900 cm^{-1}). Single element Attenuated total reflectance (ATR) contains averaged FTIR spectra from the superficial 10 microns of an entire oesophageal biopsy. We aim to extract individual cellular characteristics with molecular resolution by using FTIR and ATR on ex-vivo oesophageal biopsy specimens from patients undergoing endoscopy for BE surveillance or neoplasia assessment to detect high grade dysplasia (HGD).

Methods 731 spectra of 374 fresh biopsies from 76 patients were analysed. Biopsies were taken from visible BE. Before being placed in formalin, they were analysed by a spectrometer fitted with liquid nitrogen-cooled detector and ATR silicon microprism. For each spectrum 500 interferograms were averaged before Fourier transformation. Spectra were pre-processed using MATLAB scripts by spectrally removing liquid water and water vapour contributions, vector normalising to the 1610–900 cm^{-1} region and second derivative conversion to remove baseline artefacts. Specific cellular characteristics were first determined. Unstained 8 μm tissue sections from 1 patient were analysed with FPA (Focal Plane Array)-FTIR imaging and correlated with stained slides. It was possible to accurately describe specific features ofsquamous epithelium (SQ), columnar lined epithelium (CLE), and lamina propria (LP) with this method. These features were applied to the 374 fresh biopsies using ATR-FTIR. Combined clustering and partial least squares regression discrimination (PLSDA) was used to build a diagnostic pipeline. Biopsies were grouped according to their cellular characteristics from the prior FTIR imaging. (1. SQ vs Rest, 2. SQ only biopsies, 3. CLE only biopsies, 4. CLE and LP containing biopsies and 5. LP containing biopsies only).

Results We distinguished SQ mucosa from CLE (BE), HGD and OAC tissue at an overall sensitivity of 89% and specificity of 91%. By grouping the spectra into groups according to their cellular contents, HGD was distinguished from all other biopsies with sensitivities and specificities of 68 and 89% (CLE only), 74 and 82% (CLE and LP) and 94 and 97% (LP only) respectively.

Conclusion Combined FTIR and ATR-FTIR spectroscopy can accurately distinguish HGD arising in BE on ex-vivo biopsy specimens and might become accurate enough to exclude routine histopathological evaluation in these patients.

Disclosure of Interest None Declared.

OC-016 THE TOLL-LIKE RECEPTOR PATHWAY IS RECURRENTLY MUTATED IN OESOPHAGEAL ADENOCARCINOMA

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Introduction The interaction between the oesophageal microbiota and the inflammatory microenvironment in Barrett's carcinogenesis is poorly understood. One of the mechanisms by which microbiota may induce chronic inflammation is by triggering Toll-like receptor (TLR) signalling and activation of nuclear factor kappa B. We aimed to utilise whole genome sequencing (WGS) data to investigate TLR mutations and expression in oesophageal adenocarcinoma (OAC), with a focus on TLR9.

Methods We interrogated the mutational profiles of 66 OAC samples, with matched germline references from each case, which had undergone WGS as part of the oesophageal ICGC study. All mutations were verified using PCR and Sanger sequencing. To further explore TLR9 expression along the Barrett's progression sequence, we performed TLR9 immunohistochemistry on tissue microarray samples including normal squamous oesophagus (N=16), duodenum (N=14), non-dysplastic Barrett's (N=53), low-grade dysplasia (N=13), high-grade dysplasia (N=25) and OAC (N=338). Within the large cohort of OAC samples we binarised the intensity scores (0–1 and 2–3) and examined whether there were any significant differences in relation to clinicopathologic variables (TNM stage, histological grade, lymphovascular invasion, survival).

Results We identified missense mutations in TLR pathway genes in 8/66 (12.1%) of OAC samples, including *TLR1* (1.5%), *TLR4* (3%), *TLR7* (1.5%), *TLR9* (3%), *MYD88* (1.5%), and *TRAF6* (1.5%). TLR9 protein was expressed more highly in Barrett's and OAC than normal oesophageal squamous tissue ($p < 0.001$). The expression in Barrett's was similar to duodenum, however immunopositivity was increased in OAC ($p < 0.05$) compared with this control tissue. The staining intensity was generally consistent throughout the Barrett's progression sequence with strong immunopositivity (intensity score 3) in 7.7–14.5% of samples. Within the OAC cohort, there was no significant association between TLR9 expression and any of the clinicopathological variables tested. The only significant difference in survival was observed in a small subset of patients with metastatic disease (N = 14 patients), where median survival was significantly decreased for patients with TLR9 intensity score 2–3 (8 months ± 2.24 (standard error)) compared to patients with TLR9 intensity score 0–1 (18 months ± 6.57), $p < 0.05$.

Conclusion TLR pathway genes appear to be recurrently mutated in OAC, which given the mutational context and heterogeneity of disease[1] could represent significant involvement of the TLR signalling pathway in Barrett's carcinogenesis.

REFERENCE

1 Dulak AM, *et al*. Exome and whole-genome sequencing of esophageal adenocarcinoma identifies recurrent driver events and mutational complexity. *Nat Genet*. 2013 May;45(5):478-86

Disclosure of Interest None Declared.

OC-017 AUTOFLUORESCENCE-TARGETED OPTICAL BIOPSY ACCURATELY DIAGNOSES DYSPLASIA IN BARRETT'S OESOPHAGUS AND CAN DETECT THE FIELD OF MOLECULAR CHANGE

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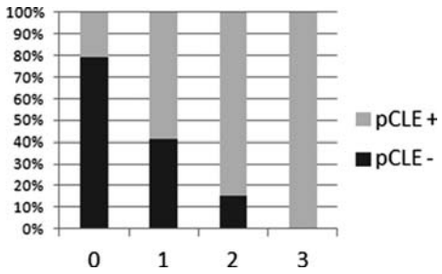
Introduction Probe-based confocal laser endomicroscopy (pCLE) allows optical biopsies in Barrett's oesophagus (BO) to predict histological outcome but it is subject to sampling error if performed in a random fashion. We used autofluorescence imaging (AFI) to direct pCLE and added molecular biomarkers to the histopathological diagnosis. The aims of this study were to assess the diagnostic accuracy for dysplasia of AFI-targeted optical biopsies and to investigate the correlation between pCLE patterns and field of molecular change.

Methods 46 patients with BO (non-dysplastic BE n = 20, indefinite for dysplasia n = 4, low grade dysplasia n = 10, high grade dysplasia (HGD) or intramucosal cancer (IMC) n = 12) were recruited at a single centre. Patients underwent high-resolution endoscopy followed by AFI and then pCLE was performed on AFI positive (AFI+) areas. Targeted biopsies were taken from



Abstract OC-017 Figure 1 Correlation between pCLE and biomarkers. X-axis: number of positive biomarkers

AFI+ areas, followed by random biopsies as per Seattle protocol. pCLE sequences were graded according to published criteria. Cyclin A and p53 expression were assessed by immunohistochemistry and aneuploidy by flow-cytometry on AFI-targeted biopsies. Statistical analyses were performed using chi-square test.

Results AFI-targeted pCLE correctly classified all the HGD/EC patients and had a sensitivity and specificity for any grade of dysplasia of 93 and 83%, respectively. The Seattle protocol had similar sensitivity for HGD/IMC and any grade of dysplasia (83 and 89%, respectively). For the per-location analysis, a total of 155 endoscopic areas were analysed with pCLE and molecular biomarkers. pCLE had a sensitivity and a specificity for HGD/IMC and any grade of dysplasia of 100/64% and 78/75%, respectively. Overall, 40% of pCLE irregular sequences corresponded to non-dysplastic areas (false positive). We found a statistically significant enrichment ($p < 0.001$) of the three molecular biomarkers in pCLE irregular areas (Figure 1). After exclusion of dysplastic areas, a significant correlation between pCLE irregularity and biomarker positivity was retained ($p = 0.008$). The presence of at least 1 positive biomarker significantly correlated with dysplasia both in pCLE irregular ($p = 0.01$) and pCLE regular areas ($p = 0.05$).

Conclusion AFI-targeted pCLE has a high diagnostic accuracy for dysplasia in BO. Tissue biomarkers are a useful adjunct to characterise the field of molecular abnormality associated with optical dysplasia. These results suggest that the presence of pCLE irregularity, even in the absence of histological dysplasia, relates to molecular changes and may warrant close follow up.

Disclosure of Interest None Declared.

OC-018 GASTRIN INCREASES MIR-222 EXPRESSION IN GASTRIC EPITHELIAL CELLS *IN VITRO* AND HYPERGASTRINAEMIC INS-GAS MICE IN VIVO

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Introduction Gastric adenocarcinoma occurs in some patients who are infected with *Helicobacter pylori*. Gastrin is a cofactor in gastric carcinogenesis and elevated serum concentrations are found in the preneoplastic condition atrophic gastritis. MicroRNAs (miRNAs) are small non-coding RNAs that post transcriptionally regulate numerous mRNAs and play critical roles in cell physiology. Previous studies have suggested that *H.pylori* infection dysregulates miRNAs to control gastric inflammation, cell cycle progression, apoptosis and cell survival. We hypothesised that gastrin would also induce alterations in gastric miRNAs and that these may influence cancer development.

Methods Human gastric adenocarcinoma cells that have been stably transfected with the human CCK2 receptor (AGS_{GR}) were treated with 0.1–100 nM gastrin for 2–48 h. Small RNAs were isolated and reverse transcribed using the Qiagen miScript PCR system kit. miRNA expression profiling was determined by qPCR using miScript PCR arrays (in triplicate) and further validated using miRNA primer assays (in quadruplicate). Cycle passing threshold (Ct) was normalised to RNU62 expression and miRNA relative expression calculated using $\Delta\Delta C_T$ method. miR-222 levels were measured in gastric mucosal scrapings from 10 week old male and female ($n = 3$ per group) wild-type FVB/N mice and transgenic hypergastrinaemic INS-GAS mice on the same genetic background. Comparisons were made using unpaired t-tests with Bonferroni correction, $P < 0.05$ was considered significant.

Results miR-376c and miR-222 were significantly overexpressed in gastrin treated AGS_{GR} cells, by 5.2-fold [$p < 0.01$] and 2.3-fold [$p < 0.0001$] respectively. However only the increase in miR-222 expression was confirmed using qPCR. Maximal increased expression of miR-222 (9-fold [$p < 0.01$]) was seen after 10 nM G17 treatment for 24 h in serum free media. Increased miR-222 expression was completely reversed by pre-treatment with the CCK-2 receptor antagonist YM022 (100 nM). miR-222 expression was also significantly increased in 10 week old female and male INS-GAS mice, compared with FVB/N mice (by 5.3-fold and 2.3-fold respectively).

Conclusion Gastrin induces gastric miRNA alterations, specifically miR-222 overexpression, both *in vitro* and *in vivo*. This was fully reversed by pre-treatment with YM022 *in vitro*. Since miR-222 overexpression has previously been linked to decreased expression of tumour suppressor proteins such as $p27^{Kip1}$ and increased oncogenesis, these data support the hypothesis that elevated gastrin may induce pathological changes via disruption of miRNA (particularly miR-222) expression. Further studies are needed to determine the mechanisms by which gastrin-induced miR-222 overexpression affects gastric pathology.

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OC-019 OPTIMISING THE PERFORMANCE OF MAGNETIC ASSISTED CAPSULE ENDOSCOPY (MACE) OF THE UPPER GI TRACT USING CT MODELLING

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Introduction Capsule endoscopy, employed to investigate the small bowel, is now being further developed to visualise the upper GI tract. In a pig model, using a hand held magnet, we have demonstrated that magnetic assisted capsule endoscopy (MACE) in the stomach is feasible. However, it is unclear what the best methodology is to achieve complete gastric luminal views in humans. Our aim was to utilise CT modelling of the abdomen to determine the optimal placements of a capsule endoscope in the stomach to allow complete mucosal visualisation and to determine the optimal placement of the hand held magnet to aid pyloric traversing.

Methods Using multiplanar reformatting, 100 good quality contrast abdominal CT scans were analysed to assess luminal visualisation by a magnetic capsule endoscope from 5 fixed stations throughout the stomach. From each station, we assessed the