region variants.

Competing interests None declared.

REFERENCES


PWE-173 INCREASED CCL20 AND CCR6+ REGULATORY T-CELL RESPONSES IN THE HELICOBACTER PYLORI INFECTED HUMAN GASTRIC MUCOSA

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Introduction *Helicobacter pylori* (*Hp*) infection may result in peptic ulcers or gastric cancer. Disease risk is associated with more virulent strains, such as those with the *cag* pathogenicity island (*cagPAI*), which induce higher levels of gastritis. Disease is also associated with an insufficient anti-inflammatory regulatory T-cell (Treg) response to *Hp*. Preliminary studies of circulating T-cell adhesion molecule expression highlighted an increased frequency of CCR6+ Tregs in the blood of *Hp*+ patients. This chemokine receptor is expressed by both T-helper 17 (Th17) cells and Tregs. It has been demonstrated that the *Hp*-infected human gastric mucosa contains significantly raised concentrations of the CCR6 ligand, CCL20. We hypothesised that CCL20 may play a role in the migration of Tregs to the infected mucosa, and therefore aimed to investigate the mechanisms by which CCL20 expression is induced by *Hp*, and to quantify and characterise gastric mucosal CCR6+ Tregs.

Methods 24 *Hp*+ and 34 *Hp*− patients attending the Queen’s Medical Centre, Nottingham, donated gastric biopsies and peripheral blood with informed consent and ethics approval. Isolated CD4 cells were stained for Treg markers (CD25+, FOXP3+, CD127+) and CCR6, prior to analysis by flow cytometry. Gastric epithelial cell lines (AGS, MKN28 and MKN45) were cultured with *Hp* (Wild type, and the following null mutants: Δ*argA*, Δ*argE*, ΔvacA and Δslt) at a range of multiplicities of infection (MOI), with or without chemical inhibitors, for up to 48 h. CCL20 in culture supernatants was quantified by ELISA.

Results CCL20 levels were threefold higher in biopsies from *Hp*+ patients than *Hp*− patients (p < 0.015). >80% of Tregs extracted from gastric biopsies were CCR6+, and 3.5-fold higher numbers of Tregs were present in samples from infected compared to uninfected patients (p < 0.050). Two-fold higher proportions of Tregs in the peripheral blood of *Hp*+ patients were CCR6+ (p < 0.021). In cell lines, *cagPAI*+ *Hp* strains induced a dose-dependent increase in anaemia (OR 3.662, p = 0.006), gastrointestinal bleeding (OR 2.532, p = 0.038) or weight loss (OR 1.052, p = 0.010) at presentation, histopathologies of poorly and moderately differentiated tumours (OR 3.632 and 1.757 respectively; p = 0.029), adjuvant therapy (OR 3.464, p = 0.000), and those requiring step-down care upon discharge (OR 5.759; p = 0.021) were at increased risk of weight loss. However, male gender (OR 0.288; p = 0.012), vomiting at presentation (OR 0.219; p = 0.014), and neo-adjuvant therapy (OR 0.225; p = 0.027) were associated with decreased risk. Age, race and operation length were not significant factors at 6 months. Of the patients who were malnourished at 6 months, 54.9% (n = 59) developed further weight loss over the subsequent 6 months. Pre-operative total lymphocyte count (OR 2.141; p = 0.053) and tumour size (OR 1.253; p = 0.054) were associated with increased risk. Age, gender and race were not significant factors at 12 months.

Conclusion Malnutrition is a significant problem post-gastrectomy. More nutritional care should be provided to patients presenting with acute presentation, poor tumour biology and those requiring adjuvant therapy and step-down care as these are risk factors at 6 months. At 1 year, patients who initially had larger tumour sizes and higher pre-operative total lymphocyte counts are at greater risk of continued weight loss, and should receive further nutritional support as part of their long-term post-operative care.

Competing interests None declared.