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 neoplasy 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 micro prism. 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 of squamous 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.

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**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 perform 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 intramucosu 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 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 clinicopathologic variables tested. The only significant difference in survival was observed in a small subset of patients with metastastic 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 could represent significant involvement of the TLR signalling pathway in Barrett’s carcinogenesis.

**REFERENCE**


**Disclosure of Interest** None Declared.
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 (AGXqα) 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 ΔΔCt 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 AGXqα 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 p27Kip1 and increased oncosogenesis, 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.

Disclosure of Interest None Declared.

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