

Hieronder vindt u de honderd abstracts die zijn ingediend en geaccepteerd om gepresenteerd te worden tijdens de Digestive Disease Days op 18 en 19 maart 2020. Helaas hebben wij de DDD op het laatste moment moeten annuleren i.v.m. COVID-19. Ruim de helft van deze abstracts zijn alsnog gepresenteerd tijdens de DDD Online in het najaar en zijn te bekijken op 29 september, 12 november en 9 december. Inschrijven voor de webinars kan via onze website.

### Feasibility of volatile organic compound in breath analysis in the follow-up of colorectal cancer

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Background: Colorectal carcinoma (CRC) has a worldwide incidence of 1.4 million patients and a large share in cancer-related mortality. After initial curative treatment, the risk of recurrent cancer is 30-65%. Early detection may result in curative treatment. However, current follow-up (FU) examinations have a low sensitivity ranging from 49-85% and are associated with high costs. Therefore, the search for a new diagnostic tool, is justified. Analysis of volatile organic compound in exhaled air through an electronic nose (eNose) is a promising new patient-friendly diagnostic tool. We studied whether the eNose under investigation, the Aeonose™, is able to detect local recurrence or metastases of CRC.

Methods: We conducted a cross-sectional study

Results: We included 62 patients, all of whom underwent curative treatment for CRC in the past 5 years. Thirty-six of them had no metastases and 26 had extraluminal local recurrence or metastases of CRC, detected during follow up after curative resection for CRC. Breath testing was performed and machine learning was used to predict extra luminal recurrences or metastases, and based on the receiver operating characteristics (ROC)-curve both sensitivity and specificity were calculated. The eNose identified extra luminal local recurrences or metastases of CRC with a sensitivity and specificity of 0.88 (CI 0.69–0.97) and 0.75 (CI 0.57–0.87), respectively, with an overall accuracy of 0.81.

Conclusion: This eNose may be a promising tool in detecting extraluminal local recurrences or metastases in the FU of curatively treated CRC. However, a well-designed prospective study is warranted to show its accuracy and predictive value before it can be used in clinical practice.

## **Pneumatic dilation for persistent dysphagia after antireflux surgery, a multicenter randomized sham-controlled clinical trial**

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**Background:** Laparoscopic fundoplication is the most effective treatment for proton pump inhibitor-refractory gastro-esophageal reflux disease (GERD). Postoperative dysphagia, a common side effect after fundoplication, is usually self-limiting and generally disappears after 2-6 weeks. In 5-10% of patients however, dysphagia persists for more than three months postoperatively. There is no evidence-based treatment for persistent dysphagia after anti-reflux surgery, retrospective data suggest that pneumatic dilation may be efficacious. The aim of this study was to evaluate the effect of pneumatic dilation on persistent dysphagia after fundoplication in a sham-controlled study.

**Methods:** We performed a multicenter, single-blind, randomized sham-controlled trial of patients with persistent dysphagia (> 3 months) after fundoplication at four medical centers. Patients with an Eckardt symptom score  $\geq 4$  were randomized between pneumatic dilation (PD) using a 35-mm balloon and sham dilation. Primary outcome was treatment success, defined as an Eckardt score < 4 and a minimal reduction of 2 points in the Eckardt score after 30 days. Secondary outcomes included change in stasis on timed barium esophagogram, change in high-resolution manometry parameters, quality of life (Short Form Health Survey - SF-36), reflux (Reflux Disease Questionnaire - RDQ) and dysphagia symptoms (Impaction Dysphagia Questionnaire - IDQ).

**Results:** A total of 41 patients (mean age, 54.6 years; 14 males) were randomized. Three patients were excluded following randomization, as they did not receive the allocated treatment (1 patient had a large diaphragmatic hernia and 2 patients withdrew consent). Pneumatic dilation and sham dilation generated equal success rates (7/20 patients (35%) in the PD group vs 7/18 patients (39%) in the sham group;  $p=0.804$ ). There was no significant difference between the groups in change of stasis on the timed barium esophagogram after 2 minutes (PD vs sham: median 0.0 cm, range 0 – 9.8 cm vs median 0.0 cm, range 0 – 6.6 cm;  $p=0.143$ ) or change in high-resolution manometry parameters (PD vs sham IRP-4: 10.56 vs 14.60 mmHg;  $p=0.062$ ). Questionnaires on quality of life, reflux and dysphagia symptoms were not significantly different between the two groups. No predictors of treatment success were identified. No adverse events occurred during this study.

**Conclusion:** Pneumatic dilation with a 35-mm balloon is not superior to a sham dilation for treatment of persistent dysphagia after anti-reflux surgery.

## **Life stage associated environmental factors influence the development of inflammatory bowel disease: a large case-control study in The Netherlands**

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**Background:** The etiology of inflammatory bowel disease (IBD), consisting of Crohn's disease (CD) and ulcerative colitis (UC) is multifactorial including host genetic and gut microbial factors. Increasingly, the role of environmental and lifestyle factors (so called exposome,) is being recognized in both disease development as well as in disease behavior. While several environmental factors like smoking and hygiene have unequivocally been proven to be involved in IBD, for other environmental factors like alcohol consumption and stress, results have been conflicting. Here, we performed a case-control study evaluating known as well as novel possibly involved environmental factors.

**Methods:** We performed a case-control study including 674 IBD patients, who filled out the validated Groningen IBD Environmental Questionnaire (GIEQ), which captures numerous exposome factors through different stages of life, using 844 items, of which 454 (53.5%) were applicable to study the role of 92 exposome factors in disease etiology. Next, cases were randomly frequency matched, based on sex and age, to 1,348 population-based controls retrieved from the Lifelines Cohort Study, The Netherlands, of whom comparable exposome data is available. For each individual environmental factor logistic regression modelling comparing cases and controls was applied to estimate the multivariable-adjusted effect (odds ratio (OR) and 95% confidence intervals (95%CI)) on both CD and UC development while correcting for the potential confounding effect of sex and age.

**Results:** In total, 92 exposome factors were examined, of which 36 (39.1%) concerned childhood, 40 (43.5%) adulthood and 16 (17.3%) lifelong exposures. In total, 9 novel exposome factors were associated with both CD and UC, including prenatal smoke exposure (OR 1.9; 95%CI 1.4-2.0 in CD, OR 1.6; 95%CI 1.2-2.2 in UC), stressful life-events (2.6; 1.7-4.0 in CD, 2.9; 1.9-4.5 in UC) and alcohol consumption (0.4; 0.3-0.6 in CD and UC), while 5 disease-specific factors were identified. Also, 9 previously described factors were replicated, among which active smoking in CD (2.6; 2.0-3.4), appendectomy (2.3; 1.5-3.5 in CD, 0.4; 0.2-0.8 in UC) and leisure sports activity (0.5; 0.4-0.7 in CD, 0.7; 0.6-1.0 in UC).

**Conclusion:** In this study, multiple novel exposome factors have been associated with development of IBD, also, previously described factors were replicated. These new insights are crucial to further the understanding of disease etiology as well as to work towards potential targets for personalized lifestyle treatments.

## **Novel fecal protein biomarker test for improved colorectal cancer screening**

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**Background:** Many colorectal cancer (CRC) screening programs are fecal immunochemical test (FIT) based. However, FIT leaves room for improvement in particular for detecting advanced adenomas. A combination of protein biomarkers including hemoglobin was measured by mass spectrometry in whole stool samples. At equal specificity, this combination yielded a higher sensitivity for advanced neoplasia (CRC and advanced adenomas) compared to hemoglobin alone. Yet, mass spectrometry and whole stool samples are not attractive for CRC screening programs. Therefore, the aim of the present study is to confirm the diagnostic accuracy of biomarkers as detected by immunoassays in FIT buffer, and to compare their accuracy to FIT.

**Methods:** FIT samples were collected from 1296 participants (1049 from a screening and 247 from a referral population) of whom 47 had CRC, 165 advanced adenoma, and 251 non-advanced adenoma as most advanced lesion. Immunoassays were developed for ten previously identified biomarkers which were analyzed in FIT buffer, just like the standard FIT. Biomarker levels of patients with advanced neoplasia were compared to biomarker levels of healthy individuals. Classification and regression tree analysis (CART) was used to identify the optimal panel of biomarkers. The sensitivity of the selected panel was compared to FIT in a paired analysis at equal specificity.

**Results:** Reliable immunoassays could be successfully developed for nine out of ten biomarkers. All nine biomarkers were expressed significantly higher in CRC patients compared to healthy individuals ( $P < 0.001$ ), while eight out of nine in advanced adenoma patients compared to healthy individuals ( $P < 0.05$ ). Multivariate CART analysis yielded a panel with three biomarkers. In the development dataset, at equal specificity of 96.8%, panel sensitivity for advanced neoplasia was 48.6% (95% CI 41.7-55.5) compared to 37.3% (95% CI 30.7-44.2) for FIT. Thus, the relative improvement was 30.3%. CRC sensitivity was 80.9% for both tests, whereas the panel detected more advanced adenomas: 39.4% compared to 24.8% for FIT. Including non-advanced adenomas as controls showed a similar improvement; sensitivity was 48.6% (95% CI 41.7-55.5) versus 38.2% (95% CI 31.6-45.1) at a specificity of 94.6%.

**Conclusion:** Performance of protein biomarkers identified with mass spectrometry in whole stool samples is maintained with immunoassays in FIT buffer. Most biomarkers show increased expression in CRC and advanced adenoma patients compared to healthy individuals. The panel of three biomarkers has an increased sensitivity for advanced neoplasia compared to FIT. A prospective validation trial is in preparation.

## **Prevalence of gastrointestinal disease in an asymptomatic population using videocapsule**

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**Background:** Although gastrointestinal (GI) diseases are common, prevalence rates of lesions in the GI tract in an asymptomatic population are difficult to assess. Colon capsule endoscopy (CCE) is a safe, minimally invasive tool that images the entire GI tract. The aim of this study is to assess prevalence of lesions in the entire GI tract in an asymptomatic population by CCE.

**Methods:** Between 2017-2019, healthy subjects participating in the Rotterdam longitudinal epidemiological study (aged 50-75 years) were invited to receive CCE with corresponding bowel preparation. Trained reviewers analyzed images of the esophagus, stomach, small bowel and colon. Abnormalities defined as significant: Barrett segment > 3 cm, severe ulceration, small bowel villous atrophy, vascular abnormalities, polyp > 10mm or ≥ 3 polyps in small bowel or colon, and cancer. Endoscopies were performed if significant lesions were found.

**Results:** Of the 2800 invited asymptomatic subjects, 462 (16.5%) agreed to participate (mean age 66.8 years, male 46.1%). 451 procedures were analyzed. In 94.4% the capsule reached the descending colon and excretion was observed in 51.2%. In 76.6% the colon cleansing score was deemed adequate. Esophageal abnormalities were found in 14.5%, with Barrett esophagus (8.3%) and esophagitis (5.5%) most common. Gastric abnormalities were reported in 28.1%, most frequently, fundic glands (18.1%) and erosions (6.6%). Small bowel abnormalities were found in 64.6%, with lymphangiectasia (30.7%) most frequent. Colon abnormalities were present in 93.3%, most commonly diverticula (81.5%) and polyps (55.9%). Significant abnormalities were found in 12%.

**Conclusion:** In an asymptomatic population, GI tract mucosal abnormalities are frequently observed, mainly in the small bowel and colon. In over 10% of the population significant lesions were found. This study provides a frame of reference on the prevalence of GI mucosal abnormalities in an asymptomatic population.

## Can lifestyle and psychosocial factors predict flares of ibd; an exploratory study using telemedicine.

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**Background:** Tight control of mucosal inflammation and prevention of disease flares are emerging treatment goals to prevent disease progression in inflammatory bowel disease (IBD). The clinical, Montreal, classification only marginally predicts flare occurrence. Mounting evidence shows that psychosocial and lifestyle factors are associated with flares. Longitudinal monitoring of these factors has been made possible by implementing a web-based telemedicine tool, *myIBDcoach*. This study explores the potential additive predictive value of patient reported outcome measures (PROMs) captured using *myIBDcoach* to predict flares.

**Methods:** Consecutive IBD patients (n= 393) were recruited from a prospective *myIBDcoach* telemedicine study cohort (ClinicalTrials.gov, NCT02173002). During a one-year follow-up, every 1-3 months, participants reported information 30 variables via *myIBDcoach*. Variables were divided into two main categories; Baseline and *myIBDcoach*. *MyIBDcoach* variables were subdivided again in the following categories: psychosocial, medication, lifestyle and nutrition, medication, infection, tool usage. The outcome of interest, a flare during follow-up, was defined as having clinical symptoms of disease activity (using the Monitor IBD At Home questionnaire) combined with either a fecal calprotectin > 250g/g, disease activity on endoscopy, MRI or CT. Stepwise group-Lasso logistic regression (G-LASSO) was used as a variable selection method to study the relationship between flares and individual variables, as well as between flares and different variable categories.

**Results:** Seven G-LASSO regressions were estimated and evaluated using the Akaike information criteria (AIC), area under the curve (AUC) and stepwise importance using 10-fold cross-validated penalty parameter. G-LASSO models, starting with baseline variables and consecutively adding *myIBDcoach* variable categories, showed a decreasing AIC (from 276.3 to 223.5) and increasing AUC (from 0.68 to 0.84). Meaning that overall model performance increased, with the largest increase for adding psychosocial and lifestyle factors. All *myIBDcoach* variable categories have a higher penalty parameter than the baseline variables, thus *myIBDcoach* variable categories were found to be more relevant compared to the baseline variable category.

**Conclusion:** This exploratory study shows that psychosocial and lifestyle factors are of superior value for flare prediction compared to clinical, Montreal, (baseline) factors. Holistic monitoring, including psychosocial and lifestyle factors, and targeted interventions are of interest for future trials and are a promising strategy to prevent flares and improve the outcome of IBD.

## Post-ercp infections caused by contaminated duodenoscopes

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**Background:** Despite compliance to extensive cleaning and disinfection protocols, duodenoscopes have been related to multiple outbreaks of multi-drug resistant organisms (MDRO) due to persistent duodenoscope contamination. Reports of duodenoscope associated infections (DAI) usually describe outbreaks of MDRO and only seldom report outbreaks caused by susceptible microorganisms. These outbreaks with susceptible microorganisms probably do occur, but are hard to recognize and thus underreported. This study aims to find all potential DAIs that occurred in a large ERCP center within a period of fifteen months.

**Methods:** This is a retrospective observational study partially based on a previous cohort study, in which all duodenoscopes in a tertiary health care center were sampled after reprocessing cycles following an ERCP procedure. Between July 2017 and October 2018, 460 duodenoscope samples were collected. This cohort was combined with patient samples acquired up to one year after ERCP. Infection is defined as positive culture from blood and liver bed (bile, drain, abscesses, etc.). Possible DAI was defined as the same microorganism at species level in the duodenoscope and in the patient culture within one year after the ERCP procedure.

**Results:** Data of 837 ERCPs was available. We found 20 patients (2.4%) with an infection within one year after ERCP with the same microorganism as found on the duodenoscope used during their ERCP. Thirteen (13) of these 20 (65%) infections were gut flora, accounting for 1.6% of all 837 ERCPs. *Enterobacter cloacae complex* was the most common cultured microorganism (7 cases). 7/20 (35%) cases were skin flora, 13/20 (65%) of all possible DAI included bacteremia.

**Conclusion:** We hypothesize that DAIs caused by susceptible microorganisms are still a blind spot in daily practice. In this study, 1.6% of the 837 ERCP procedures was associated with an infection with gut flora that potentially was transmitted by a contaminated duodenoscope based on resemblance at species level. Further molecular typing is needed to definitely match microorganisms found on the duodenoscope with patient samples. These data are of clinical relevance as these infections are caused by exogenous bacteria and potentially preventable as opposed to infections caused by patients' own flora which are an inherent risk of any endoscopic procedure.

## **Laboratory and fecal investigations have limited value in the diagnostic work-up of irritable bowel syndrome**

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**Background:** Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder with a significant social-economic burden. Diagnosis is mainly based on the Rome criteria, yet consensus about additional laboratory investigations are lacking world wide so far. The aim of this study is to investigate the added value of blood and fecal tests and in patients with suspected IBS based on the Rome-criteria.

**Methods:** This retrospective cohort study aims to evaluate the effectiveness and costs of routine laboratory tests (Hb, TSH, anti-tTG/total IgA and fecal calprotectin) in patients that visited an expert IBS outpatient clinic from January 1, 2015 till January 1, 2019. Primary outcome is the prevalence of another diagnoses than IBS explaining the symptoms followed by the number needed to diagnose (NND) and cost effectiveness analysis.

**Results:** Overall, 218 patients are included. In approximately 200 patients blood and fecal tests were performed and 47 underwent a colonoscopy. Overall, in 96.3% of patients a diagnosis of IBS was made and in 2.8% IBD, 0.5% hyperthyroidism and 0.5% coeliac disease. The NND of all included laboratory investigations is 34, and for the individual test TSH 197, for coeliac serology 199 and for FCP 50. The total costs are approximately €4.850 to diagnose one patient with another diagnosis than IBS. The optimal cut-off value of FCP is 282 µg/g, with a low sensitivity of 80% and a specificity of 99%.

**Conclusion:** IBS can be established in the far majority based on the Rome-criteria. Investigation of Hb, TSH and coeliac serology on a routinely base do not offer added value. Screening for IBD with FCP seems to be considered worthwhile, with a proposed cut-off value of 100 µg/g based on our results and other publications.



## **Cancer recurrence in patients with a pathologically complete response after neoadjuvant chemoradiotherapy and surgery for oesophageal cancer: a retrospective cohort study**

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**Background:** Neoadjuvant chemoradiotherapy (nCRT) and surgery is a standard treatment for locally advanced oesophageal cancer. After oesophagectomy, 20-50% of patients have a pathologically complete response (pCR) and this is associated with a favourable 5-year survival. The aim of this study was to assess the pattern of recurrent disease in patients with pCR.

**Methods:** All patients from the phase II and III CROSS trials (April 2001 – December 2010) and patients treated with carboplatin/paclitaxel and radiotherapy 41,4 Gy outside the CROSS-trial (January 2011-October 2017) at the Erasmus MC Rotterdam were identified from an institutional database. Patients with a pCR were included in the study. The site of recurrence as seen on the CT-scan was compared to the field of radiation. Differences in characteristics of patients with and without a recurrence were compared with two-sided Fisher's exact. Overall survival (OS) and disease free survival (DFS) were calculated according to the method of Kaplan Meier and differences in survival were tested with the log rank test. Hazard ratios were calculated with Cox regression.

**Results:** Some 143 patients had a pCR. Median (IQR) follow-up was 110 (71-138) months and 5-year OS was 74%. Some 29 of 143 patients (20%) developed recurrent disease. Locoregional recurrence was seen in 14 patients, of whom 3 had isolated locoregional recurrence. Locoregional recurrences within the field of radiation were seen in 4 patients (3%), outside in 8 (6%) and just at the border in 2 patients (1%). All recurrences within the radiation field were from adenocarcinomas. Higher cT-stage (cT3-4 versus cT1-2) was associated with overall disease recurrence ( $p=0.012$ ). Patients with a recurrence had a 5-year OS of 39% compared to 83% in patients without recurrence ( $p<0.001$ ).

**Conclusion:** Although patients with pCR after nCRT plus surgery have a good prognosis, disease recurrence still occurs in 20% of patients but is largely located outside the field of radiation. This study suggests that more effective systematic therapy is needed to prevent disease recurrence outside the radiation and surgical field.

## **Ustekinumab is associated with better effectiveness outcomes when compared to vedolizumab in crohn's disease patients with prior failure to anti-tnf: a comparative effectiveness study**

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**Background:** Both vedolizumab and ustekinumab can be considered for the treatment of Crohn's disease (CD) when anti-TNF treatment fails. However, head-to-head trials are currently not available or planned in the near future. The aim of this study was to compare vedolizumab and ustekinumab in CD patients using a quasi-experimental study design in a prospective registry specifically developed for comparative studies.

**Methods:** CD patients who failed anti-TNF treatment and started vedolizumab or ustekinumab in standard care as second-line biological and naïve to the latter two therapies, were identified in the observational prospective ICC Registry. Corticosteroid-free clinical remission (Harvey Bradshaw Index  $\leq 4$ ), biochemical remission (C-reactive protein  $\leq 5$  mg/L and fecal calprotectin  $\leq 250$   $\mu\text{g/g}$ ), combined corticosteroid-free clinical and biochemical remission, and safety outcomes were compared after 52 weeks of treatment. To adjust for confounding and selection bias, we used multiple logistic regression (correcting for: current smoker, disease duration, complicated disease (stricturing or penetrating behaviour), prior CD-related surgery, number of prior anti-TNF treatments, HBI at baseline and biochemical disease at baseline) and propensity score matching (variables include the same variables as logistic regression analysis with addition of: disease location and behaviour, corticosteroid and immunosuppressant use at baseline).

**Results:** In total, 128 vedolizumab- and 85 ustekinumab-treated patients fulfilled the inclusion criteria. By using propensity score matching, 69 vedolizumab-treated patients were matched with 69 ustekinumab-treated patients. After adjusting for confounders, ustekinumab-treated patients were more likely to achieve corticosteroid-free clinical remission (OR: 2.56, 95% CI: 1.35-4.87,  $p=0.004$ ), biochemical remission (OR: 2.22, 95% CI: 1.04-4.74,  $p=0.040$ ), and combined corticosteroid-free clinical and biochemical remission (OR: 2.58, 95% CI: 1.15-5.78,  $p=0.022$ ), while safety outcomes (infections: OR: 1.26, 95% CI: 0.63-2.54,  $p=0.517$ ; adverse events: OR: 1.33, 95% CI: 0.62-2.81,  $p=0.464$ ; hospitalizations: OR: 0.67, 95% CI: 0.32-1.39,  $p=0.282$ ) were comparable between the two groups. The propensity score matched cohort showed comparable results.

**Conclusion:** In this prospective comparative analysis ustekinumab was associated with higher effectiveness outcomes of ustekinumab when compared to vedolizumab. Safety outcomes were comparable after 52 weeks of treatment in CD patients who have failed anti-TNF treatment.

## Top-down infliximab superior to step-up in children with moderate-to-severe crohn's disease - a multicenter randomized controlled trial

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**Background:** In newly diagnosed pediatric Crohn's Disease (CD) patients current guidelines instruct to start exclusive enteral nutrition (EEN) or oral prednisolone in combination with immunomodulators to achieve remission. Infliximab (IFX) is proven to be highly effective in pediatric CD patients, but mostly used once patients are refractory, the so called step-up (SU) treatment strategy. However, evidence is emerging IFX is more effective the sooner it is initiated. We investigated whether initiation of IFX directly after diagnosis in moderate-to-severe CD, i.e. top-down (TD) treatment, results in a higher long-term remission rate than SU treatment.

**Methods:** For this international randomized controlled trial (RCT) patients aged 3-17 years, with new-onset, untreated CD with weighted pediatric CD activity index (wPCDAI) >40 were included. TD treatment consisted of 5 IFX (CT-P13) infusions of 5 mg/kg (0, 2, 6, 14, 22 weeks) combined with azathioprine (AZA). After 5 infusions, IFX was stopped while continuing AZA. SU treatment consisted of induction therapy with EEN or oral prednisolone combined with AZA as maintenance treatment. In both groups, IFX could be (re)started on predefined conditions. Primary endpoint of this study was sustained clinical remission (wPCDAI <12.5) at week 52 without need for additional therapy or surgery. Secondary endpoints included IFX use at week 52, mucosal healing (SES-CD <3) and low fecal calprotectin levels (<250 ug/g) at week 10.

**Results:** 100 patients were included in 12 centers. Three out of 100 patients did not start with the study after randomization (n=97; 49 TD vs 48 SU). At week 52, 19/46 (41%) of TD patients were in clinical remission without a need for treatment intensification or surgery, while in the SU group this number was significantly lower (7/48, p=0.004). After induction therapy, IFX was (re)started in 18/49 (36%) TD patients compared to 29/48 (60%) SU patients within 52 weeks (p=0.02).

At week 10, significantly more TD (27/44, 61%) than SU treated patients (17/44, 39%) were in clinical remission (p=0.033). 57/97 consented to endoscopy at week 10. Endoscopic remission rates were higher in TD (16/27 [59%], median SES-CD I [IQR 0-5]) than SU treated patients (5/30 [17%], median SES-CD 6 [IQR 3-16], p=0.001). Similarly, low fecal calprotectin levels were more frequent in the TD group (n=75; TD 21/40 [53%] vs SU 9/35 [26%], p=0.027).

**Conclusion:** We are the first to compare TD IFX to SU treatment in an RCT of paediatric CD patients. TD treatment was superior to SU in achieving sustained clinical remission. Therefore, we advise to start IFX directly after diagnosis in moderate-to-severe paediatric Crohn's disease patients.

## **Tofacitinib for ulcerative colitis: results of the icc registry, a nationwide prospective observational cohort study**

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**Background:** Tofacitinib is a janus kinase 1 and 3 inhibitor approved for the treatment of ulcerative colitis (UC). Real-world evidence is needed to evaluate the effectiveness, usage and safety in daily practice.

**Methods:** UC patients initiating tofacitinib were enrolled in 15 hospitals (8 academic, 7 non-academic) participating in the ICC Registry: a Dutch prospective, observational registry. Visits were planned at baseline, week 12, and 24. Patients with both clinical (Short Clinical Colitis Activity Index (SCCAI) >2) and objective disease activity (endoscopy (Mayo>0), C-reactive protein (CRP) >5mg/l or fecal calprotectin (FCP) >250µg/g) were included. In this study corticosteroid-free clinical remission (SCCAI ≤2), biochemical remission (FCP ≤250µg/g), combined corticosteroid-free clinical and biochemical remission, predictors of remission, safety outcomes, dose optimization, and effect on lipids were determined at week 24. Only patients from centers with routine endoscopic follow-up (regardless of symptoms) were analyzed to determine endoscopic remission (Mayo=0) at week 12. All analyses were done on an intention-to-treat basis.

**Results:** In total, 111 UC patients (95% anti-TNF, 60% vedolizumab, 4% ustekinumab exposed) were followed for a median of 24 weeks (IQR 12-26). All patients had both active clinical and objective disease (SCCAI 8 (IQR 5-11), FCP 1800µg/g (IQR 633-2682)). Corticosteroid-free clinical, biochemical, and combined corticosteroid-free clinical and biochemical remission rate at week 24 was 29%, 25%, and 19%, respectively. Endoscopic remission was achieved in 21% of patients (n:33) at week 12. Prior vedolizumab exposure was associated with a lower remission rate at week 24 (OR33, 95%CI 11-.94) in multivariable analysis. In total, 36 tofacitinib-related adverse events (AE) occurred (88 per 100 patient years) (most common: cutaneous lesions and headache). Six patients (18 per 100 patient years) had to discontinue treatment due to AE. No thromboembolic events were reported. We observed 4 cases of herpes zoster re-activation but no severe infections (requiring hospitalization). During follow-up we encountered 14 hospitalizations and 5 (4.5%) colectomies were performed. Cholesterol, HDL, and LDL increased between baseline and week 12 (18% (95%CI 9-26, n:35), 18% (95%CI 8-28, n:35) and 27% (95%CI 14-39, n:35), respectively). At week 24, 33% of patients used 10mg twice daily.

**Conclusion:** Tofacitinib is an effective treatment for UC after anti-TNF and/or vedolizumab failure. We did observe a relative high rate of adverse events in this refractory UC cohort.

## **Platelet-rich stroma injection (prs) as a novel surgical treatment of refractory perianal fistulas in crohn's disease: a pilot study**

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**Background:** Perianal fistulizing Crohn's disease (FCD) comes with significant morbidity and severe reduced quality of life. Treatment of FCD is challenging and includes immunosuppressive drugs, antibiotics and surgery. However, all therapies are associated with high recurrence rates. Platelet-Rich Stroma (PRS) is a combination of Platelet-Rich Plasma (PRP) and Stromal Vascular Fraction (SVF). PRS is autologous, includes stromal cells in their matrix with stimulating factors, plays an essential role in wound repair and defense mechanisms against infection and is easy to obtain and inject. PRS could be of additional value in patients with refractory FCD. This study aims to assess the feasibility, safety and efficacy of local injection of PRS in patients with refractory FCD.

**Methods:** After informed consent, 10 patients (age  $\geq 16$  years) with refractory FCD were included between March 2018 and July 2019. Exclusion criteria were rectovaginal fistulas, persistent proctitis and pelvic abscesses. All patients underwent surgery with harvesting of subcutaneous fat and venous blood sampling to obtain 6 ml of PRS (1 ml of SVF resp. 5 ml of PRP), excision of the external opening(s), fistula curettage, injection of PRS in the fistula wall and closure of the internal opening. A pre- and postoperative MRI was performed. Endpoints were clinical outcome, both closure and absence of discharge at physical examination, patient reported outcome (no, moderate or major effect), as well as radiological outcome (van Assche score). From 3 months postoperative, re-injection of PRS was considered in patients with unfavorable clinical and/or radiological outcome.

**Results:** All patients (4 female; median age 33 (IQR 22.9-38.7) had infralevatoric fistulas with a median van Assche score of 17 (range 14-20) without rectal involvement and abscesses. Median follow-up was 6 months (4-12). Median duration of FCD was 4 years (IQR 2-5). Median number of drugs given were 4 (1-7) and median number of operations 3 (2-3), including stoma formation in 2 patients. Crohn's disease activity outside the anorectum was present in all patients. 7 patients underwent 1 PRS injection and 3 patients 2 injections. There were no complications of the PRS injection. Fistula closure was present in 5 patients and open in 5 patients, of which 2 patients had no signs of discharge. One patient underwent restoration of bowel continuity. Of 7 postop MRIs available to date, median decrease of van Assche score was 4 (range 0-15). Patient reported outcome were major effect in 4, moderate effect in 3, and no effect in 3 patients.

**Conclusion:** Autologous PRS appears to be feasible, safe and promising in the treatment of refractory perianal fistulizing Crohn's disease.

## **Thioguanine and low dose thiopurines and allopurinol are both safe options after failure of conventional thiopurines: a comparative analysis of two multicenter cohorts**

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**Background:** Both thioguanine (TG) and low dose thiopurines and allopurinol (LDTA) can be considered for the treatment of inflammatory bowel disease (IBD) when conventional thiopurines fail due to intolerance or adverse events (AE). However, head-to-head trials are currently not available. The aim of this study was to compare the safety of TG and LDTA in IBD patients.

**Methods:** Adult IBD patients who failed conventional thiopurines and initiated LDTA in standard care were identified in the observational prospective multicentre ICC Registry. IBD patients who failed conventional thiopurines and initiated TG were retrospectively enrolled in three university hospitals. Patients with concomitant treatment with biologicals were excluded. Primary outcome was discontinuation of therapy due to AE. Secondary outcomes included: medication-related AE, infections (moderate: oral medication, severe: intravenously administered medication), hospitalizations, and biological- and corticosteroid-free clinical remission (physician global assessment=0) after 104 weeks of treatment. To adjust for confounding, both multiple logistic regression and propensity score matching (PSM) were used to correct for baseline characteristics associated with disease severity or therapy refractoriness.

**Results:** In total, 182 IBD patients treated with TG (n=94) or LDTA (n=88) were included with a median follow-up of 104 weeks (IQR 91-104). The median dose of TG was 0.27 mg/kg (IQR 0.22-0.32), for LDTA: 100 mg allopurinol with either 0.67 mg/kg (IQR 0.54-0.75) azathioprine (n=45) or 0.35 mg/kg (IQR 0.28-0.38) mercaptopurine (n=41) (n=2 unknown). By PSM, 64 TG patients were strictly matched with 64 LDTA patients with comparable baseline characteristics. In total, 19% (TG: 20%, LDTA: 18%) of patients discontinued therapy due to AE. After adjusting for confounders, there were no significant differences in terms of discontinuation rate due to AE (TG: n=19, LDTA=16, OR 0.50 95%CI 0.15-1.68 p=.26), other AE (TG: n=46, LDTA: n=44, OR 0.89 95%CI 0.44-1.81 p=0.75) (no cases of nodular regenerative hyperplasia of the liver were reported), infections (TG: n=13, LDTA: n=18, OR 1.05 95%CI 0.40-2.73 p=.93), hospitalizations (TG: n=5, LDTA: n=9, OR 2.00, 95%CI 0.64-6.23 p=.23), or biological- and corticosteroid-free clinical remission (OR 0.74 95%CI 0.33-1.68 p=.48). Escalation to biological treatment was comparable (TG 21% vs LDTA 24% p=.68). All these results were in line with the PSM cohort.

**Conclusion:** A relative low percentage of patients with prior failure to conventional thiopurines discontinued therapy with TG or LDTA due to AE. Both maintenance therapies may be considered after failure of conventional thiopurines before escalating to biological therapy.

## **Prediction model to safely cease anti-tnf therapy in crohn's disease: validation of a predictive diagnostic tool for cessation of anti-tnf treatment in cd in a dutch population**

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**Background:** Tools for patient identification to safely cease anti-TNF therapy in Crohn's Disease (CD) patients are urgently needed. After an individual participant data meta-analysis (IPD-MA) a predictive diagnostic tool has been developed for cessation of anti-TNF therapy in CD. This study aims to validate this tool.

**Methods:** A retrospective, multicenter study was conducted of CD patients in whom anti-TNF therapy was ceased. Inclusion criteria were anti-TNF therapy >6 months use, anti-TNF therapy due to luminal CD and remission as indication for cessation. Collected baseline demographic, clinical, biochemical, treatment and imaging data were included; age, gender, smoking, Montreal classification, disease- and remission duration, history of surgery, type of anti-TNF medication, previous or concomitant immunosuppressant, previous anti-TNF therapy, anti-TNF therapy duration, haemoglobin, leukocytes, thrombocytes, albumin, C-reactive protein, anti-TNF serum concentration, anti-infliximab/adalimumab antibodies, remission at MRI/endoscopy, additional stop reason other than remission. The outcome was documented relapse of CD that necessitated (re)introduction of biologicals, corticosteroids or immune-suppressants or surgery.

**Results:** A total of 523 CD patients (333 females (63%), median age 40 years (IQR 32–53)) were included. 293 (56%) patients experienced a relapse after anti-TNF cessation after a median follow up of 30 months (IQR 15–51). Relapse rate was 33% (95%CI 31–34) and 53% (95%CI 52–53) after 1 and 2 years. The developed predictive diagnostic tool includes: age (HR 0.99; 95%CI 0.97-0.99); no smoking (0.72; 95%CI 0.59-0.87); Montreal A2 (0.67; 95%CI 0.51-0.87); Montreal A3 (0.75; 95%CI 0.51-0.87); no Montreal L4 (0.81; 95%CI 0.59-1.09); No immunosuppressant use at baseline (1.43; 95%CI 1.40-1.46); disease duration (1.01; 95%CI 0.99-1.03); no previous anti-TNF use (0.81; 95%CI 0.59-1.11); anti-TNF type adalimumab (1.22; 95%CI 0.99-1.51); platelets baseline 1.12; 95%CI 1.00-1.25); endoscopic remission (0.92; 95%CI 0.65-1.30). The discriminative ability of the prediction model in this external validation cohort equaled that of a previous IPD-MA with a C-statistic of 0.59. An update of the model with fecal calprotectin resulted in a C-statistic of 0.60 [0.55-0.63] and a reported calibration slope of 0.69.

**Conclusion:** A previously developed predictive diagnostic tool to safely cease anti-TNF in CD has been validated, however showed moderate performance in this external cohort. A further update of the model with histological and biochemical data is necessary to improve our ability to adequately select patients for cessation of anti-TNF therapy.

**Inflammatory bowel disease (ibd) patients frequently report adverse drug reactions during biologic therapy: a multicentre, prospective, patient-reported pharmacovigilance monitoring system.**

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**Background:** The use of biologicals has improved the treatment of IBD but the understanding of adverse drug reactions (ADRs) and the knowledge of patients' perception on ADRs is poor. Patient-reporting may provide more insight in the extent and burden of ADRs in daily practice which in turn can lead to treatment optimization. This study aimed to assess systematic patient-reported ADRs during biological therapy in IBD patients.

**Methods:** This multicentre, prospective, event monitoring study enrolled adult Crohn's disease (CD) and ulcerative colitis (UC) patients treated with a biological between 1 January 2017 and 31 December 2018. Patients completed bimonthly comprehensive web-based questionnaires regarding indication and use of, description of biological induced ADRs, follow-up of previous ADRs, experienced burden of the ADR using a 5-point Likert scale, contact with a healthcare provider and therapeutic consequences due to the ADR. Patient-reported ADRs were MedDRA coded by trained pharmacovigilance assessors. MedDRA is a multi-hierarchical dictionary used to code reported ADRs into specific unambiguous terms (preferred terms). Preferred terms are subsequently grouped into high-level terms, high-level group terms and system organ classes. In total there are 26 system organ classes.

**Results:** In total, 182 patients in 4 centres (female 51%, mean (standard deviation) age 42.2 (14.2) years, CD 77%) were included and completed 750 questionnaires. At baseline, 49% used an immunomodulator (43% thiopurines, 6% methotrexate), while biologicals were documented as follows: 59% infliximab, 30% adalimumab, 9% vedolizumab, 1% ustekinumab. At least one ADR was reported by 50% of the participants, and 233 ADRs were reported in total with a median reported ADRs per participant of 2 (interquartile range, 1-3). Fatigue (n=26), headache (n=20), injection site reactions (n=16) and arthralgia (n=12) were most commonly reported, with a mean burden of 3.31, 1.63, 2.55 and 3.33, and a correlation in time with the administration of the biologic was described in 58%, 85%, 81% and 8% in these ADRs, respectively. Participants contacted a healthcare provider in 62% of all ADRs. In two out of 90 patients who reported an ADR, the biological was discontinued.

**Conclusion:** We established a patient-reported pharmacovigilance monitoring system and participants in this study frequently reported ADRs due to biologicals. Fatigue and arthralgia resulted in the highest burden. This reporting system may provide more understanding of patient-experienced ADRs which may ultimately lead to increased adherence of therapy and improved quality of life.



## Colorectal neoplasia risk in patients with inflammatory bowel disease and serrated lesions

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**Background:** The presence of serrated lesions (SL) is an established risk factor for colorectal neoplasia development in the general population. However, the impact of SLs on the colorectal neoplasia risk in inflammatory bowel disease (IBD) patients is unknown. In addition, SLs might have been misclassified in IBD patients in the past, in part due to revisions of classification systems. Presently, SLs are categorized as hyperplastic lesions, sessile SLs, and traditional serrated adenoma. We aimed 1) to compare the colorectal neoplasia risk in IBD patients with SLs versus IBD patients without SLs, and 2) to study the subclassification of SLs in IBD patients before and after histopathological review by two expert gastrointestinal pathologists.

**Methods:** We identified all IBD patients with colonic SLs from 1996 to 2019 in a tertiary referral center using the local histopathology database. Patients with neoplasia prior to SL diagnosis were excluded. Clinical data from patients' charts were retrieved until June 2019. A subgroup of 136 SLs were reviewed by two pathologists. Log-rank analysis was used to compare the cumulative (advanced) neoplasia incidence in IBD patients with SL versus IBD patients without SL undergoing surveillance in the in the same time period. Patients were censored at the end of surveillance or at colectomy.

**Results:** We identified 376 SLs in 204 IBD patients (61.9% ulcerative colitis (UC)). In the original reports, 91.9% was classified as a hyperplastic lesion. After histopathological review, 120/136 (88%) of the SLs were confirmed (16 were no SL). Of the 120 confirmed SLs, 62.2% was classified as a sessile SL, 37.0% as a hyperplastic lesion, and 0.8% as a traditional serrated adenoma. The mean time from IBD diagnosis to the first serrated lesion was 14.3 ( $\pm$ 12.3) years. A total of 41/204 (20.0%) of patients developed neoplasia (3 CRC, 3 HGD, and 35 LGD; including 2 HGD and 17 LGD at the moment of serrated lesion detection). In the 304 patients without SL (52.6% UC), 63 developed neoplasia (20.7%; 8 CRC, 5 HGD and 50 LGD). Patients who received follow-up colonoscopies after SL (n=127) had an increased cumulative risk of neoplasia ( $p < 0.01$ ), but no increased risk of advanced neoplasia ( $p = 0.50$ ) compared to the group of IBD patients without SL.

**Conclusion:** The presence of SLs in IBD patients was associated with a relatively high risk of synchronous colorectal neoplasia as well as an increased risk of subsequent neoplasia, although not with an increased risk of advanced neoplasia. Histopathological review confirmed the SL diagnosis in the majority of lesions, although a large proportion of the hyperplastic lesions was reclassified as a sessile SL.

## **Fecal microbiota transplantation as treatment for recurrent *Clostridioides difficile* infection in patients with inflammatory bowel disease: experiences of The Netherlands donor feces bank**

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**Background:** In recent years Fecal Microbiota Transplantation (FMT) is effectively implemented as an approved treatment approach of refractory *Clostridioides difficile* infection (rCDI). In patients with inflammatory Bowel disease (IBD) the prevalence of co-infection with CDI is higher than in the general population due to the use of immunosuppressive medication and dysbiosis of the bacteria in the colon. Just a small percentage of IBD patients do have an active CDI infection, not to be confused with carriage. Here we report the treatment course and efficacy of FMT provided by The Netherlands Donor Feces Bank (NDFB) for IBD patients with rCDI.

**Methods:** The NDFB was founded to facilitate FMT by providing ready to use donor feces suspensions for treatment of patients with rCDI in hospitals throughout The Netherlands. A request for FMT is evaluated by the working group (specialists in the fields of Medical Microbiology, Gastroenterology, and Infectious Diseases) to assess the indication of FMT and to formulate a treatment advise for each individual patient taking the comorbidity into account. Prior to FMT, all patients were pretreated with vancomycin 250mg for at least 4 days and bowel lavage. In patients with ulcerative colitis as comorbidity, prednisone was added when there was a IBD flare simultaneous. The results of FMT were monitored by prospective collection of outcome data by the NDFB.

**Results:** Since the start of NDFB in March 2016 until August 2019, 186 FMT requests to treat 176 (r)CDI patients were reviewed within the NDFB working group including 26 patients with rCDI and IBD. In total, 129 patients (of which 14 suffered from IBD) were treated with 143 FMTs for CDI with a cure rate of 89.9% after a single FMT (116/129).

FMT was deemed not suitable in 12 of 26 patients with IBD because patients had *C. Difficile* carriage instead of an active CDI infection.

Fourteen IBD patients were treated with FMT (9 ulcerative colitis, 2 Crohn's disease and 2 indeterminate colitis). 3/14 patients suffered from rCDI with an active episode of IBD.

Of the 14 IBD patients treated with FMT, only one patient developed a relapse of a CDI infection within two months (total cure rate 92%). This cure rate does not differ from CDI patients without IBD.

**Conclusion:** In IBD patients with rCDI, FMT is equally effective compared to other patients with rCDI. In case of concurrent activity of IBD, pretreatment with prednisolone in combination with vancomycin appears to be effective.

## **Towards personalized use of adjuvant therapy in patients with resected pancreatic cancer after neoadjuvant folfirinox: a pan-european cohort study**

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**Background:** The added value of adjuvant chemotherapy following resection of pancreatic cancer after neoadjuvant FOLFIRINOX chemotherapy is unclear. Studies in specific subgroups, aiming for personalized treatment, are lacking. This study aimed to assess the treatment effect of adjuvant chemotherapy on overall survival in different subgroups who underwent pancreatic surgery following neoadjuvant FOLFIRINOX chemotherapy.

**Methods:** Multicenter, international cohort study within the European-African Hepato-Pancreato-Biliary-Association (E-AHPBA) including patients after resection of pancreatic cancer following neoadjuvant FOLFIRINOX chemotherapy (2012-2016). The effect of adjuvant chemotherapy on overall survival (OS) was evaluated in different subgroups including interaction terms for traditional histopathologic predictors with adjuvant treatment in a multivariable Cox model. Patients who died within 3 months after surgery were excluded to minimize guaranteed time bias.

**Results:** We included data from 520 patients (54% male, median age 61 years) who underwent resection of pancreatic cancer following neoadjuvant FOLFIRINOX, collected in 29 centers from 22 countries; Of these, 330 patients (66%) received adjuvant chemotherapy. In the entire cohort, median OS was 38 months without a significant survival benefit for the adjuvant group (median OS 38 vs. 40 months, log-rank  $p=0.98$ ; univariable Cox analysis HR 1.00; 95% CI 0.78-1.29). In multivariable analysis including interaction terms with adjuvant treatment, only the interaction term for N stage reached statistical significance ( $p=0.006$ ). There was a benefit of adjuvant therapy on OS in pN+ patients ( $n=254$ , HR 0.43; 95% CI 0.23-0.78) but not in pN- patients ( $n=256$ , HR 0.92; 95% CI 0.37-2.27). Median OS was 15 months longer in patients with pN+ status who received adjuvant therapy (36 vs. 21 months,  $p<0.001$ ), whereas in the pN- patients no statistical benefit on survival was noted: 51 months (95% CI 38-not reached) in the adjuvant therapy group vs. 65 months (95% CI 50-not reached) in the non-adjuvant therapy group,  $p=0.14$ .

**Conclusion:** In this international multicenter study, adjuvant chemotherapy had a significant differential effect on overall survival, with a statistically significant survival benefit in patients with positive lymph nodes (pN+) following neoadjuvant FOLFIRINOX. This finding should be confirmed in a multicenter randomized trial.

## **Immune profiling of treatment naïve and neoadjuvant treated pancreatic ductal adenocarcinoma tissues from the preopanc-I randomized controlled trial**

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**Background:** To investigate the immune micro-environment of pancreatic ductal adenocarcinoma (PDAC) in patients after immediate surgery or neoadjuvant therapy followed by surgery.

**Methods:** We analysed tumor samples from 49 chemo-radiotherapy naïve patients and 46 patients after neoadjuvant chemo-radiotherapy. Immune profiling of FFPE tumor samples was performed using the NanoString PanCancer assay and expression of 770 immune related genes was measured. We used the two sample T-test for comparison of expression levels between the two groups.

**Results:** Total tumor infiltrating lymphocytes (TILs) are decreased in neoadjuvant treated tumor tissue compared to untreated tumors ( $p=0,01$ ). B cell infiltration is decreased significantly ( $p<0,001$ ) in the neoadjuvant group, whereas T cell infiltration did not vary between groups. In addition, CXCL12 expression was significantly ( $p<0,0001$ ) higher in neoadjuvant treated tumors. This chemokine is involved in tumor proliferation and progression. However, its receptor CXCR4 was down regulated.

**Conclusion:** The tumor immune micro-environment after neoadjuvant treatment differs from treatment naïve tumors, indicating that neoadjuvant treatment affects the immune profile. Corresponding survival outcomes of patients included in this study will be investigated using a Cox proportional hazards model, as survival data will be available soon.

## Short-term outcomes after total pancreatectomy: a european prospective modified-snapshot study

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**Background:** Studies about total pancreatectomy (TP) show acceptable mortality and morbidity, but have a wide inclusion period and include high-volume centers. This European prospective modified-snapshot study assesses short-term outcomes of elective total pancreatectomy in 2018-2019.

**Methods:** Patients who underwent elective TP for malignant or benign disease in 1 of the 42 participating centers between June 2018 and June 2019 were included. Hospitals with at least 5 TPs annually were identified as high-volume. Uni- and multivariable logistic regressions with backward step selection were performed to identify predictors for postoperative complications and 30-day mortality.

**Results:** 253 patients underwent TP and 83% was operated in a high-volume center. Median age was 67 years (IQR 57-73) and 57% was male. Most patients had malignant disease (71%). Major postoperative complications occurred in 22% and predictors were ASA score  $\geq 3$  (OR 2.08 [95%CI 1.03-4.19]  $p=0.04$ ), blood loss (OR 1.00 [95%CI 1.00-1.00]  $p=0.08$ ), and low-volume centers (OR 2.58 [95%CI 1.17-5.67],  $p=0.019$ ). Hospital stay was median 12 days (IQR 9-18) and 15% was readmitted within 90 days after surgery. Of all patients with at least 30 days or 90 days follow-up, 30-day mortality was 5% (10/210 patients) and 90-day mortality was 9% (17/183). In multivariable analysis, only age (OR 1.09 [95%CI 1.00-1.18],  $p=0.046$ ) and BMI (OR 1.15 [95%CI 1.02-1.29],  $p=0.022$ ) were predictors for 30-day mortality.

**Conclusion:** In this first multicenter study, including a large cohort of patients who underwent TP within a small time period, current practice is reflected. Major postoperative complications and 30-day mortality were still considerable.

## Minimally invasive versus open distal pancreatectomy: an individual patient data meta-analysis of two randomized controlled trials

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**Background:** Minimally invasive distal pancreatectomy (MIDP) may reduce overall complications and hospital stay as compared to open distal pancreatectomy (ODP). This study aimed to combine data of randomized trials on MIDP vs. ODP and assess treatment effects in different high-risk subgroups by conducting an individual patient data meta-analysis of available RCTs.

**Methods:** A systematic review identified two RCTs on MIDP vs ODP: the LEOPARD trial from The Netherlands and the LAPOP trial from Sweden. Individual patient data were obtained and harmonized. The primary endpoint was the overall rate of major (Clavien-Dindo  $\geq$ III) complications. Secondary outcomes included length of stay and individual major complications. Sensitivity analyses were performed in three pre-specified subgroups (i.e. BMI  $\geq$ 25 kg/m<sup>2</sup>, severe comorbidity and malignant disease).

**Results:** A total of 166 patients were included for analysis. The rate of overall major complications was 21% (17/80) after MIDP vs. 35% (30/86) after ODP (adjusted odds ratio 0.54; 95% confidence interval 0.24 to 1.24;  $p = 0.148$ ). MIDP reduced blood loss with on average 228 ml (203 vs. 450 ml,  $p = 0.002$ ), length of hospital stay with on average 2 days (6 vs. 8 days,  $p = 0.036$ ), and delayed gastric emptying with 12% (4% vs. 16%,  $p = 0.049$ ), as compared to ODP. The rates of postoperative pancreatic fistula were not significantly different between the two groups (36% vs. 28%,  $p = 0.067$ ). The 90-day mortality was 0% after MIDP vs. 2% after ODP. In the three high-risk subgroups, the rate of overall complications did not differ between MIDP and ODP.

**Conclusion:** Based on combined data from two RCTs, MIDP was associated with a non-significant 14% lower rate of major complications and significant reductions in blood loss, delayed gastric emptying, and hospital stay, as compared to ODP. In trained hands, MIDP should be the treatment of choice for benign and premalignant lesions of the pancreatic body and tail.

## **Validation of the model for end-stage liver disease sodium score in the eurotransplant region.**

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**Background:** The shortage of liver grafts results in the prioritization of the sickest patients on the waiting list for liver transplantation. Since 2006, the degree of disease severity in transplant candidates is estimated with the Model for End-stage Liver Disease (MELD) score. However, MELD does not account for the worse prognosis associated with hyponatremia. Since the prevalence of cirrhosis is on the rise, better prediction of mortality and improved allocation for liver transplantation are becoming increasingly important. This study researches the potential impact of using MELD-Na instead of MELD for the allocation of livers in the Eurotransplant region.

**Methods:** All candidates allocated through MELD with chronic liver disease on the Eurotransplant (ET) liver transplant waiting list between 2007-2018 were included. They were followed from first listing to delisting or until 90 days. The relation between MELD and Na values at listing and 90-day mortality was assessed through a multivariate Cox proportional hazard regression. A reclassification table was constructed of the relevant changes in MELD to MELD-Na score. This allowed an estimation of the lives saved if MELD-Na-based allocation would have been used.

**Results:** 5223 patients were included. After 90 days, 21.3% were transplanted, 24.2% were removed and 2.8% had died. Hyponatremia of <135, <130 and <125 mmol/L was found in respectively 28.5%, 8.8% and 2.6% of the listed patients. Between 140 to 125 mmol/L, the MELD-corrected risk of 90-day death increased by threefold (2.9; 95%CI 2.30-3.53;  $p<0.001$ ). The hazard ratio for death was 1.16 (95%CI 1.15-1.17;  $p<0.001$ ) per gained MELD point and 1.08 (95%CI 1.06-1.09;  $p<0.001$ ) per 1-unit Na decrease. The MELD-Na had a c-index of 0.847 (SE 0.007,  $p<0.001$ ). Of the deceased patients, 26.3% would have had a significantly higher chance of transplantation with MELD-Na, which equals to a 4.9% decrease in 90-day waiting list mortality.

**Conclusion:** The ET waiting list population has a relatively high prevalence of hyponatremia. For transplant candidates, a low Na increases the risk of 90-day mortality by threefold. If MELD-Na would have been used, 26.3% of the deceased patients would have had a significantly higher chance of transplantation. The 90-day waiting list mortality would have been lowered by 4.9%. Thus, MELD-Na-based allocation could reduce waiting list mortality for the ET region.

## Population-based study on preoperative imaging of colorectal liver metastases

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**Background:** In patients with colorectal liver metastases (CRLM) preoperative contrast enhanced (ce)MRI may detect additional lesions and <sup>18</sup>F-FDG-PET-CT may detect extrahepatic disease. This study assessed trends and variation between hospitals and oncological networks in the use of preoperative imaging in The Netherlands.

**Methods:** All patients who underwent liver resection for CRLM in The Netherlands between 2014 and 2018 were retrieved from a nationwide auditing database. Multivariable logistic regression analysis was used to assess case-mix factors contributing to the use of and trends in preoperative imaging and hospital and oncological network variation. The use of ceMRI, <sup>18</sup>F-FDG-PET-CT and combined ceMRI and <sup>18</sup>F-FDG-PET-CT was assessed.

**Results:** In total 4510 patients were included of whom 1562 underwent ceMRI, 872 underwent <sup>18</sup>F-FDG-PET-CT and 1293 underwent combined ceMRI and <sup>18</sup>F-FDG-PET-CT. Use of ceMRI increased over time from 9.6% to 26.2% ( $p < 0.01$ ), use of <sup>18</sup>F-FDG-PET-CT decreased (25% to 6.0%,  $p < 0.01$ ) and use of ceMRI and <sup>18</sup>F-FDG-PET-CT (17%) remained stable. Five or more lesions were positively associated with use of ceMRI (adjusted odds ratio (aOR) 2.45, 95% confidence interval (CI) 1.89-3.17,  $p < 0.01$ ). Lesions larger than 55mm were negatively associated with use of ceMRI (aOR 0.32, 95%CI 0.25–0.40,  $p < 0.01$ ) but were positively associated with use of <sup>18</sup>F-FDG-PET-CT (aOR 1.34, 95%CI 1.06–1.68,  $p < 0.01$ ). After case-mix correction, hospital and oncological network variation was present regarding all imaging.

**Conclusion:** Remarkable variation exists concerning use of preoperative imaging for CRLM between hospitals and oncological networks in The Netherlands. The use of MRI is increasing whereas use of <sup>18</sup>F-FDG-PET-CT is decreasing.



## Population-based study on practice variation regarding preoperative systemic chemotherapy in patients with colorectal liver metastases and impact on short-term outcomes

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**Background:** Indications for preoperative chemotherapy for colorectal liver metastases (CRLM) vary. Use of preoperative chemotherapy may influence postoperative outcomes. This study assessed variation in use of preoperative chemotherapy for CRLM and related postoperative outcomes in The Netherlands. **Methods:** All patients who underwent liver resection for CRLM in The Netherlands between 2014 and 2018 were included from a national auditing database. Case-mix factors contributing to the use of preoperative chemotherapy, hospital variation and postoperative outcomes were assessed using multivariable logistic regression. Postoperative outcomes were postoperative complicated course (PCC), 30-day morbidity and 30-day mortality.

**Results:** In total, 4323 (69.6%) patients were included of whom 1314 (31.1%) patients received preoperative chemotherapy and 3009 patients did not. Patients receiving chemotherapy were younger (mean (+SD) 63.3 (10.2) versus 67.1 (10.3)  $p < 0.01$ ) and had less comorbidity (Charlson scores 2+ (18% versus 27%,  $p < 0.01$ ). Unadjusted hospital variation concerning administration of preoperative chemotherapy ranged between 4% and 100%. After adjusting for case-mix factors, three hospitals administered significantly more preoperative chemotherapy than expected and six administered significantly less preoperative chemotherapy than expected. PCC was 12.1%, 30-day morbidity was 8.8% and 30-day mortality was 1.5%. No association between preoperative chemotherapy and PCC (OR 1.22, 0.97 – 1.53,  $p = 0.09$ ), 30-day morbidity (OR 1.16, 0.90 – 1.48,  $p = 0.25$ ) or 30-day mortality (OR 1.18, 0.68 – 2.01,  $p = 0.55$ ) was observed.

**Conclusion:** Significant hospital variation in use of preoperative chemotherapy for CRLM was present in The Netherlands. No association between postoperative outcomes and preoperative chemotherapy was observed.

## **Risk-stratified comparison of bridge to surgery versus emergency resection and propensity-score matched analyses of decompressing stoma versus emergency resection in patients with left-sided obstructive colon cancer: a nationwide study**

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**Background and Aims:** Most studies comparing bridge to surgery (BTS) techniques and emergency resection (ER) for left-sided obstructive colon cancer (LSOCC) did not investigate specific operative risk groups, such as elderly and frail patients. In addition, supporting evidence for decompressing stoma (DS) as BTS remains scarce. Therefore, the aims of the two current population-based studies were to 1) perform a risk-stratified comparison of BTS by either stent or DS with ER, and 2) to specifically compare DS as BTS and ER using propensity-score matching.

**Methods:** Patients with LSOCC treated between 2009-2016 were identified from the Dutch ColoRectal Audit, a mandatory, prospective national registry. Hereafter, 75 of 77 hospitals in The Netherlands retrospectively gathered additional data for each patient. For the first study, risk groups were created based on tumor characteristics (non-locally advanced [NLA] or locally advanced [LA]), age (< or ≥ 70 years), and ASA score (ASA I-II or ASA III-IV). For the second study, DS and ER patients were propensity-score matched.

**Results:** For the first study, 2587 patients were included (2013 ER, 345 DS, 229 stent). BTS patients showed significantly fewer permanent stomas than ER patients in most risk groups, with absolute risk reductions varying from 9.5% in low-risk patients (NLA, < 70 years, ASA 1-2) to 42.7% in high-risk patients (LA, ≥ 70 years, ASA 3-4). No significant differences were observed in 90-day mortality, disease free survival, and overall survival in the stratified risk groups. For the second study, propensity-score matching resulted in 236 DS and 472 ER patients. DS resulted in more laparoscopic resections (56.8% vs 9.2%,  $p < 0.001$ ) and more primary anastomoses (88.5% vs 40.7%,  $p < 0.001$ ) if compared to ER. DS patients had a lower risk of 90-day mortality (1.7% vs 7.3%,  $p = 0.006$ ) and better 3-year overall survival (79.4% vs 73.3%, HR 0.36, 95% CI 0.20-0.65,  $p < 0.001$ ). Resection-related complication (23.6% vs 37.5%,  $p < 0.001$ ), anastomotic leakage (3.4% vs 9.9%,  $p = 0.018$ ), major complication (9.0% vs 15.1%,  $p = 0.027$ ), and permanent stoma rates (18.8% vs 33.4%,  $p < 0.001$ ) were lower after DS, with shorter post-resection hospital stay (median 6 vs 11 days,  $p < 0.001$ ).

**Conclusions:** Our risk-stratified, population-based study demonstrated fewer permanent stomas after BTS than ER for LSOCC, especially in elderly frail patients, without an impact on disease free and overall survival. Secondly, our propensity-score matched analyses revealed, apart from fewer complications and fewer permanent stomas, a reduced post-operative mortality and better 3-year overall survival for DS as BTS than ER in patients with LSOCC.

## **Informing metastatic colorectal cancer patients by quantifying multiple scenarios for survival based on real-life data**

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**Background:** Reported median overall survival (mOS) in trials of metastatic colorectal cancer (mCRC) patients receiving systemic therapy has increased to over 30 months. When informing patients, many clinicians quote the mOS reported in these trials. It is uncertain whether trial results translate to real-life populations. Moreover, patients prefer presentation of multiple survival scenarios over presentation of just mOS. Therefore, we quantified multiple scenarios for survival time of real-life mCRC patients.

**Methods:** Nationwide population-based data of all stage IV CRC patients diagnosed between 2008 and 2016 were obtained from The Netherlands Cancer Registry. We calculated percentiles (scenarios) of OS per year of diagnosis for the total population, and for treatment subgroups: 10<sup>th</sup> (best-case), 25<sup>th</sup> (upper-typical), 50<sup>th</sup> (median), 75<sup>th</sup> (lower-typical), and 90<sup>th</sup> (worst-case).

**Results:** The total study population comprised 27,275 patients. Twenty-five percent these patients did not receive any antitumor treatment. From 2008-2016, mOS of the total population remained unchanged at approximately 12 months. OS improved only for the upper-typical and best-case patients; by 4.2 to 29.1 months ( $p < 0.001$ ), and by 6.0 months to 62.0 months ( $p < 0.001$ ), respectively. No clinically relevant change was seen among patients who received systemic therapy, with mOS close to 15 months and best-case scenario approximately 40 months. mOS and worst-case scenario for survival were highest in patients who underwent both metastasectomy and systemic therapy: around 48 and 15 months, respectively. A clinically relevant improvement in survival over time was observed only in patients who initially received metastasectomy without systemic treatment.

**Conclusion:** In contrast to the wide belief that mOS of mCRC patients receiving systemic therapy has improved substantially, improvement could not be demonstrated in our real-life population. Clinicians should consider quoting multiple scenarios for survival based on real-life data, instead of point estimates from clinical trials, when informing patients about their life expectancy.

## Accuracy of mri for clinical staging of early rectal cancer: a large population-based cohort study

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**Background:** MRI based staging of early rectal cancers is difficult but nevertheless used for decision making in the era of rectal conserving treatment approaches. The aim of this population-based study was to determine the accuracy of routine daily MRI reading for staging and subsequent decision making for early rectal cancer, whether or not combined with endorectal ultrasound (ERUS).

**Methods:** Patients with cT1-2 stage rectal cancer who underwent total mesorectal excision (TME) without downsizing (chemo)radiotherapy were selected from the Dutch ColoRectal Audit (DCRA), between 1 January 2011 and 31 December 2018. Accuracy of preoperative MRI ± ERUS for tumor- and nodal staging was expressed as sensitivity, specificity, and positive- and negative predicting value (PPV/NPV).

**Results:** Of 7,382 registered patients with cT1-2 stage rectal cancer, 4,847 patients were included (4,700 MRI alone, 147 MRI + ERUS; 577 cT1 and 4,270 cT2). Patients with pT1 were over staged by MRI alone in 67.7% (692/1022) and by MRI and ERUS in 74.3% (26/35). Under staging of pT2 occurred in 5.8% (131/2242) and in 20.2% (18/89), respectively. Diagnostic accuracy by MRI alone for cT1 was 80.7% and for cT2 53.7%, with a sensitivity of 32.3 and 94.2%, a specificity of 94.2 and 16.8%, PPV of 60.6% and 50.8% and NPV of 83.4 and 76.0%, respectively. MRI alone over staged pN0 stage in 17.3% (570/3303) and the positive predictive value of cN0 stage was 76.3% (2733/3583).

**Conclusion:** Current Dutch population-based analysis reveals that early-stage rectal cancer that was treated with TME surgery was mainly staged by MRI alone, with substantial over staging of pT1 lesions of 68% and false-negative rate for cN0 of 17%. These observations illustrate the opportunities to improve patient selection for organ-preserving approaches, potentially by adding a diagnostic local excision of early lesions as part of workup which can result to be curative or direct further treatment.

## **Poor diagnostic accuracy of serum igg4/igg rna ratio for discriminating igg4-related disease from pancreatic or biliary cancer (dipac): a prospective cohort study**

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**Background:** IgG4-related disease (IgG4-RD) of the biliary tract and pancreas is a benign inflammatory disease which is frequently difficult to distinguish from pancreatic or biliary cancer. Disease presentations may be similar and misdiagnosis is common, which may result in unnecessary major surgical interventions or chemotherapy. To discriminate IgG4-RD from primary sclerosing cholangitis (PSC) or pancreatobiliary cancer, a diagnostic test for IgG4-RD was recently proposed by our group. The serum IgG4/IgG RNA ratio determined by quantitative polymerase chain reaction (qPCR) was reported to discriminate IgG4-RD from PSC or biliary and pancreatic cancer with high accuracy. The present study aimed to assess the diagnostic accuracy of serum IgG4/IgG RNA ratio for the differentiation between IgG4-RD and pancreatobiliary cancer in a cohort of patients with suspicion of pancreatobiliary malignancy.

**Methods:** In this prospective, observational study, patients presenting at a specialized hepato-pancreatobiliary clinic with a suspicion of pancreatic or biliary cancer were included between February and August 2019. The IgG4/IgG RNA ratio determined by qPCR was performed in addition to standard diagnostic procedures (threshold 5.0%). Clinical and laboratory data were collected and in patients who underwent a biopsy, brush or surgery histo- or cytopathological findings were analyzed. For the diagnosis of IgG4-RD, the HISORt criteria were used as reference standard. Malignancy was defined by the presence of neoplastic cells at histo- or cytopathological examination.

**Results:** In this interim analysis, a total of 213 consecutive patients were investigated with an age of 68±11 years. Three patients were diagnosed with IgG4-RD, in two patients serum IgG4 level was markedly elevated (> 14.0 g/L). 178 patients were diagnosed with a malignancy of whom 165 patients with pancreatobiliary malignancy. 110 patients were diagnosed with pancreatic cancer, 37 with cholangiocarcinoma, 11 with carcinoma of the papilla of Vater and 7 with gallbladder carcinoma. In 3 patients (1.4%), the test was true positive whereas in 87 patients (40.8%) the test was false positive. In 123 patients (57.7%) the test was true negative. The sensitivity of the IgG4/IgG RNA qPCR was 100%, the specificity 57.7%, the positive predictive value 3.3%.

**Conclusion:** An elevated IgG4/IgG RNA ratio did not accurately discriminate pancreatic and biliary cancer from IgG4-RD as illustrated by low specificity and concordant low positive predictive value. We decided to stop the trial and advise against the use of this test for discrimination of IgG4-RD from pancreatobiliary malignancies outside a research setting.

## **Extremely sensitive next generation sequencing mutation analysis in biliary brush has a high diagnostic accuracy to distinguish benign and malignant strictures in primary sclerosing cholangitis**

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**Background:** Routine cytology of brushes from biliary strictures has a low diagnostic accuracy to distinguish benign strictures from cholangiocarcinoma (CCA). Particularly in primary sclerosing cholangitis (PSC) cytology assessment is difficult due to inflammation and heterogeneous cell populations. Next generation sequencing (NGS) mutation analysis seems a promising diagnostic tool.

**Methods:** In this case-control study, biliary brush samples obtained during ERCP in PSC patients with CCA (cases) were compared to biliary brush samples –obtained in the same period– in PSC patients without a CCA diagnosis within a follow-up period of at least one year after sampling (control group). The available brushes were processed into smears or cytospin samples. All cells on these sections were dissected for DNA isolation. NGS analysis was performed with a highly sensitive cell-free DNA gene panel including 242 hotspots in 14 genes: *AKT1*, *APC*, *BRAF*, *CTNNA1*, *EGFR*, *ERBB2*, *FBXW7*, *GNAS*, *KRAS*, *MAP2K1*, *NRAS*, *PIK3CA*, *SMAD4*, and *TP53*. The NGS analysis resulted in a limit of detection down to 0.1% with a DNA input of 20ng; samples that contained <2ng DNA were excluded from analysis. Repeated brushes within the same patients as included in the case-group were analyzed separately.

**Results:** A total of 23 brush samples (8 cases/ 15 controls) were included in the analysis, after exclusion of four PSC-CCA samples with DNA <2ng. The selected gene panel detected 8 mutations in PSC-CCA brush samples in 6/8 cases, including *TP53* (in 5 cases), *KRAS* (in 1 case), *PIK3CA* (in 1 case), and *ERBB2* (in 1 case) mutations. A *GNAS* mutation was found in 1/15 PSC-controls. The sensitivity of the 14 gene NGS panel is 75% (95% CI=41%-93%), and specificity 93% (95% CI=70%-99%); *TP53* mutation has a sensitivity of 63% (95% CI=31%-86%) and a specificity of 100% (95% CI=80%-100%). All repeated brushes (5 brushes in 3 patients) showed the same *TP53* mutation as detected in the first brush sample.

**Conclusion:** NGS mutation analysis with this 14 gene panel and a minimum threshold of only 2ng DNA has a high diagnostic accuracy to distinguish PSC-CCA from benign PSC strictures in biliary brush samples. Implementation of this analysis in routine clinical care may be anticipated.

## **CT-based radiomics for prediction of resectability in pancreatic ductal adenocarcinoma**

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**Background:** Pancreatic ductal adenocarcinoma (PDA) is a devastating disease with a 5-year overall survival of only 8%. The only curative treatment is surgical resection. However, only 15- 20% of patients is considered resectable and during surgery 10-20% of cases are considered unresectable due to unexpected locally advanced pancreatic cancer (LAPC) or metastases. Radiomics is the extraction of large amounts of quantitative image features to improve image analysis. By quantifying parameters of tumor appearance, it is possible to capture more information than by visual analysis only. This study investigates the potential value of quantitative radiomics to predict local resectability of PDAC on contrast-enhanced CT (CECT).

**Methods:** Patients from our unit with suspected PDA in the pancreatic head from 2015-2018 were included. Outcome was sensitivity and specificity of the Radiomics algorithm for resectability. For resectability (explorative) surgery was used as gold standard. For patients that were not operated on resectability status on CECT was used as reference standard. Patients with a resectable tumor and also metastases on CECT were excluded. First-order intensity, texture and shape features were calculated for manually 3D-segmented tumor volumes. Stable features were selected for further analysis after an inter-observer analysis with 3 blinded radiologists on a randomly selected subset of patients. The stable features were analysed for association to LAPC. Using all significant features ( $p < 0.05$ ), a support vector machine (SVM) was trained to predict resectability.

**Results:** 87 patients with PDA of the pancreatic head were selected. 8 patients were excluded because they had metastases with a resectable tumor on CECT. 51/79 patients (65%) underwent surgery of which 42/51 (82%) were resected. 9 were not resected due to unexpected LAPC. 28 patients did not undergo surgery due to LAPC (19/28) or LAPC combined with metastases (9/28). The inter-observer analysis was performed in 11 cases, 84/105 features were considered stable and were selected for further analysis. Univariate analysis resulted in 24 features significantly associated with LAPC. These features were used to predict resectability using a SVM. The algorithm classified 44/79 patients (56%) as resectable and 35/79 (44%) as irresectable. There were 12 false positives and 10 false negatives. This resulted in a sensitivity and specificity of 76% and 67%, with an area under curve of 0.71 for predicting resectability.

**Conclusion:** Quantitative CECT-based radiomics, based on tumor features only, seems feasible for predicting resectability in patients with PDA. With further development and validation it could be a promising tool.

## **The metastatic pattern of intestinal and diffuse type gastric adenocarcinoma - a dutch national cohort study.**

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**Background:** The Laurén classification of gastric adenocarcinoma involves three histological subtypes, the intestinal type, the diffuse type and a combination of the two, the mixed type. The subtypes differ in epidemiology, tumour biology and patients' survival. Studies on the metastatic pattern of gastric adenocarcinoma by histological subtype according to Laurén are missing.

**Methods:** All newly diagnosed gastric adenocarcinoma patients with metastatic disease at the time of diagnosis between 1999 and 2017 were identified in The Netherlands Cancer Registry (NCR). Based on pathology reports archived in the Dutch Pathology Registry the Laurén classification was determined by a syntax and then linked to the individual cases in the NCR. Differences in metastatic pattern between groups were compared using a chi-square test. Survival times were calculated with the Kaplan-Meier method and the log-rank test was used to compare survival distributions between groups.

**Results:** Between 1999 and 2017 12.759 gastric adenocarcinoma patients with metastatic disease were registered in the NCR. The location of the metastasis was known in 10.631 (83%) patients. In this group the Laurén classification could be determined in 8.140 (77%) patients. Among them, 4.632 (57%) patients had an intestinal type carcinoma, 3.149 (39%) patients had a diffuse type carcinoma and 359 (4%) had a mixed type carcinoma. Compared to diffuse type carcinomas, the intestinal type carcinomas metastasised more frequently to the liver (56% versus 28%,  $p<0.0001$ ) and lungs (13% versus 7%,  $p<0.0001$ ), whereas diffuse type carcinomas metastasised more often to the peritoneum (58% versus 29%,  $p<0.0001$ ) and bones (9% versus 6%,  $p<0.0001$ ). The median survival for patients with metastatic intestinal type gastric adenocarcinoma was 4.3 months versus 3.9 months for patients with a metastatic diffuse type gastric carcinoma ( $p<0.0001$ ). For patients with metastatic disease at a single location, patients with an intestinal type carcinoma had a better survival than patients with a diffuse type carcinoma; 5.3 versus 4.7 months for peritoneum metastasis only ( $p=0.002$ ), 3.9 versus 3.4 months for liver metastasis only ( $p=0.045$ ), 6.5 versus 5.0 months for lung metastasis only ( $p=0.029$ ) and 8.1 versus 5.0 months for lymph nodes metastasis only ( $p<0.0001$ ).

**Conclusion:** In this large national cohort study, the metastatic patterns of diffuse type and intestinal type gastric adenocarcinoma differed; the diffuse type had a predilection for the peritoneum whereas the intestinal type had a predilection for the liver. Furthermore, the results showed that also in the metastatic setting, the Laurén classification of gastric adenocarcinoma was prognostic for survival.



## **Predictive value of endoscopic esophageal abnormalities for residual esophageal cancer after neoadjuvant chemoradiotherapy**

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**Background:** Endoscopic evaluation of the esophageal mucosa may play a role in an active surveillance strategy after neoadjuvant chemoradiotherapy (nCRT) for esophageal cancer. This study aimed to investigate the yield of endoscopic abnormalities of the esophageal mucosa for detection of residual disease (RD).

**Methods:** A retrospective chart review of 156 patients was performed. All patients underwent nCRT followed by surgery for esophageal cancer. Upper endoscopy was performed six and twelve weeks after nCRT. Endoscopic records were reviewed for presence of non-passable strictures, relative strictures, residual tumor, scar tissue, or ulceration. Presence and type of endoscopic esophageal abnormalities at 6 and 12 weeks were compared between patients with RD and patients with a complete response (CR) based on the resection specimen.

**Results:** 118 of 156 (76%) patients had RD. A non-passable stricture was present in eleven patients at six weeks (RD vs CR, 5% vs 13%,  $P=0.09$ ), preventing full examination of the esophagus. In the remaining 145 patients, ulceration was the most prevalent endoscopic feature (RD vs CR at 6 weeks: 55% vs 61%,  $P=0.59$ ; 12 weeks: 40% vs 43%,  $P=0.77$ ). Comparable outcomes were found for presence of a relative stricture (18% vs 24%,  $P=0.41$ ; 18% vs 14%,  $P=0.67$ ) and scar tissue (10% vs 15%,  $P=0.39$ ; 19% vs 29%,  $P=0.31$ ). Endoscopic suspicion of residual tumor was significantly associated with RD; at 6 weeks 40/44 patients had RD (36% vs 12%,  $P=0.01$ ), while at 12 weeks all sixteen patients had RD (22% vs 0%,  $P<0.01$ ). **Conclusion:** Endoscopic esophageal abnormalities after nCRT were found to be of limited value for detection of residual esophageal cancer. Endoscopic suspicion of residual tumor was the only finding that was associated with RD. Data from prospective studies are needed to confirm its predictive value before this parameter can be implemented in clinical practice.

## 10-year follow-up of a randomised controlled trial comparing neoadjuvant chemoradiotherapy plus surgery versus surgery alone for oesophageal or junctional cancer (cross)

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**Background:** Neoadjuvant chemoradiotherapy according to the Dutch randomised controlled ChemoRadiotherapy for Oesophageal cancer followed by Surgery Study (CROSS) has become standard of care for patients with cancer of the oesophagus or oesophagogastric junction. The aim of this study was to report the long term results of the CROSS trial with a minimum follow-up of 10 years.

**Methods:** Patients with locally advanced resectable squamous cell carcinoma or adenocarcinoma of the oesophagus or oesophagogastric junction were randomised between neoadjuvant chemoradiotherapy (five weekly cycles of intravenous carboplatin [AUC 2 mg/mL per min] and intravenous paclitaxel [50 mg/m<sup>2</sup> of body-surface area]) with concurrent 41.4 Gy radiotherapy given in 23 fractions of 1.8 Gy, 5 days per week) plus surgery versus surgery alone. Primary endpoint was overall survival, defined from date of randomisation to date of all-cause death or to last day of follow-up. Analysis was by intention-to-treat.

**Results:** Between March 2004 and 2008, eight centres enrolled 368 patients. Some 178 were analysed in the chemoradiotherapy plus surgery group and 188 in the surgery alone group. After a median follow-up for surviving patients of 146.6 months (IQR 133.5-157.2), median overall survival was 48.6 months (95%CI 31.9–65.2) in the neoadjuvant chemoradiotherapy plus surgery group compared to 24.0 months (95% CI 14.3–33.6) in the surgery alone group, which was significantly different (HR 0.70 [95%CI 0.55-0.89]; log-rank p=0.004). Ten-year overall survival was 38% (95%CI 31%-45%) in the neoadjuvant chemoradiotherapy followed by surgery group compared to 25% (95%CI 20%-33%) in the surgery alone group (HR 0.68 [95%CI 0.53-0.87]). For patients with squamous cell carcinoma ten-year overall survival was 46% (95%CI 33%-64%) in the neoadjuvant chemoradiotherapy plus surgery group compared to 23% (95%CI 13%-40%) in the surgery alone group. For patients with adenocarcinoma ten-year overall survival was 36% (95%CI 29%-45%) in the neoadjuvant chemoradiotherapy plus surgery group compared to 26% (95%CI 20%-35%) in the surgery alone group.

**Conclusion:** Survival benefit of patients with locally advanced resectable squamous cell carcinoma or adenocarcinoma of the oesophagus or oesophagogastric junction receiving neoadjuvant chemoradiotherapy persists for at least 10 years compared to patients undergoing surgery alone.

## **Early changes in serum immune markers in patients who will develop hepatocellular carcinoma: promising targets for a global biomarker development initiative.**

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**Background:** Hepatocellular carcinoma (HCC) is the most frequent liver malignancy and is the second most common cause of cancer-related death worldwide. Despite, there is a clear lack of sufficiently sensitive and specific blood tests, which hampers screening efforts to detect early HCC. The most studied one is serum alpha-fetoprotein, but diagnostic performance is low in patients with cirrhosis and hepatitis. Recently, we associated levels of serum cytokines with the development of HCC in patients with hepatitis C (HCV). In this study we aimed to identify immunological signatures in serum of chronic hepatitis B (HBV) patients who eventually will develop HCC.

**Methods:** In this explorative study, immune profiles were studied using the ProcartaPlex multiplex system and Olink multiplex system for more than 100 immune markers (cytokines, chemokines and growth factors). We analyzed sera of two archived Erasmus MC cohorts, firstly a longitudinal cohort with chronic HBV patients that either had HCC (N=37), or would develop 6 (N=22) and 12 (N=25) months prior to HCC diagnosis as well HBV controls (N=28). Secondly, patients with varying liver diseases that had cirrhosis (N=20), early stage HCC with or without cirrhosis (N=48), and controls (N=20). Multivariate analyses were performed to identify HCC-specific immune profiles.

**Results:** In our cohort we identified a set of 11 immune mediators (e.g. VEGF-A, FGF-2, TWEAK, MIP-3a, CYFRA) whose levels were significantly altered in serum -6 or -12 months before visual diagnosis with imaging compared with controls. Addition of CYFRA and TWEAK to AFP levels identified patients who would eventually HCC with an area under the receiver operating characteristic curve value higher than 0.9. Using the Olink panel we identified a total of 27 biomarkers with an adjusted  $p < 0.01$  in the particular disease groups. Proteins associated with vascular mediators and inflammatory proteins were noted in patients with advanced fibrotic stage and HCC. A hierarchical clustering analysis of the Olink panel identified 2 biomarker clusters and grouped the controls and cirrhotic patients. Generally, the proteins upregulated associated with soluble endovascular mediators, including VEGF receptor 2 ( $p < 0.0001$ ), and with checkpoint proteins, such as PD-L2 ( $p = 0.0005$ ).

**Conclusion:** Early detection of HCC is important. Our data demonstrates modulation of immune profile in serum long before clinical diagnosis and in patients with cirrhosis and early HCC. These findings are highly promising, but require further validation in patients with different etiologies of HCC and ethnic backgrounds. Therefore, we are in the process of validating these markers prospectively in our global cohort.

## **Association between the adenoma detection rate and a composite quality indicator of caecal intubation, patient comfort and sedation in fit-positive colonoscopies**

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**Background:** The Performance Indicator of Colonic Intubation (PICI) has been introduced as a new measure of high-quality colonic intubation, taking three key parameters into account. An adequate PICI was defined as caecal intubation without significant discomfort and use of less than the median midazolam dose in the UK. In an UK national audit, an adequate PICI was achieved in 54.1% of colonoscopies. It was associated with higher polyp detection rates, but an association with the adenoma detection rate (ADR) was not assessed. In this study, we determined the association between PICI, using the UK and Dutch median sedation dose, and detection rates of (advanced) adenomas in the Dutch FIT-based colorectal cancer (CRC) screening program. Furthermore, we assessed the feasibility of PICI as quality indicator in this population.

**Methods:** This study was conducted within the Dutch FIT-based screening program. Colonoscopy and pathology data is prospectively collected in a national database, providing data on ADR. Data between 01-01-2016 and 01-01-2018 were analyzed. Achieving an adequate PICI was defined as a procedure with caecal intubation, a Gloucester Comfort Scale (GCS) between 1 and 3 (no to mild discomfort) and use of a maximum dose of 2.5 mg midazolam. Besides, we studied the PICI when using the median midazolam dose in this Dutch population.

**Results:** During the study period, 107.328 colonoscopies were performed in FIT-positive participants. The mean ADR was 64.2% and the median midazolam dose was 5 mg (IQR: 2.5-5 mg). Adequate PICI, using the cut-off of 2.5 mg midazolam was achieved in 49.500 (46.1%) colonoscopies. In 87.8% of the colonoscopies in which PICI was inadequate, this was solely due to a higher dose of midazolam or use of propofol. Adequate PICI was associated with higher ADR (OR: 1.05; 95%CI: 1.03-1.08), but not with advanced adenomas detection rates (OR: 1.01; 95%CI: 0.99-1.04). When using the cut-off of 5 mg, the median midazolam dose in this Dutch population, adequate PICI was achieved in 95.410 colonoscopies (88.9%) and it was associated with both higher ADR (OR: 1.52; 95%CI: 1.46-1.58) and advanced adenoma detection rates (OR: 1.19; 95%CI: 1.14-1.24).

**Conclusion:** Adequate PICI, using the UK and Dutch median sedation dose, was associated with a higher ADRs. However, in this FIT-positive population, the UK-defined PICI was inadequate in the majority of colonoscopies. This was mainly due to sedation practice in The Netherlands with a higher median midazolam dose of 5 mg. PICI appears to be an indicator that is heavily dependent on sedation practice. Therefore, the PICI should be considered in the light of regional sedation practices and related to outcome parameters, like ADR.

## Individual risk calculator to predict lymph node metastases in patients with submucosal (T1b) esophageal adenocarcinoma: multicenter cohort study

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**Background:** Surgical resection is recommended in patients with pT1b esophageal adenocarcinoma (EAC) given the risk of lymph node metastases (LNM). However, surgery is associated with morbidity and mortality and decreased quality of life. A prognostic model may help to identify patients at risk for LNM in order to stratify between a conservative approach or additional surgery, after endoscopic resection of pT1b EAC. The aim of this study was to develop a prediction model for the risk of LNM or distant metastases in patients with pT1b EAC.

**Methods:** This is a nationwide, retrospective, multicenter cohort study in collaboration with The Netherlands Cancer Registry. All patients who were diagnosed with pT1b EAC and treated with endoscopic resection and/or surgery between 1989 and 2017 were included. Primary endpoints were the presence of LNM in surgically resected specimens ( $\geq 12$  resected lymph nodes) or the development of pathologically confirmed LNM or distant metastases during 5 years follow-up. Histopathological reassessment of endoscopic and surgical resection specimens was performed by three dedicated gastrointestinal pathologists. Cox proportional hazard analysis was performed to identify independent risk factors associated with metastases. These factors were incorporated into the prediction model. The discriminative ability of this model was assessed using the c-statistic.

**Results:** A total of 283 patients were included in the study (median age 66 years [IQR: 58-72], 87% male). Endoscopic resection was performed in 100 patients and surgery in 183 patients. Ninety-three (32.9%) patients had LNM or distant metastases. The majority of LNM were found in the surgical specimen (78/93). In patients who developed metastases during follow-up (n=27), the median time to detection of metastases was 1.9 years (IQR 0.9-4.1). In multivariable analysis, the risk of developing metastases increased with worse differentiation grade (G2 vs G1: HR 3.2, 95% CI 1.2-9.0; G3 vs G1: HR 3.1; 95% CI 1.1-8.9), deep submucosal invasion (Sm3: HR 2.4; 95% CI 1.3-4.5) and lymphovascular invasion (HR 3.0; 95% CI 1.9-4.5). The c-statistic of the prediction model was 0.74 (95% CI 0.68-0.79).

**Conclusion:** One third of patients with pT1b EAC had LNM or distant metastases. Risk factors are moderate and poor differentiation grade, deep submucosal invasion and the presence of lymphovascular invasion. A personalized risk for LNM can be predicted based on the presence or absence of each of these separate risk factors with a c-statistic of 0.74.

## **Features predicting postoperative health-related quality of life in patients with esophageal cancer: results from the cross-trial.**

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**Background:** Currently, trials are investigating active surveillance in esophageal cancer compared to standard esophagectomy after neoadjuvant chemoradiotherapy (nCRT). If non-inferiority is reported, patients will be imposed on the choice between active surveillance or immediate esophagectomy. The aim of this study was to identify subgroups of patients with different clinical and tumor characteristics that could potentially benefit from such an active surveillance strategy.

**Methods:** HRQOL was measured using EORTC-QLQ-C30 and QLQ-OES24 questionnaires prior to nCRT and three, six, nine and twelve months postoperatively. Subgroups were defined from patients with different preoperative and predefined clinical (global HRQOL, WHO-status) and tumor characteristics (histology, disease stage and location of the tumor). High and low global-HRQOL were defined as global health scores  $\geq 75$  and  $< 75$ , respectively. EORTC scores were calculated according to the EORTC-scoring manual. Cohen's d effect-sizes were determined, 0.5-0.8 was considered a medium and  $> 0.8$  considered a large effect. The primary endpoints were physical functioning and eating problems. Secondary endpoints were global HRQOL, fatigue and emotional problems.

**Results:** In total, 363 patients received HRQOL questionnaires. All subgroups showed impaired HRQOL up to twelve months postoperatively for both physical functioning and fatigue with a medium to large Cohen's d effect size ( $P < 0.004$ ). No difference were found between subgroups except that patients that reported a high global HRQOL prior to nCRT had a significantly worse deterioration in HRQOL compared to patients reporting a low global HRQOL prior to nCRT on all endpoints except for physical functioning (Cohen's d 12 months postoperatively: 0.85 for eating problems, -1.25 for global HRQOL, 0.75 for fatigue and 0.65 for emotional problems).

**Conclusion:** All predefined subgroups reported impaired HRQOL for both physical functioning and fatigue up to twelve months after surgery. The results suggest that patients that report a high global HRQOL before neoadjuvant chemoradiotherapy may benefit more from active surveillance if non inferiority for active surveillance is established.

## **The role of staging laparoscopy in gastric cancer. a population-based cohort study.**

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**Background:** The value of a staging laparoscopy in gastric cancer is unclear. This study investigates the percentage unnecessary surgery (detecting metastases or local irresectability during curative gastrectomy) in patients with and without a staging laparoscopy.

**Methods:** This population-based cohort study included all patients with a potentially curable gastric adenocarcinoma, operated between 2011 and 2016, registered in the Dutch Upper GI Cancer audit. Patients with or without a staging laparoscopy were compared. The primary outcome was the rate of unnecessary surgery. Secondary outcomes were perioperative chemotherapy, surgical characteristics, postoperative complications, histopathological outcomes and (y)pTNM stage for patients with and without a staging laparoscopy.

**Results:** 2849 patients who underwent surgery with the intent of a gastrectomy were included. 414 of 2849 (14.5%) patients underwent a staging laparoscopy before initiation of treatment. The percentage of unnecessary surgery was 16.2% in the staging laparoscopy group, compared to 8.5% in the non-staging group ( $p = <0.001$ ), with a negative predictive value of 83.8%. The main reason for not executing the gastrectomy was the presence of metastases in both groups. cT and cN stage were significantly higher in patients who underwent a staging laparoscopy, as was (y)pT  $\geq$ T3 stage.

**Conclusion:** The staging laparoscopy group showed a higher cTN and pTN stage, suggesting selection of patients with a higher disease stage for staging laparoscopy. Despite the staging laparoscopy, a higher percentage of unnecessary surgery was found, suggesting a low sensitivity for detecting distant metastases in this patient group.

## **Failure to cure in patients undergoing surgery for gastric carcinoma; administration of neoadjuvant chemotherapy influences prospects for cure.**

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**Background:** In 1992, Clavien et al. proposed a novel approach towards describing negative outcomes after surgery. Failure to cure was defined as the event where the aim of the surgical procedure was not met. The current study aims to describe incidence of, and hospital variation in failure to cure in gastrectomy patients. Secondly, it investigates the influence of neoadjuvant chemotherapy (nCT) on this outcome parameter.

**Methods:** All patients registered in the Dutch Upper GI Cancer Audit (DUCA) who underwent potentially curative gastric cancer surgery were included. Primary endpoint was failure-to-cure incidence, which was defined as: 1) intra-operative metastasis or tumor ingrowth making resection impossible or infeasible, 2) macroscopic incomplete resection (R2), 3) microscopic incomplete resection (pR1), or 4) 30-day or in-hospital mortality. Association of patient and treatment characteristics with failure to cure was evaluated using multivariable logistic regression. Hospital variation was evaluated using multivariable logistic regression including baseline characteristics; a funnel plot visualized case-mix corrected hospital results. To investigate the role of nCT, analyses above were repeated for stage II or higher patients treated with or without nCT.

**Results:** Between 2011-2018, 3,461 patients were included from 28 hospitals of whom 785(22.7%) had failure to cure (ranging 14.4%-34.4% between hospitals). More preoperative weight loss, higher charlson comorbidity index, linitis plastica, higher T-stage and N-stage, performance of diagnostic laparoscopy, no nCT, and resection before 2016 were associated with failure to cure. After case-mix correction, 1 hospital had statistically significant lower than expected failure to cure rates, and 2 hospitals had higher rates.

Some 2,764 patients had stage II disease or higher. In this cohort the incidence of failure to cure was 25.5%. The incidence was 20.5% in patients treated with nCT versus 33.0% in patients not receiving neoadjuvant treatment ( $p < 0.01$ ). This remained significant after correction for confounders. A case-mix corrected funnel-plot showed two outperforming and one underperforming hospital. Another case-mix corrected funnel-plot showed the underperforming hospital administered less nCT than would be expected based on their case-mix.

**Conclusion:** Failure to cure is an important prognostic parameter which shows hospital variation in The Netherlands. This might be explained by differences in the administration of neoadjuvant chemotherapy. Non-administration of neoadjuvant chemotherapy is an important risk factor for failure to cure of which clinicians should be aware in future treatment planning.



## **Da Vinci rest-cholecystectomy: single-center experience**

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**Background:** Biliary complaints after cholecystectomy may be caused by gallstones in a residual gallbladder or cystic duct stump. Open or Laparoscopic resection of a residual gallbladder or cystic duct stump is associated with an increased risk of vascular or bile duct injury. Here we present our cohort of robot assisted laparoscopic resection and the use of fluorescence (fire fly) in rest-cholecystectomy.

**Methods:** Patients with symptomatic cholelithiasis caused by a residual gallbladder or cystic duct stump proven by imaging, were included. Surgery was performed using the Da Vinci X robot. The bile ducts were visualised by 5 mg indocyanin green in the Firefly fluorescence mode.

**Results:** From 2018 until 2019 eleven patients were included of which 4 male and 7 female. The mean age was 48 years. Mean time between complaints and diagnosis was 10 weeks and between diagnosis and surgical treatment 8 weeks. There were no pre- or postoperative complications or conversion to open surgery. The mean duration of surgery was 64 minutes. Mean hospital admittance was 1,4 nights and 4 patients could be discharged on the day of surgery. Three patients had recurrent complaints of whom 2 had a proven choledocholithiasis. At imaging none of these patients had a residual gallbladder or cystic duct stump. In all patients the residual gallbladder or cystic duct-stump was confirmed by pathology.

**Conclusion:** These results show that rest-cholecystectomy can be safely done with the da Vinci robot in combination with Firefly.

## **Safety of selective histopathological examination following cholecystectomy for presumed benign gallbladder disease: a systematic review and meta-analysis.**

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**Background:** In 2014, the Dutch guideline for gallstone disease was updated, proposing selective histopathological examination of cholecystectomy specimens. Recently, the implementation of this recommendation was evaluated and turned out to be suboptimal. The objective of this study was to provide an overview of recent literature regarding the incidence and consequences of gallbladder cancer (GBC) following cholecystectomy performed for presumed benign gallbladder disease, and to determine whether a selective policy is safe.

**Methods:** MEDLINE, EMBASE, Web of Science and the Cochrane Library were searched for studies published between January 2009 and June 2019 that reported the number of patients with GBC diagnosed during or after cholecystectomy. Main outcomes were the incidences of incidental GBC (i.e. GBC detected during or after cholecystectomy) and truly incidental GBC (i.e. GBC detected for the first time during histopathological examination), the ability of surgeons to recognise GBC intraoperatively and consequences of truly incidental GBC. Meta-analysis was performed using a random-effects model.

**Results:** 73 studies with 232,155 patients were included. Of these, 39 studies derived from countries with a low prevalence of GBC and 34 studies were performed in high risk areas. Meta-analysis of studies performed in low risk areas showed pooled percentages of incidental and truly incidental GBC of 0.32% and 0.18%, respectively. In high risk areas, the incidence of incidental GBC was 0.83% and truly incidental GBC was reported in 0.44% of patients. Subgroup analysis of studies in which the gallbladder mucosa was systematically examined by the surgeon reported pooled percentages of truly incidental GBC of 0.04% and 0.08% in low and high risk areas, respectively. Nineteen studies reported the consequences for 177 patients with truly incidental GBC. Of these, 33 patients (18.6%) received secondary surgery.

**Conclusion:** The incidence of GBC following cholecystectomy performed for presumed benign gallbladder disease is low, especially if surgeons perform a systematic macroscopic assessment of the gallbladder mucosa. GBC that is not recognised pre- or intraoperatively is usually of early stage and inconsequential. Based on these results, selective histopathological examination of cholecystectomy specimens seems safe and will likely result in significant cost savings and diminished workload for pathology Dept.s.

## Limited wedge resection for colon polyps - preliminary results of the limeric-study

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**Background:** Most colorectal polyps can be removed by endoscopy, but sometimes endoscopic removal does not seem possible leading to referral for segmental colectomy. As segmental colectomy is associated with significant morbidity and mortality, there is a need for a minimal invasive surgical technique for the colon as an alternative. We recently introduced a new technique: a limited endoscopic assisted laparoscopic wedge resection (EAWR) by using a linear stapler without anastomosis for the treatment of such polyps. Aims of our current study were to evaluate the safety and efficacy of EAWR for the treatment of complex colon polyps.

**Methods:** This prospective multicenter longitudinal cohort study was performed in 13 Dutch hospitals between January 2017 and December 2019. Patients were included in the study if (1) colonic polyps judged by an expert panel as complex polyps not easily accessible with current endoscopic resection techniques, (2) the presence of non-lifting residual adenomatous tissue in a scar after previous polypectomy or (3) positive resection margins after endoscopic removal of a low risk pT1 colorectal carcinoma (CRC). Procedural and follow-up data was prospectively collected after written informed consent. The primary end point was the 30-day morbidity after EAWR and recorded according to the Clavien-Dindo classification. Technical success was defined as a fully resected wedge with a patent lumen. A R0 resection was defined as both free lateral and vertical resection margins at histology of at least 1 mm normal mucosa.

**Results:** Of 136 screened patients, 117 patients (57% male, median age 67y (range 47-82)) were included. EAWR was performed for polyps not feasible for endoscopic removal in 68 (58%) cases, non-lifting recurrent adenoma after previous polypectomy in 29 (25%) cases, and R1/Rx resection margins after polypectomy of a pT1 CRC in 20 (17%) cases. Technical success was achieved in 93%. A R0 resection was achieved in 88% of the technical successful procedures. In lesions < 25mm there was a 90% R0 resection rate. In 10% of the cases, an additional resection was performed due to unfavorable pathological characteristics, such as tumor budding or lymphangio invasion. Minor complications (Clavien-Dindo I-II) occurred in 7 patients. There were no major (Clavien-Dindo III-V) complications. Endoscopic follow-up is currently available in 25 patients. Residual tissue was observed in 1/25 (4%) cases. None of the patients developed a colonic stenosis.

**Conclusion:** EAWR seems to be a safe and effective technique with minor complications and should be incorporated in the current armamentarium to deal with complex polyps.

## **Consensus on the definition of colorectal anastomotic leakage using a modified delphi method**

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**Background:** Despite the emerging knowledge about colorectal anastomotic leakage (CAL) through the increasing number of clinical and experimental studies, there is no generally accepted definition of CAL. Because of the wide variety of definitions used in literature, comparison of study outcomes and quality of care is complicated. This study aimed to reach consensus on the definition of CAL using a modified Delphi method.

**Methods:** The RAND/UCLA appropriateness method (RAM) was used. The expert panel consisted of international colorectal surgeons and researchers who had published three or more articles about CAL. The consensus process consisted of two online distributed questionnaires and a third round with a recommendation. In the questionnaires participants were asked to rate the appropriateness of statements using a 1-9 Likert scale. Consensus was defined as a panel median between 1-3 or 7-9 without disagreement. In the final round a recommendation was formed regarding the definition of CAL and the expert panel was asked if they agreed or disagreed.

**Results:** Twenty-three authors participated in the first round and twenty-one finished the second round. After two rounds consensus was reached on 37 items (80%) in nine different categories. The ISREC-definition is the most frequently advised general definition by our panel. Consensus was reached regarding the clinical symptoms of CAL, which serum markers contributes to the suspicion of CAL, which radiological and perioperative findings should be considered as CAL, which grading system is appropriate and if there should be a range of postoperative days (POD) in the definition. Eventually, 19 experts completed all three rounds of which 16 (84%) agreed with our final recommendations for the definition of CAL.

**Conclusion:** On the bases of our modified Delphi method, a consensus-based recommendation for the definition of CAL was formed that can be widely accepted in the field.

## **Low socioeconomic status is associated with worse outcomes after curative surgery for colorectal cancer: results from a large, multicenter study**

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**Background:** Socioeconomic status (SES) has been associated with early mortality in cancer patients. However, the association between SES and outcome in colorectal cancer patients is largely unknown. The aim of this study was to investigate whether SES is associated with short and long-term outcome in patients undergoing curative surgery for colorectal cancer.

**Methods:** Patients who underwent curative surgery in the region of Rotterdam for stage I-III colorectal cancer between January 2007 and July 2014 were included. Gross household income and survival status were obtained from a national registry provided by Statistics Netherlands (CBS). Patients were assigned percentiles according to the national income distribution. Logistic regression and Cox proportional hazard regression were performed to assess the association of SES with 30-day postoperative complications, overall survival, and cancer specific survival, adjusted for known prognosticators.

**Results:** For 965 of the 975 eligible patients (99%), gross household income could be retrieved. Patients with a lower SES more often had diabetes, more often underwent an open surgical procedure, and had more comorbidities. In addition, patients with a lower SES were less likely to receive (neo)adjuvant treatment. Lower SES was independently associated with an increased risk of postoperative complications (Odds ratio per percent increase 0.99, 95%CI 0.99–0.998,  $p=0.004$ ) and lower cancer-specific mortality (Hazard ratio per percent increase 0.99, 95%CI 0.98-0.99,  $p=0.009$ ).

**Conclusion:** This study shows that lower SES is associated with increased risk of postoperative complications, and poor cancer-specific survival in patients undergoing surgery for stage I-III colorectal cancer after correcting for known prognosticators.

## IgA coating of intestinal microbiota is associated with inflammatory bowel disease in twin pairs discordant for inflammatory bowel disease

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**Background:** The pathogenesis of Inflammatory Bowel Disease (IBD) is thought to result from an interplay between microbiota, the immune system and the environment in genetically susceptible hosts. Immunoglobulin A (IgA) produced by the immune system can be specifically directed against bacteria. The IgA-coating pattern of intestinal bacteria thus reflects interactions between the immune system and specific bacteria. Studying IBD in twins discordant for IBD reduces the impact of genetic predisposition and childhood exposures and therefore offers the unique opportunity to focus on other factors in IBD. We aimed to study intestinal microbiota-immune interactions in IBD in this setting.

**Methods:** Faecal samples from twin pairs discordant for Crohn's disease (CD) or ulcerative colitis (UC) were collected. Employing fluorescence-activated cell sorting, IgA<sup>+</sup> and IgA<sup>-</sup> bacteria from the intestinal microbiota were sorted. Subsequently, the 1) total, 2) IgA<sup>+</sup> and 3) IgA<sup>-</sup> microbial composition was determined by 16S rRNA sequencing (IgA-SEQ). We estimated the relative IgA coating per bacterial species by dividing the abundance of that species in the IgA<sup>+</sup> fraction over the abundance in the IgA<sup>-</sup> fraction, representing the IgA coating index. Linear discriminant analyses were performed with LefSE.

**Results:** We included 31 twin pairs (62 individuals) discordant for IBD (CD: 15, UC: 16). 15/31 twin pairs were monozygotic, 43/62 of participants were female, the median age was 47 years (interquartile range: 34-58.5). 7/31 participants with IBD (CD: 3, UC: 4) had signs of active inflammation based on endoscopy, Harvey-Bradshaw index or short clinical colitis activity index. Differences (log linear discriminant analysis score > 3) in the microbial composition of IgA-coated bacteria were observed between CD patients and their twin-siblings not affected by IBD: *Dorea formicigenerans* (increased in IgA coating), *Parabacteroides* sp., *Christensenellaceae* sp., *Clostridium* sp. and *Mollicutes RF39* sp. (decreased in IgA coating). In UC patients, an increase in IgA-coating was observed for *Ruminococcus gnavus* and *Dorea formicigenerans*, while *Turicibacter* sp., *Barnesiellaceae* sp. and an unclassified *Clostridiales* sp. were decreased in IgA-coating compared to their twin-siblings not affected by IBD.

**Conclusion:** In IBD twins, the pattern of IgA-coated bacteria differs between IBD and non-IBD affected individuals. These data on immune-bacteria interactions could serve as starting point for the elucidation of the immune-responses triggered by specific bacteria in IBD.

## Specific genome editing to model hypermethylation of the *SPI40* gene that associates to crohn's disease

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Background: *SPI40* (Speckled 140 KDa) encodes an epigenetic reader protein with an immune restricted expression, that binds to epigenetically modified (acetylated and methylated) histones and thereby regulates expression of large gene sets, including pro-inflammatory cytokines, in innate immune cells. *SPI40* is implicated in CD because single nucleotide polymorphisms, as well as defective protein function are associated with CD and marks anti-TNF response. Through a genome wide methylation screen of Crohn's disease (CD) patients peripheral blood, we identified two hypermethylated positions in *SPI40* locus associated with CD patients. We hypothesize that this DNA hypermethylation at the *SPI40* locus controls *SPI40* expression in CD patients contributing to their colitis development.

Methods: To address the role of *SPI40* DNA methylation, we used CRISPR "dead" Cas9 (dCas9) epigenome-editing for specifically adding methylgroups (dCas9-DNMT) or removing methylgroups (dCas9-TET) in monocyte cell line THP1. We developed guideRNAs complementary to the gene expression regulatory region of the *SPI40* gene. With lentiviral delivery, we transduced THP-1 cells with guideRNA-lentiviruses, and with dCas9-DNMT or dCas9-TET lentiviruses. We assessed the level of *SPI40* methylation using bisulfite sanger sequencing and the effect of methylation intervention of *SPI40* using qPCR and ELISA for *SPI40*, IL-6, TNF $\alpha$ , IL-1 $\beta$ .

Results: We observed that *SPI40* gene in THP-1 cells under control conditions contained little methylated CpG sites. We induced *SPI40* hypermethylation through transduction of dCas9-DNMT. We validated hypermethylation of the two *SPI40* CpGs in transduced THP1 cells, thus mimicking the observed hypermethylation in CD patients cells. *SPI40* hypermethylation in THP1 cells polarized into M1 macrophages and stimulated with lipoteichoic acid (TLR-2 ligand), displayed a decrease of TNF $\alpha$  and (p=0.042) protein levels. Similarly, we showed a decrease of TNF $\alpha$  (p=0.02) and IL-6 (p=0.03) protein release after transduction dCas9-DNMT and stimulation with LPS or Zymosan (TLR-2/4 ligand).

Conclusion: In this study we demonstrated that editing *SPI40* gene methylation through CRISPR-dCAS9 technology allows modeling of the relevance of epigenetic marks for CD etiology. Through methylome editing, we could affect the expression of CD-associated pro-inflammatory genes. Our dCas9 technique will allow us to investigate the role of DNA-methylation in etiology of CD.

## **Curdlan feeding in mice improves dss colitis and enhances bifidobacteria presence in the intestinal microbiome**

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**Background:**  $\beta$ -glucan consumption is known for its beneficial effects in reducing inflammation. Humans lack the required enzymes to digest  $\beta$ -glucans, but certain intestinal microbiota species can digest  $\beta$ -glucans and consequently trigger gut microbial changes. In this study, we assessed curdlan (a bacterial  $\beta$ -glucan) induced microbial changes, and determined its effect on intestinal inflammation in the Dextran Sodium Sulfate (DSS) colitis model.

**Methods:** C57BL/6 mice were pre-treated with vehicle (5% glucose) or curdlan (10 mg/ml) through oral gavage for 14 days. Subsequently, mice were taken off curdlan and colitis was induced by administering 2% fresh DSS daily to the drinking water for 7 days. Control (non-colitis) groups received normal drinking water for this period. To determine inflammation, colon weight, colon length, histology score, and gene expression were determined. Colon content was collected for 16S amplicon (V3-V4) sequencing of microbiota composition. Differences in amplicon sequence variance (USEARCH) composition were visualized based on the Bray-Curtis-Dissimilarity Index. Fold differences were studied using DESeq2.

**Results:** Disease activity index, weight loss and inflammation score of the curdlan pre-treated group were improved compared to the vehicle treated group. Concomitant with improved colitis, the bacterial populations exhibited a higher alpha diversity of the curdlan fed mice over vehicle treated mice. Beta diversity analysis indicated large differences ( $R^2 = 0.46$ ) in the bacterial community structure (Bray-Curtis) between the colitis and non-colitis conditions. While curdlan feeding did not induce any global community changes, specific taxa did show significant differences in relative abundance. Interestingly, a specific *Bifidobacteria* was observed to be 10 to 100-fold more prevalent in the curdlan fed group in both colitis and non-colitis conditions, respectively.

**Conclusion:** Curdlan feeding improved DSS-induced colitis and enhanced *Bifidobacteria* presence in the intestinal microbiome. Further experiments will be exploring causal relation between enhanced *Bifidobacteria* and protective mechanisms in colitis.



## **Non-invasive electrical splenic nerve stimulation ameliorates dss-induced colitis**

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**Background:** Vagus nerve (VN) stimulation has shown the potential to improve the disease development in animal models of colitis and may reduce chronic inflammation in Crohn's disease, a principle currently tested in clinical trials. However, the VN can affect respiratory and cardiovascular, endocrine and gastrointestinal physiology. The splenic nerve (SpN) has been confirmed to be the principal effector nerve for the VN-mediated immune control. In this study, we investigated the role of neural innervation of the spleen and we tested the therapeutic efficiency of stimulating the nerve plexus around the splenic artery in a mouse model of colitis.

**Methods:** Splenic nerve denervation/stimulation was performed in Dextran Sodium Sulfate-induced (DSS) colitis in mice. Splenic nerve denervation was performed by "clearing" the splenic nerve artery free of nerve tissue. For stimulation, mice were implanted at day -10 with a micro-cuff electrode (CorTec) onto the SpN. DSS was added to the drinking water at day 0 until day 5, thereafter the animals received normal drinking water for an additional 7 days. At day 0 splenic nerve stimulation (SNS) was started and applied until the end of the experiment. SNS was applied as rectangular charged-balanced biphasic pulses with 650  $\mu$ A pulse amplitude, 200  $\mu$ s pulse width at 10 Hz frequency for 2 min 6 times a day using a Plexon stimulator. Sham-stimulated mice were undergoing the same procedure but did not receive stimulation. Disease parameters included Disease Activity Index (DAI), histology score and colon weight/length ratio. Gene and protein expression of colonic cytokines were determined by quantitative PCR and cytometric bead array.

**Results:** Splenic nerve denervation aggravated DSS-induced colitis, represented by an increased DAI compared to sham-operated animals (3 vs. 2 respectively,  $p=0.03$ ). Inflammatory parameters were generally unaffected. In contrast, colitis was ameliorated when SNS was applied. At sacrifice, the stimulation group demonstrated decreased colon edema, reflected by colon weight/length ratios (40 vs. 52 mg/cm,  $p=0.011$ ), improved DAI (0 vs. 1.5,  $p=0.057$ ) and lower colon histology scores (4 vs. 11.5;  $p=0.18$ ) compared to sham-stimulated mice. Relative mRNA expression of tumor necrosis factor (TNF)- $\alpha$  was decreased in the STIM group (0.44 vs 1.00,  $p<0.01$ ). SNS further led to decrease of colitogenic pro-inflammatory proteins (monocyte chemoattractant protein 1 (MCP)-1, interleukin (IL)-6 and interferon (IFN)- $\gamma$ ), although this decrease was not significant.

**Conclusion:** Splenic nerve stimulation improved the outcome of DSS-induced colitis. Further studies will address the role of the splenic innervation in colitis disease process.

## **Macrophages in crohn's disease mesentery are predominantly inflammatory and produce calprotectin**

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**Background:** Alterations in the mesentery of Crohn's disease (CD) patients have long been described, although the functional importance of this tissue is less clear. Some studies hypothesize an anti-inflammatory role for enhanced mesentery, whereas others consider a more pathologic function, given its close proximity to the inflamed intestine. Better phenotypic and functional evaluation of the cells present in this tissue is warranted to improve our understanding of its role in disease. We have previously shown a disproportionate presence of macrophages in CD mesorectum. In this study we aimed to better characterize the phenotype and function of the macrophages in the mesentery in CD.

**Methods:** We collected mesenteric specimens from Crohn's disease, Ulcerative Colitis (UC) and non-Inflammatory Bowel Disease (IBD) patients, undergoing surgical bowel resections. In IBD patients, mesentery was collected contiguous to the inflamed region of the intestine, in non-IBD patients, mesentery adjacent to the resection margin. Macrophages were sorted by flow cytometry as CD45<sup>+</sup> CD66b-CD14<sup>+</sup>CD11b<sup>+</sup> cells. Sorted cells were analysed by expression profiling and functional analysis.

**Results:** Macrophages were present in all analysed tissues. Within the population, a discriminating expression pattern for CD11b was present, dividing CD14<sup>+</sup> macrophages in a CD11b<sup>high</sup> and a CD11b<sup>dim</sup> subset. Transcriptional profiling and gene set enrichment showed the CD11b<sup>high</sup> population to be consistent with IFN induced pro-inflammatory macrophages, while the CD11b<sup>dim</sup> population was most consistent with IL4 induced regulatory macrophages. Among the top upregulated genes in CD11b<sup>high</sup> macrophages were S100A8 and S100A9, the two heterodimeric subunits for calprotectin. Flow cytometry confirmed higher expression of calprotectin in the CD11b<sup>high</sup> population also on the protein level. Functionally, CD11b<sup>dim</sup> macrophages showed higher phagocytic capacity, again consistent a role in tissue repair. Strikingly, the CD11b<sup>dim</sup> subset was diminished significantly in the mesentery of CD patients, with a near absence in some patients, while the profile in UC was comparable to non-IBD patients.

**Conclusion:** Mesenteric macrophages contain two populations, CD11b<sup>high</sup> with a pro-inflammatory profile and CD11b<sup>dim</sup> with a regulatory profile. In the mesentery of CD patients, the CD11b<sup>dim</sup> population was strongly decreased, consistent with a pro-inflammatory role for this tissue.

## High-dimensional mass cytometry after local mesenchymal stromal cell treatment in patients with refractory proctitis shows an effect on the myeloid compartment: preliminary data

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**Background:** Ulcerative Proctitis (UP) causes chronic rectal mucosal inflammation. A subset of patients does not respond to one or more medication options. Mesenchymal Stromal Cells (MSCs) show beneficial effects in animal models and patients with immune diseases. However, the effects of MSCs on the mucosal immune composition in patients with Inflammatory Bowel Disease (IBD) is not unraveled yet.

**Methods:** This phase IIa study on allogeneic bone marrow derived MSCs (bmMSCs) in the treatment of UP was registered in The Netherlands National Trial Register under study number NTR7205. Patients with UP (N=7) were locally treated with bmMSCs. A total of  $20 \times 10^6$  MSCs was injected if the inflammation was restricted to 7 cm and  $40 \times 10^6$  if inflammation exceeded 7 cm. Medication was stable in all patients from at least 2 weeks before treatment up to week 6 after treatment. Biopsies were taken from the affected and unaffected colon before (t=0) and 6 weeks (t=6) after treatment from the same locations and fecal calprotectin (FCP) was determined. Single-cell suspensions from the biopsies were stained with a 41 antibody panel and analysed with a mass cytometer to identify and characterise possible effects of MSCs on immune subsets. The generated dataset was analysed with Hierarchical SNE (HSNE) in the Cytosplore analysis and visualization tool.

**Results:** FCP decreased significantly in 4 out of 7 patients (419→191; 365→40; 1744→346; 3270→291), “responders”, while in 3 patients FCP increased, “non-responders”. Unbiased hierarchical clustering of the subsets showed no clear differences in the major immune lineages before and after treatment. In some patients, however, a prominent myeloid population was present in the affected colon, whereas the CD8<sup>+</sup>- and CD3-CD7<sup>+</sup> populations in affected tissue were in general less frequent compared to unaffected tissue from the same patients. We then zoomed into every major lineage separately, and observed changes in the frequencies of 2 myeloid clusters (Cluster 1=CD11b<sup>+</sup>CD11c<sup>+</sup>CD66b<sup>+</sup>CD15<sup>int</sup>CD16<sup>+</sup>CD45RA<sup>-</sup>CD45RO<sup>+</sup> cells, Cluster 2= CD11b<sup>+</sup>CD11c<sup>+</sup>CD66b<sup>+</sup>CD15<sup>-</sup>CD16<sup>-</sup>CD69<sup>+</sup>CD45RA<sup>+</sup>CD45RO<sup>+</sup> cells) in the affected tissue of “responders” compared to the “non-responders”.

**Conclusion:** This preliminary data show differences between “responders” and “non-responders” in the myeloid compartment in the inflamed tissue after MSC therapy. Since CD11b<sup>+</sup> and CD11c<sup>+</sup> populations mediate the tolerogenic effect of MSC in mouse models, we will explore the regulatory potential of the observed populations in MSC-treated UP patients in future studies. The inclusion of patients in a second cohort (N=7) is currently ongoing.

## **Interleukin-28a induces epithelial barrier dysfunction in ibd patient-derived intestinal organoids**

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**Background:** The intestinal barrier is recently designated as another hallmark of IBD pathogenesis. Interleukin-28A (IL-28A) is a newly identified member of the IL-10/interferon cytokine family, with its most implicated function being antiviral and anti-proliferative properties. However, the role and underlying mechanisms of IL-28A in the regulation of the epithelial barrier in IBD remain so far unexplored.

**Methods:** Levels of IL-28A were measured in the plasma of 11 healthy subjects, 15 active CD, 12 active UC, 14 inactive CD and 13 inactive UC patients using ELISA assay. 3D intestinal organoids were generated from proximal colon biopsies of inactive CD patients (n = 7), characterized by the expression or differentiation gene markers using qPCR or immunofluorescence staining. Intestinal organoids were exposed to TNF- $\alpha$ , IFN- $\gamma$  and IL-1 $\beta$  (20 ng / mL) or LPS (100 ng / mL), or IL-28A (500 ng / mL) for 24 h with or without GLPG0634. Epithelial permeability was assessed by the flux of FITC-D4 from the basal to the luminal compartment. Expression of IL-28A, IL-28AR, IL-10R2, and junctional components was analyzed by qRT-PCR, immunofluorescence staining or western blotting. JAK-STAT pathway activity was analyzed using western blotting.

**Results:** Plasma levels of IL-28A were significantly increased in active CD and UC patients when compared to healthy subjects. IL-28A and its receptor complex IL-28AR/IL-10R2 were detected in CD patient-derived intestinal organoids and showed a selective response to the stimulation of IFN- $\gamma$ . IL-28A triggered epithelial barrier disruption, accompanied by reduced expression of ZO-1 and E-Cadherin. This effect was mediated by the activation of JAK-STAT1 signaling. Pre-incubation with the JAK1 inhibitor GLPG0634 ameliorated the barrier dysfunction induced by IL-28A.

**Conclusion:** These results identified cytokine IL-28A as a novel contributor to the pathogenesis of IBD through converging epithelial barrier function and could be a putative target for IBD treatment. We also provide new basic evidence that restoring the intestinal barrier is a potential mechanism that contributes to the clinical benefits of JAK1 inhibitor in CD patients.

## Characterization of gut-homing molecules in non-endstage livers of patients with primary sclerosing cholangitis and inflammatory bowel disease

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**Background:** The co-occurrence of inflammatory bowel disease (IBD) in up to 80% of patients with primary sclerosing cholangitis (PSC) has led to the hypothesis that gut-homing memory T-cells that are originally primed in intestinal environment can migrate to the liver via aberrantly expressed homing molecules in the liver. The expression of Mucosal Addressin Cell Adhesion Molecule-1 (MAdCAM-1) in PSC livers is one of the main findings supporting this hypothesis. Expression of these markers in early PSC remains unclear. The aim of this study was to investigate expression patterns of chemokines and adhesion molecules in PSC-IBD liver tissue, and to study whether changes are already present in early stages of PSC-IBD.

**Methods:** Needle biopsies of 20 PSC patients with short-term PSC (PSC-IBD<sub>ST</sub>) as well as explant liver biopsies of 8 patients with long-term PSC (PSC-IBD<sub>LT</sub>) were collected (median disease duration 0 and 22 years, respectively). Only patients with concomitant IBD were included (89% ulcerative colitis and 11% Crohn's disease). Expression and distribution of MAdCAM-1, integrin  $\beta$ 7, CC- Chemokine Ligand 25 (CCL25), CCL28, CXCL12,  $\alpha$ E (CD103) and E-cadherin were assessed using immunohistochemistry and quantitative PCR. Control groups consisted of liver tissue from obstructive cholangitis in resection specimens for Klatskin tumors or resection specimens from hepatic metastases, liver tissue of patients with hepatitis C virus (HCV) and of patients with primary biliary cholangitis (PBC).

**Results:** We found increased expression of MAdCAM-1 in livers of PSC-IBD<sub>LT</sub> patients compared to controls. The proportion of  $\beta$ 7 positive T-cells did not differ between PSC-IBD<sub>ST</sub> and control groups, but was significantly higher in liver tissue of PSC-IBD<sub>LT</sub> patients compared to PSC-IBD<sub>ST</sub>, predominantly in the portal areas. There was no difference in  $\alpha$ E<sup>+</sup> T-cells among PSC-IBD groups and control livers. CCL28 was highly expressed in biliary epithelial cells. This intense staining pattern was more pronounced in PSC-IBD<sub>ST</sub> compared to PSC-IBD<sub>LT</sub>, but did not differ from control livers.

**Conclusion:** Our findings support the hypothesis of a role for aberrant gut-lymphocyte homing to the liver in PSC, linking gut and liver disease pathology in PSC-IBD.

## **Pd-I expressing t cells in patients with different types of colitis**

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**Background:** Immunotherapy-related colitis is a frequent adverse event in patients with malignancies, treated with inhibitors targeting programmed death-1 (PD-1). This treatment leads to enhancement of lymphocyte activity, thereby generating antitumor T-cell activity. The role of PD-1<sup>+</sup> T cells in the pathophysiology of different types of colitis is unclear. Therefore, we aimed to study the presence of PD-1<sup>+</sup> T cells in inflammatory bowel disease (IBD), infectious colitis and healthy controls compared to anti-PD-1-related colitis.

**Methods:** We performed a prospective cohort study. Newly diagnosed patients with ulcerative colitis (UC, n=51), Crohn's disease (CD, n=29), infectious colitis (n=4), anti-PD-1-related colitis (n=5) and healthy controls (n=6) were included. Baseline colonic biopsy specimens were collected for immunohistochemistry identifying PD-1<sup>+</sup> and PD-L1<sup>+</sup> lymphocytes in the epithelium and lamina propria separately and for immunophenotyping by flow cytometry identifying PD-1<sup>+</sup> T-cell subsets.

**Results:** Using immunohistochemistry, PD-1 was not present on lymphocytes in the epithelium of patients with any type of colitis, nor in healthy controls. Of all lymphocytes in the lamina propria, % PD-1 expression was 40% in UC, 5% in infectious colitis, 3% in anti-PD-1-related colitis and 0% in healthy controls. PD-L1 was expressed on lymphocytes in the epithelium and lamina propria of UC patients (12.5% and 40%) and in infectious colitis (1% and 30%), whereas in anti-PD-1-related colitis (0% and 15%) and healthy controls (0% and 15%) no PD-L1<sup>+</sup> lymphocytes were demonstrated in the epithelium.

Flowcytometry showed higher percentages of PD-1<sup>+</sup> T cells in biopsy specimens of UC patients (25.2% (IQR 17.9-35.6)) compared to all other groups; CD patients (13.5% (5.0-25.3), p=0.001), infectious colitis (9.8% (4.7-17.4), p=0.005), anti-PD-1-related colitis (1.5% (1.1-2.1), p=0.001) and healthy controls (14.3% (4.9-28.2), p=0.08). In IBD and infectious colitis the majority of PD-1<sup>+</sup> T cells were CD4<sup>+</sup> (84.8% (76.5-90.5)), while in anti-PD-1-related colitis the majority of PD-1<sup>+</sup> T cells were CD8<sup>+</sup> (76.2% (68.5-82.1)). PD-1<sup>+</sup> T cells in all patient groups were mainly effector T cells (CD45Ro<sup>+</sup>).

**Conclusion:** In patients with different types of colitis and in healthy controls, PD-1 was only expressed on T cells in the lamina propria and not in the epithelium. The percentage of PD-1<sup>+</sup> T cells was significantly higher in patients with UC compared to patients with CD or infectious colitis and healthy controls. As expected PD-1<sup>+</sup> T cells were nearly absent in patients with anti-PD-1-related colitis.

## **Autophagy regulates rhoGTPase homeostasis in intestinal epithelium**

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**Background:** The T300A variant of the ATG16L1 gene that reduces autophagy is one of the few highly prevalent risk factors associated specifically with Crohn's disease, but not with ulcerative colitis. Interestingly, patients carrying the T300A allele suffer more postoperative complications, suggesting a reduced wound healing capacity. Wound healing is an active process requiring both proper immune control and regeneration of the epithelium. We have previously shown a regulatory role for autophagy during dendritic cell (DC) - T cell interactions and T cell activation. As the T300A variant is a body wide mutation, we now investigated the role of autophagy and RhoGTPases homeostasis in intestinal epithelium.

**Methods:** Knockdown of relevant autophagy related proteins was achieved by lentiviral shRNA transduction using 2 independent constructs per gene. Migratory capacity was assessed in time lapse scratch assays. Protein expression and activity were determined by Western Blot and G-Lisa respectively. **Results:** ATG16L1 knockdown (KD) in the intestinal epithelial cell lines HT29 and Caco-2 resulted in reduced migration upon wounding. To confirm that this was due to reduced autophagy as a process, we tested knockdown of a second autophagy protein (ATG5) as well as a potent pharmacological inhibitor, Bafilomycine A1. In both cases, migration was reduced significantly.

RhoA is an important regulator of cellular motility, and RhoA activity was reduced in HT29 ATG16L1 KD cell lines, ATG5 KD cell lines and in HT29 WT cell lines treated with Bafilomycine. However, whole protein levels of RhoA were not altered, suggesting regulation at the activation level. Indeed, ARHGAP18, a specific RhoA inactivator, was increased in ATG16L1 KD as well as ATG5 KD cell lines. Confirming the physiological relevance, primary colonic tissue of homozygous ATG16L1 T300A carriers also showed higher levels of ARHGAP18.

**Conclusion:** Reduced autophagy impacts RhoGTPase homeostasis and thereby decreases migration capacity in intestinal epithelium. This may result in defective epithelial regeneration and thus contribute to the defective wound healing and the known association between the ATG16L1 T300A variant and Crohn's disease.

## **A human 2d organoid model to study gut barrier maturation and host-pathogen interaction in the small intestine**

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**Background:** The intestinal barrier consists of a single layer of polarized epithelial cells, covered by a layer of mucins and separates the intestinal content from the rest of the body. To maintain its integrity and ward off luminal treats such as viruses and bacteria, the intestinal epithelium is constantly renewed from a pool of stem cells. In infants the intestinal barrier is underdeveloped and consequentially infants are more susceptible to conditions such as infectious diarrhea, necrotizing enterocolitis and allergic gastroenteropathy. In contrast, microbial signals are also necessary to stimulate epithelial maturation.

**Methods:** To study the influence of host-pathogen interactions at the epithelial barrier during early stages of life, we have established and validated human fetal intestinal primary cells as a source of polarized epithelium using transwell inserts. Specifically, we generated proximal and distal gut monolayers and characterized this model with respect to epithelial cell types (qPCR, immunofluorescence), epithelial polarization (immunofluorescence), epithelial barrier function (TEER, FITC-Dextran) and gene expression profiles. In addition, the potential of viral replication and bacterial translocation after apical infection of the monolayers with enterovirus A71 and *listeria monocytogenes*, both enteric pathogens, was evaluated.

**Results:** Our data reveal that the fetal intestinal organoid-derived monolayer preserved all the characteristics of *in vivo* epithelium, including a functional epithelial barrier, cell heterogeneity, epithelial polarization and gene expression profiles that match proximal and distal fetal intestinal tissue of origin. Enterovirus A71 was able to replicate in the epithelial monolayer, with similar kinetics for proximal and distal cultures. Translocation of *listeria monocytogenes* was observed in this model, with a higher translocation in distal cultures compared to proximal, which led to a distinct pro-inflammatory IL8 response.

**Conclusion:** In conclusion, the human 2D organoid model described here will be a valuable tool in the field of nutrition, gastroenterology and infectiology to study for example early life gut (barrier) maturation and host-pathogen interactions. Furthermore, with respect to animal ethics in research, this human intestinal organoid model could replace and thereby reduce the use of animals in medical and nutrition research.



## **Fibroblast specific loss of the bone morphogenetic protein signalling initiates polyp formation in the mouse intestine**

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**Background:** The bone morphogenetic protein (BMP) pathway is a crucial signalling pathway in the maintenance of intestinal tissue homeostasis. Dysregulation of BMP signalling may lead to cancer as is seen in Juvenile Polyposis Syndrome (JPS) patients. JPS patients, characterized by their multiple gastrointestinal juvenile polyps, harbour a germline mutation in essential signalling components of the BMP pathway such as BMP receptor 1a (BMPRIa) or SMAD4. Interestingly, fibroblast specific BMPRIa conditional knockout (cKO) mice also develop intestinal polyps. In this study we aimed to elucidate how fibroblast specific loss of BMPRIa contributes to the polyp formation. Furthermore, we set out to investigate the mechanism underlying the polyposis. We hypothesize that the loss of BMPRIa causes the fibroblasts to upregulate BMP antagonist and mitogens, which in turn drive epithelial proliferation.

**Methods:** The Cre-LoxP system was exploited for generating different knockout mouse models in which BMPRIa could be conditionally and specifically knocked out using cell specific promoter driven CRE expression in fibroblast (Collagen1a2 or Coll1a2-Cre) and myofibroblast/ smooth muscle cells (SM22-Cre). Six months after induction, mice were sacrificed and intestines were collected for (immune)histochemical and transcriptional analysis.

**Results:** Histologically, these polyps showed strong resemblance to serrated polyps seen in patients, which are thought to be the precursor lesions for the Consensus Molecular Subtype (CMS) 4 colorectal cancer. These polyps had expansion of the mesenchymal compartment, accompanied with a significant increase in epithelial Ki67 staining, Olfm4<sup>+</sup> intestinal stem cells and the BMP antagonist Gremlin in polyp regions. Additionally, a 2-fold increase in CXCL12 and its receptor CXCR4 was found in the induced BMPRIa<sup>fl/m</sup>;Coll1a2-Cre mice 6 months upon loss of BMPRIa. Further co-culture experiments with normal colonic organoids and autologous fibroblasts are ongoing to further elucidate the molecular mechanism.

**Conclusion:** Our data suggest that BMP-signalling in (myo)fibroblasts contributes to intestinal homeostasis and a disturbance of fibroblast dependent BMP-signalling may lead to polyposis, potentially via dysregulation of Gremlin expression and CXCL12 induction. We speculate that both CXCL12 and Gremlin could prevent the proliferating cells from losing their stemness by PTEN, the protein converging the BMP and Wnt pathway, mediated inhibition of BMP signalling in epithelial cells thereby driving their proliferation. This data could help to better understand how polyps arise as a consequence of BMPRIa inactivation as is observed in JPS patients.

## **Transforming growth factor- $\beta$ signalling in cancer-associated fibroblasts drives an il6 family cytokine dependent pro-metastatic inflammatory program in hepatocytes**

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**Background:** Colorectal cancer (CRC) is characterized by a metastatic pattern that shows tropism toward the liver. The cellular actors and molecular mechanisms of this organotropism are starting to be unravelled, indicating a crucial role for cancer associated fibroblasts (CAFs). Current molecular classifications of CRC, the Consensus Molecular Subtypes (CMS), suggest an association between TGF $\beta$  signalling, CAFs and the risk of metastasis. In this study we identified key downstream effectors of TGF $\beta$  signalling in CAFs and their role in hepatogenic metastasis.

**Methods:** CAFs from primary CRC tissues and CRC associated liver metastasis were stimulated with recombinant TGF $\beta$ . TGF $\beta$  signalling qPCR arrays were performed to identify differentially expressed genes and verified with ELISA. Results were cross-referenced with RNAseq expression data of CMS-classified CRC. Stimulation of hepatocyte cell lines with the identified factors was performed and the effect on pro-inflammatory gene expression was determined with qPCR. Subsequently, blockade of these pathways via chemical inhibition and genetic ablation (CRISPR/Cas9) was done to study the exact mechanism involved in the induction of this pro-inflammatory program.

**Results:** TGF $\beta$  signalling in CAFs leads to increased expression of different IL-6 cytokine family members. Moreover, publically available RNAseq data also shows high expression of these factors in the subset of CRC with the highest risk of hepatogenic metastasis. To investigate their effect on the liver, we stimulated hepatocytes with recombinant IL-6 family cytokines or CAF conditioned medium. Stimulation of hepatocytes with either cytokines or CAF conditioned medium led to upregulation of known myeloid chemoattractants and also increased neutrophil-to-hepatocyte migration *in vitro*. Finally, chemical blockade and genetic ablation (CRISPR/Cas9) of the IL-6 family cytokine signalling pathway showed the critical role of the GPI30 co-receptor in the regulation of this inflammatory response in hepatocytes.

**Conclusion:** TGF $\beta$  signalling in CAFs leads to increased expression of IL-6 cytokine family members that induce a pro-inflammatory response in hepatocytes, characterized by the expression of myeloid chemoattractants and increased neutrophil migration *in vitro*. This indicates a potential role for CAFs in regulating the influx of pro-metastatic myeloid cells in the liver as high CAF content and active and active TGF $\beta$  signalling are also seen in the subset of CRC (CMS4) with the highest risk of hepatogenic metastasis. Our data suggests a feedback loop between TGF $\beta$  signalling in CAFs, hepatocytes and neutrophils that might play a role in hepatogenic metastasis.

**An *in vitro* model that combines digested infant nutrition and gut epithelial cells provides a physiologically relevant model to study gut maturation.**

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**Background:** The neonatal gastrointestinal tract is immature, and appropriate maturation can be supported by early life nutrition. Bioactive components that are liberated during digestion likely play a role. A combined digestion-gut epithelial cell model that accurately represents the nutrient state and environment during intestinal transit, allows to study the impact of early life nutrition on gut maturation under relevant physiological conditions. Establishing such a model is hindered by active digestion components that cause cytotoxicity in *in vitro* cell models. The aim of this study was therefore; 1) To establish a modified digestion model yielding lower toxicity to combine with cells. 2) To demonstrate the efficacy of this combined model using an infant formula (IF) containing prebiotics and postbiotics, i.e. bioactive compounds produced by food-grade micro-organisms in a fermentation process that supports health and well-being.

**Methods:** Infant digestion conditions were simulated in a semi-dynamic model, which was modified by employing trypsin, chymotrypsin and pancreatic lipases, and mixed micelles of biliary lipids to replace the commonly used pancreatic and bile extracts. During and after IF digestion, samples were taken and protease and lipase inhibitors were added to stop digestive enzyme activity. The impact of the modifications on proteolysis and lipolysis were investigated by measuring peptide bond and fatty acid ester hydrolysis. C2BBel gut epithelial cell monolayers were incubated with digested IF and cytotoxicity was measured. Subsequently, non-cytotoxic concentrations of undigested and digested IF containing prebiotics and postbiotics (PRE+POST) or control IF (CTRL) were applied to C2BBel cells and activity of the functional gut maturation marker alkaline phosphatase (ALP) was measured.

**Results:** The modified digestion model reduced cytotoxicity of digested IF (>75%), and proteolysis and lipolysis were unchanged compared to the common digestion model. Upon digestion, PRE+POST IF stimulated ALP activity ( $p < 0.05$ ) compared to digested CTRL. In contrast, undigested PRE+POST IF did not stimulate ALP activity compared to undigested CTRL.

**Conclusion:** Modifications to the digestion model resulted in a substantial reduction in cytotoxicity without affecting digestive capacity and allowed to combine digested IF with a gut epithelial cell model. Using this approach, PRE+POST IF appeared to stimulate gut maturation, i.e. ALP activity. Undigested PRE+POST IF did not stimulate ALP activity, suggesting that digestion of IF liberated specific bioactive components. This approach can bridge the gap between testing intact IF concepts and individual IF components in *in vitro* cell models.

## Planting the seeds in preventing necrotizing enterocolitis

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**Background:** Necrotizing enterocolitis (NEC) is a devastating inflammatory condition in preterm neonates thought to be induced by an exaggerated immune response to a dysregulated microbial colonization after birth. The oral administration of probiotic bacteria has been suggested to reduce microbial dysbiosis and help to prevent NEC. However, little is known about the impact of the administration of probiotic bacteria shortly after birth on the establishment of the microbiota.

**Methods:** Therefore, a longitudinal cohort study (natural experiment) on the postnatal establishment of the enteric microbiota in neonates was performed. Preterm human neonates (<31 gestational week) received oral the probiotic bacteria *Lactobacillus* and *Bifidobacterium* (10<sup>9</sup> CFU per day) starting at day 3 after birth until completion of the corrected gestational week 32 (probiotic group). Another group of neonates did not receive probiotics (n=20) (control group). Fecal samples were collected weekly directly after birth during hospitalization until 3 months of age. We analyzed the microbiota composition changes over time and between the probiotic group and control group by sequencing of the V4 16S rDNA region. The analysis included microbiota composition and diversity as well as tracking of the presence of the probiotic strains.

**Results:** We observed significant changes in microbial community structure both during and after probiotic supplementation. Although not all probiotic strains appeared to colonize the neonatal gut, the relative abundance of *Bifidobacteria* was enhanced in the probiotic group as compared to the control group. In addition, we observed an increase in the incidence of patients that developed NEC among the neonates that did not receive probiotics.

**Conclusion:** Together, we were able to characterize qualitative and quantitative changes in the microbiota composition upon administration of probiotic bacteria that persisted upon cessation of probiotic supplementation. The lower incidence of NEC among probiotic supplemented preterm neonates suggests a potential benefit for preterm neonates in the prevention of NEC.

## **Supramolecular structure of dietary fat in early life modulates small intestinal gene expression related to epithelial barrier function and defence in mice.**

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**Background:** In early life the intestinal epithelium matures structurally and functionally, establishing proper digestive and barrier functions. Early life nutrition can modulate the process and timing of gut maturation, and mother's milk with its unique composition and structure is considered the gold standard. Here, we tested the hypothesis that early life exposure to a concept infant milk formula (IMF) with a supramolecular lipid structure similar to mother's milk stimulates gut maturation and function closer to the mother-fed situation than standard IMF.

**Methods:** From postnatal day (PN) 14.5 to PN18.5, mouse pups were separated from dams and received either 'concept' IMF containing large lipid globules (mode diameter 3-5µm) coated with bovine milk-derived phospholipids (Nuturis®) or 'control' IMF *ad libitum* from dishes in the cages. Age-matched pups suckled by the dam served as 'mother-fed' reference. Next to assessing morphological and functional markers for gut maturation on PN18.5, we characterized gene expression profiles in small intestinal tissue using microarrays and comprehensive data analysis to determine the molecular effects and their functional implications.

**Results:** Both administered IMFs did not affect gross gut morphology and gut barrier markers. Activity of the brush border disaccharidases lactase, sucrase and maltase was uniformly increased in the concept IMF group. Small intestinal gene expression was altered by both IMFs compared to mother-fed, with a similar effect size (# differentially expressed genes) but largely affecting different sets of genes. Principal component analysis on the gene set significantly changed by control IMF revealed a clear separation of the control and mother-fed groups, while the concept group lay in between the two clusters, indicating higher similarity between concept IMF and mother-fed groups. Individual down-regulated genes of this gene set are implicated in regulation of epithelial barrier function and epithelial defence (incl. Reg3b, Reg3g, Ptk6, Tifa). Furthermore, functional gene entities within this gene set are related to immune homeostasis.

**Conclusion:** Early life exposure to IMF with a lipid structure similar to mother's milk did not affect gut morphology or functional gut barrier maturation in the present model. Yet, it stimulated activity of all assessed brush border disaccharidases and thereby potentially increased carbohydrate digestive capacity. On molecular level, concept IMF mitigated the gene expression effects induced by control IMF overall, including epithelial barrier, epithelial defence and immune-related changes, rendering mice receiving IMF with a more natural lipid concept closer to the mother-fed situation.

## **Complex inheritance explains infantile hypertrophic pyloric stenosis development in patients with esophageal atresia best**

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**Background:** Patients born with esophageal atresia (EA) appear to have a 30 times higher prevalence of infantile hypertrophic pyloric stenosis (IHPS). This makes sense from a developmental perspective as both the esophagus and the pyloric sphincter are foregut derived structures. We hypothesized that genetic defects, disturbing foregut morphogenesis, are responsible for the specific combination of EA and IHPS.

**Methods:** Patients with both EA and IHPS born between 1970-2017 and where possible their parents were included. Ethical approval had been obtained. After parental written informed consent we determined genetic profiles with whole exome sequencing and SNP-array based copy number variation analysis. We focused on (1) genetic variation in known EA and IHPS disease genes, (2) pathways important for foregut morphogenesis, (3) shared rare genetic variation and (4) ultra-rare variants in variant-intolerant genes which have a high chance of being de novo. Segregation analysis of possible candidate variants was performed.

**Results:** Twenty-seven out of 664 patients (4.1%) born with EA during the study period developed IHPS, of which 15 cases were analyzed. As none of the parents were affected, we considered dominant (de novo) or recessive and X-linked inheritance models. Unfortunately, we could neither identify rare de novo mutations, nor variants fitting a recessive model. We did however identify inherited putative deleterious heterozygous variants in genes either known to be involved in EA or IHPS (e.g. COL7A1, TNXB, WDR11, GDF6) or important in foregut morphogenesis (e.g. GLI3, NKX2.1) in all patients. Moreover, seven genes were affected by rare variation in  $\geq 2$  patients (ADAMTSL4, ANKRD26, CNTN2, HSPG2, KCNN3, LDB3, SEC16B) and expressed in the developing foregut, esophagus or pyloric sphincter in mice between E8.25 and E18.5. However, burden analysis did not show a significant difference with unaffected controls.

**Conclusion:** Although the presence of genetic variation in likely candidate genes suggests a genetic component, genetic factors alone could not explain the abnormalities seen in these patients. Therefore, we propose a multifactorial model in which the combination of high impact genetic, mechanical and environmental factors together can shift the balance from healthy to abnormal development.

## Early life antibiotics influence *in vivo* and *in vitro* mouse intestinal epithelium maturation and functioning

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**Background:** Antibiotic (AB) use in early life has been associated with short and long-term effects, such as infantile colic, allergy risk, childhood IBD, obesity and diabetes. In mice, consequences of AB treatment have only been studied during adulthood. However, the neonatal and adult intestine differ significantly. For example, the neonatal intestinal epithelium is more permeable and is characterized by vacuolated cells that allow the passage of macromolecules present in the milk. Three to four weeks after birth, the intestine achieves a mature state comparable to the adult state. We have recently developed an *in vitro* organoid model, which uses primary cells isolated from mouse fetal intestine, to study this epithelial maturation process *ex vivo*. Thereby, we aimed to investigate, both *in vivo* and *in vitro*, how AB treatment in early life influences the maturation and functioning of the neonatal intestine.

**Methods:** Mice were treated orally with an AB mix (amoxicillin, metronidazole and vancomycin) or PBS from postnatal day (P)10 to P20. At P20, mice received FITC-dextran orally and 4 hours later blood and tissue were collected for subsequent analysis. Epithelial cells were FACS-sorted and global gene expression was analyzed. Organoids were isolated from late fetal stage (embryonic day 19) and cultured with the same AB mix for one month. Maturation and differentiation of intestinal tissue and organoids were assessed by immunohistochemistry and qRT-PCR. Metabolic status of organoids was followed in real-time using Seahorse.

**Results:** After AB, P20 mice showed a strong reduction in intestinal permeability. Vacuolated cells were no longer visible in the distal intestinal epithelium of AB mice compared to control mice. Global expression analysis revealed that the most significantly changed genes were related to intestinal epithelial maturation. In addition, genes typically expressed by enteroendocrine cells were upregulated in AB mice. Interestingly, a subset of the differentially expressed genes identified in the AB mice *in vivo* behaved in similar manner in mouse fetal organoids cultured in the presence of AB *in vitro*, demonstrating a direct effect of AB on gut epithelial cells. Finally, AB treated organoids presented restricted glycolytic capacity.

**Conclusion:** Early life antibiotics change gut morphology and gene expression and decrease gut permeability in mice reflecting a precocious maturation of the neonatal intestinal epithelium. In addition to the widely known effects of antibiotics on bacteria, our data in mouse organoids show that antibiotics can directly affect epithelial cell maturation and function, which may play a role in the effects seen in human infants.

## **A model to study ischemia-reperfusion injury in human intestinal organoids**

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**Background:** Intestinal ischemia-reperfusion (IR) leads to damage of the intestinal epithelium, which functions as a physical and immunological barrier and is therefore crucial in maintaining intestinal homeostasis. Our aim is to model IR injury in human intestinal organoids, in order to investigate potential therapeutic targets that may reduce IR injury, and promote regeneration of intestinal epithelium after injury. Objectives are 1) to differentiate organoids in crypt-like and villus-like organoids, and 2) to investigate whether the response to hypoxia-reoxygenation (HR) in the organoid model mimics findings of human intestinal IR *in vivo*.

**Methods:** To differentiate organoids in crypt-like and villus-like phenotypes, organoids were cultured in growth (GM) and differentiation medium (DM) respectively. The presence of cell types was evaluated by qPCR for IFABP, MUC2, LYZ, and OLFM4, and staining of alkaline phosphatase, mucus (Alcian Blue), lysozyme and Ki67. A human experimental IR model was used for temporal gene expression profiling of the *in vivo* IR response. To simulate IR, organoids were subjected to 12 hours of hypoxia (1% O<sub>2</sub>) followed by 120 minutes of reoxygenation. Unfolded protein response (UPR) activation was assessed by qPCR for XBPIs, CHOP and GADD34.

**Results:** We observed a significant increase in IFABP and MUC2 gene expression and increased staining for enterocytes and goblet cells in DM-cultured organoids compared to GM, indicating an increase of villus-like cell types. In contrast, increased LYZ and OLFM4 gene expression and increased lysozyme and Ki67 staining in GM-cultured organoids compared to DM, indicated a crypt-like phenotype. The UPR was the top perturbed pathway during intestinal IR *in vivo*. Subjecting intestinal organoids to HR significantly increased mRNA expression of UPR-related genes XBPIs, CHOP and GADD34 compared to organoids not subjected to hypoxia.

**Conclusion:** Human intestinal organoids can be differentiated into crypt-like and villus-like phenotypes. In line with findings in the *in vivo* human IR model, HR in intestinal organoids induced significant UPR activation, which was higher in crypt-like organoids compared to villus-like organoids.



## **Colonic mucosal kinase activity, cytokine and chemokine profiles in inflammatory bowel disease**

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**Background:** With the approval of tofacitinib, an oral Janus Kinase (JAK) inhibitor, modulation of kinase activity has been added to the therapeutic armamentarium of inflammatory bowel disease (IBD). Despite its established efficacy, at least a third of patients will not respond to this or other therapeutic options such as anti-tumour necrosis factor (TNF), anti-interleukin (IL)23/IL12, or anti- $\alpha$ 4 $\beta$ 7 integrin compounds. A better understanding of the inflammatory profile could aid in tailoring drugs to individual patients. We therefore explored mucosal cytokine, chemokine, and kinase activity profiles in IBD.

**Methods:** Colonic mucosal biopsies were collected from 1) patients with Crohn's disease (CD, N=8), 2) patients with ulcerative colitis (UC, N=8), and 3) healthy controls (N=4). IBD samples were collected both from inflamed and non-inflamed tissue from the same patients. All IBD patients were biological-naïve and had not used corticosteroids in the past 3 months. Biopsies were snap frozen for later kinase activity determination or directly used in a 24 hour explant culture. Whole biopsy kinase activity (tyrosine, serine and threonine kinases) was assessed using the Pamgene platform. A 64-analyte panel was examined in the supernatant of the cultured biopsies employing a multiplex assay (Luminex).

**Results:** Whole-biopsy kinase activity differed between inflamed and non-inflamed mucosa of IBD-patients. The kinase activity profiles when comparing inflamed mucosa and non-inflamed mucosa within patients was different between UC and CD. The kinase activity in inflamed mucosa in UC was mostly increased for tyrosine kinases, while in CD the increase was mainly in serine/threonine kinases. The kinase activity profile of non-inflamed mucosa of CD and UC patients was different from mucosa of healthy control participants, with large overlap between the CD and UC non-inflamed mucosa in the kinases that were differently active. The cytokine and chemokine profile of inflamed biopsies differed from non-inflamed IBD biopsies and healthy control biopsies, with higher levels of S100A8, TNF $\alpha$ , IL-6, oncostatin M (OSM), and triggering receptor expressed on myeloid cells-1 (TREM-1), amongst others.

**Conclusion:** In IBD, inflammation in the mucosa can be characterized both by explant-culture and kinase activity assessment. The difference in kinase activity between non-inflamed IBD mucosa and healthy control mucosa suggests the presence of sub-clinical alterations in cell signalling. The observed differences in the kinase, cytokine and chemokine profiles underscore the importance of this approach to gain a better understanding of the pathophysiology of IBD.

## **In vitro lipid digestion of infant formula with large phospholipid coated fat droplets is slower than standard infant formula and closer to human milk.**

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**Background:** Breastfeeding is considered the gold standard of infant nutrition. Infant nutritional lipids supply ca 50 % of the infants energy need and support optimal brain development and growth. Lipid utilization is dependent on digestion and absorption, a process known to be modulated by lipid structure. Human milk (HM) lipids are delivered to the gastrointestinal tract as large (mode diameter; 4µm), phospholipid coated fat globules, whereas standard infant milk formula (sIMF) lipid structure consists of small fat droplets (0.5µm) coated with milk protein. To mimic HM structure more closely, we have developed a concept infant milk formula (cIMF), Nuturis®, with large milk phospholipid coated fat droplets (3-5µm). In the current study, *in vitro* lipid digestion and clotting behavior of HM, sIMF and cIMF are compared.

**Methods:** HM donations (n=3) were collected after written consent and macronutrient content was measured using a MIRIS human milk analyzer™. Lipid content of HM ranged from 3.1-3.5 g/100ml, lipid content of sIMF and cIMF was 3.4 g/100ml. Infant digestion conditions were mimicked in a semi-dynamic *in vitro* model that included: a consecutive gastric (120 min), and intestinal phase (120 min), pH control, and gradual addition of simulated digestive fluids. Free fatty acids released in time were quantified by gas chromatography and expressed as proportion of total fatty acids: degree of hydrolysis (%DH). Further, clotting behavior under acidic gastric conditions was evaluated by light microscopy.

**Results:** Total gastric hydrolysis was low; on average ca 4% and during gastric digestion, sIMF %DH was higher than HM (from 30-120 min) and higher than cIMF (at 30 and 90 min). cIMF %DH was also higher than HM (at 30 and 120 min). During intestinal digestion, %DH of sIMF was higher than HM throughout the entire phase, and higher than cIMF from 30 min onwards. Intestinal cIMF %DH was not different from HM. Total gastrointestinal hydrolysis was on average ca 44%. Aggregate size in the gastric phase was different between the three groups, sIMF aggregates were the largest followed by cIMF and HM.

**Conclusion:** Gastrointestinal digestion rate could be ranked as sIMF > cIMF ≥ HM. This study highlights the difference in gastrointestinal lipid digestion between HM and sIMF and demonstrates that cIMF fat droplet structure modifies lipid digestion and gastric clotting to be closer to HM. In infants different rates of lipid digestion and aggregate size might lead to different availability of nutrients, and lipids in particular, that are important for growth and brain development.

## **Four weeks intake of galacto-oligosaccharides did not affect immune markers in healthy adults and prefrail elderly**

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**Background:** Ageing is associated with a decline of physiological function, including immunosenescence, contributing to frailty and comorbidity. Prebiotics may improve immune function, e.g. by bacterial production of short-chain fatty acids. Our aim was to compare immune markers in healthy adults and prefrail elderly and to study the impact of galacto-oligosaccharides (GOS) in both groups.

**Methods:** In this double-blind, randomized, placebo-controlled, cross-over trial, 24 healthy adults (33.3% male, age 38.1±7.8 yrs) and 20 prefrail elderly (Fried criteria; 55.0% male, age 74.3±3.7 yrs) received 21.6 g/day GOS and placebo for 4 weeks. CRP by immunoturbidimetry, and cytokine production after 24h stimulation by 10µg/ml LPS or PHA were assessed pre- and post-intervention. IL-1β, IL-6, IL-8, TNF-α, IL-10, IL-12p70, IL-13, IFN-α and IFN-γ were measured using CBA and FACS analyses. Gastrointestinal Symptom Rating Scale and Bristol Stool Scale were completed weekly to monitor tolerance of GOS. Healthy adults and prefrail elderly were compared by independent-samples T-tests, the effects of GOS by linear mixed model analyses.

**Results:** Stimulated cytokine levels and serum CRP did not differ between age groups (all  $P \geq 0.255$ ), nor between GOS and placebo interventions (all  $P \geq 0.130$ ). Mean change between pre- and post GOS-intervention on stimulated cytokine levels ranged from -8.5-9.5% in prefrail elderly and from -12.5-16.5% in adults. GI symptom scores and stool characteristics did not differ between GOS and placebo intervention in adults, nor in prefrail elderly (all  $P \geq 0.058$ ).

**Conclusion:** Immune markers did not differ between prefrail elderly and adults. Four weeks GOS intake did not affect immune markers in prefrail elderly, nor in adults. GOS was tolerated well by both groups. Analysis of microbiota composition is underway.

## **Fecal water from crohn's disease patients does not lead to increased paracellular permeability in vitro**

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**Background:** Crohn's disease (CD) is a chronic inflammatory gastro-intestinal disease with a variable disease course among patients. Impaired intestinal integrity and microbial dysbiosis seem to play a role in the pathophysiology and occurrence of exacerbations. The question rises whether microbial perturbations may contribute to impaired barrier function. Therefore, this *in vitro* study aims to investigate the impact of bacterial products on intestinal epithelial barrier function comparing active CD with remission.

**Methods:** Six healthy subjects and twelve CD patients were included in this study. Disease activity was based on the Simple Endoscopic Score for CD. Fresh fecal samples were collected within one week prior to endoscopy and processed to obtain fecal water within six hours after production. Outer membrane vesicles (OMVs) were isolated from frozen samples using an ultrafiltration and size exclusion chromatography-based protocol. Fecal water and OMVs were applied luminally on differentiated Caco-2 cell monolayers. After 24 h incubation, the difference in transepithelial electrical resistance (TEER) and fluorescein-isothiocyanate dextran 4 kDa (FITC-D4) was determined to detect paracellular junction disruption.

**Results:** Fecal water (50 % v/v) resulted in up to 84 % decrease of TEER in 3 remissive ( $p < 0.001$ ) samples and in up to 126 % increase of TEER in one active ( $p < 0.05$ ) and one remissive sample ( $p < 0.001$ ). No changes were found in FITC-D4 permeation, nor in TEER values after exposure to OMVs ( $10^8$ /ml).

**Conclusion:** Although some CD samples resulted in altered TEER values, this was not associated with changes in FITC-D4, indicating that fecal bacterial products of the investigated patients did not lead to increased paracellular permeation as compared to controls. The changes in TEER values may be associated with altered ion fluxes or increased nutrient exposure, which warrants further study.

## **Whole genome dna methylation profiling identifies neuroendocrine tumor origin.**

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**Background:** Determining the origin of a neuroendocrine tumor (NET) of unknown primary can be challenging. Liver metastases can originate from any organ in the body, while pulmonary NETs can be metastases but also primary tumors. This especially holds true for Multiple Endocrine Neoplasia Type I patients, who often have multiple primary pancreatic and gastro intestinal NETs. It is important to know the origin of the primary tumor since resection or ablation is crucial in case of treatment with curative intent. Furthermore, the site of origin determines prognosis, treatment options and eligibility for clinical trials. DNA methylation profiles are highly tissue specific and can be used to determine tumor origin. The use of whole genome methylation profiling to determine NET origin is explored.

**Methods:** A training cohort was built with publicly available Illumina 450k data of 67 pulmonary, pancreatic and ileal NETs. As test cohort, formalin-fixed and paraffin-embedded (FFPE) tissues of 8 primary NETs (4 pancreatic, 3 pulmonary, 1 ileal NET), 3 liver metastases (2 pancreatic, 1 ileal NET) and 1 lymph node NET of unknown primary were analyzed with the Illumina 850k EPIC array. A random forest model built on the 5000 most variable CpG sites in the training cohort was used to predict tumor origin in the test cohort.

**Results:** This approach was able to determine NET origin with perfect accuracy in the training (0% out of box error) and test cohort (100% accuracy). The NET metastasis of unknown primary was predicted to be of ileal origin.

**Conclusion:** This technique has the potential to be used as tool to determine NET origin, and thereby aid diagnostics and treatment decision-making. Importantly, these data were generated from FFPE material which highlights the clinical applicability of this technique. Validation on additional NET cases and more sites of origin is warranted.

## **Targeting gitr enhances human tumour-infiltrating t cell functionality in primary mismatch repair-proficient colorectal carcinoma and liver metastases**

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**Background:** Immune checkpoint blockade (ICB; e.g. anti-PD-1/-CTLA-4) has been proven to be clinically effective in mismatch repair (MMR-) deficient colorectal carcinoma (CRC). Yet, no studies have shown any beneficial population-based effects of ICB in MMR-proficient CRC, compromising the majority of patients. Here, we studied the effect of immune checkpoint stimulation via GITR ligation on human tumour-infiltrating lymphocyte (TIL) functionality in primary MMR-proficient CRC and liver metastases (CRLM).

**Methods:** Human TILs were isolated from freshly resected tumours of patients with primary MMR-proficient CRC (stage I-3) or liver metastases. Intra-patient GITR expression on TILs was determined using flow cytometry and compared to leukocytes isolated from blood (PBMC) and tumour-free surrounding tissues (tumour-free colon/liver, resp. TFC and TFL). Ex vivo polyclonal functional assays were used to assess TIL expansion and activation upon GITR co-stimulation.

**Results:** GITR expression was highest on intra-tumoural CD45RA<sup>-</sup> FoxP3<sup>hi</sup> CD4<sup>+</sup> regulatory T cells, CD45RA<sup>-</sup> FoxP3<sup>int</sup> CD4<sup>+</sup> activated T helper cells, and CD8<sup>+</sup> cytotoxic T cells compared to PBMC and TFC or TFL compartments. Within intra-tumoural CD8<sup>+</sup> T cells, GITR expression was higher on CD103<sup>+</sup> CD39<sup>+</sup> tumour reactive TILs compared to its single positive and double negative counterparts. GITR expression was associated with impaired T cell effector cytokine production upon ex vivo PMA/ionomycin stimulation. Recombinant GITR ligation reinvigorated ex vivo T cell responses by significantly increasing TIL numbers, interferon-gamma, perforin and granzyme B secretion compared to controls. Dual treatment with GITR ligand and nivolumab (anti-PD-1) further enhanced CD8<sup>+</sup> TIL responses compared to GITR ligand monotherapy, whereas nivolumab alone did not show to have any effect.

**Conclusion:** Agonistic targeting of GITR enhances ex vivo human TIL functionality in CRC and might therefore be a promising approach for novel mono- or combinatorial immunotherapies in primary MMR-proficient CRC and CRLM.

## **The gastrointestinal endoscopy satisfaction questionnaire captures patient satisfaction as a key quality indicator of gastrointestinal endoscopy**

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**Background:** Patient satisfaction is a crucial indicator of gastrointestinal endoscopy quality. The gastrointestinal endoscopy satisfaction questionnaire (GESQ) was recently validated for assessment of patient satisfaction undergoing endoscopy in English-speaking countries with good internal and face validity. We translated and validated the GESQ in The Netherlands.

**Methods:** The original GESQ was translated in Dutch according to the World Health Organisation (WHO) linguistic validation guidelines. First, internal validation of the Dutch GESQ (D-GESQ) was established by application of the think-aloud method and subsequent expert panel analysis. Next, the D-GESQ was embedded in the computer-based education (CBE) program in our unit, with a 30-day interval after endoscopy. Adult patients, who were informed via CBE and had undergone endoscopy, were included. Exclusion criteria were conscious sedation, limited Dutch language skills, no e-mail address available, dementia and visual impairment. For statistical analysis, several psychometric analyses of the questionnaire were performed to identify the underlying dimensions and assessed the questionnaire for reliability and validity.

**Results:** In total, 227 of 1065 patients completed the D-GESQ, a response rate of 21.3%. Men comprised 52.6% (N=129) of patients. Mean age was  $62.7 \pm 11.54$  years. In total 180 patients (79.3%) had previously undergone endoscopy, with 157 (87.2%) of them two or more times. The exploratory factor analysis showed that the 21 questions could best be clustered into five clusters instead of four in the original GESQ. The D-GESQ had an overall Cronbach  $\alpha$  of 0.88, confirming the high internal validity of the tool.

**Conclusion:** The Dutch version of the GESQ showed high internal validity and practicality. We recommend the D-GESQ for routine use in daily clinical practice to improve quality of patient care in daily endoscopic practice.

## Duodenal mucosal resurfacing combined with glp-1 receptor agonism may eliminate insulin treatment in type 2 diabetes while improving glycaemic control and overall metabolic health

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**Background:** Duodenal mucosal resurfacing (DMR) is an endoscopic intervention in which the duodenal mucosa is ablated by hydrothermal energy. DMR improves glycaemic control in type 2 diabetes mellitus (T2DM), probably through altered entero-endocrine signaling from the duodenum causing insulin sensitization. We studied the feasibility of eliminating insulin therapy in T2DM by combining DMR with glucagon like peptide-1 receptor agonism (GLP-1RA) and lifestyle counselling.

**Methods:** Single arm, single center study in 16 insulin treated T2DM patients (long-acting insulin, HbA1c  $\leq 8.0\%$ , c-peptide  $\geq 0.5$  ng/ml). After a single DMR, insulin therapy was discontinued. After a 2-week post-procedural diet GLP-1 RA (liraglutide) was introduced. Lifestyle counselling was provided throughout the study. The primary endpoint was the percentage of patients free of insulin with an HbA1c  $\leq 7.5\%$  at 6 months (responders). Secondary endpoints were change in hemoglobin A1c (HbA1c), homeostatic model assessment of insulin resistance (HOMA-IR), fasting plasma glucose (FPG), mixed-meal postprandial glucose levels, metabolic parameters (body mass index [BMI]), total body fat percentage, liver proton density fat fraction [PDFF]) at 6 months and percentage of responders at 12 months. A sub analysis was performed to assess change in insulin dose in non-responders after DMR compared to baseline.

**Results:** All 16 patients underwent successful DMR without procedure-related serious adverse events during follow-up. At 6 months, 75% of patients (12/16) (95% CI: 0.48 – 0.93) were off insulin therapy with an HbA1c  $\leq 7.5\%$ . This was associated with significant improvement of all glycaemic and metabolic parameters in the responders. Median HbA1c improved from 7.4 to 6.7% ( $\Delta -0.6\%$  (95% CI: -0.9 to -0.2;  $p=0.009$ ), HOMA-IR from 8.9 to 2.5 ( $\Delta -5.9$  (95% CI -9.9 to -2.6);  $p=0.006$ ) and peak postprandial glucose from 10.1 to 7.6 mmol/l ( $\Delta -2.5$  mmol/l (95% CI -3.2 to -1.5);  $p=0.003$ ). PDFF improved significantly from 8.1 to 4.6% ( $\Delta -3.7\%$  (95% CI -6.6 to -0.5);  $p=0.016$ ). BMI and total body fat percentage also decreased significantly. In the intention-to-treat population all glycaemic and metabolic parameters, except for HbA1c and PDFF, improved significantly. At 12 months, 56% of patients were responders with a median HbA1c of 6.7%. The median insulin dose decreased from 36 to 17 units per day in non-responders with a median HbA1c of 7.9%.

**Conclusion:** A single endoscopic DMR procedure, combined with GLP-1 RA and lifestyle counselling can effectively eliminate the need for insulin therapy in the majority of our T2DM patients while improving glucose regulation and overall metabolic health.



## Comorbidity is associated with safety outcomes in vedolizumab and ustekinumab treated inflammatory bowel disease patients

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**Background:** The effect of comorbidity on treatment outcomes of vedolizumab and ustekinumab in Inflammatory Bowel Disease (IBD) patients is currently unknown. We aimed to assess the impact of comorbidity on safety and effectiveness of therapy in vedolizumab and ustekinumab treated IBD patients. **Methods:** In this multi-center prospective ICC Registry study, IBD patients from 10 IBD centers in The Netherlands starting vedolizumab or ustekinumab in regular care were enrolled. Prior to therapy initiation, comorbidity prevalence was assessed using the Charlson Comorbidity Index (CCI), a weighted index accounting for 16 comorbid diseases among which cardiovascular and pulmonary disease. The effect of an increased CCI was determined on safety outcomes (infection, all-cause hospitalization and adverse events) during treatment and effectiveness outcomes (clinical response: reduction of  $\geq 3$  points in HBI/SCCAI, clinical remission (HBI  $\leq 4$ /SCCAI  $\leq 2$ ), corticosteroid-free remission and biochemical ((CRP  $\leq 5$  mg/L and FCP  $\leq 200$   $\mu$ g/g) combined with clinical remission) at 52 weeks of follow-up. Confounders were accounted for using multiple logistic regression.

**Results:** We included 203 vedolizumab and 207 ustekinumab treated patients: mean age 42.2 (SD 16.0) and 41.6 (SD 14.4) years, median HBI 7.0 (IQR 5.0-10.0) or SSCAI 5.0 (IQR 3.0-8.0) and median HBI 7.0 (5.0-11.8). Median treatment duration 54.0 (IQR 19.9-104.0) and 48.4 (IQR 24.4-55.1) weeks, median follow-up time 104.0 (IQR 103.1-104.0) and 52.0 weeks (IQR 49.3-100.4), respectively. Twenty vedolizumab treated patients (9.9%) had cardiovascular disease at baseline (congestive heart failure, myocardial infarction, peripheral vascular disease and cerebrovascular accidents/transient ischemic attacks) compared to seven (3.4%) ustekinumab treated patients ( $p=0.031$ ). Sixteen (7.9%) vedolizumab treated patients had pulmonary disease at baseline compared to seven (3.4%) ustekinumab treated patients ( $p=0.048$ ). CCI associated independently with safety outcomes in vedolizumab and ustekinumab. In vedolizumab, CCI associated with infection (OR 1.387, 95% CI 1.022-1.883,  $p=.036$ ) and hospitalisation (OR 1.586, 95% CI 1.127-2.231,  $p=.008$ ). In ustekinumab, CCI associated with hospitalisation (OR 1.621, 95% CI 1.034-2.541,  $p=.035$ ). CCI did not associate with time to treatment cessation or effectiveness.

**Conclusion:** Comorbidity associated with all-cause hospitalization in vedolizumab and ustekinumab treated IBD patients. A significant impact on infections was found only in vedolizumab treated patients. This study underlines the importance of comorbidity assessment in these IBD patients.

## Geriatric impairments in older ibd patients are associated with higher disease burden-results of a multicentre cohort study

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**Background:** The population of older patients with Inflammatory Bowel Diseases (IBD) is expanding. Knowledge on the prevalence and impact of geriatric impairments is scarce in this heterogenous group. In other fields of medicine, it has been established that geriatric impairments associate with adverse outcomes. Therefore, the aims of our study were to assess the prevalence of geriatric impairments in older IBD patients and to evaluate the association between geriatric impairments and disease burden of IBD through the short Inflammatory Bowel Disease Questionnaire (sIBDQ).

**Methods:** Consecutive IBD patients aged  $\geq 65$  years were included at outpatient Dept.s and infusion centers of four hospitals in The Netherlands. Comorbidity, polypharmacy and malnutrition (somatic domain), cognitive impairment and depressive symptoms (mental domain), handgrip strength and gait speed (physical domain) and impairments in (instrumental) activities of daily living ((I)ADL) (functional domain) were assessed. Disease activity was assessed through HBI or pMS (remission: HBI $<5$  or pMS $<2$ ); The sIBDQ was used to assess IBD disease burden. Association between geriatric impairments and sIBDQ was assessed using multiple linear regression, including confounders age, sex, IBD type, disease duration and disease activity.

**Results:** We included 336 patients: median age 70.0 (IQR 67.0-73.0); 161 CD (47.9%); 71 active disease (21.1%); mean sIBDQ 59.7 (SD 8.1). An impaired somatic domain was present in 158 patients (47.0%); 12.5% multiple comorbidities, 32.1% polypharmacy and 18.8% at risk of malnutrition. An impaired mental domain was present in 54 patients (16.9%): 10.1% cognitive impairment and 11.9% depressive symptoms. Seventy-nine (25.2%) were impaired in their physical domain: 18.8% low grip strength and 7.4% low gait speed. An impaired functional domain was present in 147 patients (43.8%): 30.0% impaired ADL and 23.2% impaired IADL. The number of impaired geriatric domains were statistically significantly and independently associated with a higher disease burden (lower sIBDQ). One impaired domain associated with -1.660 in sIBDQ score (95% CI -3.631310,  $p=.098$ ), two impaired domains with -2.625 in sIBDQ score (95% CI -4.986, -.264,  $p=.029$ ), three impaired domains with -6.303 in sIBDQ score (95% CI -9.084, -3.523,  $p<.001$ ) and four impaired domains with -19.258 in sIBDQ score (95% CI -24.121, -14.395,  $p<.001$ ).

**Conclusion:** In an older IBD patient population, geriatric impairments are frequently encountered and associate with a higher disease burden. These results are a call for more research on the relationship between geriatric impairments and IBD disease burden to improve patient care in older IBD patients.

## Chronic mesenteric ischemia, a horse instead of a zebra

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**Background:** Chronic mesenteric ischemia (CMI) is an invalidating disease. Unfortunately, CMI is still underappreciated, resulting in diagnostic delays. This is partially caused by the assumed rarity of CMI and by a lack of knowledge and awareness among physicians. Incidence rates of CMI are unknown, but especially the incidence of atherosclerotic CMI could be substantial, since risk-factors of atherosclerosis are prevalent in western populations. Aim of the current study was to determine the incidence of all cause CMI, atherosclerotic CMI, median arcuate ligament syndrome (MALS) and chronic non-occlusive mesenteric ischemia (chronic NOMI).

**Methods:** This study is based on a prospectively collected database, containing all patients suspected of having CMI referred to Medisch Spectrum Twente, a renowned mesenteric ischemia expert center. Patients residing within the well-defined region of the expert center, between January 2015 and December 2018, were included. Patients not residing within the region were excluded. The region of the expert center consisted of 262,645 inhabitants. Patients were classified as having CMI when symptoms improved or resolved and the treated vessels were patent at 3 months after treatment. Data concerning the number of inhabitants of the region were derived from the Dutch central bureau of statistics. Annual incidence rates are given per 100,000 inhabitants.

**Results:** A total of 262 patients suspected of having CMI were identified in the database, 103 patients (39%) had been diagnosed with CMI. The median age of CMI patients was 70 (IQR 52-78), 62% of the CMI patients was female. Presenting symptoms in patients with CMI were abdominal pain (83%), post prandial abdominal pain (68%), weight loss (77%) and an adapted eating pattern (81%). The median duration of symptoms at first presentation was 5.5 months (IQR 2.8-9.0). Risk factors of cardiovascular disease (CVD) in CMI patients were smoking (76%), a history of CVD (48%), dyslipidemia (34%), hypertension (43%) and diabetes mellitus (18%). CMI was caused by atherosclerosis in 78 patients, by MALS in 20 patients and by chronic NOMI in 5 patients. The annual incidence of all cause CMI was 9.8; annual incidence rates were 7.4 for atherosclerotic CMI, 1.9 for MALS, and 0.5 for chronic NOMI.

**Conclusion:** CMI is not as rare as stated in literature. The incidence of CMI is higher than the incidence of occlusive acute mesenteric ischemia (4.5) and only slightly lower than the Dutch incidence of Crohn's disease (10.5). The results of this study underline that CMI should be considered in patients with chronic abdominal pain. Awareness and knowledge among physicians should, therefore, be raised.

## **Value of serum amylase and lipase in patients with acute pancreatitis and severe hypertriglyceridemia**

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**Background:** Patients with severe hypertriglyceridemia are at risk for acute pancreatitis. High serum triglycerides interfere with serum amylase and lipase measurement. We hypothesize that despite current laboratory techniques serum amylase and lipase measurement is negatively influenced because of high serum triglycerides, leading to a diagnostic challenge.

**Methods:** We retrospectively reviewed levels of serum amylase and lipase in all patients presenting with severe hypertriglyceridemia ( $>22.6$  mmol/L) at our center between 2005-2018. We assessed if criteria for acute pancreatitis were met. We obtained serum levels of amylase and lipase at presentation including the upper limit of normal (ULN) at time of measurement. All serum measurements were performed in our center. NTR ID: NTR7282.

**Results:** We identified 179 patients presenting with severe hypertriglyceridemia. A total of 11% ( $n=19$ ) presented with acute pancreatitis (median age 42 years [IQR 38-46], 63% male, 16% diabetes, 68% alcohol use, 32% statin use). In 63% ( $n=12$ ) of patients presenting with acute pancreatitis (based both on clinical and imaging criteria), serum level of amylase or lipase at presentation was less than three times ( $<3\times$ ) ULN. In these patients, median time until acute pancreatitis diagnostic criteria were met was 1 day [IQR: 0-8 days]. Age, gender, diabetes, alcohol use, statin use or time between start symptoms and serum amylase/lipase measurement were not significantly different between groups  $<3\times$  ULN and  $\geq 3\times$  ULN.

**Conclusion:** In patients presenting with acute pancreatitis and severe hypertriglyceridemia, serum levels of amylase and lipase are frequently  $<3\times$  ULN despite current laboratory techniques. Therefore these patients are at risk for a delay of diagnosing acute pancreatitis.

## **Risk estimate of duodenoscope-associated infections (dai) in The Netherlands**

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**Background:** The likelihood of endoscopy-associated infections (EAI) is often referenced from a paper published in 1993 by Kimmerly and co-workers in which a risk of 1 exogenous infection for every 1.8 million endoscopies (0,00006%) is proclaimed. Even though Ofstead et al. pointed out in 2013 that this was at least an underestimation by 6-fold because of erroneous assumptions and mathematical errors, the original calculation is still often referred to. In the past decade, multiple outbreaks of multi-resistant microorganisms (MDRO) related to contaminated duodenoscopes have been reported worldwide. This leads to the assumption that the former risk calculation is indeed incorrect. The aim of our study is to calculate the duodenoscope-associated infection (DAI) risk for the Dutch ERCP practice.

**Methods:** We searched and consolidated all Dutch patients reported in the literature to have suffered from a clinical infection linked to a contaminated duodenoscope between 2008 and 2018. From a national database the number of ERCP's performed per year in The Netherlands were retrieved. Actual numbers were available from 2012 to 2018. Numbers from 2008 to 2011 were estimated and assumed to be equal to 2012.

**Results:** In the period 2008 to 2018, three MDRO outbreaks in Dutch hospitals were reported in the literature with 21 patients suffering from a clinical infection based on a microorganism proven to be transmitted by a duodenoscope. In that time period, approximately 203.500 ERCP procedures were performed. Hence, for every 1 out of 9690 procedures one patient developed a clinically relevant infection amounting to a DAI risk of 0.010%.

**Conclusion:** The risk of developing a DAI is at least 30 to 180 times higher than the risks that were previously reported for all types of endoscopy-associated infections. Importantly, the current calculated risk of 0.010% constitutes a bare minimum risk of DAI because endoscope related infections are under-reported. Apart from DAI risk there is also the risk of patients becoming colonized with MO through contaminated endoscopes but without developing symptoms of a clinical infection. These data call for concerted action of medical practitioners, industry and government agencies to minimize and ultimately ban the risk of exogenous endoscope associated infections and contamination. As a first step, the FDA recently recommended health care facilities and manufacturers begin transitioning to duodenoscopes with disposable components.

## **Long-term outcomes after endoscopic resection without subsequent ablation therapy for barrett's esophagus (be) with early neoplasia.**

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**Background:** After endoscopic resection (ER) of neoplastic lesions in BE, it is generally recommended to ablate the remaining flat BE to minimize the risk for metachronous disease. However, the majority of patients will not develop metachronous disease, and if it occurs it is generally detected at early stages that allow re-ER. Ablation is still accompanied by complications and requires multiple hospital visits. We report the long-term outcomes for all patients treated in NL between 2008-2018 who did not undergo ablation after ER.

**Methods:** Endoscopic therapy for BE neoplasia in NL is centralized in 9 expert centers with specifically trained endoscopists & pathologists. Uniformity is further ensured by a joint protocol and regular group meetings. Prospectively collected data are registered in a uniform database. We report all patients who underwent ER for a neoplastic lesion and in whom subsequently no ablation therapy was applied. We report progression rates during endoscopic FU and mortality after endoscopic FU was stopped. Data on date and cause of death were extracted from Statistics Netherlands (CBS).

**Results:** Of the 2,098 BE patients with early neoplasia, 1,305 underwent ER for a visible neoplastic lesion and 95 (7%) entered endoscopic surveillance without additional ablation. 78% was male, mean age 74(±10)yrs, BMI 27(±6)kg/cm<sup>2</sup>, ASA classification II(64%) or ≥III(29%). Median BE was C4M6. ER was performed for LGD(12%), HGD(23%) or EAC(65%). Reasons for not proceeding to ablation therapy were: age, comorbidity and extent of residual BE(88%); anticipated poor response upon ablation therapy (e.g. BE regeneration of the ER-scar)(14%); other treatment protocols(13%); patient preference(7%); and/or complications after ER(4%). After ER, median BE was C2M5 with IM(52%), LGD(31%) or HGD(6%) (no histology obtained: 12%). During median 25mo of endoscopic FU(IQR 12-53) with median 4 endoscopies per pt, 0 progressed to advanced cancer. 17 pts (18%) developed HGD/EAC after median 29 mo: 14 with a visible lesion were successfully treated with ER for HGD(6) or EAC(8). The other 3 had flat HGD and were successfully treated with ablation. Of the 78 non-progressors, 4 had successful ablation for persistent multifocal LGD. In 62 patients (64%), endoscopic surveillance was stopped median 20mo after ER (5-59) because of comorbidity and anticipated limited life expectancy. During median 44mo (28-77) after endoscopic FU was stopped, no patient developed symptomatic disease or had a tumor-related deaths, whilst 45% died of unrelated causes.

**Conclusion:** In selected patients, ER monotherapy with endoscopic surveillance of the residual BE is a valid alternative to prophylactic ablation therapy.

## **The effect of an intragastric balloon on non-alcoholic fatty liver disease (naflD) in obese patients (pts)**

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Background: Non-alcoholic fatty liver disease (NAFLD) is an emerging health problem worldwide. Dietary and lifestyle interventions resulting in weight reduction are the cornerstones of treatment but are often challenging. The aim of the current study is to assess the prevalence and severity of NAFLD with the aid of Fibroscan in pts opting for treatment in a Dutch private health clinic for obesity and to explore potential beneficial effects of intragastric balloon therapy (IB).

Methods: Pts were treated with detailed nutritional advice, psychological counseling and option to insert an intragastric balloon during 6 months. Main inclusion criteria were: Age  $\geq$  18 years and BMI 27-48 kg/m<sup>2</sup>. Transient elastography (Fibroscan) was used to diagnose steatosis and fibrosis. Steatosis was defined as: S1 (no steatosis) CAP < 215 db/m; S2 (possible steatosis) CAP 215-30 db/m; S3 (definite steatosis) CAP > 300 db/m. Fibrosis stage was as follows: F0/I (no or minimal fibrosis) < 7.1 kPa; F2 (moderate) 7.2-9.4 kPa; F3 (severe) 9.5-12.4 kPa; F4 (cirrhosis) > 12.5 kPa. Pts with definitive steatosis or at least moderate fibrosis were offered a second Fibroscan after six months.

Results: 60 of 153 consecutive pts were excluded, mainly because of no consent. Of the included 69 pts, 45 (65%) received IB and 24 (35%) dietary therapy only (DT). In both groups baseline characteristics were comparable: age 45 $\pm$ 9 vs 47 $\pm$ 11 yrs for IB vs DT; Female 73 vs 74%; median ALT 26 vs 27 U/L; GGT 25 vs 33 U/L; none had diabetes. At baseline definite, possible and no steatosis were present in 44%, 49% and 7% of IB group and in 63%, 29% and 8% of DT group respectively (P= 0.224). Fibrosis stage was F4, F3, F2 or F0/I in 0%, 7%, 7% and 86% in IB group and in 4%, 8%, 8% and 79% of DT group (P= 0.38). Until now, 10 pts had 6-month FU (8 IB, 2 DT). Weight and BMI decreased markedly in all pts, with a reduction of 13% (13kg) vs 5% (6kg) in IB and DT groups resp. BMI decreased with 12% vs 7% in IB and DT groups resp. Also steatosis and fibrosis score improved in all pts. Steatosis score reduction of 22% (73 dB/m) vs 12% (40 dB/m) in IB resp. DT (p=0.0090). Fibrosis score reduction of 17% (1.1 kPa) vs 25% (2.3 kPa) in IB resp. DT groups. There were no severe adverse effects.

Conclusion: Steatosis is highly prevalent in otherwise healthy obese pts opting for weight loss therapy in private care setting in The Netherlands. Despite relatively young age, significant fibrosis was not uncommon. 6-month intragastric balloon therapy generally improved steatosis and fibrosis, and may be an alternative to more invasive treatment options.

## **Dye-based chromoendoscopy versus standard-definition and high-definition white-light endoscopy for endoscopic adenoma detection in lynch syndrome: meta-analysis of individual patient data from randomized trials**

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**Background:** The additional diagnostic value of dye-based chromoendoscopy (CE) compared to standard-definition and high-definition white-light endoscopy (SD-WLE and HD-WLE) for surveillance of Lynch syndrome patients is subject to debate.

**Methods:** To clarify this debate, an individual patient data (IPD) meta-analysis was performed according to the PRISMA-IPD guidelines. Randomized controlled trials (RCTs) comparing the efficacy of dye-based CE to WLE (SD and HD) for the detection of adenomas in Lynch syndrome patients were included. Only individuals with a proven Lynch syndrome associated gene mutation (*MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*) were included in the IPD meta-analysis. The primary outcome measure was the adenoma detection rate (ADR) (i.e. proportion of patients with at least one adenoma detected during colonoscopy). Patients were subdivided in two groups: (1) SD equipment and (2) HD equipment. Mixed-effect logistic regression models were used to estimate ADR across studies. To account for heterogeneity between-trials a random intercept for study was used.

**Results:** Two RCTs and one randomized tandem colonoscopy study were included, comprising 533 Lynch syndrome patients with a proven gene mutation. HD equipment was used in 363/533 (68%) of the colonoscopy procedures. The ADR was 74/265 (28%) in patients randomized to WLE compared to 83/266 (31%) patients randomized to CE (odds ratio [OR] 1.17; 95% CI 0.81-1.70,  $P=0.41$ ). There was no difference in ADR for either imaging modality within the HD equipment group (OR 1.20, 95% CI 0.77-1.90,  $p=0.42$ ) or the SD equipment group (OR 1.17; 95% CI 0.60-2.32,  $P=0.65$ ). The mean number of adenomas per patient detected with CE was 0.52 compared to 0.47 with WLE (incidence rate ratio [IRR] 1.09; 95%CI 0.78-1.52,  $P=0.60$ ). Subgroup analyses showed no significant differences between CE and WLE for proximal adenomas (OR 1.40, 95%CI 0.92-2.14,  $P=0.11$ ), flat adenomas (OR 1.34; 95%CI 0.80-2.24,  $P=0.26$ ) or diminutive adenomas (OR 1.21, 95%CI 0.81-1.81,  $P=0.34$ ). CE was more time consuming than WLE with a mean extubation time of 19 minutes for CE versus 12 minutes for WLE ( $p<0.01$ ).

**Conclusion:** In this IPD meta-analysis of RCTs in Lynch syndrome patients, dye-based CE did not improve the ADR or mean number of adenomas per patient detected compared to WLE. As dye-based CE is associated with a prolonged procedural time and prior studies showed that HD equipment increases adenoma detection compared to SD equipment, we suggest to use HD-WLE as the preferred image modality for the surveillance of Lynch syndrome patients.



## Development and clinical implementation of an endocytoscopy scoring system of dysplasia in the barrett's esophagus: preliminary results.

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**Background:** As the precursor stage of esophageal adenocarcinoma (EAC), Barrett's esophagus (BE) has a 0.5% to 1.0% annual risk of progression to EAC [1]. Current biopsy strategy may miss early EAC lesions as it only samples around 5% of the entire BE segment [2], leading to less than 5% of patients with EAC diagnosed by surveillance endoscopy [3]. Imaging by endocytoscopy (ECS) allows real-time *in vivo* histological identification of suspected areas. Thus, it allows targeted biopsy and so potentially reduces the risk of sampling errors and increases the diagnostic yield of surveillance endoscopy [4]. To evaluate the diagnostic capability of endocytoscopy (ECS) [model GIF-H290 EC, Olympus] in detecting dysplasia in Barrett's esophagus (BE).

**Methods:** For each procedure, the BE segment was at first evaluated for lesion localization by high definition-white light endoscopy. This was followed by further tissue characterization by assessment of the vascular network through magnifying narrow band imaging. Then the tissue of interest was rinsed with approximately 8 ml N-acetylcysteine, prior to spraying the staining mixture of 0.05% crystal violet (10ml) and 1% methylene blue (1ml). After 90 seconds of absorption time, ECS was performed to assess architectural and cytological features of the tissue. A comparable procedure was conducted *ex vivo* for imaging the EMR specimens.

**Results:** This on-going study of ECS in BE patients included at the moment 30 patients. We imaged 43 sites with ECS, of which we have 32 targeted biopsies containing all stages of dysplasia in BE. Imaging was considered to be classifiable in 56% (n=24) and unclassifiable in 44% (n=19). Nevertheless, poor resolution of images (51%) due to patient-related factors and low quality of staining (56%) often made it hard to interpret *in vivo* ECS images. Overall, we are able to classify images into three categories including BE without dysplasia, BE with dysplasia and EAC *in vivo* and *ex vivo*.

**Conclusion:** The ECS could provide clear images in which distinct architectural and cytological features could be identified. However, certain patient-related as well as procedure-related factors can trouble clear ECS imaging and thus diagnoses in BE patients. In order to implement ECS into clinical practice, we will build an *in vivo* atlas with representative images of each category and investigate the use of an artificial intelligence-aided diagnostic system that could help to enable a highly accurate diagnosis.

## **Endoscopic follow-up of radically resected high-risk mucosal adenocarcinoma and low- and high-risk submucosal adenocarcinoma arising in Barrett's esophagus, results of 120 patients from the dutch barrett expert center cohort**

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**Background:** After radical endoscopic resection(ER) of an esophageal adenocarcinoma(EAC) in Barrett's esophagus with high-risk(HR) features, optimal management is unclear. This concerns three groups: HR-T1a-EAC (poorly(G3)/undifferentiated(G4) cancer a/o lymphovascular invasion(LV+)); low-risk(LR) T1b-EAC (submucosal invasion <500um, well/moderate differentiation, LV-); HR-T1b EAC (invasion >500um, G3/4 cancer a/o LV+). Endoscopic follow-up (FU) to detect lymph node metastases(N+) at a curable stage is considered an option in selected cases, however, optimal FU strategy is unclear. Aim was to evaluate outcomes of endoscopic FU in all patients treated by radical ER for HR-T1a EAC or T1b-EAC. **Methods:** Endoscopic therapy for Barrett's neoplasia in The Netherlands is centralized in 9 expert centers with specifically trained endoscopists and pathologists. In an ongoing registry, treatment/FU data of all patients treated endoscopically for BE neoplasia in The Netherlands, is collected in a dedicated database. We identified all patients who underwent radical ER for HR-T1a EAC or T1b EAC, followed by endoscopic FU. The decision to follow patients endoscopically was made by the treating physician, taking co-morbidity and patient's wish into account. Endoscopic FU consisted of gastroduodenoscopy(GDS) ± endoscopic ultrasound(EUS). Outcome parameters were number of patients with N+, distant metastases(M+) and tumor related death(TRD).

**Results:** From Jan 2008 to Oct 2019, 120 patients (median 74years) underwent radical ER of HR-T1a (n=27), LR-T1b (n=55) or HR-T1b EAC (n=38) and endoscopic FU (median 29 months (IQR 15-48)). Nine patients were diagnosed with N+ (n=4;3%) and/or M+ (n=5;4%) after median FU of 27 months (IQR 23-38), diagnosed by EUS-FNA (n=5), or CT performed for symptoms (n=4). N+/M+ was found in 22% of HR-T1a, 2% of LR-T1b and 5% of HR-T1b patients. The 4 patients with N+-disease were treated with curative intent; 1 was cured, 1 is still treated, 2 died of complications. Seven patients (6%) died from EAC (of which 2/7 from treatment complications). Non-EAC related mortality was 8,3%.

**Conclusion:** In our cohort of selected patients treated and followed endoscopically for a high risk EAC, we found an unexpected high risk of lymph node metastases associated with mucosal cancer with poor differentiation or lymphovascular invasion. In patients diagnosed with N+ during follow-up, treatment with curative intent was still an option in almost half of patients, and TRD was lower than non-TRD. Thus, in selected patients with high risk EAC, endoscopic follow-up may be justified, yet the optimal FU regimen to detect N+ at a curable stage is yet to be established.

## **Cusum analysis is a valuable tool to monitor quality in eus guided tissue acquisition of solid pancreatic lesions in community hospital practice**

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**Background:** Endoscopic ultrasound (EUS) guided tissue acquisition (TA) is the method of choice to establish a pathological diagnosis of solid pancreatic lesions. EUS guided TA is a complex multistep procedure involving efforts of both endosonographers and cytopathologists. Practice variation regarding quality and yield of these procedures amongst community hospitals is an unwanted but existing phenomenon (1). Diagnostic Yield of Malignancy (DYM) is one of the ASGE defined quality indicators for these procedures with its minimum target value set at 70% (2). Cumulative sum (CUSUM) analysis is an established tool to evaluate endoscopy trainees by visualizing their performance development over time (3). Similarly, CUSUM can be used to visualize DYM over time as a tool to continuously measure quality. **Methods:** We prospectively collected data of EUS guided TA procedures from 5 regional community hospitals from 2015-2019. A total of 454 consecutive EUS guided TA procedures was included. CUSUM curves of DYM, using 70% as a cut off, were created for all hospitals combined and per hospital.

**Results:** The overall CUSUM curve of DYM show a gradual improvement over the years. The CUSUM curve of one of the participating hospitals however revealed a temporal but remarkable negative change in the quality of DYM during an episode of 4 months in 2017. Analysis of potential explanations for this change revealed a sudden increase in the number of diagnoses of atypia related to a temporal absence of one of the more experienced cytopathologists.

**Conclusion:** CUSUM analysis is an easy-to-use tool to monitor quality and to guide quality-improvement of EUS guided TA of solid pancreatic lesions. Variations in the curve, in particular a negative trend, may trigger further analyses to instigate corrective actions. In particular, a change in dedicated staff involved in the delicate multistep processes of EUS guided TA of solid pancreatic lesions may have a clinically relevant negative impact on quality. In anticipation of such possibility, performance should be monitored closely using CUSUM analysis.

## Utility of routine esophageal biopsies in patients with refractory reflux symptoms

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**Background:** In the most recent update of the Rome criteria, it is recommended to obtain esophageal biopsy samples in all patients with refractory reflux symptoms, in order to rule out eosinophilic esophagitis (EoE). However, obtaining biopsies in every suspected reflux patient would indicate an enormous volume of biopsies, while the prevalence of EoE in this patient group is thought to be extremely low. Moreover, biopsies will not help in the differentiation of functional heartburn from reflux disease. The main objective of this study was to assess the additional diagnostic yield of esophageal biopsy sampling in patients with PPI-refractory reflux symptoms.

**Methods:** Consecutive patients with reflux symptoms refractory to standard dose PPI therapy were prospectively enrolled. All patients underwent upper endoscopy with biopsy sampling in accordance with current guidelines. Endoscopic features suggestive of EoE and other endoscopic abnormalities were routinely recorded by the endoscopist. The Reflux Disease Questionnaire, the Straumann Dysphagia Index, and a complete medical history were carried out to assess comorbidities and esophageal symptoms prior to endoscopic evaluation. EoE was defined as the presence of >15 eosinophils per microscopic high-power field in at least one esophageal biopsy specimen, accompanied by symptoms of esophageal dysfunction.

**Results:** Of the 260 included patients (mean age 54.0±18.6; 101 males), 11 patients (4.2%) met the clinicopathological diagnostic definition of EoE. Dysphagia and symptoms of food impaction were significantly more common among EoE patients compared to patients without EoE (100% versus 64%;  $p < 0.001$  and 73% versus 17%;  $p < 0.001$ , respectively). Three EoE patients (29.3%) had a history of food impaction that required endoscopic bolus removal, in contrast to 1.6% of the non-EoE group ( $p < 0.001$ ). When only patients with reflux symptoms and dysphagia were analyzed, the prevalence of EoE was 8.7% (11/127). This further increased to 32.0% when patients with endoscopic features of EoE were taken into account. Routine biopsies in patients that presented with dysphagia but lacked endoscopic features only led to a diagnostic yield of 2.94% (3/102). Atopic background (OR 18.8%; 95%-CI 3.9-90.1) and a history of endoscopic dislodgement of food bolus impaction (OR 23.0; 95%-CI 4.4-120.1) were other factors being strongly associated with presence of EoE.

**Conclusion:** In patients with PPI-refractory reflux symptoms, esophageal biopsy sampling for EoE has a very low diagnostic yield (prevalence of 4%). Dysphagia was present in all EoE patients. Our data suggests that biopsies should only be obtained if patients have dysphagia or other typical EoE hallmarks.

## **Applicability of colon capsule endoscopy as pan-endoscopy: from bowel preparation, transit times and completion rate to rating times and patient acceptance**

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**Background:** Despite its noninvasive character and its potential to explore the entire gastrointestinal tract, implementation of colon capsule endoscopy (CCE) as pan-endoscopy has not yet been achieved. The applicability of CCE as pan-endoscopy is highly dependent on several quality parameters. The aim of this study was to evaluate these parameters to determine which factors need optimization.

**Methods:** Participants received CCE with corresponding bowel preparation (5mg bisacodyl, 2L PEG and 2L water split-dose) and booster regimen (10mg metoclopramide and 0,5L Eziclen - half directly after and half three hours after small bowel recognition). Different quality parameters were assessed. Patient acceptance was measured by questionnaires.

**Results:** A total of 462 people ingested the colon capsule. Bisacodyl was taken in 99,5%, complete PEG intake was achieved in 98,5% and complete Eziclen intake was achieved in 96,9% of the participants. Due to 11 technical failures (signal interference), 451 procedures were analyzed. The overall colon cleansing score was adequate in 76.6% and the bubbles effect scale was insignificant in 74.7%. The Z-line was objectified in 44.8%. The proportion of visualized stomach mucosa was good (>90%) in 69.6%. The small bowel cleansing was adequate in 99,1%. Median transit times were 55 minutes for the stomach, 47 minutes for the small bowel and 392 minutes for the colon. The capsule reached the descending colon in 95%. Total completion was achieved in 51.2% of the participants. Median staff reading time was 3 minutes for the stomach, 10 minutes for the small bowel and 55 minutes for the colon. Participants graded the procedure with a 7.8 (scale 0-10). There were no procedure-related serious adverse events.

**Conclusion:** CCE is a safe procedure with good patient acceptance. However, technical developments are necessary to achieve complete observation of the gastrointestinal tract and bowel preparation and booster regimen need to be improved.

## Endoscopic submucosal dissection for barrett's related neoplasia in The Netherlands: results of a nationwide cohort of 140 cases

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**Background:** Endoscopic resection (ER) of visible lesions followed by RFA is the standard of care for early neoplasia in Barrett's esophagus (BE). Generally (piecemeal-)EMR is used for ER yet the use of endoscopic submucosal dissection (ESD) is gradually expanding. Experience with ESD in BE is limited and efficacy and safety data in this setting is scarce. We aimed to report the outcomes of all ESDs for BE neoplasia, performed in a setting of centralized care in NL.

**Methods:** Endoscopic therapy for BE neoplasia in NL is centralized in 9 expert centers with specifically and jointly trained endoscopists & pathologists. Uniformity is further ensured by a joint protocol and regular group meetings. ESD is restricted to 5 centers and is only performed for large and bulky lesions that cannot be removed with cap-based ER and in case of suspicion for submucosal (sm) invasion. Prospectively collected treatment/FU data are registered in a uniform database. We report efficacy and safety outcomes of all ESD-BE cases treated since 2008. En-bloc resection was defined as complete resection of the delineated target lesion in a single piece, R0-resection as absence of cancer in the vertical and lateral margin.

**Results:** Of the 2098 BE patients with ER for BE neoplasia, 140 (7%) underwent ESD. BE segment was median CIM4 (IQR 0-5; 2-7) with a lesion of 30mm length (20-40) over 30% of the circumference (25-50). During median 125min (90-180), 130/140 lesions were removed en-bloc (93%). In the other 10, ESD was done in piecemeal (6) or was terminated due to deep invasion (4). Pathology was HGD (6%), m-EAC (42%) or sm-EAC (51%; 36% sm1 and 64%  $\geq$ sm2). 51% had  $\geq$ 1 high-risk feature ( $\geq$ sm2, poor differentiation or lymphovascular invasion). Of the en-bloc resections, 77% (100/130) was R0. Stratified for invasion depth, R0 rate was 89% for m-sm1 and 52% for  $\geq$ sm2. The combined en-bloc/R0 resection rate was 71% (100/140). In 2 pts (1.4%) a small perforation occurred; both were managed endoscopically during ESD. Bleeding occurred in 4pts (2.9%), all classified as mild-moderate. Overall, 20pts (14%) developed a stenosis that was resolved after median 4 (3-8) dilatations. Of 100pts with en-bloc/R0 resection, 90 underwent endoscopic FU. 2 pts (2%) with a persisting lesion in the scar were directly treated with EMR/APC. 56 pts were treated with RFA. During median 15mo FU (9-24), 0 pts developed local recurrence.

**Conclusion:** In a tertiary setting with specifically trained endoscopists and under a common treatment protocol, ESD for bulky lesions and/or lesions with sm-invasion is safe and results in an en-bloc resection with R0 in 71%. The majority of procedures results in resection of submucosal cancer, often with other high-risk histology.

## Colonoscopy quality assurance in an organized fit-based colorectal cancer screening program

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**Background:** In The Netherlands, average risk individuals aged 55-75 years are invited for colorectal cancer (CRC) screening by biennial fecal immunochemical test (FIT). Positive FIT is followed by colonoscopy. High quality of colonoscopy is essential for optimal performance of a screening program. Therefore, endoscopists performing within the Dutch CRC screening program have to receive accreditation and fulfill minimum standards. In this study we assessed the quality of colonoscopies performed by the certified endoscopists.

**Methods:** Structured data on endoscopy and pathology were obtained for all first colonoscopies after a positive FIT within the first five years of the Dutch CRC screening program (2014-2018). Quality indicator performance was assessed for each endoscopist with  $\geq 50$  procedures. We determined quality indicators regarding completeness of visualization (cecal intubation rate, bowel preparation and withdrawal time), detection rates (cancer detection rate, adenoma detection rate [ADR], mean number of adenomas per procedure and per positive procedure), removal rates (polyp removal rate) and patient satisfaction (Gloucester comfort score). In colonoscopies in which no lesions were detected, withdrawal time was assessed.

**Results:** In total 431 endoscopists performed 237,092 first colonoscopies. In these colonoscopies, cecal intubation rate was 97.0%, bowel preparation was at least sufficient in 97.5% and withdrawal time was  $\geq 6$  minutes in 96.7%. CRC detection rate was 7.3% and ADR was 64.2%. Mean number of adenomas was 1.7 per procedure and 2.5 per positive procedure. In 96.4% of colonoscopies all polyps were removed. In 4.2% of procedures the patient experienced moderate or severe discomfort. Of all endoscopists, 401/431 (93.0%) performed  $\geq 50$  colonoscopies. In total 365 (365/401, 91.0%) endoscopists had an unadjusted cecal intubation rate  $\geq 95\%$ . Sufficient bowel preparation was achieved in  $\geq 90\%$  of patients for 390 endoscopists (390/401, 97.3%). Withdrawal time was  $\geq 6$  minutes in  $\geq 90\%$  of colonoscopies for 366 endoscopists (366/401, 91.3%). ADR was  $\geq 30\%$  for all endoscopists (401/401, 100%), moreover ADR was  $\geq 40\%$  for 399 endoscopists (399/401, 99.5%) and  $\geq 50\%$  for 396 endoscopists (396/401, 98.8%). In total 388 endoscopists (388/401, 96.8%) removed all polyps in  $\geq 90\%$  of the screening colonoscopies.

**Conclusion:** Colonoscopies, performed after a positive FIT in the Dutch CRC screening program, are of high quality. All minimum standards are met by over 90% of endoscopists. ADR is much higher than the current minimum standard, so the minimum standard for ADR should be increased to  $\geq 40\%$  for optimal quality assurance in FIT-based CRC screening programs.

## Endoscopic submucosal dissection of malignant non-pedunculated colorectal lesions: results from 2 western endoscopy centers

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**Background:** Endoscopic submucosal dissection (ESD) is a promising local resection technique for malignant non-pedunculated colorectal cancers with superficial invasion into the submucosa. The majority of the data on efficacy of ESD in removing such cancers comes however from Asian endoscopy centers. We evaluated clinical outcomes of intentional ESD for malignant non-pedunculated colorectal lesions at 2 high-volume Western endoscopy centers.

**Methods:** Medical records of all patients who underwent ESD for malignant non-pedunculated colorectal lesions between 2011 and 2019 at 2 Dutch academic hospitals, were reviewed. ESD was considered curative when no high-risk features (i.e. high-grade tumor budding, lymphovascular invasion, RI (< 0.1 mm) or Rx resection margins for the invasive part, invasion depth  $\geq$  Sm3 or poor differentiation) were found. **Results:** Among the 305 patients treated with ESD between 2011 and 2019, 99 had an invasive adenocarcinoma. Median size of these lesions was 30 mm (range 10-140). Most lesions were located in the rectum (46.5%), followed by the left-sided (43.4%) and right-sided colon (10.1%). Median ESD duration was 135 minutes (range 15-660). The immediate (micro)perforation rate was 7.1%. Three (3.0%) patients had a delayed perforation, of which 2 required emergency surgery. *En bloc* resection was achieved in 76 patients (76.8%): 12 ESDs were converted to piecemeal resection and 11 ESDs were terminated without macroscopically complete removal of the lesion due to signs of deep invasion. Of all *en bloc* ESDs, the resection margins for the invasive part were free in 54 cases (R0 resection rate: 71.1%). *En bloc* ESD was curative for 36 malignant lesions (47.4%). The other 40 patients were referred for additional surgical treatment, because of incomplete resection of the invasive part (12 cases), presence of high-risk features (16 cases) or both (12 cases). Of these 40 patients, 5 (2 incomplete resection of the invasive part, 3 presence of high-risk features) refused additional treatment. Among the 48 patients with data on follow-up after *en bloc* ESD (median follow-up time 11.5 months, range 1-52), no local recurrences were observed.

**Conclusion:** ESD seems to be a feasible resection technique for non-pedunculated malignant colorectal lesions in Western endoscopy centers. However, careful patient selection is warranted to increase the rate of curative ESDs.



## **Diagnosis and treatment of pancreatic duct disruption or disconnection: an international expert survey and case vignette study**

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**Background:** A disrupted or disconnected pancreatic duct is an often overlooked and potentially severe complication of necrotizing pancreatitis. There are currently no guidelines available to inform the evaluation and treatment of this condition. We aimed to evaluate current expert opinion regarding the diagnosis and treatment of pancreatic duct disruption and disconnection in patients with necrotizing pancreatitis to assist in developing future guidelines and help design prospective studies.

**Methods:** An online survey consisting of 6 general questions and 3 case vignettes was sent to 124 international expert pancreatologists. Experts were selected based on publications on pancreatic duct disruption and disconnection in the last 5 years, participation in the development of IAP/APA and ESGE guidelines on acute pancreatitis or the Dutch Pancreatitis Expert Panel. Consensus was defined as agreement by at least 80% of the respondents.

**Results:** The response rate was 35%; 20 surgeons (47%), 19 gastroenterologists (44%) and 4 radiologists (9%) responded. Of the respondents, 38 (88%) had over 10 years of experience in treating patients with necrotizing pancreatitis. Seventeen respondents (40%) always evaluate pancreatic duct integrity in patients with necrotizing pancreatitis, 12 (28%) usually, 11 (25%) sometimes and 3 respondents (7%) never. Thirty-five respondents (81%) prefer evaluation of a disrupted or disconnected pancreatic duct by MRI/MRCP over other imaging modalities. Endoscopic transluminal drainage is the preferred intervention in patients with infected necrotizing pancreatitis and a disrupted duct (37 respondents, 86%) or disconnected duct (39 respondents, 91%). When drained endoscopically, 15 respondents (35%) would prefer plastic pigtailed, 21 (49%) lumen-apposing metal stents and 7 (16%) no preference. In patients with persistent percutaneous drain production and duct disruption, 15 respondents (35%) would perform EUS-guided drainage to internalize the external drain, 13 (30%) endoscopic transpapillary drainage and 3 respondents (7%) upfront surgery.

**Conclusion:** This international survey demonstrated that MRI/MRCP is the preferred diagnostic modality and endoscopic transluminal drainage the preferred intervention for pancreatic duct disruption or disconnection following necrotizing pancreatitis. Consensus is lacking regarding when to look for a disrupted duct and regarding the treatment of patients with persistent percutaneous drain production.

## **Effectiveness and safety of laparoscopy-assisted transgastric endoscopic retrograde cholangiography in a large population of patients with roux-and-y gastric bypass**

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**Background:** Conventional endoscopic retrograde cholangiopancreatography (ERCP) is anatomically challenging in patients with a Roux-and-Y gastric bypass (RYGB). Laparoscopic-assisted transgastric endoscopic retrograde cholangiography (LAERC) is an alternative as it allows access to the biliary tree via the gastric remnant. We investigated the effectiveness and safety of LAERC in patients with a RYGB. **Methods:** We retrospectively reviewed all charts from RYGB patients who underwent a LAERC between January 2009 and August 2019 in a non-academic referral center for bariatric surgery. Patients who underwent pancreatic therapy were excluded. We collected demographic, clinical and outcome data. An adverse effect was defined as any complaint related to the LAERC up to 30 days after the procedure and graded according to the ASGE lexicon.

**Results:** We identified 100 LAERC in 86 patients with RYGB. Median age at LAERC was 54 years of whom 70% female. Simultaneous cholecystectomy was performed in 35 LAERC (35%). The therapeutic success rate was 95%. Stone extraction succeeded in 88.8% and sphincterotomy was performed in 96.7%. We identified 30 adverse effects regarding 28 procedures, whereof 8 endoscopy-related, 14 laparoscopy-related and 8 non-specified. In total, 6 severe adverse effects were seen concerning post-ERCP pancreatitis (n=2), laparoscopy-related hemorrhage (n=1), abscess (n=1), shock (n=1) and pneumonia (n=1). No patient died because of a LAERC-associated cause.

**Conclusion:** LAERC is a safe and effective approach for biliary diseases in patients with RYGB if performed by an experienced gastroenterologist.

**Recurrent neoplasia after endoscopic treatment for barrett's neoplasia is rare and random biopsies do not contribute to its detection: results from a nationwide cohort including all 1,154 patients treated in The Netherlands between 2008 and 2018.**

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**Background:** Radiofrequency ablation(RFA) +/- endoscopic resection(ER) is the standard of care for treatment of early neoplasia in Barrett's esophagus(BE). We report durability outcomes for all patients treated in The Netherlands (NL) from 2008-2018, with uniform treatment and follow-up(FU) in a centralized care setting.

**Methods:** Endoscopic therapy for BE neoplasia in NL is centralized in 9 expert centers with specifically trained endoscopists&pathologists. Uniformity is further ensured by a joint protocol and regular meetings. Treatment/FU data are registered in a uniform database.

Patients with low/high grade dysplasia (LGD/HGD) or low-risk adenocarcinoma (EAC) had visible lesions removed by ER, followed by RFA until complete remission of BE and intestinal metaplasia (CR-IM).

FU consisted of HD-endoscopy and was initially done every 3mo in year 1, followed by yearly endoscopies until year 5, then every 2-3 years. In 2015, FU endoscopies within year 1 were abandoned. Initially, 4Q-random biopsies(RBx) were obtained from neosquamous epithelium(NSE) and cardia at every FU endoscopy. These were abandoned in 2013 and 2016, resp.

**Results:** 1,154 patients with median BE length C2M4 and LGD(27%), HGD(31%) or EAC(42%) achieved CR-IM. Median FU was 4(IQR 2-6) years with 4 endoscopies per pt. 370 pts had FU >5yrs and 112 >8yrs. 2% was lost to follow-up. 1,114 (97%) pts had sustained complete remission of neoplasia(SCR-N). 38(3%) developed recurrent neoplasia (14 LGD; 7 HGD; 17 EAC), median 30mo after CR-IM. 33/38(87%) were successfully managed endoscopically, 5 (0.4% of all pts) progressed to advanced cancer (2 curative surgery, 3 developed metastasis). At baseline, these 5 pts were already identified as highly complicated due to multifocal HGD/EAC and/or severe reflux stenosis. Overall annual recurrence risk was 0.81%, with a relatively low risk within year 1(0.18%) and after year 5(0.37%). All HGD/EAC recurrences were detected in targeted Bx of endoscopically visible abnormalities. None of the 13,184 NSE RBx contributed to detection of recurrent neoplasia. 9,746 RBx from a normal cardia detected LGD in 9 pts (0.8%) and IM in 124(11%); none of which progressed to HGD/EAC.

**Conclusion:** In a setting of centralized BE care, the 2-step approach of ER and RFA has remarkably low rates of neoplastic recurrence after CR-IM. These recurrences are amendable for curative endoscopic treatment. Progression to advanced disease is rare and generally manifests as "out-of-protocol" cases at baseline. Our data support more lenient FU intervals, with emphasis on careful endoscopic inspection whilst RBx biopsies can be abandoned.

## Improving optical diagnosis of colorectal polyps using computer-aided diagnosis (cadx)

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**Background:** Optical diagnosis is the endoscopic prediction of histopathology of colorectal polyps detected at colonoscopy. Optical diagnosis remains challenging with accuracies of 71-90% in the Dutch bowel cancer screening program, exposing patients to risks of incorrect optical diagnosis. We propose a new methodology to improve the diagnostic accuracy of optically diagnosing colorectal polyps by Computer-Aided Diagnosis (CADx). Correct optical diagnosis may lead to: faster diagnosis, less complications, shorter duration of the colonoscopy and reduction in healthcare costs. We therefore examined the accuracy of CADx in comparison to experts and novices in optically diagnosing colorectal polyps.

**Methods:** We prospectively compared the optical diagnosis of colorectal polyps made by CADx with experts from the international BLI-expert group and Dutch novices. Images of colorectal polyps were conducted with White Light Endoscopy, Blue Light Imaging and Linked Color Imaging. The optical diagnosis was first made based on intuition, with a time limit of 30 seconds. After a washout period of four weeks, the same set of polyps was optically diagnosed based on a clinical classification model; BASIC (BLI Adenoma Serrated International Classification). The CADx algorithm classified colorectal polyps by exploiting machine learning.

**Results:** In total 60 colorectal polyps were included consisting of the following histopathology: hyperplastic polyp (n=15), adenoma (n=39), sessile serrated adenoma (n=4) and adenocarcinoma (n=2). Five experts, with a mean colonoscopy experience of 16.0 years and nine novices (mean 2.3 year) participated. The CADx algorithm was based on benign (hyperplastic polyps) versus premalignant (adenomas and sessile serrated adenomas). A subgroup analyses for experts and novices was performed to allow for an adequate comparison with CADx. The diagnostic accuracy of experts (81.0%) was significantly higher in comparison to novices (64.2%) based on BASIC and based on intuition (78.6% vs 63.8%, respectively). CADx had a significantly higher overall diagnostic accuracy of 93.8% ( $p < 0.001$ ). Sensitivity (91.7% vs. 62.7% and 52.6%) and specificity (100.0% vs. 95.3% and 93.3%) were also significantly higher for CADx compared to both experts and novices, respectively.

**Conclusion:** The clinical classification model BASIC increased the diagnostic accuracy of experts and novices compared to intuitive optical diagnosis. CADx diagnosed colorectal polyps significantly better in comparison to both experts and novices. These findings stress the need for further validation of CADx systems for future implementation into daily endoscopy practice.

## **Artificial intelligent algorithm detects barrett neoplasia with high diagnostic accuracy during live endoscopic procedures.**

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**Background:** Early neoplastic lesions in Barrett's esophagus (BE) are often subtle, focally distributed, and poorly visible endoscopically, resulting in a high miss rate by endoscopists. Artificial intelligent systems have the potential to overcome these limitations related to Barrett-surveillance. We aimed to evaluate the feasibility of a recently developed computer aided detection (CAD) algorithm for detection of Barrett neoplasia.

**Methods:** Our CAD algorithm (*de Groof et al. Gastroenterology; in press*) was assessed during endoscopic procedures of 10 patients with non-dysplastic Barrett's esophagus (NDBE) and 10 patients with confirmed Barrett neoplasia. At every 2-centimeter level of the Barrett's segment, three endoscopic images were obtained. These images were analyzed by the CAD system, providing instant feedback to the endoscopist. Ground truth of our endoscopic images was established by both expert assessment and corresponding histopathology using targeted biopsies or endomucosal resection. The CAD prediction was considered positive when at least 2/3 images surpassed the neoplasia threshold of 60%. Outcome measures were: 1) diagnostic performance of the CAD algorithm, defined as accuracy, sensitivity and specificity, assessed per-level and per-patient, 2) delineation performance of the CAD algorithm, and 3) consistency of three sequential CAD predictions.

**Results:** In total, 48 endoscopic levels were analyzed, of which 11 had a visible lesion and 37 contained no visible abnormalities. Diagnostic accuracy, sensitivity, and specificity of the CAD algorithm per level were 90% (43/48), 91% (10/11), and 89% (33/37), respectively. In a per-patient analyses, 90% (9/10) of the neoplastic patients were correctly diagnosed. The only lesion not detected by the CAD algorithm, showed NDBE in the endoscopic resection specimen. False positive predictions were present in 1 NDBE patient only, resulting in a specificity of 90% (9/10). The algorithm correctly localized and delineated 100% (9/9) of the true neoplastic lesions. In 75% (36/48) of all endoscopic levels, the CAD algorithm produced 3 concordant predictions (e.g. 3x NDBE or 3x neoplastic).

**Conclusion:** This is the first study to evaluate a CAD algorithm for Barrett neoplasia during live endoscopic procedures. The algorithm detected neoplasia with high accuracy, with only a small number of false-positive predictions, and with a high concordance rate between separate predictions. The CAD algorithm is thereby ready for testing in larger, multicenter trials.

## Patency of eus-guided gastroenterostomy in the treatment of malignant gastric outlet obstruction

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**Background:** Endoscopic ultrasonography-guided gastroenterostomy (EUS-GE) using a Lumen Apposing Metal Stent (LAMS), is a novel, minimally invasive technique in the palliative treatment of malignant gastric outlet obstruction (GOO). Several studies have demonstrated feasibility and safety of EUS-GE. However, evidence on long-term durability is limited. The aim of this study is to evaluate long-term patency of EUS-GE with a LAMS in the treatment of malignant GOO.

**Methods:** A multicenter international retrospective study was performed in seven centers from four European countries. Patients who underwent EUS-GE with a LAMS (Hot AXIOS™ stent) between March 2015 and March 2019 for palliative treatment of symptomatic malignant GOO were included. Primary endpoint was recurrent obstruction, secondary endpoints were technical success, clinical success, adverse events and survival.

**Results:** A total of 45 patients (48.9% male; mean age 69.9 ± 12.3 years) were included in this study. Median follow-up was 59 days (IQR 41-128). Recurrent obstruction after initial clinical success occurred in two patients (6.1%), after 33 and 283 days of follow-up. Technical success was achieved in 38 (84.4%) patients. Clinical success was achieved in 33 (73.3%) cases. Adverse events occurred in 12 (26.7%) patients, five (11.1%) of which were fatal.

**Conclusion:** Patency of EUS-GE seems good once performed successfully and relief of symptoms is durable with a low rate of recurrent obstruction. The relatively high number of fatal complications indicates a careful implementation in daily practice.

## Oral or intragastric delivery of the bitter tastant quinine does not influence food intake

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**Background:** Stimulation of taste receptors, which are expressed along the entire gastrointestinal tract, has been shown to influence food intake behavior. Previous studies demonstrated that intraduodenal infusion of tastants leads to increased satiety and reduced food intake, whereas intraileal infusions of tastants did not influence satiety or food intake. Currently, it is unknown whether oral or intragastric activation of taste receptors could induce a larger inhibitory effect on food intake. The aim of the present study is to investigate the effects of oral- versus intragastric administration of the bitter tastant quinine on food intake, satiety feelings, and heart rate variability (HRV).

**Methods:** Thirty-four healthy volunteers (24 female, age  $25.0 \pm 3.9$  years, BMI  $22.5 \pm 1.9$  Kg/m<sup>2</sup>) underwent four regimens with at least one-week washout in-between in a double-blind randomized fashion: oral placebo and intragastric placebo (OPGP), oral quinine and intragastric placebo (OQGP), oral placebo and intragastric quinine (OPGQ), and oral quinine and intragastric quinine (OQQQ). On test days, 150 minutes after a standardized breakfast, participants ingested a capsule containing quinine or placebo and were sham-fed a mixture of quinine or placebo orally. Fifty min after the intervention, participants received an *ad libitum* pasta meal to measure food intake. Visual analogue scales (VAS) for satiety feelings were collected and HRV measurements were performed over the course of the test day. **Results:** Oral and/or intragastric delivery of the bitter tastant quinine did not alter food intake compared with placebo (OPGP:  $782.4 \pm 31.5$  kcal, OQGP:  $734.4 \pm 31.6$  kcal, OPGQ:  $786.1 \pm 31.7$  kcal, OQQQ:  $765.8 \pm 31.8$  kcal,  $p = 0.69$ ). Intervention effects were found for desire to eat and hunger scores (respectively  $p < 0.01$ ,  $p < 0.01$ ), but not for satiety and fullness and HRV.

**Conclusion:** Oral or intragastric activation of bitter taste receptors decreased desire to eat and hunger scores, without influencing food intake, satiety and fullness scores, and HRV.

## Effect of elemental nutrition added to four-food elimination in adult eosinophilic esophagitis patients: preliminary analysis of a randomized controlled trial

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**Background:** Dietary therapies such as empiric elimination of common causative foods or elemental nutrition with amino acid-based formulas (AAF) are often prescribed for the management of eosinophilic esophagitis (EoE). However, an exclusive AAF diet is difficult to adhere to long-term, and elimination diets are not always effective. A combination of empiric elimination with AAF might improve adherence and therefore efficacy of dietary management.

**Aim:** To evaluate whether addition of AAF to a Four-Food-Elimination-Diet (FFED) is more effective than a standard FFED in decreasing Peak Eosinophil Counts (PEC), symptoms and endoscopic signs.

**Methods:** This prospective randomized controlled trial enrolled 38 (out of the 40 subjects in the original study design) adult patients with active EoE, defined as having symptoms related to esophageal dysfunction and  $\geq 15$  eosinophils per microscopic high-power field (HPF) on baseline biopsy. Patients were randomized to a standard FFED or FFED with addition of AAF (FFED+AAF) providing 30% of their daily energy needs. Histological disease activity, clinical response (Straumann Dysphagia Instrument patient-reported outcome (SDI-PRO)) and endoscopic signs (Endoscopic reference score (EREFS)) were measured at baseline and after 6 weeks of dietary intervention.

**Results:** Thirty-eight patients (62% male, mean age  $36.5 \pm 10.8$  years) were randomized to FFED (n=20) or FFED+AAF (n=18), 37 patients completed the diet. Complete histological remission ( $< 15$  eos/HPF) was achieved in 50% (9/18) of patients treated with FFED+AAF compared to 21% (4/17) with FFED,  $P=0.09$ . Median PEC decreased after FFED (63 interquartile range (IQR) 45-80) – 28 (IQR 20-50);  $P=0.011$ ) and FFED+AAF (50 (IQR 40-100) – 13.5 (IQR 3-31.5);  $P=0.002$ ). Trends signified an association between decrease in PEC and addition of AAF ( $P=0.103$ ). A significant reduction of the SDI-PRO scores was observed in both groups, FFED (5 (IQR 3.75-7) – 2 (IQR 0.75-4.25);  $P=0.001$ ) and FFED+AAF (5 (IQR 3-6) – 3 (IQR 0.75-3.25);  $P=0.006$ ), respectively. Change of SDI-PRO scores did not differ between both groups ( $P=0.768$ ). A significant reduction of the EREFS after FFED (4 (IQR 3-6) – 4 (IQR 1-4);  $P=0.001$ ) and FFED+AAF (5 (IQR 3-6) – 3.5 (IQR 1-4);  $P=0.005$ ) was observed. Change of the EREFS was similar in both groups ( $P=0.639$ ).

**Conclusion:** Although statistical significance was not reached ( $P=0.09$ ), these preliminary results suggests that AAF added to FFED results in a higher number of patients achieving complete histological remission. A possible association between decrease in PEC and addition of AAF ( $P=0.103$ ) was observed. SDI-PRO scores and EREFS showed no significant difference between both groups.



## Psychological well-being and distress among adult eoe patients

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**Background:** Data on psychological well-being and prevalence of psychological distress among adult eosinophilic esophagitis (EoE) patients are scarce. Also, the degree to which clinical and socio-demographic variables are related to symptoms of psychological distress is unknown.

**Methods:** Adult EoE patients were invited to complete standardized measures on quality of life (SF-36), anxiety and depressive symptoms (HADS), psychopathology (SCL-90-R) and coping strategies (UCL). All scores were compared to General Population (GP) norms, stratified by age and sex. Socio-demographic and clinical factors were assessed by means of a self-reported questionnaire. Factors with p-value < 0.2 in univariable analysis, or factors that were considered clinically relevant, were entered into multivariable logistic regression.

**Results:** In total, 124 adult EoE patients (63% males, mean age 42.2 ± 14.3 years) were included (*table 1*). 38/124 (31%) patients reported to have current or past mental problems, 18/38 (47%) patients felt that this was related to EoE. The SF-36 Mental Component Scale (MCS) was significantly lower compared to the GP (48.4 ± 9.9 vs. 50.2 ± 1.5; P = 0.049), whereas the Physical Component Scale (PCS) was similar (50 ± 8.9 vs. 51.4 ± 3.2; P = 0.101). No difference with GP values was found for total anxiety and depressive symptoms (7.5 ± 6.2 vs. 8.3 ± 0.22; P = 0.125). A total of 31/124 (25%) patients reported high levels of anxiety and/or depressive symptoms (HADS-A: 28/124 (22%) and HADS-D: 10/124 (8%) ≥ 8), indicative of a psychiatric disorder. Trends signified a possible association between high anxiety levels (HADS-A ≥ 8) and history of severe food impactions, multiple endoscopic interventions with food bolus extraction and younger age. In a multivariate analysis, age between 18-35 years was independently associated with anxiety (Odds Ratio (OR) 3.67 | 95 % Confidence Interval (CI) 1.4 – 9.8). SCL-90-R dimensions; somatization, obsessive compulsive, depression, hostility and sleeping disturbance were significantly affected compared to the GP (P < 0.001). High levels of somatization were reported in 53/119 (45%) patients, while coping styles of EoE patients actually showed significantly more active problem solving and less passive reaction compared to the GP (P < 0.001).

**Conclusion:** EoE has a pronounced impact on QoL and symptoms of anxiety and/or depression are prevalent among one quarter of the EoE patients. In the management of EoE a pro-active approach towards these symptoms seems warranted.

## **Pathophysiology of the inability to belch syndrome: observations made with prolonged esophageal pressure and impedance monitoring**

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**Background:** Symptoms of inability to belch are occasionally reported in gastrointestinal and otorhinolaryngological clinical practices. Although the phenomenon has previously been associated with dysfunction of the cricopharyngeal muscle, its underlying etiology is unclear. Esophageal and pharyngeal air transport patterns and the role of the upper esophageal sphincter (UES) have never been objectively investigated. The aim of this study was to evaluate pathophysiological mechanisms underlying symptoms of inability to belch using prolonged esophageal pressure and impedance monitoring.

**Methods:** Consecutive patients with symptoms of inability to belch were included. VAS scores were collected to evaluate esophageal symptoms. All patients underwent prolonged stationary esophageal high-resolution impedance manometry (HRIM) to assess UES and lower esophageal sphincter (LES) pressures and relationships with air transport patterns in the pharyngoesophageal region. Patients drank 500 mL of carbonated water to stimulate belching. A subsequent ambulatory pH-impedance study was performed to assess gas reflux patterns and esophageal air presence time over 24 hours. Statistics were presented as medians with range.

**Results:** We included five patients, age 24 (19–27) years. Bloating (100%; VAS 7.2 (6.4-9.1)) and gurgling noises from the throat (80%; VAS 7.5 (6.0-10.0)) were the most frequently reported symptoms. In 4 out of 5 patients, motility was classified as ineffective or absent (distal contractile integral 359.3 (28.1-390.2) mmHg×cm×s), and UES resting and residual relaxation pressures during wet swallows were normal in all cases (114.0 (71.7-154.0) mmHg and 2.7 (0-9.5) mmHg, respectively). After ingestion of the carbonated water, a median number of 20 (14-51) gas reflux episodes up to the level of the lower border of the UES were observed, but none resulted in UES opening. Most events (76%) were followed by a secondary peristaltic contraction. During 24-h pH impedance monitoring, patients reported 8 (5-174) symptom episodes of inability to belch. The majority of these episodes (85% (50-100)) were associated with gas reflux impedance patterns. Moreover, periods of continuous high impedance levels, indicating air entrapment, were observed in all patients. The 24-h esophageal air presence time was 8.1% (2.3-29.3). In all patients, the number of liquid reflux episodes and total acid exposure time were normal (15 (1–32) and 1.8% (0.7–2.6), respectively).

**Conclusion:** Prolonged HRIM recordings confirm the existence of a syndrome characterized by an inability to belch and support the hypothesis that ineffective UES relaxation, and consequent esophageal air entrapment, may lead to pharyngoesophageal symptoms.

## **Towards a visceral hypersensitivity-associated microbiome signature: analysis of a clinical cohort indicates, independent of intervention, baseline differences and compositional shifts related to visceral sensitivity changes**

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**Background:** The functional disorder Irritable Bowel Syndrome (IBS) is characterized by recurrent abdominal pain. Associations between increased sensitivity to colonic stimuli (visceral hypersensitivity) and altered luminal gut microbes has previously been shown. To evaluate longitudinal changes of the microbiome associated with visceral sensitivity, we examined the microbiota of hypersensitive IBS patients who participated in a probiotics-intervention randomized control trial (Ludidi et al, 2014). No differences in visceral sensitivity were observed after probiotic treatment as compared to placebo. However, visceral sensitivity decreased in a sub-set of patients. In this current study, we aim to define a microbial signature associated with a decrease of visceral sensitivity, independent on the intervention.

**Methods:** Sixteen hypersensitive Rome III IBS patients were selected for microbiome analysis (7 probiotics; 9 placebo), of which visceral sensitivity decreased in several patients after 6 weeks of treatment (3 vs. 5 respectively). Microbial DNA was isolated from frozen fecal sample scrapings, both at baseline and after treatment. DNA amplicons for bacteria-specific (16S) and fungi-specific (*Internal Transcribed Regions*, ITS-1) regions were sequenced. Microbial richness and compositional diversity were analyzed by employing linear mixed models (LME) and PERMANOVA on Bray-Curtis dissimilarity. Multilevel principal component analysis (PCA) was used to identify compositional shifts.

**Results:** Fungal diversity and composition could not be linked to improvement of visceral sensitivity. However, bacterial richness metrics are associated with the change from hypersensitive to normosensitive (FPD, linear mixed effect (LME);  $p=0.017$ ; Observed species,  $p=0.021$ ). Bacterial composition could not be related to visceral hypersensitivity while multilevel PCA did show a compositional shift associated with a decrease of visceral sensitivity (MANOVA;  $p=0.002$ ). This compositional shift was marked by a significant increase of *Akkermansia* (LME;  $p=0.007$ ) in patients that had reduced visceral sensitivity.

**Conclusion:** Taken together, our initial analysis indicated that transition from hyper- to normosensitivity associated with moderate diversity- and compositional changes of luminal bacteria. Low microbiome richness at baseline may be a predictor for improvement, independent of the intervention. The association between increased *Akkermansia* and reduced visceral sensitivity confirms earlier reports on inverse correlations of this mucus associated bacterium with abdominal pain. Future analyses will focus on differently abundant species or a consortium of microbes as predictors of decreasing of visceral sensitivity.

## **Assessment of small bowel motility in chronic intestinal pseudo-obstruction using caloric stimulation and cine-mri**

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**Background:** Chronic intestinal pseudo-obstruction (CIPO) is a rare and severe digestive disorder, characterized by failure of intestinal motility. MRI has emerged as a non-invasive method for evaluating bowel motility. This study aimed to gain insight in fasted and fed small bowel motility in CIPO patients using cine-MRI.

**Methods:** Eight CIPO patients underwent a cine-MRI protocol after an overnight fast, comprising fasting state scans and subsequently postprandial scans after orally ingesting a small-volume, high-caloric-density, test meal (Nutridrink, 300 kcal). Small bowel motility was visually scored by an experienced abdominal radiologist as well as quantified by a validated post-processing technique (GIQuant, Motilent, UK) with a new edge-detection technique to account for dilated bowel, resulting in a motility score in arbitrary units (AU). Motility scores were compared with 16 healthy volunteers that previously underwent a similar cine-MRI protocol.

**Results:** Visually, small bowel motility was increased in four out of eight CIPO patients, six showed distended bowels filled with air and/or intestinal content. Motility quantitation demonstrated a median fasted small bowel motility of 0.21 AU (IQR 0.15-0.30) and directly after intake of the meal 0.23 AU (IQR 0.15-0.27). In healthy volunteers, corresponding fasted and fed motility were 0.18 AU (IQR 0.14-0.24) and 0.25 AU (IQR 0.20-0.29), respectively.

**Conclusion:** Surprisingly, we found hyperactive small bowel motility in half of the CIPO patients, suggestive of uncoordinated intestinal motility. Quantitation showed a wide variation in motility patterns, with both higher and lower motility than in healthy subjects, and an absence of postprandial activation. Dynamic MRI helps to gain insight in this complex disease and can potentially impact treatment decisions in the future.

## Overall Health, Daily Functioning, and Quality of Life in Acute Hepatic Porphyria Patients: ENVISION, a Phase 3 Global, Multicenter, Randomized, Double-Blind, Placebo-Controlled Trial

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**Introduction:** Acute Hepatic Porphyria (AHP) is a family of rare genetic diseases leading to an enzyme deficiency in the heme biosynthesis pathway, causing accumulation of neurotoxic heme intermediates, resulting in neurovisceral attacks and chronic manifestations. Givosiran, an investigational RNAi therapeutic, is being evaluated for its ability to reduce the levels of neurotoxic intermediates thus decreasing attacks and disease manifestations.

**Aim:** ENVISION (NCT03338816), a Phase 3 global, multicenter, randomized, double-blind, placebo-controlled trial, evaluated the efficacy and safety of givosiran in AHP.

**Methods:** The primary endpoint was composite annualized attacks over six months. Secondary endpoints included worst daily pain, and the QoL Physical Component Summary, Short Form-12 (PCS SF-12). Exploratory endpoints included EuroQoL Visual analog scale (EQ-VAS), Patient Global Impression of Change (PGIC), Porphyria Patient Experience Questionnaire (PPEQ), and missed days of work.

**Results:** Ninety-four AHP patients enrolled. Givosiran significantly reduced composite attacks relative to placebo ( $p=6.04 \times 10^{-9}$ ), as well as the cardinal symptom of pain ( $p=0.0493$ ). Givosiran led to greater change in PCS SF-12 scores from baseline (givosiran=5.4; placebo=1.4,  $p=0.0216$ ), and to higher change in EQ-VAS scores (givosiran=5.2; placebo=-1.3). More givosiran patients (89%) reported greater improvements in overall health since study-start, as measured by PGIC, than placebo (37%). Givosiran led to greater improvement in PPEQ (traveling, social activities, planning future events, household chores, exercise, and treatment satisfaction) and fewer missed work days, compared to placebo.

**Conclusions:** In a Phase 3 study, givosiran treatment resulted in clinically meaningful efficacy and marked improvements in AHP patients' overall health status, daily functioning, and quality of life.