Abstracts of Invited Lectures
Poster Abstracts

Symposium 212

IBD AND LIVER:
EAST MEETS WEST

Kyoto, Japan
September 7 – 8, 2018

Scientific Organization:
T. Hibi, Tokyo (Japan)

Scientific Co-Organization:
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M. Imawari, Kanagawa (Japan)
G. Rogler, Zurich (Switzerland)
H. Tilg, Innsbruck (Austria)
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156. Development of hepatocellular carcinoma in patients infected with hepatitis B virus genotype B or C in Japan

157. Is zinc effective for the prediction and the treatment of hepatic fibrosis in patients with autoimmune hepatitis?

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162. Linker phosphorylation of Smad 3 promotes fibro-carcinogenesis in non-alcoholic steatohepatitis of hepatocellular carcinoma

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164. Effects of a SGLT2 inhibitor on growth and metabolisms on hepatocellular carcinoma
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165. ALA improves mitochondrial function and prevents lipid accumulation, oxidative stress, and diet-induced steatohepatitis
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166. Acute liver failure due to HBV reactivation associated with immunosuppressive or anticancer therapies in Japan (2010–2016)
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167. Accuracy of selected CCL and CXCL chemokines in the assessment of patients with alcoholic liver disease
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168.* Combination effect of canagliflozin and exercise training in non-alcoholic fatty liver disease
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169. The optimal exercise regimen for patients with NAFLD: Aerobic or resistance exercise?
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170. DPP-4 inhibitor suppresses the progression of hepatocellular carcinoma through activation of chemotaxis of NK cells in mice
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171. Evaluation of ballooned hepatocytes as a risk factor for future progression of fibrosis in patients with non-alcoholic fatty liver disease
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172. Allogenic transplantation of MUSE cell administration ameliorates liver function in pig models of chronic liver injury

173.* Clinical management of primary sclerosing cholangitis in Japan 2017
174. A multicentre prospective study: Ballooning biomarker in patients with non-alcoholic fatty liver disease

175. Systemic profile of neutrophil-derived mediators and its association with the severity of alcoholic liver disease

176.* Characteristics and risk factors of fatty liver disease development and non-alcoholic steatohepatitis recurrence following liver transplantation
K. Tokodai, A. Karadagi, F. Kjaernet, A. Romano, B.G. Ericzon, G. Nowak (Stockholm, SE)

177. Promising antiviral combination therapy targeting HBV eradication with a novel compound derived from spice

178.* Relevance of FXR-p62/SQSTM1 pathway for survival and protection of mouse hepatocytes and liver with steatosis
M. Ozaki, S. Haga (Sapporo, JP)

179. Effective biomarkers for advance fibrosis NASH and reflect biomarkers as changes of liver fibrosis with NASH

180. Beneficial effects of agomelatine on obesity associated liver inflammation in mice

* = Posters of Distinction
Regeneration of intestinal epithelial cells and IBD

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Recent studies have expanded our knowledge of gastrointestinal stem cell biology. We have been studying colonic epithelial stem cells (Nat Med 2002, Gastroenterology 2005 & 2007). In the series of our research, we developed a novel culture method that maintains colonic stem cells in vitro. The crypt cells formed a round cystic structure consisting of epithelial monolayer of multilineage cells and could be propagated without losing their properties. Importantly, expression of Lgr5 was significantly up regulated and then constantly maintained for a long time period. Moreover, successful, long-term engraftment was observed even with the transplantation of organoids that were derived from a single Lgr5 colon stem cell after extensive in vitro expansion in mice (Nat Med 2012). Transplanted cells readily integrated into the colonic tissues covering the area that lacked epithelium, and accelerated the recovery of recipients from acute colitis. Donor-derived cells constituted single-layered epithelium forming self-renewing donor-derived crypts that were functionally and histologically normal. We also showed that cultured cells derived from fetal gut-derived cells (Cell Stem Cell 2013) and small intestinal stem cells (Genes Dev 2014) can be transplanted in colonic tissues as stem cells. Also, our recent data have shown that our collagen-based organoid culture could faithfully recapitulate the regenerative-phase mucosal environment and induce reprogramming of cells into a primitive state (Cell Stem Cell 2018). We developed our original method for human colonic epithelial stem cell culture from normal and IBD patients. Our data for the first time demonstrate that tissue stem cell therapy by in vitro expansion and transplantation of gastrointestinal stem cells could be an option for patients with severe IBD patients in humans. A new way for the treatment of ulcerative colitis (UC) by colonic epithelial stem cell organoids has been developed for 6 years, under the support of a 10-year grant from the Japan Agency for Medical Research and Development. After extensive studies, we have established a method to generate patient-derived clinical-grade (GMP-grade) organoids. These clinical-grade organoids are validated to be pathogen-free, and also free of tumorigenicity. Also, targeted delivery of those organoids by using GI endoscopes has been established. Depending on these new technologies, we are planning a first-in-human study (FIH study) for UC patients, and have already gone through several approval steps such as those of RB in our university hospital and Ministry of Health, Labor and Welfare.
Session I

Optimization of clinical practice in IBD: What can we learn from the differences between Asian and Western countries?
Epidemiology – Trend in inflammatory bowel disease in Asia

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The changing epidemiology of inflammatory bowel disease (IBD) across time and geography suggests that environmental factors play a major role in modifying disease expression. As the incidence and prevalence of IBD may have stabilized in high-incidence areas such as North America and northern Europe, they continue to rise in previously low-incidence areas such as southern and eastern Europe, Asia, and much of the developing world. In these countries, ulcerative colitis (UC) has emerged first followed by Crohn’s disease (CD) after a variable period of time. Improved physician awareness and diagnostic methods are unlikely to fully account for this rapid increase. At the turn of the 21st century, the evolution of IBD has spread globally with newly industrialized countries in Asia, Africa, and South America reporting rapidly rising incidence. For example, the incidence of Crohn’s disease and ulcerative colitis rose by 14% and 10%, respectively in Seoul, South Korea from 1991 to 2005. The explanation for the dramatic rise in the incidence of IBD outside the Western world is multifactorial including increase awareness of IBD, advances in healthcare infrastructure and improved access to healthcare, development of disease surveillance systems to track incidence of IBD, and exposure to environmental risk factors of IBD associated with westernization of a newly industrialized society. Nonetheless, IBD, today, is a global disease. This epidemiological shift likely relates to increased contact with the West, changes in diet, increasing antibiotics use, improved hygiene, vaccinations, or changes in the gut microbiota, as part of the socioeconomic development in Asia. Genetic factors for IBD differ between Asia and the West. NOD2/CARD15 mutation, repeatedly observed in the Caucasian populations, is not associated with CD in Asian populations. There is a male predominance of CD in Asia, but a trend towards equal sex distribution for UC. A positive family history is much less common than in the West, as are extra-intestinal disease manifestations. These differences may relate to time factor, genetic background and environmental factors. There are clear ethnic differences in incidence within countries in Asia, and an increased incidence in IBD in migrants from Asia to the West. Research in Asia, an area of rapidly changing IBD epidemiology, may lead to the discovery of critical etiologic factors that lead to the development of IBD.

References:


The best way of using anti-TNF and immunomodulators considering the Asian-specific situation

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The efficacy and safety profiles of simultaneous thiopurine use for inflammatory bowel disease (IBD) have been rigorously investigated in various clinical trials and cohort studies. It has become evident that a combination of infliximab and thiopurine is superior to infliximab monotherapy for the treatment of Crohn’s disease. However, the profiles in Asian population are different from those of Western population. It has also become evident that in Asian population NUDT 15 gene is closely associated with severe adverse events related to thiopurines. We previously attempted a prospective, randomized, multicenter, open-labeled trial evaluating the efficacy of adalimumab with and without azathioprine in Japanese patients with Crohn’s disease. An intention-to-treat analysis with non-responder imputation demonstrated that the remission rate at 26 weeks was not significantly different between the monotherapy group and the combination group (71.8% vs. 68.1%; OR = 0.84, p = 0.63). The rate of endoscopic improvement at 26 weeks was significantly higher in the combination group (84.2%) than in the monotherapy group (63.8%) (p = 0.019). Withdrawal from the study was more frequent in the combination group (16.5%) than in the monotherapy group (16.5% vs. 2.4%, p < 0.01). Pharmacokinetic analyses revealed trends towards higher trough levels of adalimumab and lower prevalence of antibody to adalimumab in the combination group than in the monotherapy group. These findings suggest that simultaneous use of thiopurine has marginal efficacy for the management of Crohn’s disease treated by adalimumab, and that prediction of adverse events related to thiopurine is inevitable for Asian IBD patients.
New strategy in the surveillance colonoscopy for colitic cancer

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There is a growing awareness of the importance of detecting colitis-associated cancer in inflammatory bowel diseases. With the advent of new agents for the treatment of inflammatory bowel diseases, colectomy can be avoided in a subset of cases with severe or fulminant ulcerative colitis (UC) in which colectomy was previously considered to be inevitable. As a result, the relative proportion of cases with neoplasia among the colectomized patients is higher than before. The incidence of neoplasia excluding sporadic adenoma in patients with UC was 12.1% and 21.8% at 20 and 30 years, respectively, after the onset of UC in our surveillance cohort, whereas that of invasive colorectal cancer was 3.2% and 5.2%. These results are almost comparable to those reported for western surveillance cohorts.

According to European guidelines, it was usually recommended to perform more than 33 random biopsies, but recently targeted biopsy under chromoendoscopy is gaining attention. However, there had been no randomized controlled trial (RCT) directly comparing the two approaches, until we conducted an RCT comparing the targeted and random biopsy approaches. The mean number of biopsies with neoplasia per colonoscopy was the primary endpoint of this RCT. The detection rate of neoplastic lesions found to contain neoplastic tissue per colonoscopy was 0.211 (24 of 114) in the target group and 0.168 (18 of 107) in the random group, which was considered to be comparable. More biopsies were obtained from the random biopsy group (34.8 specimens), which were examined for a longer time (41.7 minutes), in contrast to that in the targeted biopsy group (3.1 specimens, 26.6 minutes), and these differences were statistically significant (p < 0.001). Moreover, the results suggest that random biopsies from areas without inflammation can be omitted, because none of the biopsy samples from areas without any signs of present or past inflammation showed signs of neoplastic tissues in this RCT.

In this presentation, the current status of surveillance colonoscopy for colitic cancer, including new strategies such as magnifying colonoscopy and image enhanced endoscopy will also be presented.
Session II

Personalized health care in special situations in IBD
Very early onset IBD

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Advancements in human genetics now poise the field to illuminate the pathophysiology of complex genetic disease and guide the development of mechanism-targeted treatment strategies. Collective discoveries from genome wide association studies, fine mapping, and exome sequencing have generated novel insights into the mechanisms driving inflammatory bowel disease (IBD) and implicated common sets of genes shared by multiple immune pathologies. Although several autoimmune diseases share overlapping genetic risk factors with IBD, each disease is associated with a unique genetic risk profile. Moreover, emerging genetic evidence indicates that IBD can be further classified into clinical subtypes with distinct genetic risk profiles. For example, very early onset IBD is associated with polymorphisms in $IL10$ and/or its receptor. Thus, a subset of severe IBD (phenotype: very early onset) is associated with a distinct genetic risk profile (genotype: IL10 pathway polymorphisms) and mechanistic underpinnings that precipitate loss of tolerance to commensal microbes (mechanism: IL10-mediated dyshomeostasis). In this mechanistic subtype of IBD, insights from genetics and functional genomics suggest a pathway-focused therapeutic approach to re-establish immune tolerance by restoring function in the IL10 pathway.

We will discuss this and additional examples of “pathway medicine” wherein human genetics and functional genomics implicate cellular and molecular pathways underlying IBD subtypes and guide the development of mechanism-targeted therapeutics.
IBD in elderly patients

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As the incidence of IBD increases in the developing world the number of newly diagnosed elderly patients is increasing. Further, with improved health standards in developing countries where IBD is emerging and with an ongoing relatively high burden of IBD in western countries, there is an increasing prevalence of elderly with IBD worldwide. Middle aged populations initiated on biological therapy up to twenty years ago will be elderly now and still on their biological therapy. The elderly pose different challenges in terms of tolerance of risks posed by different immunomodulating drugs. This stems from senescence of the immune system, changes in pharmacometabolism and the increased burden from comorbid illnesses. The elderly has less aggressive disease with less progression from inflammatory to penetrating or fibrotic disease in Crohn’s disease and less progression to pancolitis or need for colectomy over time in UC. Nonetheless, when they do need biologic therapy they are at greater risk for cancer and infection. When they do undergo colectomy they are at greater risk of postoperative death. Despite the fears of serious infection in the elderly, when biological therapy is indicated, untreated IBD may pose as much, if not more of a health threat. Among biological therapies, vedolizumab and soon other integrin blocking therapies, provide very safe options with less systemic immune effects. With no evidence that combination therapy enhances outcomes with integrin blockers, these may be relatively simple and safe choices in the elderly with IBD. On the other hand, despite the proven efficacy of combination therapy with anti-TNF use, to enhance the safety of anti-TNF therapy in the elderly, monotherapy may be the best approach. For the elderly with penetrating Crohn’s disease anti-TNF therapy may still be the first choice, just as in younger patients. Considering its relatively short availability on the market for Crohn’s disease there is less known as to the long term safety of ustekinumab in the elderly but it may present a less lifestyle-intrusive approach. Finally, with tofacitinib on the market, the interest in oral therapy will undoubtedly drive its use. The increased risk of herpes zoster infections with tofacitinib may pose an added challenge for the elderly who are already at increased risk for this infection. Hence, clinicians must be extra vigilant with supportive health care in the elderly with IBD who require immunomodulating therapy. This means vigilance with annual influenza vaccines, pneumococcal vaccines and herpes zoster vaccines. With the recent availability of a non live herpes zoster vaccine, more liberal use of it despite ongoing immunomodulating therapy may be warranted. Vigilance with Pap smears in females and regular skin surveillance for cancers especially if skin cancers had previously been identified is important. Finally, for the elderly with several years of biological therapy, with several years of deep remission, and with an increasingly senescent immune system withdrawal of biological therapy should also be seriously considered.
Pregnancy in inflammatory bowel disease

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Key points:
• Women with IBD in remission have the same chance of conceiving as women without IBD unless pelvic surgery
• Once pregnant, increased risk of adverse outcomes so follow as high-risk obstetric patient
• Majority of medications low risk for use during pregnancy and compatible with lactation
• Multi-disciplinary approach involving the gastroenterologist, obstetrician and pediatrician is needed to provide optimal care

Preparing for pregnancy:
• Prior to conception, women should be in a durable remission on stable maintenance medications. Confirm by colonoscopy, fecal calprotectin, etc.
• Laboratory testing to evaluate for correctable deficiencies
• Healthcare maintenance should be up to date:
  o Surveillance colonoscopy
  o Cervical cancer screening
  o Vaccinations

Fertility:
• Women with IBD have similar fertility to age-matched women without IBD
• Fertility affected by:
  o Prior pelvic surgery, particularly ileal pouch-anal anastomosis (IPAA)
  o Active inflammation
• IBD patient with difficulty conceiving after 6 months, refer to infertility specialist

Inflammatory bowel disease during pregnancy:
• Women with UC more likely to flare in pregnancy than Crohn's disease
• No post-partum increase in flares
• Higher rates of complications of pregnancy, follow as high risk obstetric patients.
• All pregnant women with IBD are at increased risk for:
  o Preterm birth
  o Small size for gestational age
  o Miscarriage
  o Complications of labor and delivery including pre-eclampsia and thromboembolism

Labor and delivery:
• Mode of delivery at discretion of obstetrician
• Cesarean section considered in patients with:
  o Active perianal disease
  o Ileal pouch-anal anastomosis
**Medication use during pregnancy:**
Stopping medications can lead to significant risk of flare which is more harmful overall to pregnancy and impairs mother’s ability to care for child after delivery.

**Aminosalicylates:**
- Compatible with lactation
- Caveats:
  - Avoid dibutyl phthalate
  - Sulfasalazine given with folic acid 2 mg daily

**Corticosteroids:**
- Compatible with lactation
- Low risk for birth defects
- Increased risk of:
  - Gestational diabetes, macrosomia
  - Preterm birth

**Methotrexate:**
- Contraindicated - known teratogen
- Discontinue 3-6 months prior to attempting conception

**Azathioprine/6-Mercaptopurine:**
- Considered low risk
- Compatible with lactation

**Biologics/Monoclonal Antibodies:**
- Compatible with Lactation
- Infliximab, adalimumab, golimumab, vedolizumab, and ustekinumab (IgG1 antibodies) actively cross the placenta in the 2nd and 3rd trimester
- Certolizumab only passively crosses
- Continue biologics through pregnancy with last dose timed for one interval prior to delivery i.e. q 8 weeks given 8 weeks prior to due date

**Minimizing risks to infants exposed to biologics in utero:**
- No documented association with biologic exposure in utero and increase in infections or immune system dysfunction.
- Infants exposed in utero to biologics should not receive live vaccine for the first 6 months of life.
- All other standard vaccines can be given on schedule

**Post-partum dosing:**
Resume dosing after delivery if interval appropriate and no evidence of infection:

**Tofacitinib:**
- Limited human data
Gender issues in inflammatory bowel disease

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**Background:** Inflammatory bowel disease (IBD) is characterized by chronic inflammation of the gastrointestinal tract. It comprises two distinct entities, Crohn’s disease (CD) and ulcerative colitis (UC), which differ in type, site and extent of inflammation, disease history and extraintestinal manifestations. The pathogenetic mechanism is not fully understood but the inflammation characteristic for the disease is thought to result from a chronic up-regulation of the enteric mucosal immune system. Data from clinical epidemiological studies indicate the importance of gender-related differences in the clinical course of the disease as well as in the underlying pathogenesis.

**Clinical Aspects of Gender-Related Issues in IBD**

*Quality of life*

IBD course is characterized by flare-ups and remissions and no existing therapeutic approach can cure the disease. The improvement of the quality of life represents a primary goal for therapeutic strategies. It has been shown previously that female gender in CD is predictive for a reduction of quality of life (1). Studies analyzing the quality of life in IBD patients demonstrated a lower quality of life during the use of immunosuppressants, hospitalization and disease relapse (1–4) but none of these studies focused on exclusive female health care problems, such as effect of the disease on the menstrual cycle, fertility and pregnancy. Results of a survey we conducted in 1067 Dutch patients (CD/UC 617/450, 703 females and 364 males) demonstrated distinct concerns of females and males in terms of body image, health related and quality of life related issues (5). Thus, it is presumable that the results of quality of life studies in general cannot be extrapolated to female patients and the development of female-specific quality of life assessment is of extreme importance for daily practice.

*Natural history and phenotype of the disease*

The possibility to predict disease evolution would be helpful in determining the right strategy for a particular patient. Previous studies demonstrated that initial steroid use, smoking, age under 40 years and perianal disease are predictive factors of complicated disease course (6–8). Interestingly, reports focused on IBD genetics suggest that there might be a sex-linked genetic background in (9–12) and it has been shown that particular genetic background correlates with disease phenotype. Indeed, it has been shown that uveitis, skin lesions and lower bone density occur more often in female patients (13–15). Identifying phenotypes of female IBD and determining their predictive value for a complicated course of the disease will be necessary for therapy optimization.

*Gender-related therapy outcomes and side effects*

It has been shown that the sex of the patient can have profound influences on drug metabolism and efficacy. For instance, in cardiovascular disease, the commonly used
beta-blocker metoprolol has significantly higher drug concentrations in women than in men (16). These differences are likely caused by sex-specific differences in body composition (e.g., proportion of body fat) and drug metabolism (e.g., cytochrome P450 [CYP] enzyme activity). Although a biological agent such as adalimumab (ADA) is not metabolized by CYP enzymes, sex-specific differences have been seen with biological drugs in other fields of medicine. Specifically, on a pharmacokinetic level several oncological-biological agents such as bevacizumab, cetuximab, and rituximab are cleared at different speeds between men and women (17). Although the clinical implications of these pharmacokinetic differences are unclear, these differences again probably result from differences in body composition.

In rheumatology female sex was found to be a significant negative predictor for longer ADA drug survival (i.e., the continued use of ADA). Particularly, lower drug survival was seen in women compared with men with arthritic psoriasis and rheumatoid arthritis (18, 19). In a prospective CD cohort, we studied possible sex differences in the outcome to ADA treatment (20). At baseline, several differences already existed, chief among them a difference in previous therapies. In addition, we observed several sex differences concerning response, primarily a greater proportion of treatment in male patients than in female patients, of 48.1% and 30.8% respectively. Survival analysis in this cohort also underscored the effect of sex on ongoing ADA treatment, along with baseline disease activity. Furthermore, we found that female patients report more side effects and also cease ADA treatment more often due to side effects than male patients. This difference in side effects was illustrated by a retrospective study where sex differences in the frequency and types of adverse drug reactions (ADR) to immune suppressive medication in IBD patients were studied (21). In contrast to thiopurines and methotrexate with similar rates of ADR in both sexes, a sex dimorphic profile of ADR to anti-TNF agents with higher frequency of ADR among female IBD patients compared with male patients was observed. With regard to particular types of ADRs, females experienced more often allergic reactions to the most frequently used anti-TNF agents, IFX and ADA. In addition, these ADR have led to discontinuation of treatment more frequently in females than males, thus substantially limiting the long term use of anti-TNF agents by female patients.

Gender and IBD Pathogenesis

Sex-linked genetic susceptibility to IBD

Several findings provide arguments for existence of sex-linked susceptibility in IBD. CD is more frequent in women than in men. Furthermore, there are indications of pertinent imprinting in IBD with predominance of mother/child affected pairs (22) and studies provided evidence for suggestive IBD susceptibility locus on the chromosome X. The evidence for sex-linked susceptibility for IBD is however limited. In a Dutch familial IBD cohort, female predominance as a feature characteristic for familial inflammatory bowel disease was observed. In CD, the female predominance may be related to the imprinting of the disease predisposition with a specific female to female transmission pattern. This transmission pattern suggests existence of female-specific modifier of genetic predisposition for the disease. The epigenetic female transmission of CD is a major contributing factor to the familial presentation of disease and might explain the epidemiologically-detected female bias in the risk for contracting IBD (23).
The role of sex hormones in the disease pathogenesis

In IBD female patients, it has been demonstrated that women experience fluctuations in their gastrointestinal symptoms across the menstrual cycle, and have higher level of IBD symptom severity than men (24–29).

References:


Session III

Optimization of IBD treatment
Predictors of low adherence to therapy in patients with IBD

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For most patients with inflammatory bowel disease (IBD) medical treatment is fundamental for inducing and maintaining remission, preventing flares and reducing the risk of colorectal cancer. Non-adherence is defined as a deviation from the mutually agreed treatment plan and may affect patients’ quality of life resulting in unfavourable treatment outcomes, more hospitalizations and higher healthcare-related costs. Recognising and improving adherence is therefore a primary aim for the treatment of IBD.

Non-adherence is notoriously difficult to detect in routine clinical encounters but can be objectively assessed by drug metabolite levels, self-report scales, and prescription data. The rates of non-adherence vary wildly depending on the studied cohorts but for most settings range from 30–45%. Non-adherence to mesalazine and immune-modulators occurs more frequently than non-adherence to biological agents.

Predictors of non-adherence can be divided into non-modifiable factors (age, sex, marital status, disease type, etc) and modifiable factors (frequency of drug administration, anxiety & depression, patient knowledge, patient views on medication and side effects). While the former can help identify non-adherent patients the latter form the basis for potential intervention to improve adherence. I will discuss the evidence with a focus on patients’ beliefs in necessity and concerns about potential side effects as these have been consistently associated with non-adherence across many studies. I will review the current evidence on interventions to improve adherence and the gaps in evidence, which should inform future studies.
Risk management in thiopurine therapy

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Thiopurines are a group of immune modulators that includes azathioprine (AZA), 6-mercaptopurine (MP), and thioguanine (TG). These drugs are used either to maintain remission in inflammatory bowel diseases (IBD) patients, or as co-therapy along with biological treatment to reduce immunogenicity. These clinical benefits have been shown in selected groups of patients in multiple studies and a Cochrane review. Additional benefits of thiopurines in daily practice include the decades of clinical experience, relatively low costs, the option of measuring metabolites to optimize therapy, and the lack of immunogenicity.

Unfortunately, over half of thiopurine-treated IBD patients discontinue treatment within two years. This is frequently related to the therapy’s safety profile. For example, bone marrow depression resulting in leucopenia is a well-known complication. Strategies to assess this risk include measuring 6TGN levels and thiopurine S-methyltransferase (TPMT). Genetic variants of NUDT15, found in both Asian and European cohorts, may identify individuals predisposed to leucopenia.

TG therapy but also conventional thiopurines are associated with nodular regenerative hyperplasia of the liver (NRH). This risk was initially identified in patients on high doses of TG (≥ 40 mg daily). Current risk estimates for lower doses of TG appear reassuring. New data on the clinical cases of thiopurine-induced NRH and related non-cirrhotic portal hypertension show that this condition is partially reversible following discontinuation of therapy.

Thiopurines reduce the risk of colorectal cancer but are also associated with an increased risk for other types of cancer. The risk of various types of lymphomas is increased during thiopurine therapy, including the post-transplant-like, post-mononucleosis, and hepato-splenic T cell lymphoma. Strategies to reduce this risk may include assessment of EBV serology and discontinuation of thiopurines in high-risk patients. Additionally, the risk of basal cell carcinoma and squamous cell carcinoma (non-melanoma skin cancer) is significantly increased in IBD patients on long-term thiopurine therapy. Regular dermatological screening and sun protection should be recommended as preventative measures. Finally, recent data indicates an increased risk of urinary tract cancer in thiopurine-treated IBD patients. This risk occurred predominantly in older (> 65 years) patients.

Prior to initiating thiopurine therapy, physicians and patients should engage in a discussion to weigh the clinical benefits against drug safety. Age, gender, comorbidities, IBD characteristics, available alternative therapies, as well as the patients’ view on benefit vs risk should be taken into account in order to come to a shared decision for an individualized IBD treatment strategy.
Therapeutic drug monitoring (TDM): Its role in anti-TNF therapy

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In the last decades, several pharmacological test have become available, including monitoring of serum levels of monoclonal antibodies such as the anti-tumor necrosis factor (anti-TNF) agents, infliximab, adalimumab and golimumab. Furthermore, different assays became available to measure anti-drug antibodies. Although these tests are commercially available, their implementation in daily clinical practice is still variable, due to costs and varying availability, and partly also due to ignorance. Based on three clinical scenario’s we aim to show the clear added value of measuring TNF serum levels and anti-drug antibodies in daily clinical practice.

Reactive therapeutic drug monitoring

In a patient with secondary loss of response to biological therapy, objectifying disease activity by measuring C-reactive protein and/or fecal calprotectin, performing endoscopy or transmural imaging has been well implemented. However, these analyses will only confirm disease activity but will not be sufficient to guide therapeutic management. In contrast, therapeutic drug monitoring will provide a clear algorithm to decide on unaltered treatment continuation, treatment optimization, switching of therapy within class, or switching of therapy out of class (see Table underneath). Treatment optimization can be achieved by increasing the dose of the biological agent, decreasing the interval between two doses, or adding an immunosuppressive agents. However, before making a therapeutic decision one should always check adherence to treatment, especially for subcutaneous therapies.

Proactive therapeutic drug monitoring

The use of therapeutic drug monitoring in a patient in clinical remission is still debated. Although the primary endpoint was not met in the TAXIT trial (Vande Casteele, Gastroenterology 2015), the pre-randomization phase showed that less than half of the patient with durable response to infliximab had a serum level within the therapeutic range. One quarter had sub-therapeutic serum levels (9% even undetectable) and benefited treatment optimization, while another quarter had supra-therapeutic serum levels and could be dose de-escalated without a risk of clinical deterioration. Furthermore, patients randomized to the serum level guided group experienced clinical relapse significantly less frequent than patient randomized to the clinical guided group.

Retrospective studies have shown a clear association between anti-TNF serum levels after induction and long-term outcome. However, the prospective TAILORIX study failed to show a clear benefit of infliximab optimization based on infliximab serum levels (D’Haens, ECCO Congress 2016). Based on the currently available data, a yearly measurement of drug serum levels in an asymptomatic patient seems justifiable.
Therapeutic drug monitoring can be helpful in making decision on treatment discontinuation. For example, in a patient on combined therapy with infliximab and a thiopurine, discontinuation of the thiopurine did not lead to loss of response in patients with an infliximab serum level above 5 µg/ml (Drobne, *Clin Gastroenterol Hepatol* 2015). In STORI, higher infliximab serum levels were associated with clinical relapse after discontinuation of infliximab therapy (Louis, *Gastroenterology* 2012).

**General remarks**

In general, one should always take into account the type of assays provided, since therapeutic ranges of anti-TNF serum levels may vary between different assays. Furthermore, drug tolerant or drug resistant antibody assays are clearly more informative than a drug sensitive antibody assay, since they are able to measure anti-drug antibodies in presence of (high) anti-TNF serum levels.

One of the major limitations with the current ELISA assays is the fact that they are quite time consuming. However, new platforms for drug monitoring with a fast turn-around time (less than 20 minutes) are becoming available and will make a huge difference in daily clinical practice.

<table>
<thead>
<tr>
<th>Drug serum levels</th>
<th>Anti-drug antibodies</th>
<th>Suggested intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>Absent</td>
<td>Objectify loss of response&lt;br&gt;<strong>If confirmed switch out of class</strong></td>
</tr>
<tr>
<td>Adequate*</td>
<td>Absent</td>
<td>Objectify loss of response&lt;br&gt;<strong>If confirmed switch out of class</strong></td>
</tr>
<tr>
<td>Low</td>
<td>Intermediate or absent</td>
<td>Check compliance&lt;br&gt;<strong>Increase serum levels by decreasing the interval or increasing the dose or adding an immunomodulatory drug</strong></td>
</tr>
<tr>
<td>Absent</td>
<td>Intermediate or absent</td>
<td>Check compliance&lt;br&gt;<strong>Increase serum levels by decreasing the interval or increasing the dose or adding an immunomodulatory drug</strong></td>
</tr>
<tr>
<td>Absent</td>
<td>High</td>
<td><strong>Switch to another drug within class or out of class</strong></td>
</tr>
</tbody>
</table>

* Therapeutic ranges based on the ELISA platforms developed by the Laboratory of Pharmaceutical Biology, KU Leuven, Leuven, Belgium

For infliximab 3–7 µg/ml  
For adalimumab probably 4–10 µg/ml  
For golimumab probably > 1.5 µg/ml  
For vedolizumab probably > 14 µg/ml  
For ustekinumab no clear cut-off yet
Post-operative management of Crohn’s disease patients

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Postoperative recurrence (POR) of Crohn’s disease is common after surgical resection. Diagnosing POR early and preventing the clinical relapse are crucial tasks in the management of this condition.

I will first describe the natural course and the best modalities to diagnose this surgical sequela. I will then focus on the potential risk factors for relapse and the medications that have been used to prevent it. Anti-TNF agents are the most effective therapy to prevent POR in Crohn’s disease. Patient risk stratification and active monitoring with scheduled ileocolonoscopy and non-invasive markers of inflammation are cornerstones of optimal POR management. However, there might be limitations. While many gastroenterologists are aware of the recent progress in the field there is still uncertainty on when to start therapy and how to manage POR in the long term. In addition, studies are needed to address the role of therapeutic drug monitoring in this setting and the role – if any – of new biologics when patients lose response to the primary agents. Finally, I will propose a global strategy to address the management of POR.
Session IV

Mechanism-based treatment strategy for IBD: How to use new medicines properly
IBD pathogenesis and novel therapeutic targets

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Recent years have provided us with important insights into IBD pathogenesis. Genome-wide association studies highlighted the role of the innate immune system for the onset of IBD. Environmental factors could be identified that induce or perpetuate intestinal inflammation and last but not least an important role of the intestinal microbiota was demonstrated.

Recent evidence indicates that meanwhile established therapies such as anti-integrin antibodies may not mainly target T-cells but may also influence innate immune mechanisms and involve macrophages and dendritic cell alterations. New small molecules that specifically target pro-inflammatory pathways have been investigated. One group of small molecules that has been studied are Janus kinase (JAK) inhibitors. The JAK protein family comprises four intracellular tyrosine kinases: JAK1, JAK2, JAK3 and tyrosine kinase 2 (TYK2). These kinases are intracellular signal transducers for many pro-inflammatory cytokines. Subsequently, JAK inhibitors block several cytokine and inflammatory pathways simultaneously. The most advanced development in the area of JAK inhibitors is for Tofacitinib (Xeljanz, Pfizer). Tofacitinib inhibits JAK1, JAK3 and JAK2. However, further JAK inhibitors with more JAK1 specificity of selective TYK2 inhibitors are under current clinical or development.

A second group of small molecules developed are S1P receptor modulators. Sphingosine-1-phosphate (S1P) is a lipid that binds to specific G-protein-coupled receptors (S1PRs). Lymphocyte trafficking is regulated by these S1PRs. Ozanimod is a novel, orally administered small molecule that selectively modulates S1P1 and S1P5 receptors. Ozanimod is currently evaluated for the treatment of immune-mediated inflammatory diseases, such as multiple sclerosis and IBD. In a double-blind, phase II RCT (TOUCHSTONE), ozanimod showed a higher clinical remission rate at week 8 compared with placebo (16% vs 6%, p = 0.048). Two more S1PR modulators (etrasimod and amiselimod) are currently tested in IBD. Whether the target cell of Ozanimod is really the lymphocyte needs to be investigated in the future. It may also change migration of innate immune cells in the mucosa.

In addition to the mentioned small molecules, more orally administered new compounds are currently being investigated in clinical trials in both UC and CD. Besides monotherapy also combination therapies may be interesting and will be addressed in future trials. A combination therapy between the oral small molecules and biologics may be a strategy in complicated disease courses. Current data from animal models indicate that not all combinations may have additive effects.

Microbiota therapy also is in an early stage of development. Several clinical trials indicated a role for fecal microbiota transplantation (FMT) in ulcerative colitis therapy. However, we just begin to understand the complex ecosystem of the intestinal microbiota. The strategies so far have not been optimized and adapted to specific patient’s needs.
Anti-TNF and cytokine inhibitors

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Inflammatory bowel diseases (IBD) such as ulcerative colitis (UC) and Crohn’s disease (CD) are believed to arise from a complex interplay of environmental factors, genetic susceptibility, epithelial barrier defects and dysregulation of the intestinal immune system. Besides anti-inflammatory, immunosuppressive and targeted anti-cytokine strategies, inhibition of lymphocyte gut homing by blockade of anti-adhesion molecules has arisen as a new and promising therapeutic approach lately.

Within the field of cytokine blockers, TNF blockers remain a key strategy for suppression of inflammation in IBD. In addition, the p40 blocker ustekinumab has been approved for patients with Crohn’s disease and is currently being tested in ulcerative colitis. Moreover, p19 blockade is a promising new field for therapy of inflammatory bowel diseases and phase 3 studies are ongoing. Another important development in the field of cytokine inhibition consists of the Jak inhibitors that target several proinflammatory cytokines simultaneously. Finally, several alternative strategies to suppress cytokine functions including DNAzymes will be presented and discussed.

It is expected that cytokine inhibition will remain an important strategy for treatment of IBD.
Use of inhibitors of cellular adhesion molecules and leukocyte trafficking in IBD

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Inflammatory bowel disease (IBD) is characterized as a chronic inflammatory disease of the gastrointestinal tract caused by immune dysregulation in genetically susceptible individuals. Intestinal lymphocyte trafficking involves a complex and tightly regulated network of different effectors and is primarily mediated by interaction between α4 integrin and its specific ligands. Integrins are cell transmembrane heterodimeric (α/β) glycoproteins that mediate external cell-cell and cell-matrix interactions and signaling pathways. The development of monoclonal antibodies against α4 integrin allows targeting of lymphocyte trafficking into the intestine as a novel therapeutic intervention. Vedolizumab is a humanized immunoglobulin monoclonal antibody against α4β7 integrin that selectively inhibits the adhesion of α4β7+expressing lymphocytes to MADCAM-1 that is preferentially expressed in the gut endothelium. This prevents trafficking into the mucosa and thereby has the potential to modulate inflammation in the gastrointestinal tract without inducing systemic immunosuppression. Randomized controlled trials have confirmed efficacy and safety of vedolizumab in patients with ulcerative colitis and CD both as first line therapy as well as after anti-TNF failure. Vedolizumab is associated with improved clinical outcomes in UC and CD including symptomatic remission, endoscopic mucosal healing and improved quality of life. Real world experience and long-term extension studies have confirmed the efficacy and safety of vedolizumab. Other lymphocyte trafficking molecules in development include etrolizumab, a humanized monoclonal antibody to the β7 subunit of the integrins α4/β7 and αE/β7, anti-MAdCAM-1 antibodies, and selective antagonists of CCR9 (chemo-kine receptor 9) expressed by a significant proportion of circulating α4β7+ T cells and B cells.

Sphingosine-1-phosphate (S1P) is a membrane-derived lipid signaling molecule involved in the regulation of several cellular processes though the activation of a G protein-coupled receptor family known as the S1P receptors. S1P modulation is a key regulator of lymphocyte migration from lymph nodes and its blockade may control aberrant leukocytes migration into the mucosa in IBD by forcing sequestration of lymphocytes in secondary lymphoid organs, indirectly impeding them from entering the gut. Phase 2 clinical trials of ozanimod, an oral S1P modulator, has confirmed efficacy and safety in patients with moderate-to-severe ulcerative colitis. Phase 3 studies are in progress. Enhanced understanding of mucosal immunology and lymphocyte trafficking in IBD should usher in a new era of biologic and small-molecule immune modifiers with highly gut-selective targets promising enhanced efficacy and superior safety.
JAK inhibitors

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Treatment options for ulcerative colitis (UC) remain limited because conventional therapies cannot successfully control the disease in a considerable percentage of patients, while up to 30% of those receiving biologics are primary non-responders and 10–20% lose response per year, requiring a dose increase or switch to a different drug. The Janus Kinase (JAK) inhibitor is one of the new treatment options with a different mechanism of action.

Cytokines play key roles in the pathogenesis of inflammatory bowel diseases (IBD). The JAK family comprises four intracellular tyrosine kinases (JAK1, JAK2, JAK3, and TYK2), which activate STAT through their phosphorylation. JAK-STAT pathways regulate signaling for multiple cytokines, including interferon-γ, and interleukins 2, 4, 6, 7, 9, 12, 15, 21, 23, and 27.

The first JAK inhibitor conducting clinical trials was tofacitinib. Tofacitinib is an oral, small-molecule JAK inhibitor, which was first approved for rheumatoid arthritis before its approval for UC. It inhibits all JAKs but preferentially JAK1 and JAK3 was confirmed to be effective for UC in the OCTAVE clinical trial programs (Sandborn WJ et al. NEJM 2016). In the OCTAVE Induction 1 trial, remission at week 8 was achieved in 18.5% of the patients in the tofacitinib group versus 8.2% in the placebo group (p = 0.007); in the OCTAVE Induction 2 trial, remission occurred in 16.6% versus 3.6% (p < 0.001). In the OCTAVE Sustain trial, remission at week 52 was achieved in 34.3% of the patients in the 5-mg group and 40.6% in the 10-mg group versus 11.1% in the placebo group (p < 0.001).

JAK inhibition by tofacitinib can lead to opportunistic infections, and viral infections seem to be especially frequent. Among them, particular safety concerns are raised for herpes zoster infection and its incidence is significantly higher in East Asians than other populations. Although no malignancy signals have been identified to date, long-term follow-up and further research are needed to understand the risk of malignancy associated with JAK inhibitors.

Tofacitinib and a number of other JAK inhibitors including upadacitinib and filgotinib are currently being tested in phase II and III trials for the treatment of a variety of autoimmune inflammatory diseases including IBD.

Herein, how this new molecule could be inserted into the therapeutic algorithm of patients with UC will be discussed.
Session V

Gut flora and metabolism in human health care and disease
The impact of intestinal epithelial barrier function

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Intestinal mucosa is protected from microbiota as well as pathogenic bacteria by several types of barriers. One of these barriers is constructed by mucus layers, composed of inner and outer mucus layers in the colon. Microbiota is present in the outer mucus layer, whereas there is no microbiota in the inner mucus layer. Separation of microbiota from the intestinal epithelial cells contributes to prevention of intestinal inflammation. Indeed, invasion of bacteria into the colonic epithelial surface was shown in several mouse models of intestinal inflammation. However, the precise mechanisms by which the inner mucus layer is free of microbiota in the colon remained unknown.

Ly6/PLAUR domain-containing protein 8 (Lypd8), which was selectively expressed on the uppermost layer of colonic glands, was a highly glycosylated GPI-anchored protein and secreted into the colonic lumen, particularly the inner mucus layer. In mice lacking Lypd8, bacterial free space in the inner mucus layer disappeared and they were highly susceptible to intestinal inflammation. On the intestinal epithelial cell layer of the colon of the mutant mice, flagellated bacteria such as Escherichia, Helicobacter and Proteus were present. Depletion of these bacteria by antibiotics restored the bacterial free space in the inner mucus layer and ameliorated the intestinal inflammation of the mutant mice. Lypd8 bound to bacterial flagella and suppressed motile activity of flagellated bacteria. Thus, Lypd8 mediates segregation of microbiota from the intestinal epithelial layer in the colon, and thereby contributes to the prevention of intestinal inflammation.

We will also discuss the sensitivity of Lypd8-deficient mice to intestinal inflammation in a dysbiotic condition as well as to infection with pathogenic bacteria.
The gut microbiota form a highly complex ecological community together with host intestinal cells. The so-called gut ecosystem has a profound influence on human physiology, immunology, and nutrition. It has been reported that imbalance in the structure of gut ecosystem could be a risk factor in human disorders including not merely gut-associated disorders such as inflammatory bowel disease and colonic carcinogenesis, but also systemic diseases such as metabolic disorders and allergy. However, the molecular mechanisms of the host-microbial crosstalk remain obscure. To this end, we firstly established a highly integrated omics-based approach involving genome, transcriptome, and metabolome analyses to elucidate the molecular basis of the host-microbial crosstalk, and to regulate the function of gut microbiota. Applying this novel method to mice models, we found that acetate produced from carbohydrate metabolism by probiotic bifidobacteria largely contributes to the protection of mice from enterohaemorrhagic E. coli O157:H7 lethal infection through enhancement of gut epithelial barrier function. In addition, we showed that lactate derived from dietary fiber fermentation by lactic acid bacteria accelerates colonic epithelial cell turnover in starvation-refed mice. Furthermore, we demonstrated that butyrate produced from dietary fiber metabolism by microbial order Clostridiales progresses the induction of regulatory T cell differentiation from naïve T cells through epigenetic modification, which suppress colonic inflammation. Moreover, metabologenomic approach revealed that the aging-related alterations in the intestinal luminal metabolism lead to gut microbial dysbiosis with a potential to induce obesity and impaired glucose tolerance. Additionally, we found that lubiprostone (commonly used for the treatment of constipation) ameliorates the progression of chronic kidney disease by reduction of gut microbiota-derived uremic toxin accumulation through intestinal luminal metabolism alteration. Taken together, gut microbiota-derived metabolites are considered to be crucial factors to shape host physiological homeostasis.
Impact of smoking on the gut microbiota in IBD patients

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²Kanagawa Institute of Industrial Science and Technology, Japan
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Introduction: Smoking has been observed to exert protective effects on ulcerative colitis (UC), but not on Crohn’s disease (CD), however underlying molecular mechanisms have not been well understood. We hypothesized that smoking could affect oral and/or gut microbial composition, which in turn affects the UC pathology.

Methods: 16S rRNA gene amplicon sequencing-based microbial composition of feces, saliva and colonic aspirates during colonoscopy were compared among UC and CD patients with/without smoking history. GC/MS-based metabolome analysis of fecal samples were also done.

Results: We found that colonic aspirates represented mucus-associated bacteria. Microbial compositions of colonic aspirates could be divided into four distinct groups, and that UC smokers were significantly concentrated into one of the cluster compared to UC non- and ex-smokers. Interestingly, this cluster showed an increased abundance of orally found bacteria.
Metabolome analysis showed that short-chain fatty acids (SCFA) such as butyric acid and acetic acid were significantly increased in feces from UC smokers compared to those from UC ex-smokers.

Discussion/Conclusion: We showed that orally found bacteria abundantly existing in the microbial cluster of colonic aspirates where UC smokers were significantly concentrated compared to UC non- and ex-smokers. Interestingly, previous observations suggest the involvement of mucosa-associated oral bacteria for pathogenesis of IBD. We are now trying to isolate oral bacteria in this cluster to elucidate the mechanisms how these bacteria are involved in UC pathogenesis.
The role of diet and small bowel microbiota in health and metabolic diseases

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Several studies have shown that high fat (HF) diets profoundly, rapidly, and sustainably alter microbial communities that affect membership and function. Very few of these studies have considered the regional and functional differences of regional gut microbiota, choosing to focus on fecal microbiota which is subject to change during intestinal transit and mostly representative of microbiota of the distal bowel. In this regard, the role of small bowel microbiota has been neglected as a factor that can affect macronutrient absorption and energy acquisition of the host. While less diverse and abundant than that in the colon, the small intestine microbiota is complex and routinely subjected to dietary variation and host factors such as low pH, faster transit time, bile acids, and antimicrobial peptides.

Although some past studies in germ-free (GF) mice and zebrafish have shown an association between gut microbiota and fat malabsorption, few mechanistic insights are available. We therefore hypothesized that small bowel microbes have a role in regulating intestinal lipid digestion and absorption, allowing the host to adapt to dietary fluctuations. Furthermore, we posited that western diets perturb microbial composition and function to alter capacity for lipid processing in ways that contribute to metabolic disease and obesity. Thus, the goals of this study were to 1) determine if microbes are required for proper digestion and absorption of dietary lipid using GF animals, 2) examine the impact of HF diets on small intestinal microbiota membership using 16s rRNA gene profiles, 3) determine the functional impact of HF diet-induced jejunal microbiota on fat absorption, and 4) test proof-of-concept that microbially-derived metabolites or products from specific microbial strains directly affect lipid absorption. We demonstrate that gut microbes act as indispensable signal transducers of dietary lipid, allowing the host to adapt by regulating capacity for fat digestion and absorption (Martinez-Guryn K, et al. Cell Host Microbe. 2018). Consumption of a HF diet profoundly alters the microbiota in the small intestine, and this community increases fat absorption in conventionalized animals. Lastly, we show that these effects may be promoted by specific bacterial strains through microbe-derived components or small molecules. These findings indicate that proximal gut microbiota play key roles in host adaptability to dietary lipid variations through mechanisms involving both the digestive and absorptive phases and that these functions may contribute to conditions of overnutrition as well as undernutrition.
Obesity and cellular senescence: A gut microbial connection

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Multiple epidemiological studies have revealed that obesity is a major risk factor for not only diabetes and cardiovascular diseases but also cancer. Therefore, effective strategies for obesity prevention are needed for cancer prevention. However, since the prevalence of excess bodyweight in most developed countries has been increasing markedly over the past several decades, alternative approaches are also required to conquer obesity-associated cancer. Although several phenomena have been proposed to explain how obesity increases cancer risk, the exact molecular mechanisms underlying obesity-associated cancer have remained largely obscure. Recently, we have traced the association between obesity and increased risk of hepatocellular carcinoma (HCC) development to gut microbiota communities that provoke cellular senescence in hepatic stellate cells (HSCs) through increasing the levels of deoxycholic acid (DCA), a gut bacterial metabolite known to cause DNA damage. The enterohepatic circulation of DCA provokes SASP in hepatic stellate cells (HSCs), which in turn secretes various inflammatory and tumor-promoting factors in the liver, thus facilitating HCC development in mice after exposure to chemical carcinogen. However, it remains unclear exactly how DCA provokes SASP in HSCs in obese mice and which bacteria are involved in DCA production in obese mice. Here, I report recent progress on the link between cellular senescence and obesity-associated HCC. I believe that a better understanding of the molecular mechanisms involved will lead to new strategies for the prevention of obesity-associated cancer.
Session VI

NAFLD
Pathogenesis of NAFLD

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Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in the world. The proportion of patients with NAFLD that have non-alcoholic steatohepatitis (NASH) might range from 10–20%. NASH patients commonly present with metabolic inflammation characterized by elevated circulating levels of C-reactive protein, ferritin, interleukin 6 (IL-6), or IL-1 receptor antagonist. Inflammation and fibrosis determine the long-term prognosis of this disease. Lipotoxicity is of crucial importance and evidence is increasing that multiple lipid intermediates are cytotoxic and pro-inflammatory. Another consistent feature of NAFLD is presence of insulin resistance, which is also the target of several therapeutics. Metabolic liver inflammation is a prognostically highly relevant factor also in driving hepatic insulin sensitivity. Cytokines, gut-derived products including metabolites and other inflammatory mediators might substantially affect liver outcome in NAFLD. Besides pro-inflammatory mediators, a large variety of lipid metabolism abnormalities have been described in insulin-resistant states and certain lipids such as free fatty acids might interfere with insulin signaling thereby contributing to insulin resistance. Farnesoid X receptor (FXR) agonists negatively regulate bile acid synthesis, decrease hepatic lipogenesis and gluconeogenesis and improve peripheral insulin sensitivity. All those inflammatory responses provoke regenerative responses resulting in progressive scarring and cirrhosis. To summarize, the current pathophysiology suggests that a complex array of multiple-parallel hits underlie NAFLD pathogenesis and especially drive evolution of NASH. Targeting various pathways might be necessary for an effective NASH therapy.
The role of the microbiome in the pathogenesis of NAFLD

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Up to 25% of non-alcoholic fatty liver disease (NAFLD) patients develop a progressive inflammatory liver disease termed non-alcoholic steatohepatitis (NASH) that may progress towards cirrhosis, hepatocellular carcinoma, and the need for liver transplantation. In recent years, several lines of evidence suggest that the gut microbiome plays important roles for the pathogenesis of NASH. We demonstrated *faecalibacterium* (FB) was significantly decreased with exacerbated liver fibrosis in human study. The aim of our study was to assess if FB improves NASH pathogenesis in mice and mechanism between FB and improving NASH-pathogenesis. Using NAFLD model, histological findings revealed that hepatic triglyceride contents and number of neutrophil elastase positive cells, fibrotic area were significantly improved in a high-fat high-fructose high-cholesterol diet plus administrated FB (F) group compared with those in a high-fat high-fructose high-cholesterol diet (H) group. The serum endotoxin, AST, ALT T-cho, Cho-VLDL levels, and HOMA-R were significantly decreased in F group in comparison to H group. In OGTT test, blood glucose level in 90 min and 120 min were ameliorated in F group compared with H group. Gene expression levels of TNFα, collagen1a1, PPARα, CPT1a1, MTP, IRS1, IRS2, PEPCK, and G6P in the liver were significantly ameliorated in group F compared with H group. Also, in gut-permeability analysis, significant increase in gut permeability was observed in H group in comparison to that in group B, whereas the increased gut permeability was abrogated in F group in comparison to that in group H. In colon mucosa, gene expression levels of TNFα, OCLN, CLDN4, 8, 15 were improved in F group in comparison to those in H group. Gene expression levels of FOXP3 and TGF-β were significantly increased in F group compared with those in H group. In flow cytometry, significantly increased in CD4+CD25+FOXP3+ cells in lamina propria lymphocyte (LPL) was observed in F group compared with H group. Administration of anti-FR4 antibody on B group leads Treg depletion, resulted in the increasing intestinal permeability in B group.

In conclusion, our study indicated that FB-administration improves NASH pathogenesis via ameliorating gut-permeability by inducing Treg in colon. Our results suggest that FB could be a candidate agent for the treatment of NASH through the improving leaky-gut.
Non-invasive assessment of fibrosis in NAFLD

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Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in the United States (US) afflicting more than 30% of the US population.(1, 2) It is becoming the major cause of liver disease related morbidity and mortality.(3, 4) NAFLD is a spectrum of disease ranging from nonalcoholic fatty liver, non-progressive subtype of NAFLD, to nonalcoholic steatohepatitis (NASH), the progressive subtype of NAFLD, and cirrhosis. It is patients with advanced fibrosis (stage 3 and 4 fibrosis) who are at the highest risk for adverse outcomes such as hepatocellular carcinoma and hepatic decompensation.(5, 6) Owing to the sheer volume of at-risk patients, there is substantial unmet need for efficient and cost-effective means for risk-stratification of patients with NAFLD. Liver biopsy, the gold-standard for this purpose, is impractical to satisfy these needs. Expensive, risky, and frequently refused by patients,(7–9) liver biopsy is further limited by sampling error(10) and poor inter-rater reliability.(11) Spurred by these limitations, validated non-invasive alternatives to the liver biopsy are an area of intense research interest in the field.

Among the non-invasive biomarkers of disease severity in NAFLD, imaging-based biomarkers are emerging to be the lead candidates in clinical development. There are several imaging-based biomarkers but the most promising and also most widely applied are techniques that perform elastography – or liver stiffness measurements (LSM). These include magnetic resonance elastography (MRE), virtual contrast transient elastography (VCTE), acoustic radiation forced impulse wave imaging (ARFI), and shear wave elastography (SWE). Serum and blood based biomarkers such as European Fibrosis Panel and Fibrospect 2 are emerging as useful tools. Data are limited however on how they compare, their tradeoffs and how they complement each other in the context of real-world clinical practice. Herein, we will aim goal to review each modality with reference to their inherent properties, diagnostic performance, and caveats associated with their clinical application. We will also compare their performance and pitfalls and lay out our view on how to utilize them based upon published data.

References:


Current and future treatment strategies of non-alcoholic steatohepatitis

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is rapidly becoming the leading cause of chronic liver disease, worldwide. The global prevalence of NAFLD is estimated to be approximately 25%. Although NAFLD is highly prevalent in the general population, its progressive subtype of non-alcoholic steatohepatitis (NASH) is more clinically important. In the United States (U.S.), an estimated 1.5–6.45% of the general population has NASH. Longitudinal studies of NAFLD have demonstrated that it is associated with increased all-cause mortality, cardiovascular mortality, and liver-related mortality. Furthermore, NASH is the subtype primarily associated with liver-related mortality. In this context, stage of hepatic fibrosis appears to be the most important determinant of mortality in patients with NAFLD and the risk increases with increasing stage of fibrosis. Other important outcomes of NAFLD are development of hepatocellular carcinoma (HCC) and listing for liver transplantation. A recent study has suggested that patients with NAFLD had a higher incidence of cancers than those without NAFLD. In addition, NASH has become the fastest-growing and the second- or third-leading indication for liver transplantation in the US.

It is important to note that patients with diabetes are highest risk for NASH. In fact, the prevalence of NASH in diabetics (DM) is estimated to be about 65%. In addition to the high prevalence of NASH and related fibrosis, presence of DM increases the risk of advanced fibrosis and mortality. In fact, each additional component of metabolic syndrome further increases the risk of adverse outcomes in NAFLD. Given the growing global epidemic of obesity and DM, prevalence of NASH is projected to double in the next two decades leading to a projected 800,000 excess liver deaths.

There is strong evidence to suggest that NASH is a phenotype with multiple pathogenic pathways. Although hepatic fat deposition is the initial "hit", it is followed by multiple other insults leading to liver cell injury and hepatic fibrosis. The basic abnormality of NAFLD and NASH are related to the systemic consequences of caloric overload. This abnormal calorie-rich environment promotes visceral obesity and insulin resistance leading to proinflammatory and a steatogenic state. Additionally, this pro-inflammatory milieu is accompanied by altered bile acid profile and intestinal permeability, associated with a decrease in hepatic FXR activation, altered gut microbiome, increased oxidative stress and other abnormalities that can ultimately lead to the activation of hepatic Stellate cell and hepatic fibrosis. Challenges in understanding the triggers of the complex pathways involved in NASH have led to a lack of effective treatment and absence of a fully validated non-invasive test for NASH or related fibrosis.
In 2018, lifestyle modification is the first line treatment for NASH. Although several dietary models have been proposed, the Mediterranean diet seems to be most promising. In addition to diet, increased physical activity may also have beneficial effect for NASH. Although about 5% weight loss through diet can reduce hepatic fat, higher degrees of weight loss (> 10%) is required to improve hepatic fibrosis. In this context, less than 10% of individuals are able to achieve and sustain this amount of weight loss. Additionally, exercise alone may be beneficial for hepatic steatosis but its impact on necroinflammation or fibrosis has not been established.

A number of medications have been used to treat NASH. However, currently only vitamin E and pioglitazone are recommended by the AASLD clinical guidance document. It is important to note that neither one of these drugs have received approval for treatment of NASH. Vitamin E is only recommended for non-cirrhotics, non-diabetics with biopsy proven NASH. Pioglitazone can be considered in patients with NASH and diabetes. Nevertheless, the risk and benefit of these drugs must be clearly discussed with each patient. Despite lack of approved drugs, currently, there are over 85 drug regimens that are being tested for treatment of NASH. Of these, only 4 are in phase 3 clinical trials. Cenicriviroc (CVC) is an immunomodulatory agent that can inhibit both CCR2 and CCR5. The results of a phase Ib, randomized, double-blind, placebo-controlled, multinational study of CVC has been published. The data from CENTAUR showed that despite the inability to meet the pre-defined primary outcome of NASH resolution, CVC did have a positive impact on fibrosis reduction. A large phase 3 clinical trial of CVS (AURORA) is currently underway.

Another important pathway that can result in apoptosis of hepatocytes leading to the development of fibrosis is the activation of apoptosis signal-regulating kinase 1 (ASK1). ASK1 activation results in the production of inflammatory cytokines, chemokines, expression of matrix remodeling genes and promotion of apoptotic cell death. Selonsertib (SEL) is an ASK1 inhibitor and has been evaluated in a phase II clinical trial. Results of this trial showed a potential efficacy and good safety for SEL. In fact, the efficacy of these regimens is being assessed in 2 large phase III clinical trials (STELLAR 3 and 4) for patients with NASH with advanced fibrosis (stage 3 and stage 4).

As noted previously, peroxisome proliferator-activated receptors (PPAR) are ligand-activated transcription factors that regulate genes important in cell differentiation, as well as metabolic processes, including glucose and lipid homeostasis. PPARs (PPAR-α, PPAR-β/δ, and PPAR-γ) are a family of ligand-activated nuclear hormone receptors, which means after interaction with specific ligands, nuclear receptors are translocated to the nucleus and regulate gene expression. In a double-blind, placebo-controlled, randomized, international phase IIb trial (GOLDEN-505 trial), the potential impact of elafibranor (dual PPAR-α/δ agonist) in patients with NASH without cirrhosis was assessed. Although the initial analysis did not show significant difference from placebo, the subsequent analysis showed that elafibranor at 120 mg daily was significantly superior to placebo. RESOLVE-IT is a large, randomized, placebo-controlled, double-blind, multicenter phase III clinical trial which is currently enrolling subjects to assess the impact of elafibranor in patients with NASH.
Finally, FXR is an important target for treatment of NASH. In this context, obeticholic acid (OCA) is a modified bile acid, derived from chenodeoxycholic acid, which is the natural ligand for the farnesoid X receptor (FXR). OCA increases glucose-stimulated insulin secretion, augments peripheral glucose uptake, inhibits hepatic lipid synthesis, and induces lipid uptake by adipocytes. Data from a multicenter, double-blind, placebo-controlled randomized clinical trial (FLINT) reported on the efficacy of OCA in adult patients with biopsy-proven NASH. The data showed that patients who received OCA for 72 weeks had significantly higher histologic improvement than patients in the placebo arm. REGENERATE is ongoing double-blind, placebo-controlled, randomized, multicenter phase III trial designed to assess the long term evaluation of OCA for NASH and fibrosis.

In addition to these regimens, a non-steroidal FXR (GS-9674)a, recombinant FGF19 agonist (NGM282), a recombinant FGF21 agonist (BMS986036), a synthetic lipid molecule (aramchol), THRβ agonists (MGL-3196), an acetyl Co-A to malonyl Co-A inhibitor (GS-0976), a nalmefene (JKB-121) and a GLP-1 receptor agonist (liraglutide) are all being tested in phase 2 clinical trials with some encouraging data.

In addition to these regimens, a number of anti-fibrotic strategies have been used to treat NASH with advanced fibrosis. These agents have either failed or have provided less than impressive data (simtuzemab, emricasan, GR-MD-02). Although some of these agents are not being pursued as monotherapy (simtuzemab), others are still being considered for additional clinical trials.

Finally, the most exciting opportunity for clinical trials of NASH is combination of multiple drugs. Of these, three combination regimens are already being tested or considered. These include combination of ASK1 inhibitor (SEL) with non-steroidal FXR agonist (GS-9674) and/or ACC inhibitor (GS-0976). Another regimen uses the combination of PPAR alpha and delta agonist (elafibranor) and an FXR agonist. Finally, a combination of chemokine CCR2/CCR5 receptor blocker (CVC) and a FXR agonist is being considered. The results of these regimens are not fully available.

Despite a great deal of enthusiasm for treatment of NASH with monotherapy or combination therapy, it is unlikely that NASH will be managed with a short course of treatment. In this context, it is important to remember that NASH is another manifestation of metabolic syndrome such as insulin resistance, dyslipidemia or hypertension. Unless the underlying obesity fueling NASH is addressed, most patients with NASH will require long-term treatment. In fact, it is most probable that patients with NASH and significant fibrosis are first treated with a regimen for a limited period of time, followed by a long-term maintenance regimen to prevent them from further progression or induce regression of their liver disease. Therefore, these future treatment regimens should not only have evidence for efficacy but also long-term safety.

In summary, NASH is rapidly becoming the most common and important cause of chronic liver disease worldwide. Although approved drug treatment regimens are not available, a number of agents are being tested. It is important to note that these regimens should not only improve clinical outcomes but also PROs and be cost-effective. Furthermore, international efforts are underway to develop validated non-invasive test to establish the diagnosis, monitoring and prognosis of NASH.
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Session VII

Viral hepatitis
Innate and adaptive immunity in hepatitis B and hepatitis C virus infection – Aiming at the virus elimination by 2030

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Hepatitis B virus (HBV) and hepatitis C virus (HCV) are among the most endemic pathogens worldwide. WHO set the target of the elimination of transmission of both viruses by 2030. These pathogens can cause acute and chronic hepatitis that progress to liver cirrhosis or hepatocellular carcinoma (HCC). Mild and pervasive immune cell dysfunction, but not fully compromised, is a hallmark of chronic HBV or HCV infection, the fundamental mechanisms of which are yet to be clarified.

HBV has developed specific strategies to evade recognition by the innate immune system and is acknowledged to be a stealth virus. However, extensive research has revealed that HBV is recognized by macrophages, dendritic cells (DCs) and natural killer (NK) cells. Indoleamine-2,3-dioxygenase (IDO) is an enforcer of sequential immune reactions in acute hepatitis B, and this molecule has been shown to be induced by the interaction of HBV-infected hepatocytes, DCs, and NK cells. Functional cure of HBV, or HBsAg loss, from chronically-infected patients is a desirable clinical target of reducing the risk of liver cancer. In cases of acute resolving hepatitis, or ideal functional cure, we demonstrated that sequential chemokine and cytokine activation is involved to HBsAg seroconversion (Yoshio S, Hepatology 2016). These results shed light on an active role of DC, macrophages and follicular helper T cells in attaining functional HBV cure.

We reported that human DCs consist of three functionally distinctive subtypes and play unique and substantial roles in HBV or HCV infection (Kanto T. JI 1999, JID 2004, Yoshio S. Hepatology 2013). Disabled DCs potentially give negative impact on adjacent cells, such as NK cells, NKT cells and T cells. However, lack of evidence for active viral replication in DCs or blood cells imply the presence of undisclosed contrivances that are independent of infection. Real-world clinical data have proven that all oral agents against HCV, or direct anti-viral agents (DAAs) successfully eradicate HCV from more than 95% of the infected patients. HCV eradication by DAAs are considered to restore host immunity either indirectly by reducing viral burden or directly by immune modulation. However, the risk of HCC could not be completely erased from aged patients with advanced fibrosis even after HCV clearance, the impact of DAA-mediated immune alteration on which is still undisclosed.

Here we review the current status of research on immune responses against HBV and HCV infection. A comprehensive study of clinical samples, based on cutting edge technologies, is urgently needed to improve our understanding of the immune mechanisms associated with viral control in the liver and thus to develop novel immune modulatory therapies to cure chronic HBV and HCV infection.
Current treatment of hepatitis B

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Entecavir and tenofovir are the recommended first line antiviral therapies due to their high antiviral potency and low risk of drug resistance. The estimated annual incidence of HCC is significantly reduced among entecavir-treated patients as compared to historic untreated controls in a few studies in Taiwan, Hong Kong and Japan. Most benefit is seen among patients with liver cirrhosis. Complete viral suppression is associated with better HCC prevention. Approximately 20% of entecavir treated patients cannot have complete viral suppression at 3 years. There is no evidence that combination therapy can improve viral suppression among incomplete responders.

As antiviral therapy cannot clear cccDNA inside the liver, most patients require long-term antiviral therapy. This poses a challenge to drug adherence and safety, particularly renal and bone safety for tenofovir disoproxil fumarate. The introduction of tenofovir alafenamide is a solution. HBsAg seroclearance is an acceptable timing to stop antiviral therapy, but it rarely develops in Asian patients. HBV DNA suppression alone is an insufficient condition to avoid viral relapse after stopping treatment. Approximately 90% of HBeAg negative patients will have viral relapse and 50% have clinical relapse after stopping antiviral treatment according to the APASL criteria.
Hepatitis C therapy: Everything solved?

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Hepatitis C virus (HCV) infection is responsible for chronic hepatitis, cirrhosis, decompensation of cirrhosis and hepatocellular carcinoma. The mortality related to HCV is increasing globally. Active research and development efforts have led to the approval of several combination regimens based on the use of direct-acting antiviral drugs with different viral targets, ensuring potency and a high barrier to resistance of the combinations. The latest approved combination regimens have pangenotypic activity and a very high barrier to resistance. These therapies yield high rates of infection cure (> 95% in most patient groups) and they are safe and well-tolerated over 8 to 16 weeks of administration. HCV infection cure was shown to be associated with a reduced incidence of hepatocellular carcinoma, of liver-related clinical events and death. However, the story is not over. Indeed, the World Health organization aims to eliminate viral hepatitis as a public health threat. This means reducing new chronic HCV infections by 90%, treating 80% of eligible persons with chronic HCV infection and reducing mortality rates by 65% on a global scale. To achieve this huge challenge, the cascade of care must be substantially improved. This implies that infections be diagnosed in patients who are not aware of their status and that these patients be proactively linked to efficient care. Only with such measure will the goal of HCV elimination as a public health threat be achieved.
Hepatitis E: The new epidemic?

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Hepatitis E virus (HEV) is a member of the *Hepeviridae* family, genus *orthohepevirus*. HEV is a significant cause of viral hepatitis globally but has two distinct epidemiological and disease patterns in developing and industrialized countries. In developing countries, HEV infections are the cause of epidemic and endemic acute hepatitis, occurring along the fecal-oral route via contaminated water, while in industrialized countries, zoonotic food-borne transmission of HEV is considered to be an important cause of autochthonous hepatitis E. In Japan, hepatitis E had long been considered to be a rare liver disease which can be accidentally imported from endemic countries in Asia and Africa, where the sanitation conditions are suboptimal. However, since the identification of the first autochthonous hepatitis E case and hepatitis E viremic domestic pigs in Japan in 2001, our understanding of HEV infection in this country has been changing markedly. This has largely been due to the development of serological and gene-based diagnostic assays, accumulation of molecular epidemiological findings on HEV infection in humans and animals (as potential reservoirs for HEV in humans) and the recognition of the importance of zoonotic food-borne and other routes of transmission of HEV, including blood-borne transmission. Chronic hepatitis E has also been diagnosed in liver, kidney and heart transplant recipients and patients with hematological disorders in Japan. Of note, the annual number of reported hepatitis E cases has increased 5- to 6-fold since the implementation of anti-HEV IgA assay system covered by the government insurance program. However, clinical and subclinical HEV infections indigenous to Japan remain underdiagnosed and their prevalence is still underestimated due to the presence of unknown transmission routes and a low awareness of the infection status by many physicians in Japan. My presentation focuses on the features of acute and chronic HEV infection in humans in Japan.
Session VIII

Autoimmune liver diseases
Clinical management of PBC

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Primary biliary cholangitis (PBC) is a potentially progressive disease leading to death or liver transplantation. After the wide spread of disease entity, the incidents of PBC were in rise until 2000s, although this increase seems to be stabilized for recent decades. Meanwhile, the prevalence of PBC consistently increase globally. This observation could result from the improved survival of the disease, especially after the introduction of ursodeoxycholic acid (UDCA) in 1990s. The effects of UDCA upon survival or transplantation-free periods were consistently better in treated-patients, especially in early disease stage. Alkaline phosphatase and bilirubin levels correlate with the risk of liver transplantation or death, and approximately 30–40% of UDCA-treated patients do not respond UDCA treatment. Thus, the second line treatment was feasible in these patients group. Recently, obeticholic acid, a farnesoid X receptor agonist, has been reported to be beneficial for improving serum liver enzymes (POISE trial). However, this treatment has been associated with the increased pruritus. More recently, the results of the BEZURSO trial (bezafibrate in combination with ursodeoxycholic acid) has demonstrated the improved serum markers in bezafibrate-add-on group. Although the adverse effects were not remarkable in this study, the elevated serum creatinine levels were documented in other clinical trials in longer observation period. Moreover, the long-term improvement such as transplantation-free survival, were not proven in these new second-line treatments. Thus, in spite of recent improvement of prognosis, there are still unmet needs for developing new treatment drugs for subpopulation of patients with PBC.
Autoimmune hepatitis (AIH)

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Autoimmune hepatitis (AIH) is an immune-mediated and chronic destruction of hepatocytes by mononuclear cells infiltrated into the liver. Middle-aged women are at highest risk but development in childhood or presence in male is not uncommon. In most cases, presentation of AIH is insidious followed by slow progression, eventually leading to cirrhosis and even hepatocellular failure when lacking appropriate treatment. Autoimmune reactions against hepatocytes definitely play a crucial role because of 1) presence of autoantibodies including anti-nuclear antibodies (ANA) and anti-smooth muscle antibodies (AMSA), 2) elevation of serum IgG, 3) marked inflammation of mononuclear and plasma cells in liver histology, and 4) excellent treatment effect of immunosuppressive drugs. AIH is not a rare disease in Asia. Indeed, recent epidemiological study of AIH in Japan revealed the prevalence of patients with AIH was 23.9 per 100,000, comparable to those in Western countries. Prevalence is increasing in Japan as also observed in the West.

In this presentation, I would like to address three issues to be urgently solved in the Asia-Pacific region regarding AIH. First, it is an urgent need to widely disseminate the recognition, diagnosis and management of AIH in this region. The proportion of cirrhotic patients in newly-diagnosed AIH patients is around 6% in Japan, but more than 70% in South Asia, indicating AIH at early stage might be largely misdiagnosed. It is crucial to appropriately diagnose AIH before progression and to promptly initiate treatment. In this regard, the clinical practice guidelines (CPG) of AIH should be established in the Asia-Pacific region. Second, establishment of the second-line treatment is needed for patients refractory or intolerant to prednisolone. Azathioprine, budesonide, mycophenolate mofetil, all of which are listed as second-line drugs in the CPG in the West, have not been approved and scarcely used for AIH in the East. Well-designed clinical trials of these agents as well as new drugs are definitely needed; and finally, acute-onset AIH, frequently lacking typical features of AIH, should not be overlooked. Acute-onset is not uncommon, observed in almost 25% of AIH patients in Japan, and could get exacerbated and even mortal without prompt diagnosis and appropriate treatment. It is very likely that this type of AIH might be present among etiology-unknown acute liver failure.

Finally, I would like to shortly introduce diagnostic criteria and CPGs of primary sclerosing cholangitis (PSC) established in Japan.
Primary sclerosing cholangitis (PSC) results from an interplay between unknown environmental triggers and an individual genetic background comprising multiple susceptibility alleles. In Europe, PSC most frequently occur in the context of inflammatory bowel disease (IBD), and even in Japan a distinct subgroup of younger patients have a high frequency of IBD. Over the years, genetic studies in PSC and IBD as well as several autoimmune diseases, have revealed hundreds of relevant susceptibility alleles. Ongoing efforts aim to translate these genetic findings into pathophysiological insight, as well as to help identify relevant environmental co-factors and novel treatment targets. The lecture will summarize our current knowledge on the genetics of PSC, and comparisons will be made with the genetic landscape of IBD and autoimmunity in Europe and Japan. Ongoing translational research deriving from the genetic findings will be described, to illustrate how findings in genetics may directly and indirectly inform therapeutic opportunities. In parallel, several phase II clinical trials have been performed in PSC over the same period, and the results from these trials will be discussed in the context of preclinical data from the genetically informed studies. A paradox is evident, since immunosuppressive therapy is mostly not considered efficacious, whereas the genetic appearance of PSC is similar to that of many prototypical autoimmune diseases. In clinical trials, biochemical signals of efficacy in PSC are mostly seen for drugs targeting bile acid homeostasis and the gut microbiota, and possible reasons for this observation will be discussed, alongside an elaboration of possible ways forward towards effective therapeutics in PSC based on the current insights.
IgG4-related sclerosing cholangitis

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IgG4-related sclerosing cholangitis (IgG4-SC) is a characteristic type of sclerosing cholangitis with an unknown pathogenic mechanism. IgG4-SC patients show increased levels of serum IgG4 and dense infiltration of IgG4-positive plasma cells with extensive fibrosis in the bile duct wall. IgG4-SC is one of the common organ manifestations of IgG4-related disease (IgG4-RD). IgG4-SC is frequently associated with type 1 autoimmune pancreatitis (AIP). Various cholangiographic features of IgG4-SC are similar to those of primary sclerosing cholangitis (PSC), pancreatic cancer, and cholangiocarcinoma (CC). Therefore, it is not easy to discriminate IgG4-SC from these progressive or malignant diseases on the basis of cholangiographic findings alone, and accurate diagnosis of IgG4-SC not associated with AIP is particularly difficult.

IgG4-SC can be diagnosed based on the clinical diagnostic criteria for IgG4-SC proposed in 2012. The characteristic features of IgG4-SC can be classified into 4 types based on the regions of stricture as revealed by cholangiography and differential diagnosis. Type 1 IgG4-SC shows stenosis only in the lower part of the common bile duct, and it should be differentiated from AIP, chronic pancreatitis, pancreatic cancer, and CC. Type 2 IgG4-SC, in which stenosis is diffusely distributed throughout the intrahepatic and extrahepatic bile ducts, should be differentiated from PSC. Type 2 is subdivided into 2 further types: type 2a, with narrowing of the intrahepatic bile ducts with prestenotic dilation; and type 2b, with narrowing of the intrahepatic bile ducts without prestenotic dilation and reduced bile duct branches, which is caused by marked lymphoplasmacytic infiltration into the peripheral bile ducts. Type 3 IgG4-SC is characterized by stenosis in both the hilar hepatic lesions and the lower part of the common bile duct. Type 4 IgG4-SC shows strictures of the bile duct only in the hilar hepatic lesions. Cholangiographic findings of types 3 and 4 need to be discriminated from those of CC. Intraductal ultrasonography (IDUS) findings such as circular-symmetric wall thickening, a smooth outer margin, a smooth inner margin and a homogeneous internal echo at the stenotic area are useful for the diagnosis of IgG4-SC.

The treatment of IgG4-SC is basically similar to that for type 1 AIP. Oral prednisolone therapy is started at 0.6 mg/kg/day for 2 or 4 weeks, and the dose is then gradually tapered. Disease relapse occurs in approximately 20–40% of patients either during the steroid taper or after the discontinuation of steroids.
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POSTER ABSTRACTS

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IL-33 delays recovery of inflammation via down-regulation of homeostatic colonic ABCG5/8

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Introduction: IL-33 influences intestinal inflammation and is likely involved in the pathogenesis of ulcerative colitis (UC). ABCG5/8 are known cholesterol transporters in the small intestine, whereas their role in the colon is totally unknown. We investigated colonic ABCG5/8 in IL-33-mediated mucosal injury.

Methods: Acute colitis was induced in wild-type (WT) and IL33−/− mice by administration of a 3% dextran sulfate sodium (DSS) solution. 7 days after DSS-treatment, recombinant IL-33 (rIL-33) or PBS was selectively injected. The mice were evaluated for colitis severity and microarray analysis was performed. In vitro, Toll-like receptor (TLR)-stimulated Caco2 and HCT-15 cell lines were cultured with various doses of rIL-33, with siRNAs for ABCG5/8 selectively added. RNA expressions and cytokine levels were assessed. In addition, human biopsy samples from healthy volunteers and UC patients were obtained and assessed by histology and PCR.

Results: WT mice had more severe colitis than IL33+/− mice, while rIL-33-treatment delayed recovery from colitis in DSS-treated IL33−/− mice. Microarray demonstrated that the ABC transporter and tight junction pathways in the colon were significantly down-regulated by rIL-33-treatment. In vitro, TLR-stimulation upregulated Abcg5/8 RNA expressions in cell lines, with subsequent down-regulation by rIL-33. Abcg5/8 siRNA down-regulated tight junction genes and increased TLR-stimulated IL-8 production in the examined cells. In human UC, colonic ABCG5/8 are inversely correlated with IL-33 and histological inflammation.

Discussion/Conclusion: IL-33 down-regulates colonic ABCG5/8 in a colitis recovery phase, indicating their involvement in the pathogenesis of UC and a potential therapeutic target for UC.
Reduction of infliximab treatment after dose escalation in patients with Crohn’s disease

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Background: When dose escalation of infliximab (IFX) is effective to overcome loss of response, most patients with Crohn’s disease (CD) continue intensified treatment from a concern of flare. However, part of the patients are supposed to successfully reduce the dose.

Methods: In patients who continued double dose of IFX for more than 1 year, the dose was reduced to standard regimen with informed consent. SUCCESS was the group of patients who remained in sustained remission for more than 16 weeks after dose reduction. FAILURE was the group of the patients who had symptom, and/or high CRP level, and/or active endoscopic findings, and needed intensification of treatment.

Results: Total of 110 patients who continued double dose of IFX for more than 1 year and underwent more than 3 reduced dose of IFX infusion were included in the study. Observation period after dose reduction was 46.9 ± 4.2 weeks. As a result 49 (44.5%) were SUCCESS, 61 (55.5%) were FAILURE. A significant difference of patient background between the two groups was: IFX was the second biologics, 0 vs. 6 (SUCCESS vs. FAILURE, p = 0.024), combo therapy with immunomodulator, 13 vs. 30 (p = 0.016), need for steroid, 5 vs. 15 (p = 0.052). In FAILURE, 29 patients shorten infusion interval, 23 patients re-escalated IFX dose, 9 patients started another biologics.

Conclusion: Overall about a half of patients successfully reduced IFX to standard regimen. IFX as the first biologics, remission free of steroid or immunomodulator seemed favorable for successful reduction.
Effectiveness and associated prognostic factors of granulocyte and monocyte adsorptive apheresis for patients with ulcerative colitis

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Introduction: We investigated the effectiveness and associated prognostic factors of Granulocyte and monocyte adsorptive apheresis (GMA) for patients with ulcerative colitis (UC).

Methods: Data were retrospectively collected from UC patients who received GMA at our hospital between April 2010 and September 2017. Patients with a Lichtiger clinical activity index (CAI) score of ≤4 were excluded. GMA was performed using Adacolumn®. Each patient received two GMA sessions a week for five weeks, and the maximum number of GMA session was 10. Remission was defined as a CAI score of ≤4 within 10 GMA sessions. The remission rate and mean duration required for remission following the first GMA session were calculated. Prognostic factors related to the remission rate were evaluated using univariate and multivariate logistic regression analysis.

Results: Of the 138 patients (median age, 40.0 years), 58 were female. The median duration of disease was 2.6 years. At baseline, the median CAI scores was 9.0. Remission was achieved in 74 patients (54%). The median duration required for remission was 13.5 days. In the univariate analysis, the median CAI score at baseline was significantly higher in patients who had not achieved remission (9.5) than in those who had (8.0). In the multivariate logistic regression analysis, a higher CAI score was identified as an independent predictor of a lower rate of remission.

Discussion/Conclusion: Remission was achieved in approximately 50% of UC patients treated by GMA within 2 weeks on average. Higher CAI scores at baseline might indicate a lower likelihood of remission.
In addition to the adenocancer events, the risk of neuroendocrine tumor (NET) development is increased in patients with Crohn’s disease

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**Introduction:** The incidence of colorectal (adeno) cancer is increased in patients with inflammatory bowel diseases (IBD) and mainly due to the long duration of IBD. However, neuroendocrine tumor (NET) risk was not fully evaluated in IBD, particularly Crohn’s disease (CD). We aimed to determine whether there is an increased risk for the development of NET in CD. We also questioned whether the incidence of any cancer in patients with IBD is increased.

**Materials and methods:** We studied on patients with ileocecal resection at the Surgical Clinic of Bezmialem Vakif University Medicine Faculty Hospital between 2011 and 2017. Of the 246 patients performed ileocecal resection for any reason (15–98 and average 59 years) with pathology results, 56 were due to CD with non-malignant reasons such as fistula and or stricture and abcess.

**Results:** Of the 246 patients, 16 with NET, 6 with carcinoid tumor, 164 had adenocancer and 60 were non-malignant reason. In subgroup analysis, of the 56 patients with CD, 2 had NET, 4 had carcinoid tumor, 4 showed adenocancer. Of the 190 non-IBD patients, 14 had adenocancer with NET differentiation, 2 had carcinoid tumor, 160 with adenocancer and 14 with non-malignant reason.

**Conclusion:** Our study showed that patients with CD had an increased NET development risk, besides increased colon adenocancer frequency.

**References:**


Retrograde contrast study and SES-CD through double-balloon enteroscopy can predict the risk of bowel resections in Crohn’s disease patients with small intestinal strictures

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Introduction: We reported that retrograde contrast study through double-balloon enteroscopy (DBE) could examine small intestine of the Crohn’s disease patients (CD) with adhesion or small bowel stenosis and predict the risk of small bowel resection (Inflamm Bowel Dis. 2017). We compared the predictability of bowel resections of retrograde contrast and SES-CD scoring though DBE.

Methods: The findings of retrograde contrast and SES-CD though DBE in 48 CD patients with small intestinal strictures were examined.

Results: Of the 48 patients, 16 (33%) underwent surgery for small intestinal strictures during a median observation period of 4.9 years. According to the results of the multivariate analysis, mSES-CD (≥ 10) and ratio of maximum diameter of prestenotic dilations to the diameter of the normal small intestine (≥ 1.45) were independent risk factors of surgery for small intestinal strictures (risk ratio = 30.2 [95% CI: 2.2–423], p = 0.01; and risk ratio = 22.3 [95% CI: 2.8–180], p = 0.0004, respectively). Cumulative surgery-free rates were discriminated significantly according to the presence or absence of these two risk factors (log-rank test: p < 0.001).

Discussion/Conclusion: Findings of retrograde contrast and mSES-CD through DBE are helpful to predict risk of surgery in CD patients with small intestinal strictures.
The prevalence of liver, biliary tract and pancreas abnormalities by ultrasound and then confined by EUS in 835 patients with inflammatory bowel disease

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Background: It was reported that involvement of the pancreas, liver and biliary tract, including the gallbladder is rare in patients with inflammatory bowel disease (IBD). More specifically, pancreas pathologies were not well defined in patients with IBD, so far. Our aim to find the prevalence of these involvements by using transabdominal ultrasound (US). Additionally, we further characterized pancreas abnormalities by echoendoscopy (EUS).

Methods: We evaluated our IBD clinic's records which includes 2700 patients with IBD. We used US to show pancreas, liver and biliary tract abnormalities. Then, patients with pancreas abnormalities were further examined by EUS. Patients with recent onset dyspepsia were used as a control group.

Results: Of the 2700 patients with IBD, 835 had documented US Results. There was 162 patients without IBD as a control. All of the patients, 59% in IBD and 58% in control were male. The prevalence of abnormalities as follows: liver steatosis, 40% in IBD vs. 45% in controls (p > 0.05); gallbladder polyps, 2.4% in IBD vs. 8% in controls (p > 0.05); gallbladder sludge and stones, 8.4% in IBD vs. 9.9% in controls (p > 0.05); hepatomegaly, 9.7% in IBD vs. 25.3% in controls (p < 0.001); gallbladder operation, 4.4% in IBD vs. 7.4% in controls (p < 0.001); gallbladder pathologies (polyp, sludge, and operation), 15.1% in IBD vs. 22.2% in controls (p = 0.024); gastric antrum wall thickness, 0.5% in IBD vs. 1.9% in controls (p > 0.05); hepatic simple cyst, 1.6% in IBD vs. 2.5% in controls (p > 0.05); hemangioma, 2.8% in IBD vs 1.9% in controls (p > 0.05); pancreas parenchymal abnormalities, 5.3% in IBD vs 0.6% in controls (p = 0.009); chronic liver disease findings, 2.2% in IBD vs. 0% in controls (p: 0.057); hepatic calcification, 1.0% in IBD vs. 0.6% in controls (p > 0.05). Of the 44 patients with parenchymal changes in pancreas, EUS investigation was performed in 13. EUS showed major A or B with minor single finding according to the Rosemont classification. The presentation of the disease was autoimmune pancreatitis (AIP) in 3 patients; acute pancreatitis in 2; without any symptom in 6 patients. Echoendoscopy findings as follows: The size of the main duct was dilated up to 5.0 mm; pancreas atrophy in 2 patients; sausage-shaped enlargement in 2 patients with AIH; honey comb appearance in 10 patients, hyperechogenic stria in the head of the pancreas in 6 patients.

Conclusion: Our results showed that involvement of the pancreas, liver and biliary tract not frequent, but also not a rare finding in patients with IBD. Chronic parenchymal changes of the pancreas are underestimated and should be followed for any progress in clinical practice.
Treatment adherence of patients with inflammatory bowel disease is dependent on the form of 5-aminosalicylic acid

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Background/Aims: It is unclear whether 5-ASA formulation is associated with treatment adherence in ulcerative colitis (UC). Thus, we aimed to investigate the adherence rate after switching from 5-ASA tablets to granules.

Methods: This prospective study included 121 UC outpatients treated using 5-ASA tablets. They were grouped based on choice: Group 1 (continued with tablets) and Group 2 (switched to granules without regimen change). Group 2 was further divided into Group 3 (returned to tablets) and Group 4 (continued with granules). The patients completed a self-administered questionnaire regarding the treatment. The primary endpoint was change in adherence after switching to granules.

Results: Seventy-nine patients continued with tablets, while 42 patients switched to granules. The adherence rate to the tablet was not significantly different between Group 1 and Group 2 before switching. In Group 2, switching to granules did not affect adherence. However, in Group 4, adherence significantly improved from 94.2 ± 10.9% to 97.3 ± 7.0% (p = 0.008) after switching from tablet to granules. Group 3 showed no significant change in adherence before and after switching from tablets. Full-time employment and difficulty taking the tablet were significant predictors of continuing with granules in Group 4 (hazard ratio [HR] = 53.1; 95% confidence interval [CI]: 1.908–1479.324; p = 0.019 and HR = 193.8; 95% CI: 1.967–19085.427; p = 0.025, respectively).

Conclusion: The present study reflected real-world clinical care, and the patients who continued 5-ASA granules by choice demonstrated a significant increase in adherence, suggesting that patient-tailored formulations and dosing regimens could be useful for improving adherence.
Proton-pump inhibitors associated with a markedly increased risk of microscopic colitis

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Introduction: Microscopic colitis (MC) is a chronic inflammatory bowel disease with unknown etiology. Proton-pump inhibitor (PPI) use has been consistently linked to an increased risk of MC. The specific role of the different classes of PPI is unknown. Based on nationwide Danish registries we wanted to explore the effect of recent use of different classes of PPI and in addition the effect of the dose on the risk of MC.

Methods: In a 10-years period (January 2004 to December 2013) we identified 10,652 patients with a first-time recorded diagnosis of MC including 6254 (59%) with collagenous colitis (CC) and 4398 (41%) with lymphocytic colitis (LC) in the Danish Pathology Registry. Information on PPI use was obtained from the Danish Prescription Register. The association between PPI exposure and the risk of obtaining a diagnosis of MC was studied in a case-control design with population-based controls.

Results: We found an association between the prescriptions for PPI and both CC (adjusted OR = 8.75; 95% CI: 8.12–9.43) and LC (adjusted OR = 5.03; 95% CI: 4.61–5.49). Odds ratios of CC and LC by the current prescription of different classes of PPIs are shown in Table 1. We found a strong and highly significant association between the use of lansoprazole and both CC (adjusted OR = 20.10; 95% CI: 18.05–22.37) and LC (adjusted OR = 7.70; 95% CI: 6.79–8.73). This observed OR for lansoprazole was higher than observed for other classes of PPIs. Secondly we demonstrated that the increased risk was primarily related to a current use of PPI and was not found to be dose-dependent. The use of NSAID was associated with a modest increased risk of CC (adjusted OR = 1.70; 95% CI: 1.55–1.87). However, the combination of NSAID and PPI and was associated with a higher risk for CC (adjusted OR = 9.72 CI; 8.65–10.92) compared to the use of one of the drugs alone.

Conclusions: In a large comprehensive case-control study based on nationwide Danish registries we found an increased risk of MC associated with the use of PPI and a specific increased risk connected with the use of lansoprazole. The combination of NSAID and PPI and was associated with a high risk for CC.
Associations between the Prognostic Nutritional Index and morbidity/mortality during intestinal resection in patients with ulcerative colitis

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Introduction: Ulcerative colitis (UC) is well known as a gut immune disorder and is often treated with immunosuppressive therapies. Currently, besides the patient’s background information and the above risk factors, which cannot be avoided in the clinical course and especially in urgent/emergent surgery, we have no other predicting factor for mortality and morbidity with a definite index. Onodera’s Prognostic Nutritional Index (O-PNI) is a well-known predictor for the prognosis of several surgeries. The aim of this study was to evaluate the association between O-PNI and surgical outcome during surgery for ulcerative colitis.

Methods: This was a single-institution retrospective cohort study conducted in the Department of Inflammatory Bowel Disease at Hyogo College of Medicine, Japan. All patients who underwent surgery for UC at our institution. The pre-operative predictive factors that were associated with mortality, morbidity and pouch-related complications (PRC) were examined distinct from surgical procedure.

Results: 1151 patients with UC who underwent surgery between January 2000 and December 2015 were included. Total colectomy (TC) alone, ileal-pouch anal anastomosis (IPAA) with ileostomy, and IPAA without ileostomy were performed in 254 patients, 736 patients and 161 patients, respectively. Mortality and morbidity were found in 9 (0.8%) and 320 (27.8%) patients, respectively. The significant predicting factors for mortality among TC alone were elder patients (p = 0.03, odds ratio [OR] = 6.8), higher C-reactive protein (CRP) (p = 0.02, OR = 14.5), and O-PNI < 24.9 (p = 0.04, OR = 5.6). Among IPAA with ileostomy, American society of anesthesiologist score ≥ 3 (p = 0.01, OR = 2.3), PSL dose just before surgery ≥ 14 mg/day (p = 0.04, OR = 1.8), and O-PNI < 35.5 (p < 0.01, OR = 2.1) were selected as predictors for PRC.

Discussion/Conclusion: Lower O-PNI may predict the prognosis in patients with UC. O-PNI may be useful indicator for decision making for surgical timing and procedure. In addition, in patients with O-PNI below 35, total colectomy alone without pouch reconstruction may be better for avoiding from PRC.
Crohn’s disease patient-derived small intestinal organoids reveal disease status-related modification of stem cell properties

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**Introduction:** Intestinal stem cells (ISCs) play indispensable roles in the maintenance of the intestinal epithelium, and their dysfunctions can deeply relate to diseases including Crohn’s disease (CD). Studies have shown that patient-derived intestinal organoids can maintain its disease-related cell properties in vitro. This time, we performed a single-cell level analysis of the organoids that were established from CD patients, to reveal the possible modification of their ISC properties.

**Methods:** CD patient-derived small intestinal organoids were established from enteroscopic biopsy specimens taken from active lesions (aCD-SIO), or from mucosa under remission (rCD-SIO) by following the method described by Sato T. et al (Gastroenterology, 2011). Expressions of ISC-marker genes in those organoids were examined by microfluid-based single-cell multiplex gene expression analysis. For its functional analysis, organoid cells were subjected to a single-cell organoid reformation assay using a 3D-scanner system.

**Results:** Single-cell gene expression data of 12 ISC-markers were acquired from a total of 1215 organoid cells and its analysis showed that aCD-SIOs, rCD-SIOs or those of non-IBD controls (NI-SIOs) showed a comparable level of OLFM4 and SLC12A2 expression in all organoids. t-distributed stochastic neighbour embedding (tSNE) analysis identified a cluster of candidate ISCs in aCD-SIO, characterized by modified expression pattern of Smoc2 and Lgr5. In addition, 3D-scanner assisted single-cell organoid reformation assays showed significantly higher reformation efficiency of aCD-SIO-derived cells, compared to others.

**Discussion/Conclusion:** In conclusion, small intestinal organoids established from active lesions of CD maintain potential ISCs with modified marker expression profiles, and also with high organoid reformation ability.
Effect of a *Hibiscus sabdariffa* extract in liver inflammation and intestinal epithelial permeability in obese mice

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**Introduction:** Although liver disease is not fully considered as a complication of obesity, the prevalence of non-alcoholic fatty liver disease (NAFLD) in obese patients is high. Obesity has been related to intestinal dysbiosis, characterized by altered intestinal permeability that can result in endotoxemia, systemic inflammation and NAFLD. Different phenolic plant extracts have been reported to exert beneficial effects in experimental models of metabolic syndrome. In the present study a well-characterized extract from *Hibiscus sabdariffa* has been evaluated in diet-induced obesity in mice, to investigate its impact on the liver inflammatory status and intestinal epithelial function.

**Methods:** Male C57BL/6J were distributed into seven groups: control, control-treated, obese, obese Hibiscus-treated (1, 10 and 25 mg/kg/day) and obese metformin-treated (250 mg/kg/day) for 6 weeks. Control and control-treated mice were fed with normal chow diet, whereas obese mice received a high-fat diet. Animal weight and food consumption were periodically measured. At the end of the experiment, the liver inflammatory status was evaluated, as well as different markers of intestinal epithelial barrier function, by RT-qPCR.

**Results:** The administration of the *H. sabdariffa* extract resulted in a reduction of body weight gain, associated with reduced fat deposition. The extract significantly ameliorated the altered expression of key adipogenic genes in the inflamed liver from obese mice, like PPARs, the protein kinases JNK1 and JNK2, the receptor for leptin, as well as inflammatory cytokines (TNFα, IL-1β, IL-6) and TLR4. The markers of altered intestinal permeability (MUC-3 and ZO-1) were also improved in mice treated with the extract.

**Discussion/Conclusion:** *H. sabdariffa* showed an anti-inflammatory activity in the liver of obese mice, maybe related to an improvement in the intestinal epithelial barrier function.
Analyzing psychological structure of patients with inflammatory bowel disease and medical staff for better patient relations

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Introduction: To know the psychological structure of the patients as well as medical stuff is important for better clinical care.

Methods: Using TEG II (Tokyo University Egogram New Version II) we performed transactional analysis of 4 groups comprising 71 patients with Crohn’s disease (CD), 70 with ulcerative colitis (UC), 70 medical staff (MS), and 70 healthy volunteers (HV).

Results: One-way analysis of variance showed deviation among 4 groups concerning the ego-state of critical parent (CP), nurturing parent (NP), and free child (FC). Post hoc analysis by Turkey-Kramer method revealed significant difference between CD and HV in CP, NP, and FC, between UC and HV in FC, between MS and HV in NP. The result can be interpreted to mean that both UC and CD are not good at changing mood or at expressing emotion than HV. CD tend to be more passive and more unsocial than HV. Surprisingly, MS has less devotion or interest to others than HV.

Discussion/Conclusion: From this study we can say that MS need to interact with patients with IBD more proactively and carefully.
A novel small-caliber deep enteroscope can overcome technical difficulty of balloon dilatation for stricture in patients with Crohn’s disease

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Introduction: Balloon enteroscopy is a useful modality for the evaluation of small intestinal lesions in patients with Crohn's disease (CD). Not infrequently, deep insertion of the enteroscope carries difficulty due adhesion, stricture, or other causes. Deep insertion is required especially for accurate assessment of mucosal healing and treatment of stricture in CD. The novel device (SIF-Y0006, Y-0018: Olympus Co. LTD., Tokyo) is a single balloon enteroscope, with distal tip diameter of 5.4 mm and active/passive bending mechanism. We have previously reported the improvement of insertion ability with these new devices, as compared to the standard single balloon enteroscope.

The aim of this study is to investigate the feasibility of balloon dilation with the new devices.

Methods: Thirteen CD patients with small intestinal strictures undergoing endoscopic balloon dilatation with the new scope were analyzed. All patients had insertion difficulty with standard single balloon enteroscopy. The balloon dilatation (EBD) was performed by the over-the-wire method. The rate of technical success and complication was assessed.

Results: EBD was succeeded in 11 patients (85%) without any complication. The average number of dilated stricture was 2.1 (1–5).

Discussion/Conclusion: Although this study has limitation such as retrospective single center observational study, the new small caliber enteroscopes is useful in the patients with small bowel insertion difficulty for both diagnostic and therapeutic purpose.
Relationship between endoscopic mucosal healing and histologic inflammation during remission maintenance phase in ulcerative colitis

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Introduction: Ulcerative colitis (UC) is an unidentified chronic inflammatory disease limited to the colon mucosa. The achievement of mucosal healing (MH) is recognized as an important factor in the maintenance of remission. Recently it has been suggested that histologic inflammation is an important predictor for relapse. To investigate whether histological remission of colon mucosa can be a predictor for maintenance of remission in patients, we retrospectively compared the histological inflammation and endoscopic findings in UC patients who are in the clinical remission phase with MH.

Methods: Mayo endoscopic subscore (MES) was used to evaluate the mucosal findings. Endoscopic MH was defined as an overall MES of 0 or 1. Inflammation in biopsy specimens was evaluated according to the Matts histopathological grade (Matts). We categorized Matts 1+2 as histological healing group (HH group), Matts 3–5 as non-histological healing group (NHH group). Comparative study was conducted on the relapse-free survival due to histological inflammation in patients who are in clinical remission phase with MH.

Results: The remission maintenance rate was significantly higher in the MES 0 group compared to the MES 1 group (p = 0.004), while the HH group in both of them came up with better results than the corresponding NHH group (p = 0.003). We put a special interest on the MES 1 group, finding that the HH group showed a significantly higher rate of remission maintenance than the NHH group (p = 0.030).

Discussion/Conclusion: The rate of remission in the subgroup of the MES 0 and MES 1 showed an equivalent outcome, while the NHH subgroup of the MES 1 has a significantly lower remission rate. Considering these results, histologic remission is an important predictor for maintenance of remission. Therefore, especially those patients belonging to MES 1 group should undergo further histological examination clarify their chances on remission.
Anastomotic ulceration in patients after small bowel resection: Crohn’s disease, bacterial overgrowth or... what?

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Introduction: Anastomotic ulceration (AU) in the late period after small bowel (SB) resection could be a rare source of obscure bleeding. The reasons for the formation of ulcers debatable, not very clear and still insufficiently studied. Most gastroenterologists mistakenly believe it is a clinical manifestation of Crohn’s disease. The aim is to evaluate the clinical features, etiology and treatment outcomes of the patients with AU of SB.

Methods: From 14.02.2007 to 30.10.2017 AU was revealed in 8(3.7%) patients (m-7, f-1, mean age 38.0 ± 14.4 years, range 19–59) from 213 patients who admitted to our hospital with suspected SB bleeding. The interval between SB resection (made for different reasons except IBD) and first signs of bleeding varied from one to 28 years (mean 11.2 ± 8.4 years). There were seven (87.5%) patients with obscure overt and 1 (12.5%) with obscure occult bleeding. Recurrent bleedings occurred in 7 patients. VCE was performed in six (75.0%) patients; BAE in all patients.

Results: VCE and BAE revealed ulcers from 5 to 25 mm in size at the anastomotic area in 5 patients and in the long blind loop nearby anastomosis in 3 patients; including stenosis of the lumen in 2 patients. It was confirmed that IBD wasn’t the reason for the ulceration. Small bowel reresection was performed in 7 patients (including 2 patients with unsuccessful conservative treatment considered to be Crohn’s disease); iron supplementation – in 1 patient who refused surgery. Histology showed acute and chronic ulcers, including suture material in the ulcer bases of 2 patients. All patients have been free of relapse and anemia from 1 to 7 years after surgery.

Discussion/Conclusion: AU with obscure bleeding usually have no connection with IBD and conservative treatment is useless. One of possible factors leading to AU is long blind loop and suture material. Surgical reoperation tends to be an effective treatment option.
Detection of gut dysbiosis due to reduced *Clostridium* subcluster XIVa based on the serum bile acid profile

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**Introduction:** Dysbiosis in gut microbiota has been proposed as an important factor in the pathogenesis of numerous disorders. Especially, a reduced proportion of *Clostridium* subcluster XIVa has been reported in several diseases. Since *Clostridium* subclusters XIVa has been reported to be the main microbes that metabolize primary bile acids (BAs) into secondary or tertiary BAs, we hypothesized that the BA profile in feces, and possibly in serum, could be a convenient biomarker for the intestinal proportion of *Clostridium* subcluster XIVa.

**Methods:** Twenty patients, including 6 with Crohn’s disease (CD), 6 with ulcerative colitis (UC) and 8 with other gastrointestinal diseases, and 26 healthy controls were studied. The fecal microbiota profile in each subject was determined by terminal restriction fragment length polymorphism analysis. BA compositions in serum and feces were quantified by HPLC-MS/MS.

**Results:** The fecal proportion of *Clostridium* subcluster XIVa was decreased significantly in CD (15.4 ± 6.8%, mean ± SD, p < 0.0001), UC (8.4 ± 10.1%, p < 0.0001) compared with healthy controls (33.2 ± 9.2%). In all 46 subjects, correlations between the fecal proportion of *Clostridium* subcluster XIVa and both fecal and serum were DCA/(DCA+CA) (r = 0.52, p < 0.001), LCA/(LCA+CDCA) (r = 0.43, p < 0.01), 3-oxo BAs/(3-oxo BAs + 3α-OH BAs) (r = 0.51, p < 0.001), 12-oxo BAs/(12-oxo BAs + 12α-OH BAs) (r = 0.38, p < 0.01), 7β-OH BAs/(7β-OH Bas + 7-oxo BAs) (r = 0.48, p < 0.001), and 12β-OH BAs/(12β-OH BAs + 12-oxo BAs) (r = 0.31, p < 0.05).

**Discussion/Conclusion:** Decreased *Clostridium* subcluster XIVa exhibits a strong correlation with reduced intestinal bile acid metabolism. These changes were found in both feces and serum. Thus, the serum bile acid profile could be a surrogate marker for the intestinal proportion of *Clostridium* subcluster XIVa.
Heme-induced Spi-C in macrophages prevents colitis by inhibiting expression of NF-kappaB/IRF5-dependent genes

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Introduction: One of the mechanisms for the modulation of intestinal CX3CR1high macrophages activity is the IL-10-dependent suppression of Toll-like receptor (TLR) responses, which is common to many types of macrophages. However, a unique mechanism that modulates activation of intestinal innate immune cells remains poorly understood.

Methods: Transcription factor Spi-C was selectively expressed in CX3CR1high macrophages in the colon. To assess the physiological role of Spi-C in intestinal CX3CR1high macrophages, myeloid lineage cell-specific Spic-deficient (Lyz2-cre; Spicflox/flox) mice were generated and analyzed the sensitivity to dextran sodium sulfate (DSS)-induced colitis. In addition, comprehensive gene expression profiles were analyzed in control and Spic-deficient bone marrow macrophages stimulated with LPS following heme treatment.

Results: Lyz2-cre; Spicflox/flox mice showed severe intestinal inflammation during DSS-induced colitis. LPS-induced production of IL-6 and IL-1alpha, but not TNF, by large intestinal CX3CR1high macrophages from Lyz2-cre; Spicflox/flox mice was markedly enhanced. The interaction of Spi-C with IRF5 was linked to disruption of IRF5-NF-kappaB p65 complex formation, thereby abrogating recruitment of IRF5 and NF-kappaB p65 to the IL-6 and IL-1a promoters.

Discussion/Conclusion: Collectively, these results demonstrate that Spi-C is a key molecule for the non-inflammatory signature of intestinal CX3CR1high macrophages by suppressing the induction of a subset of TLR-inducible genes through binding to IRF5.
Probiotic-derived super-long-chain polyphosphate induces mucosal healing in patients with refractory ulcerative colitis

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Introduction: The goal of the treatment of ulcerative colitis (UC) is considered to be “mucosal healing”, which a condition sufficiently possessing intestinal barrier function, leading to a significantly long-term remission. However, more than half of UC patients fail to achieve mucosal healing, even when they receive anti-TNF alpha treatments. Probiotics confer a health benefit to the host and possess sufficient safety; however, their efficacy in the treatment of UC remains controversial. We identified super-long-chain polyphosphate from conditioned media of Lactobacillus brevis SB885 and show that the molecule improves the intestinal barrier function and inflammation in in vitro and mouse models. We then conducted a clinical trial to determine the safety and efficacy of super-long-chain polyphosphate in the treatment of patients with refractory UC.

Methods: We designed an investigator-initiated, open-label study of super-long-chain polyphosphate. Ten UC patients with steroid resistance or dependence, who showed moderate disease activity with obvious hematochezia were enrolled. The first 5 patients took 300 mg of super-long-chain polyphosphate orally, once daily for 4 weeks; the second 5 patients took 900 mg of the drug orally on the same schedule. Anti-TNF alpha treatments were fixed at 20 weeks and other medications were fixed at 2 weeks before the start of the study. The disease activity was assessed according to the Mayo score.

Results: Seven of the 10 patients acquired clinical remission and four achieved endoscopic remission. The effect of the drug was unaffected by the history of mesalamine, steroid, anti-TNF alpha agents or calcineurin inhibitor administration. No adverse events were observed in association with the administration of the drug.

Discussion/Conclusion: Super-long-chain polyphosphate is an effective and safe drug for the treatment of refractory UC. The next phase of the study has been prepared and will provide stronger evidence concerning the safety and efficacy of this drug.
Effectiveness of Multi Matrix System mesalazine for the induction of remission in patients with ulcerative colitis who insufficiently respond to other mesalazine formulations: A Japanese single-center study

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Introduction: Multi Matrix System (MMX) mesalazine has been approved in Japan for the treatment of ulcerative colitis (UC). We evaluated the effectiveness of MMX mesalazine for the induction of remission in patients with UC who insufficiently respond to pH- or time-dependent mesalazine.

Methods: Retrospective data were collected from active UC patients who switched to MMX mesalazine 4.8 g/day because of an insufficient response to 3.6 g/day of pH- or 4.0 g/day of time-dependent mesalazine between December 2016 and December 2017. Patients with a partial Mayo score (pMS) of ≤ 4 and those who received other remission induction therapy at baseline were excluded. The effectiveness of switching to MMX mesalazine was evaluated by the decrease in pMS, which was calculated at baseline, 4 weeks, and 8 weeks. Remission was defined as a decrease in pMS to ≤ 2. Prognostic factors related to the remission rate were evaluated using univariate analysis.

Results: Of the 46 patients included in this study (mean age, 39.1 years), 34 were female. The mean duration of disease was 7.9 years, and the mean pMS was 4.9 at baseline. Sixteen patients had pancolitis, 19 had left-sided colitis, and 11 had proctitis-type colitis. Concomitant treatment with immunomodulators and local mesalazine was administered in 8 and 20, respectively. Previous treatment included pH- and time-dependent mesalazine in 32 and 14 patients, respectively. The remission rate at 4 weeks was 63% and at 8 weeks was 67%. In the univariate analysis, concomitant treatment with local mesalazine was a significant prognostic factor for a higher remission rate at 8 weeks in patient with left-sided colitis.

Discussion/Conclusion: Switching to MMX mesalazine 4.8 g/day in UC patients insufficiently responding to 3.6 g/day of pH- or 4.0 g/day of time-dependent mesalazine is effective and should be considered, especially for left-sided colitis patients with concomitant treatment with local mesalazine.
The efficacy of infliximab treatment after the surgical treatment of perianal fistula in Crohn’s disease patients

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Introduction: Crohn’s disease (CD) patients with fistulising region are frequently treated with Infliximab (IFX), but are also treated with IFX combined with the surgical procedure. The influence of the IFX treatment after the surgical treatment of perianal fistula in CD patients was investigated in the present study.

Methods: Crohn’s disease patients who were surgically treated for perianal fistula at Tokyo Yamate Medical Center from January 2005 to September 2014 were enrolled. The patients who were treated with IFX within 2 months after the surgical treatment were designated as IFX(+) group, and patients who were not treated with any biologic reagents as IFX(-) group. Three years after the surgery, rates of re-operation of perianal fistula, stoma construction, and abdominal perineal resection were compared between these two groups. Patients who had colorectal cancer during observation period were excluded from the analysis.

Results: Thirty five patients were included in IFX(+) and 179 were in IFX(-). Rates of re-operation of perianal fistula were 25% (n = 9) in IFX(+) and 18% (n = 32) in IFX(-), respectively (Fisher’s exact test, \( p = 0.3462 \), odds ratio 1.59, 95% confidence interval: 0.68–3.72). The constructing rate of stoma in IFX(+) was tended to be lower than IFX(-) (n = 1 [3%] vs. n = 27 [15%], \( p = 0.0551 \), odds ratio 0.17, 95% confidence interval: 0.02–1.26). The rates of abdominal perineal resection was 0% (n = 0) in IFX(+) and 1% (n = 2) in IFX(-), respectively (\( p = 1.0000 \), odds ratio 1.00, 95% confidence interval: 0.05–21.27).

Discussion/Conclusion: After IFX treatment, the constructing rate of stoma following the surgical treatment for perianal fistulas in Crohn’s disease patients was tended to be lower.
Short- and long-term outcomes of infliximab treatment for patients with ulcerative colitis and associated prognostic factors

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Introduction: There are few reports on the effectiveness of infliximab (IFX) treatment for ulcerative colitis (UC). The aim of this study was to analyze the short- and long-term outcomes of IFX treatment in patients with UC and related prognostic factors.

Methods: Retrospective data were collected from 172 patients with UC who had received IFX treatment from 2005 to 2017. Patients with the Lichtiger clinical activity index (CAI) scores of ≤ 4 were excluded. Remission was defined as a CAI score of ≤ 4. The CAI scores were calculated at baseline, 2 weeks and 6 weeks following IFX administration. Cumulative remission-maintenance rate in patients who achieved remission at 6 weeks was estimated using Kaplan-Meier method. The prognostic factors related to the remission rate at 6 weeks and the cumulative remission-maintenance rate were evaluated using a multivariate logistic regression analysis and a multivariate Cox regression analysis, respectively.

Results: Of the 172 patients (mean age: 38.5 years), 68 were female. The mean CAI score at baseline was 9.7. One hundred four patients had pancolitis, 58 had left-sided colitis and 2 had proctitis-type colitis. Remission rates at 2 weeks and 6 weeks were 45%, 59%, respectively. Cumulative remission-maintenance rates at 1-, 3-, 5-year were 75%, 59%, 54%, respectively. In the multivariate analysis, previous treatment with calcineurin inhibitors was identified as an independent predictor for a lower remission rate at 6 weeks. Concomitant treatment with immunomodulators was a predictor for a higher cumulative remission-maintenance rate.

Discussion/Conclusion: Approximately 60% of UC patients receiving IFX treatment achieved remission at 6 weeks. Remission was maintained for five years in 54% of patients who achieved remission at 6 weeks. Previous treatment with calcineurin inhibitors was a prognostic factor for a poor short-term outcome of IFX treatment. Conversely, concomitant treatment with immunomodulators was a prognostic factor for a good long-term outcome.
Long-term clinical study of perianal lesions with Crohn’s disease

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Introduction: Anorectal lesions are frequently complicated with Crohn’s disease (CD). These complications include fistula, abscess, ulcer, skin tag, stricture and carcinoma. Perianal fistulas are the most commonly observed condition and exhibit multiple incidence and intractable characteristics. In this study, the long term outcome of perianal lesions with CD was studied in our department.

Methods: The subject were 298 patients with CD who received successive observation over the 10 years in our departments. We studied the long-term clinical course and postoperative outcome of perianal lesions.

Results: At initial observation, 247 (82.9%) of the 298 patients had perianal lesions, and fistula or abscess was the most common (61.4%), and following skin tag (26.2%), ulcerating lesion (24.5%), stricture (15.8%), carcinoma (1.3%) etc. Moreover, the mixture of various anal lesions was found frequently (54.7%). In 115 (38.6%) of the cases, perianal lesions were found the initial manifestation. During the mean follow-up period of 212.3 months (120~402), all lesions increased and exacerbated, particularly with complex fistula and anal stricture. Finally, anorectal carcinoma was complicated 16 patients (5.4%). After conventional fistulotomy (92 cases) for perianal fistula, relapse was frequent (58.7%) with the risk of anal sphincter damage (23.9%). Seton drainage (81 cases) effectively improved symptoms in the long term (good course 34.6%, controllable 34.6%). Rectal amputation was performed for 49 patients including 13 patients with carcinoma and 36 patients with intractable lesions such as complex fistula, vaginal fistula, and incontinence.

Discussion/Conclusion: It is thought, in surgery for perianal lesions with CD, the main goal is to alleviate the symptoms and improve the quality of life, successive careful management is important, with consideration of anal function and complicated carcinoma in the long term.
Protective effect of autophagy on endoplasmic reticulum stress-induced apoptosis of intestinal epithelial cells in chronic colitis model

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Introduction: Inflammatory bowel disease (IBD) results from a complex series of interactions between susceptibility genes, the environment, and the immune system. Recently, some studies provided strong evidence that the process of autophagy affects several aspects of mucosal immune responses. Recent studies have identified susceptibility genes involved in autophagy, such as \textit{NOD2}, \textit{ATG16L1}, and \textit{IRGM}, and active research is ongoing all over the world. In this study, we examined the role of autophagy in chronic colitis model.

Methods: \textit{Atg5}^{flox/flox} villin-Cre mice in which Atg5 is deficient in intestinal epithelial cells were prepared by crossing \textit{Atg5}^{flox/flox} mice and villin-Cre mice. Mice (6–8 weeks of age) were placed on three five-day cycles of 3% DSS with 15 days of recovery between each cycle. Mice were sacrificed after the final 15-day rest period. The severity of DSS colitis was assessed using the disease activity index (DAI). The protein associated with ER stress was examined by immunoblot analysis and immunohistochemistry.

Results: The ratio of weight to length, which is an index of intestinal tissue edema, was significantly higher in \textit{Atg5}^{flox/flox} villin-Cre mice than \textit{Atg5}^{flox/flox} mice. Histological inflammation scores were significantly higher in \textit{Atg5}^{flox/flox} villin-Cre mice than \textit{Atg5}^{flox/flox} mice. The expression of XBP1s/XPB1u ratio was significantly higher in \textit{Atg5}^{flox/flox} villin-Cre mice than \textit{Atg5}^{flox/flox} mice. The expression of phosphorylated (p-) IRE1α and p-JNK was higher in \textit{Atg5}^{flox/flox} villin-Cre mice than \textit{Atg5}^{flox/flox} mice. The number of apoptosis in epithelial cells was significantly increased in \textit{Atg5}^{flox/flox} villin-Cre mice than \textit{Atg5}^{flox/flox} mice.

Discussion/Conclusion: We suggested that autophagy may have a protective effect on endoplasmic reticulum stress induced apoptosis of intestinal epithelial cells in chronic colitis.
The immunomodulatory and antimicrobial properties of PTSO contribute to its intestinal anti-inflammatory effect in experimental colitis

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Introduction: Propyl-propane thiosulfonate (PTSO) is a component isolated from garlic (*Allium sativum*), a plant used as seasoning and in traditional medicine for its health benefits, related to their potent antioxidant, anti-inflammatory, immunomodulatory and antimicrobial properties. This study evaluated the impact of PTSO in the dextran sodium sulfate (DSS) model of mouse colitis.

Methods: Intestinal inflammation was induced in male C57BL/6J mice by incorporating DSS in the drinking water (3%) for 5 days. Mice were administered by oral gavage PTSO (0.01, 0.05 or 0.1 mg/kg day) from the day of colitis induction and sacrificed after 10 days of treatment. The inflammatory status was evaluated by the disease activity index (DAI), histological assessment and colonic gene expression of inflammatory markers. Colonic microbiota composition was determined by pyrosequencing. PTSO immune-modulatory properties were assessed in vitro in Caco-2 (colon adenocarcinoma) and THP-1 (monocytic) cells.

Results: PTSO treatment ameliorated the DSS colonic damage, which was evidenced by a reduction in DAI values in comparison with the control group. It was confirmed by the histological and biochemical evaluation. PTSO treatment ameliorated the expression of pro-inflammatory cytokines (TNFα, IL-1β and IL-6), the adhesion molecule ICAM-1 and the enzyme iNOS. Moreover, PTSO enhanced the colonic expression of occludin and MUC-2, improving the impaired intestinal barrier function. Moreover, PTSO enhanced the altered gut microbiota composition observed in colitic mice. In vitro assays revealed that PTSO reduced the production of pro-inflammatory mediators in Caco-2 and THP-1 cells, and down-regulated MAPKs signaling pathways, involving both p42/44 and p38 phosphorylation.

Discussion/Conclusion: PTSO has shown beneficial effects in the DSS model of experimental colitis, which can be ascribed to its immunomodulatory and antimicrobials effects, thus supporting its future development in human IBD.
Hospital-based IBD epidemiological trends in a Romanian tertiary referral center

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Background: Eastern Europe was previously considered a low incidence and prevalence area for IBD. However, new data confirm that incidence and prevalence are quickly increasing in some regions, reaching Western European countries. In 2006 we have initiated a hospital-based registry (IBDPROSPECT), including IBD cases admitted in our Tertiary gastroenterology Center, the largest in Romania in terms of number of yearly admissions.

Aim: In order to identify recent trends in IBD epidemiology, we have analyzed our Hospital-based registry using IBDPROSPECT variables framework, comparing two patient cohorts, 2012 vs. 2016. We have included in the analysis all IBD cases admitted in our Gastroenterology Unit during the specified time frames.

Methods: Variables concerning sex distribution, new confirmed IBD cases, residence areas, first degree relatives with IBD, Crohn’s disease (CD) and ulcerative colitis (UC) phenotypical characteristics, disease severity and treatment options have been compared between the two cohorts. Qualitative variables have been compared using Chi-squared test, with a two-tailed p value < 0.05 considered for statistical significance. There were 427 patients in 2012 group, with CD:UC ratio of 1.17:1 and 659 patients in the 2016 cohort respectively, with a CD:UC ratio of 0.97:1.

Results: The mean hospital-based prevalence of IBD during the study period was 344.2 cases per 10,000 yearly admissions, 170.8 for CD and 173.2 for UC respectively. There was no significant difference in distribution between the two cohorts with regard to sex, residence area or presence of first degree relatives with IBD. In CD patients there was an increase of stenosing behavior in 2016 (22.7% vs. 14.29%, p = 0.001), an increase in extensive ileo-colonic disease (37.7% vs. 25.5%, p = 0.002), increased diagnosis of intestinal complications (25.2% vs. 14.7%, p = 0.0004) but a decreased hospitalization for moderate-severe flares (19.4% vs. 33.8%). With regard to treatment in CD patients, there was a significant decrease in corticosteroids use in 2016 (21.1% vs. 36.8%, p < 0.0001) and increase in biologicals prescription (53.2% vs. 30.3%, p < 0.0001). In UC patients no significant changes were registered with regard to disease extension but a significant decrease in corticosteroids use in 2016 (21.1% vs. 36.8%, p < 0.0001) and an increase in biologicals prescription (53.2% vs. 30.3%, p < 0.0001). In UC patients no significant changes were registered with regard to disease extension but a significant decrease in corticosteroids use in 2016 (21.1% vs. 36.8%, p < 0.0001) and an increase in biologicals prescription (53.2% vs. 30.3%, p < 0.0001). In UC patients no significant changes were registered with regard to disease extension but a significant decrease in corticosteroids use in 2016 (21.1% vs. 36.8%, p < 0.0001) and an increase in biologicals prescription (53.2% vs. 30.3%, p < 0.0001). In UC patients no significant changes were registered with regard to disease extension but a significant decrease in corticosteroids use in 2016 (21.1% vs. 36.8%, p < 0.0001) and an increase in biologicals prescription (53.2% vs. 30.3%, p < 0.0001). In UC patients no significant changes were registered with regard to disease extension but a significant decrease in corticosteroids use in 2016 (21.1% vs. 36.8%, p < 0.0001) and an increase in biologicals prescription (53.2% vs. 30.3%, p < 0.0001).
Conclusions: The hospital-based IBD prevalence in our tertiary gastroenterology Center has not changed during the last 5 years; however, significant epidemiological trends could be registered with a direct link to therapeutic options. In CD patients, despite a significant increase in stenosing disease behavior and extensive ileo-colonic disease, there was a decrease in hospitalization for moderate to severe cases, probably due to increased use of biologics. The same significant decrease in hospitalization for severe flares was noted also for UC cases, paralleled by a low need for surgery and an increased use of biological treatment.
Elevated risk of stoma outlet obstruction following colorectal surgery in patients undergoing ileal pouch-anal anastomosis

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Introduction: Stoma outlet obstruction (SOO) is a troublesome complication following colorectal surgery requiring stoma. We aimed to clarify the incidence of SOO and to identify risk factors for the development of SOO.

Methods: Patients with colorectal cancer, ulcerative colitis (UC), familial adenomatous polyposis, or diverticulitis who underwent colorectal surgery requiring stoma from 2012 to 2017 were included in the study. A total of 410 patients were included in the present study, and data on age, sex, diagnosis, type of stoma (ileostomy vs. colostomy, loop vs. end), surgical approach (open vs. laparoscopic), past medical history (coronary artery disease, chronic obstructive pulmonary disease, hypertension, diabetes mellitus, and appendectomy), body mass index, and blood hemoglobin concentration were retrospectively reviewed. Fisher’s exact test, chi-square test or Student’s t-test was used for univariable analysis, and logistic regression was used for the multivariable analysis.

Results: The overall incidence of SOO was 7.6% (n = 31). Univariable analysis indicated that UC, ileal pouch-anal anastomosis, ileostomy, loop stoma, rectal cancer, laparoscopy approach, and age were significant higher risk factors for SOO, and abdominoperineal resection was a lower risk factor for SOO, whereas multivariable tests further identified IPAA as an independent risk factor [p = 0.0007, OR = 31.8 (95% CI: 4.3, 232.5)]. Most of the patients who developed SOO were successfully managed by tube drainage through stoma, whereas stoma closure was performed earlier than the originally planned timing in two patients. Twenty-two of the 31 patients (77.4%) developed SOO within 2 weeks postoperatively, with a median time of 6 days (range, 3–41 days).

Conclusion: Patients who received IPAA had a significantly higher risk of postoperative SOO.
Laparoscopy combined with enhanced recovery pathway in ileocecal resection for Crohn’s disease: A randomized study

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Background: Laparoscopic approach is recommended as the first-choice option for simple ileocecal resections. However, there are no randomized trials that have focused on patients with Crohn’s disease [CD] treated by laparoscopy and enhanced recovery pathway. The aim of the present study is to prospectively evaluate the feasibility, safety, and short-term outcomes of laparoscopy with enhanced recovery pathway for CD patients undergoing ileocecal resection.

Methods: A consecutive cohort of 32 CD patients who underwent laparoscopic ileocecal resection between December 2015 and December 2016 were randomized to enhanced recovery after surgery [ERAS] group or standard care group. Primary outcome was total postoperative hospital stay. Secondary outcomes were time to first flatus and stool, pain scores, morbidity, reoperation rate, readmission rate, and in-hospital costs.

Results: Compliance with the ERAS was almost 100% except the items of abdominal drains and early fluid intake. A significantly earlier return of bowel function was observed in the ERAS group. Compared with the standard care group, patients in the ERAS group had shorter postoperative hospital stay and lower in-hospital costs [5.19 ± 1.28 versus 9.94 ± 3.33 days, p < 0.001; 2.70 ± 0.50 versus 3.73 ± 0.75 10,000 RMB, p < 0.001, respectively]. Other parameters did not show any significant differences between the two groups.

Conclusions: Laparoscopic approach within a ERAS perioperative care program is the safe and effective treatment combination for CD patients requiring for ileocecal resection.

This study was registered at Clinical Trial [NCT02777034].
Keys to having a normal pregnancy and delivery in IBD patients

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Introduction: The pregnancy rate in females with IBD is lower compared to general population, mainly because voluntary childlessness, fear of pregnancy and inappropriate information.

Methods: We retrospectively analyzed a group of 51 females who experienced at least one pregnancy after being diagnosed with IBD. The data were extracted from IBDPROSPECT, a hospital-based registry. We evaluated the difficulty in obtaining and keeping a pregnancy, the impact of pregnancy on IBD status and treatment, and the effects of IBD on pregnancy, delivery, health status of the newborn and lactation.

Results: The mean age (SD) at IBD diagnosis (35 UC, 16 CD) was 27.97 (5.18) and the mean age (SD) at the 1st pregnancy was 31.4 (3.95). The group was mainly composed of patients with mild and limited disease: 75% of CD patients had colonic involvement without perianal disease and inflammatory pattern (62%). 83% of UC patients were classified as E1 or E2. At the conception point, 91% of UC and 62% of CD females were in clinical remission.

The group experienced minimal difficulty in obtaining and keeping a pregnancy (19.6% needed > 1 year to obtain a pregnancy, 17.6% had spontaneous abortions, 9.8% had premature births). 83% of the newborns had an APGAR score of 9 or 10. Otherwise, most were indicated a cesarian surgery (72%) and limited their experience to 1 child (60.8%).

The multivariate analysis found significant correlation between CD location and the rate of spontaneous abortions (-0.626, p = 0.009), CD pattern and premature births (-0.623, p = 0.010), CD activity at conception point (CDAI) and number of pregnancies (0.603, p = 0.013), worsening of disease during pregnancy (0.716, p = 0.002), rate of flares in postpartum (0.523, p = 0.034), and reduced lactation (-0.6555, p = 0.021).

The extension of UC correlated with the type of delivery (-0.419, p = 0.014) and the rate of flares in postpartum (0.618, p = 0.014).

The rate of flares and use of corticoid drugs in postpartum correlated with reduced lactation (R = -0.526, p = 0.015; 0.288, p = 0.042).

Discussion/Conclusion: Limited extension, no perianal involvement and inactivity of the disease at conception point are the keys for having a normal pregnancy, delivery and lactation with minimal acute events rate.
Importance of the findings of rectal sparing for the decision of severity of ulcerative colitis

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Introduction: Ulcerative colitis (UC) is known as a disease repeating relapse and remission. In general, inflammation of UC arises from distal proctitis and extends to the proximal colon. However, an atypical distribution of inflamed mucosa, such as rectal sparing (RS), has been previously noted. Furthermore, it is considered that rectal sparing may be the predictive factor of increased recurrence rates and future colectomy.

Methods: We examined the importance of rectal sparing for the decision of severity by analyzing our two cases retrospectively.

Results: We analyzed 31-year-old man and 56-year-old woman diagnosed as UC. Durations of their diseases after the onset of UC are within one year. Both cases were diagnosed as severe UC (total Mayo scores were over 10). Endoscopic examinations revealed the findings of RS (rectum were evaluated as 0 to 1 by Mayo endoscopic subscore (MES), and proximal colons were MES 3 with longitudinal ulcer). Although disease recurrences and remissions in both cases were repeated during short periods even with medications such as corticosteroid, RS was always found at any colonoscopy examinations despite of not taking any topical treatment.

Discussion/Conclusion: It was reported that UC patients with RS have relatively short duration of disease after the onset, and exhibit medical treatment resistance and high recurrence rates. Our cases were similar in the duration after the onset and in the severity. Therefore we should be careful because the risk of future colectomy might be high. The findings of RS were found at any colonoscopy during both recurrence and remission phase in both cases. Taken together, it is important to evaluate not only rectum but also proximal colon to judge the severity correctly when performing colonoscopy.
Infliximab biosimilar in the treatment of ulcerative colitis: A Japanese single cohort observational study

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Introduction: There are no studies about a comparison of Infliximab biosimilar (IFXBS) and the originator infliximab infusion therapies for patients with ulcerative colitis (UC) in Japan.

Methods: We evaluated the efficacy and safety of IFXBS retrospectively compared with the originator in a single cohort analysis in 77 patients with UC in Chiba University hospital from July 2010 to September 2016. Patients received infusions at a dose of 5 mg/kg of body weight at Weeks 0, 2, and 6, and then every eight weeks. We assessed Lichtiger index score (LIS) and defined remission as LIS ≤ 3. Laboratory data and LIS were measured at every infusion time point.

Results: In all 24 patients who received IFXBS, 29.2% (n = 7) were female, 91.7% (n = 22) were bio-naïve and 79.2% (n = 19) were treated with immunomodulators in combination. Median age at disease onset was 29 years old (interquartile range [IQR], 23–40) and median disease duration was 5.5 year (IQR, 2–8). Similarly, in all 53 patients who received the originator, 35.8% (n = 19) were female, 5.7% (n = 3) were bio-naïve and 41.5% (n = 22) were treated with immunomodulators in combination. Although there were significantly more patients received immunomodulators as concomitant drug in IFXBS cohort compared with the originator cohort (p = 0.002), other baseline characters did not show the significant difference between the two groups. LIS and laboratory data at each infusion time point did not show the significant difference, and remission rate at Weeks 14 and 30 were also similar (p = 0.411 and 0.061, respectively). Infusion reaction occurred in two patients (8.3%) received IFXBS and 9 patients (17.0%) received the originator (p = 0.315) and drug-induced adverse events occurred in one patient (4.2%) and 3 patients (5.7%), respectively (p = 0.784).

Discussion/Conclusion: IFXBS is considered to be efficacious and safe compared with the originator infliximab in Japanese UC patients.
Correlation between macroscopic severity of Crohn’s disease in resected intestine and bowel wall thickness as evaluated by water-immersion ultrasonography

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Introduction: Transabdominal ultrasonography (TAUS) is a commonly used and accurate tool in managing Crohn’s disease (CD); however, the significance of the data obtained is poorly understood. This study was performed to determine the association between wall-thickness evaluated by water-immersion US and macroscopic severity of CD in surgically resected specimens.

Methods: One-hundred intestinal segments from 27 patients with CD were evaluated retrospectively. The resected specimens were placed in saline immediately after surgery and bowel wall thickness (BWT) measured by water-immersion US and compared with macroscopic findings. Also to confirm the accuracy of water-immersion US, findings of water-immersion US and TAUS performed preoperatively were compared. Correlations between BWT and macroscopic findings were assessed using analysis of variance (ANOVA) and receiver operating characteristic (ROC) analysis. Pearson correlation analysis was employed to assess the relationship between water-immersion US and TAUS.

Results: The mean BWT of macroscopically intact areas was 4.1 mm (n = 12), and in areas with longitudinal ulcer scars 5.4 mm (n = 8), with longitudinal ulcers 6.0 mm (n = 22), with large ulcers: 6.4 mm (n = 31), with cobble-stone-like lesions 7.1 mm (n = 8), and with fibrotic strictures 7.4 mm (n = 19). For all types of lesion except longitudinal ulcer scars, BWT was significantly thicker than that of macroscopically intact areas (p < 0.001). Additionally, BWT of fibrotic strictures was significantly thicker than that of longitudinal ulcers and ulcer scars (vs. open ulcers, p = 0.009, vs. ulcer scar, p = 0.002). According to ROC curves, BWT > 4.5 mm is associated with CD lesions (area under ROC curve [AUC], 0.911; sensitivity 97.6%; specificity 71.4%), and > 5.5 mm with more severe lesions (AUC, 0.843; sensitivity, 73.7%; specificity, 69.6%). Pearson correlation analysis identified a strong positive correlation between water-immersion US and TAUS (r = 0.784, p < 0.001).

Discussion/Conclusion: BWT of CD lesions evaluated by water-immersion US correlates with macroscopic disease severity.
Effects of acesulfame potassium on indomethacin-induced enteritis

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Introduction: Acesulfame potassium (ACK), a kind of the artificial sweetener, has been used for various processed foods as food additives worldwide. However, the alteration of gut microbiota and induction of metabolic syndrome by artificial sweeteners were reported. We hypothesized that artificial sweeteners affect the gut immunity and aimed to examine the influence of ACK on the indomethacin (IND)-induced enteritis of mice.

Methods: We used male C57BL/6J mouse (8 weeks). ACK (150 mg/kg w/v) was administered to some groups by free dirinking. To induce small intestinal injury, IND (10 mg/kg) was administered intraperitoneally 1 day before the sacrifice. Histological damage was evaluated and mRNA expressions of inflammatory cytokines were examined.

Results: No histological change was observed in HE staining between control and ACK group, but surprisingly mRNA expressions of TNFα, IFNγ, IL1β, ICAM, VCAM, and MAdCAM were significantly increased in ACK group. MPO activity was significantly increased in ACK and IND group compared with IND group. But histological damage by IND was not altered by the additional administration of ACK.

Discussion/Conclusion: We found that administration of ACK increased inflammatory cytokines and adhesion molecules, although there was no histological change, suggesting that ACK individually might have effects on gut immunity partly via alteration of lymphocyte homing. Those effects were exaggerated by administration of IND. Our study suggests that daily use of artificial sweeteners might alter gut immunity. Since it is reported that MAdCAM is aberrantly expressed in the intestinal tract of the inflammatory bowel diseases, increased expression of MAdCAM might be related with the onset of intestinal inflammation.
Development of a novel transabdominal ultrasound disease activity score in patients with ulcerative colitis (UCUS score)

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Introduction: Colonoscopy (CS) is the gold standard technique for determination of treatment for Ulcerative Colitis (UC). However, CS can be burdensome to patients. Transabdominal ultrasonography (TAUS) may become an alternative to CS because it is non-invasive, and has excellent spatial resolution. This study was performed to identify the US parameters that can predict UC endoscopic activity and develop a simple US score.

Methods: 116 patients with UC underwent both TAUS and CS within a 1-week period. Four TAUS parameters were assessed: bowel wall thickness (BWT), bowel layer structure, color Doppler, and white moss echo. Endoscopic activity was graded with the UC Endoscopic Index of Severity (UCEIS). All assessments were performed by advanced experts in a blinded fashion. Correlations between the US and CS variables were assessed using Spearman’s rank correlation coefficient. Multiple regression analysis was employed to develop a predictive TAUS score.

Results: BWT showed the strongest correlation with the UCEIS (Spearman 0.767, p < 0.0001). The wall layer structure (Spearman 0.524, p < 0.0001) and color Doppler (Spearman 0.725; p < 0.0001) and white moss echo (Spearman 0.662, p < 0.0001) were also correlated with the UCEIS. Based on the multiple regression analysis results, we developed a US activity score as follows. BWT: 0 (≤ 3 mm), 8 (> 3 mm, ≤ 5 mm), and 16 points (> 5 mm); wall layer structure: 0 (preserved), 1 (obscure), and 2 points (disappearing); and color Doppler: 0 (Limberg 0), 4 (Limberg 1), 8 (Limberg 2), and 12 points (Limberg 3); and white moss echo: 0 (none), 5 (presence). This novel US score of UC (“UCUS score”) showed a strong correlation with the UCEIS (Spearman 0.845, p < 0.0001).

Discussion/Conclusion: We developed a UCUS score that accurately identifies UC endoscopic activity. Non-invasive TAUS may be a very useful alternative to CS for quantitative evaluation of bowel activity.
Clinicopathological features of nivolumab-induced colitis

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Introduction: Nivolumab is efficacious for the management of patients with metastatic melanoma and other solid tumors. However, immune-related adverse events (irAEs) are known as serious side effects of immune-checkpoint inhibitors. The purpose of this study is to clarify the clinicopathological characteristics of colitis occurring as an irAE in patients treated by nivolumab.

Methods: During a period from December 2015 until February 2018, we encountered 7 patients who were suspected of having nivolumab-induced colitis. There were 2 women and 5 men with ages ranging from 51 to 81 years (mean, 66 years). We retrospectively investigated clinical features, endoscopic findings and pathologic characteristics of the patients. Nivolumab-induced colitis was defined as 1) diarrhea occurring during nivolumab treatment, 2) exclusion of other causes of diarrhea, and 3) histological confirmation of apoptosis colonic epithelial cells.

Results: The underlying tumor was malignant melanoma in three patients, non-small cell lung cancer in two patients and renal cell carcinoma in two patients. In addition to diarrhea, two patients complained of hematochezia. Three of seven patients fulfilled the diagnostic criteria of nivolumab-induced colitis. The remaining four patients manifested diarrhea and they were free from other causes of diarrhea, apoptosis was not evident in these patients. Colonoscopic findings of nivolumab-induced colitis included friable erythematous mucosa of the rectum and granular mucosa of the colon, which were apparently compatible with ulcerative colitis. These patients were treated by prednisolone, which was effective in patient. Two patients intractable to prednisolone were treated by infliximab, but a patient failed to respond to infliximab. Four patients without nivolumab-induced colitis had normal colonoscopic findings and they continued nivolumab without any specific treatment.

Discussion/Conclusion: Colitis mimicking ulcerative colitis is one of irAEs induced nivolumab. Our diagnostic criteria, which include apoptosis of colonic epithelial cells, may be appropriate in consideration of mucosal damages and subsequent clinical course.
Berberine chloride, an enhancer of IL-10 production in macrophages, ameliorate an experimental colitis in mice

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Introduction: Abnormalities of intestinal innate immune functions have been regarded as key properties in immunogenetic profile of inflammatory bowel disease (IBD). Intestinal macrophages play pivotal roles in the regulation of immune homeostasis in the intestine. We have recently reported that the enhancement of IL-10 production in the intestinal macrophages has the potential to be a novel therapeutic mechanism against IBD. Thus, to address the development of new therapeutic medicines for IBD, we have screened 96 compounds derived from medicinal herbs for the ability to enhance IL-10 production in the intestinal macrophages.

Methods: Male BALB/c mice were used. Bone marrow-derived macrophages (BMDMs) were cultured with macrophage colony-stimulating factor (100 ng/ml) for 7 days. Experimental acute colitis in mice was induced by giving 3% dextran sulfate sodium (DSS) in drinking water for 7 days.

Results: Among the 96 compounds, we found that Berberine chloride significantly increased the LPS (100 ng/ml)-stimulated IL-10 production in BMDMs. Berberine chloride (1–30 µM) significantly increased the LPS-stimulated IL-10 production of BMDMs in a concentration-dependent manner. Further, Berberine chloride increased the expression of IL-10 mRNA and inhibited the expression of proinflammatory cytokines such as TNF-α, IL-1β and IL-6 mRNA in the colonic lamina propria cells. Importantly, the administration of Berberine chloride (100 mg/kg, po) markedly suppressed the development of DSS-induced colitis with a concomitant increment of IL-10 mRNA expression in the colons of colitis mice. Further, Berberine chloride significantly inhibited the upregulation of TNF-α, IL-1β and IL-6 mRNA expression in the colon of the DSS-induced colitis mice.

Discussion/Conclusion: These results can lead to the hypothesis that enhancers of IL-10 production in intestinal macrophages suppress the development of colitis.
Correlation between fascin-1 and caspase-8 proteins expressions in ulcerative colitis

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Introduction: Fascins are a group of globular proteins responsible for maintaining the normal structure of the cellular cytoskeleton. Fascin-1 builds structures responsible for the migration of cells, which are filopodia. It has been shown that fascin-1 in normal colon epithelial cells is absent, while the presence of fascin-1 in the inflammatory cells and tumor cells has been confirmed. Overexpression of this protein is associated with filopodia formation, increased ability to cells migration and increase of cells proliferation, especially of cancer cells. On the other hand, caspase-8 is one of initiators of apoptosis, a programmed cell death. Its classic role is to detect the signal and activate the cascade leading to apoptosis. Currently, caspase-8 is now known to have a seemingly misleading opposing effect in securing cell survival. Both proteins may play a role in inflammatory bowel diseases. Therefore, the purpose of the research was to evaluate and compare the expressions of fascin-1 and caspase 8 proteins in ulcerative colitis.

Methods: The study included a group of 30 patients with ulcerative colitis. The expressions of fascin-1 and caspase-8 in tissue material was evaluated and determined by the immunohistochemical technique. The staining reaction was observed in details in the surface epithelium, normal and dysplastic glands and in inflammatory cells.

Results: Patients with ulcerative colitis showed no expression of fascin-1 in epithelial cells in 45% cases, weak, medium and strong in 55% (12.5%, 20% and 22.5%, respectively). In inflammatory cells of UC we observed rather positive reaction of this protein (72.5%). The weak and the medium expression of caspase-8 was observed in the surface epithelium of ulcerative colitis (38.7% and 38.7%, respectively), the absence and the weak expression in normal glands of UC (41.9% and 32.3%, respectively), predominant weak and medium reactions in dysplastic glands (33.3% and 50%, respectively), and weak in the inflammatory cells of UC (58%). Moreover, higher expression of caspase-8 in dysplastic glands correlated with decreased expression of fascin-1 in glandular epithelium (p = 0.040) and inflammatory cells of UC (p = 0.030). But fascin-1 expression didn’t correlate with grade of dysplasia in UC.

Conclusion: Fascin-1 and caspase-8 proteins seem to play a role in ulcerative colitis development. But the high expression of caspase-8 in dysplastic glands of UC in correlation with low expression of fascin-1 seems to play rather pro-apoptotic than pro-survival function in UC.
IL-4 receptor-deficient mice are resistant to the development of DSS-induced colitis

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Introduction: Ulcerative colitis (UC) patients have been reported to show Th2-like responses in the colonic mucosa. The major Th2 cytokines, IL-4 and IL-13 exert their function through common IL-4 receptor alpha (IL-4R). However, the precise role of IL-4R signaling in UC remains unclear. In the present study, we examined the role of IL-4R signaling in the experimental colitis using IL-4R knockout (KO) mice.

Methods: IL-4R KO mice (BALB/c background) and their littermate WT mice were used. Experimental acute colitis was induced by giving 3% DSS in drinking water for 7 days. Colitis symptoms such as body weight loss, diarrhea occurrence and rectal bleeding were observed daily. After 7 days, the colons of mice were excised for histological analysis and quantitative real-time PCR analysis.

Results: The treatment with DSS for 7 days caused damage in the colon, with body weight loss, diarrhea occurrence and rectal bleeding as well as shortening of colon length. These manifestations of DSS-induced colitis were significantly reduced in IL-4R KO colitis mice compared with WT colitis mice. The histological examination of colonic mucosa showed a loss of epithelial integrity and crypt architecture as well as submucosal edema in WT colitis mice and these histological abnormalities were diminished in IL-4R KO colitis mice. Further, the expression of proinflammatory cytokines such as IL-1β and IL-6 mRNA in the colons of WT colitis mice was markedly upregulated, which were significantly higher in those of IL-4R KO colitis mice.

Discussion/Conclusion: These results demonstrate that IL-4R KO mice are resistant to the development of DSS-induced acute colitis. The present finding suggests the possibility that IL-4R signaling is involved in the pathogenesis of UC.
Luminal protease activity is increased in pouch inflammation of ulcerative colitis patients

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Introduction: Restorative proctocolectomy ileal pouch anal anastomosis (IPAA) is the surgical therapy for reconstruction of bowel continuity in refractory ulcerative colitis (UC) patients requiring proctocolectomy. Up to 50% of these patients develop pouch inflammation (pouchitis). Pouchitis may occur through compromise of the epithelial barrier function through disruption of tight junction proteins. Increased luminal protease activity was associated with exacerbation of colitis and may originate from dysbiotic microbiome. We aimed to study whether luminal proteolytic activity is increased in UC pouchitis patients and whether their fecal supernatants mediate epithelial permeability.

Methods: Fecal supernatants were extracted from: normal pouch (NP), active pouchitis (AP) and healthy controls (HC) participants. Fecal protease activity was determined using FITC-casein florescence assay. Caco-2 cells monolayers were exposed to fecal supernatants. Epithelial integrity and permeability were determined by measuring trans-electrical epithelial resistance (TEER) and permeability of a 4 kDa FITC-dextran across the monolayers. Immunofluorescence and Western blot were performed on Caco-2 cells to assess for tight junction proteins integrity (ZO-1, occludin) post exposure to fecal supernatants.

Results: Fecal supernatants derived from twenty-five patients were analyzed: 6 NP, 10 AP and 9 HC participants. AP patients exhibited 4.3-fold (p < 0.05) greater proteolytic activity compared to NP patients and HC. Most proteolytic activity was inhibited by specific bacterial protease inhibitors. Fecal supernatants from AP disrupted occludin and ZO-1 (Western blot and immunofluorescence). TEER was reduced by 1.6 fold change (p < 0.05) and epithelial permeability to FITC-dextran was increased by 18.36 (p < 0.05) and 73 fold (p < 0.01), compared to fecal sups from NP and HC, respectively.

Discussion/Conclusion: Pouch inflammation is associated with increased luminal proteolytic activity, probably of bacterial origin. Fecal supernatants from pouchitis patients disrupt tight junction proteins of Caco-2 cell monolayers and increase epithelial cells permeability, implicating a mechanism through which pouch inflammation may be initiated.
Intestinal anti-inflammatory effects of *Kalanchoe brasiliensis* and *Kalanchoe pinnata* extracts exert intestinal anti-inflammatory effects in experimental colitis

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**Introduction:** Different plant extracts have been reported to exert beneficial effects in experimental models of colitis, most probably due to the presence of phenolic compounds that exert antioxidant, antiinflammatory and immunomodulatory properties. The aim of the present study was to compare the effects of two extracts obtained from the aerial parts of *Kalanchoe brasiliensis* and *K. pinnata*, which have been traditionally used in Brazil for their antiinflammatory properties, in the dextran sodium sulfate (DSS) model of mouse colitis.

**Methods:** Intestinal inflammation was induced in male C57BL/6J mice by incorporating DSS in the drinking water (3%) for 6 days. From the day of colitis induction, colitic groups were administered the *Kalanchoe* extracts (100–200 mg/kg day), while colitic and healthy control groups were given water. Mice were sacrificed after 9 days of treatment. The inflammatory status was evaluated by determining the disease activity index (DAI), macroscopic analysis of the colonic segments and colonic gene expression of inflammatory markers.

**Results:** *Kalanchoe* extracts improved the colonic damage induced by DSS, as evidenced by a reduction in DAI values in comparison with the control group, which was confirmed when colonic macroscopic analysis was performed. Biochemically, both extracts ameliorated the expression of pro-inflammatory cytokines (TNFα, IL-1β and IL-6), the adhesion molecule ICAM-1, the chemokine MCP-1 and the inducible enzyme iNOS. However, only *K. brasiliensis* extract enhanced the intestinal barrier function restoring the expression of TFF-3 and villin.

**Discussion/Conclusion:** Both *K. brasiliensis* and *K. pinnata* showed beneficial effects in the DSS model of experimental colitis, mainly related to their ability to improve the altered immune response in the inflamed colon, and *K. brasiliensis* also enhancing the intestinal barrier function.
Accuracy of transabdominal ultrasound in assessing disease activity in ulcerative colitis

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Introduction: A paradigm shift in the treatment of ulcerative colitis (UC) has emerged with recent medical advancements. Beyond clinical remission, endoscopic mucosal healing has become a major therapeutic goal of UC and is associated with better long-term prognosis. In addition, it is known that clinical symptoms do not accurately reflect the luminal inflammation. Therefore, endoscopic evaluation is considered indispensable, however, frequent colonoscopy (CS) could be difficult due to its invasiveness. Transabdominal ultrasonography (TAUS) is a non-invasive imaging technique which enables us to frequently monitor the disease and its utility has been previously confirmed. This study precisely examined the usefulness of Doppler TAUS in patients with UC comparing with CS.

Methods: Retrospective chart review of 14 patients with UC who were examined both CS and Doppler TAUS from July 2017 to April 2018 was conducted. Patients in whom CS and TAUS were conducted within the interval of 1 month without the therapeutic intervention of induction treatment between them were recruited. Severity was defined by the Mayo endoscopic subscore (MES) for colonoscopy and Limberg score. Limberg score in TAUS was graded from Grade 0 to 4. MES and Limberg score were compared per-colonic segment (ascending, transverse, descending, sigmoid and rectum) and the sum of both scores were calculated. Finally, the association with Limberg score with between activity scores (Lichtiger index [LI] and partial Mayo score [PMS]), C-reactive protein (CRP), and MES was assessed by non-parametric Spearman rank correlation (rs) and receiver operating characteristic analysis.

Results: Limberg score was significantly associated with MES (rs = 0.61, p = 0.02). The sum of Limberg scores also well-correlated with the sum of MES (rs = 0.68, p < 0.01). CRP and clinical severity scores such as LI and PMS did not correlate with MES (CRP: rs = 0.44, p = 0.12, LI: rs = 0.25, p = 0.39, PMS: rs = 0.41, p = 0.15). Per-colonic segment analysis demonstrated a significant correlation between Limberg score and MES (rs = 0.69, p < 0.01). Association was significant in ascending (rs = 0.92, p < 0.01), transverse (rs = 0.83, p < 0.01), and descending (rs = 0.60, p = 0.02) whereas not significant in sigmoid (rs = 0.43, p = 0.13) and rectum (rs < 0.01, p = 0.98). Limberg score = 0 accurately predicted MES = 0 (sensitivity 77.6%, specificity 90.5%, AUC = 0.86) and MES ≤ 1 (sensitivity 87.9%, specificity 70.3%, AUC = 0.83).
**Discussion/Conclusion:** Doppler TAUS, but not clinical symptoms or biomarker reflects endoscopic severity. TAUS is a useful monitoring tool alternative to CS, however, is less accurate in the assessment of distal colon.
Clinical usefulness of bowel ultrasonography for patients with ulcerative colitis

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Introduction: During follow-up, some patients with ulcerative colitis (UC) are reluctant to undergo repeat colonoscopy (CS). In such patients, ultrasonography (US) of the bowel can be a useful, non-invasive tool for assessing disease activity. Because few studies have assessed this option, this study aimed to compare the accuracy of bowel US with CS for patients with UC.

Methods: We retrospectively reviewed the medical records of patients with UC who underwent total CS and bowel US from 2013 to 2017. We categorized the colon into five segments, ascending colon, transverse colon, descending colon, sigmoid colon and rectum. We assessed the endoscopic severity with the Mayo endoscopic subscore, using a score of 2 or 3 to define active UC. In addition, we evaluated the maximum bowel wall thickness (BWT) of the colon as the US parameter, with a thickness ≥ 4 mm (≥ 6 mm in the rectum) defined as active UC, and also assessed the loss of wall stratification. Furthermore, we evaluated the median BWT and the loss of wall stratification per Mayo endoscopic subscore, followed by the sensitivity and specificity of bowel US to detect active UC lesions.

Results: Of 160 patients enrolled in this study. Mayo endoscopic subscores of 0, 1, 2 and 3 corresponded to median BW Ts of 2.4, 3.9, 4.8 and 6.3 mm, respectively. US findings of the complete or partial loss of wall stratification occurred in 12%, 10%, 25% and 51% of patients with respective Mayo endoscopic subscores of 0, 1, 2 and 3, respectively. The respective sensitivity and specificity of bowel US for detecting active UC lesions were 77% and 70%, respectively.

Discussion/Conclusion: The BW Ts and the loss of wall stratification of the colon increased as the endoscopic severity became higher. Bowel US may be a useful follow-up tool for patients with UC.
TNF-α and IFN-γ induce an anti-inflammatory phenotype in intestinal mesenchymal stromal cells


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Introduction: There is increasing evidence that exosomes secreted by mesenchymal stromal cells (MSC) are one of the main mediators of their physiological/therapeutic effects. The immunomodulatory properties of MSCs from bone marrow and adipose tissue have been extensively investigated, few studies focusing on intestinal MSCs (IMSC) and their exosomes have been performed. They could participate both in the development of autoimmune diseases like IBD and also be used as therapeutic tools for treating these conditions. Thus, the aim of the study is to evaluate the effect of different stimuli present in the gut on the expression of immune mediators produced by IMSCs as well as to better characterize the exosomes they secrete.

Methods: IMSCs were isolated with an enzymatic cocktail from resections of intestinal human samples and cultured. At passage 3-5, their phenotype was checked by FACs. IMSCs at passage 7 and 70–80% confluency were treated with different stimuli: (1) LPS (10 ng/ml); (2) poly I:C (1 μg/ml); and (3) TNF-α (3 ng/ml) and IFN-γ (10 ng/ml) during 1 and 24 h. IMSCs without stimulation were used as negative control. After the treatment, the medium was replaced with new free serum medium and 24–48 h later, the supernatant was collected. The expression of immune mediators was analyzed by RT-qPCR, and polarization towards MSC1 and MSC2 phenotypes was assessed.

Results: Cells isolated from the intestinal resections showed a fibroblastic like shape and a mesenchymal phenotype (CD90+, CD73+, CD105+ and CD45−). With regards to immune mediators expression, 1 h and 24 h of stimulation with TNF-α and IFN-γ showed the most promising results – the expression of indoleamine 2,3-dioxygenase (IDO) was significantly increased when comparing it with the negative control. Moreover, this combination did not induce the expression of proinflammatory cytokines such as IL-6 and IL-8. Conversely, LPS (1 h and 24 h) and poly I:C (1 h) did not induced the expression of IDO and the expression of IL-8 was induced after 1 hour stimulation with LPS. Poly I:C stimulation for 24 h showed similar results to TNF-α and IFN-γ, unless to a lesser extent.

Discussion/Conclusion: Independently of the stimulation time, TNF-α and IFN-γ can polarize IMSCs towards an anti-inflammatory MSC2 phenotype, as well as 24 h stimulation with poly I:C. It is very likely that the exosomes released by these cells display similar immunomodulatory properties and could be used for the development of a new therapy for IBD.
A comparison of thiopurines versus anti-TNF-α therapy in steroid-dependent ulcerative colitis using propensity-score matched analysis

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Introduction: Ulcerative colitis (UC) can be successfully treated by 5-aminosalicylates and corticosteroids, however, their efficacy has been inadequate and corticosteroid refractoriness or dependence is a clinically important problem. Up to 20% of UC patients were reported to become steroid-dependent during their clinical course. Although immunomodulators (IM) such as thiopurines (azathioprine or 6-mercaptopurine) are recommended in steroid-dependent UC, the occurrence of thiopurine-related adverse events has been reported relatively high (15–20%). Anti-tumor necrosis factor (TNF)-α therapy has proven effective for the induction and maintenance of remission in UC. As most studies of these therapies for UC included patients with both steroid-refractory and steroid-dependent, it is unclear which therapy is more effective for maintenance therapy in steroid-dependent UC. Aims of this study were to compare the effectiveness and safety of thiopurines with anti-TNF-α therapy in steroid-dependent UC.

Methods: Consecutive patients with steroid dependent UC treated with thiopurines (IM) or anti-TNF-α therapy between May 2007 and December 2015 were retrospectively enrolled. The patients treated with IM and anti-TNF-α combination therapy were excluded. We evaluated sustained clinical response and adverse events in patients who had IM or maintenance anti-TNF-α therapy using propensity score matching and Cox proportional hazards analysis.

Results: We enrolled 101 patients (IM group; 75 patients and anti-TNF-α group; 26 patients). As treatment was discontinued owing to adverse events or primary non-response (within 8 weeks), 87 patients (IM; 64 and anti-TNF-α; 23) were analyzed for long-term outcome. Concomitant corticosteroid was required in 82.8% of IM group and 43.5% of anti-TNF-α group. 1-year and 3-year relapse-free rates were 54.8% and 39.3% in IM group and 67.9% and 57.5% in anti-TNF-α group, respectively (p = 0.135 by Log-rank test). Univariate and multivariate Cox proportional hazards analysis showed that no significant difference in the risk of relapse between groups [anti-TNF-α/IM; hazard ratio (HR) = 0.579, 95% confidence interval (CI): 0.280–1.196, p = 0.140], [anti-TNF-α/IM; HR = 0.741, 95% CI: 0.321–1.712, p = 0.483], respectively. Multivariate analysis also showed that serum albumin level was independently associated with the risk of relapse [HR = 0.476, 95% CI: 0.271–0.836, p = 0.033]. On propensity-score matched analysis, there was no significant difference in the risk of relapse between groups [anti-TNF-α/IM; HR = 0.752, 95% CI: 0.320–1.763, p = 0.522]. Adverse events were observed in 11 patients (14.7%) in IM group, while no adverse events were observed in anti-TNF-α group.
Discussion/Conclusion: Anti-TNF-α therapy for maintenance of remission in patients with steroid-dependent UC had similar effectiveness and less adverse events compared to thiopurines.
On the clinical course of anti-TNFα agent in ulcerative colitis (UC)

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Introduction: Biologics were effective in patients with UC. But the efficacy was lost in some patients. In this study, we examined the prediction factors of loss of response (LOR) and the efficacy of 2nd biologics.

Methods: We consecutively assigned 305 patients (Men 185, Women 120) with UC who were received anti-TNFα agent (infliximab [IFX], adalimumab [ADA]) in our hospital for the first time from March 2009 to July 2017. The research design is a retrospective review of a prospective database. We examined the short-term remission rate, the cumulative continuous administration rate, the prediction factors of LOR, the efficacy of 2nd biologics, and adverse events. Clinical remission was assessed using partial Mayo score (pMayo score). Primary LOR was defined as non-remission at 14 weeks, Secondary LOR was defined as relapse after remission at 14 weeks.

Results: In the patient background, the pMayo score was slightly higher in the IFX group than in the ADA group. Short-term remission rates at 14 weeks were 48% in the IFX group and 39% in the ADA group. 130 of 250 patients in the IFX group and 33 of 55 patients in the ADA group became primary LOR. The efficacy of switching cases between both agents in primary LOR was about 30%. 23 of 120 patients in the IFX group and 1 of 22 patients in the ADA group became secondary LOR. The efficacy of switching cases between both agents in secondary LOR was about 70%. About half of 305 patients had continued the agent for a long time.

Discussion/Conclusion: Anti-TNFα agent was an effective treatment not only for short term but also for long term against UC. Switching between anti-TNFα agents was one of effective treatment for secondary LOR.
**Introduction:** Small GTPase Ral regulates tumorigenesis and invasion/metastasis in some cancers, however, the role of Ral in colitis-associated cancer (CAC) has not been investigated. We aimed to elucidate the role of Ral in the mechanism of CAC.

**Methods:** Immunohistochemical (IHC) staining was performed on normal colonic epithelium and human CAC samples to investigate the expression of RalGAPα2. We used RalGTPase-activating protein α2 (RalGAPα2) knockout (KO) mice that could activate Ral. CAC was induced in wild-type (WT) mice and RalGAPα2 KO mice by intraperitoneal injection of azoxymethane following adding of dextran sulfate sodium. Colon tissues were collected from both mice and analyzed by histology, polymerase chain reaction, and immunoblotting. Colon26 cells were transfected with RalGAPα2 siRNA to examine the effect of RalGAPα2 knockdown on their migratory and invasion capacity. Intestinal epithelial cells were isolated from colonic tissues of both mice, and we performed cDNA microarray analysis.

**Results:** IHC staining on human CAC samples showed lower expression of RalGAPα2 than normal colon epithelium. RalGAPα2 KO mice had a significantly larger number and size of tumors with higher proportion of tumors invading the submucosa than WT mice. Colon26 cells transfected with RalGAPα2 siRNA had the increased migratory and invasive capacity. Significant expressions of matrix metalloproteinase (MMP)-9 and -13 were observed in RalGAPα2 KO mice in comparison with WT mice. The expressions of IL-1β, NLRP3, ASC, and Caspase-1 were significantly elevated in tumors of RalGAPα2 KO mice than those of WT mice.

**Discussion/Conclusion:** Ral activation is involved in the mechanism of CAC development through Ral-NLRP3 inflammasome pathway.
Short- and long-term efficacy of 5-aminosalicylic acid for intestinal Behçet disease


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Introduction: Behçet disease (BD) is a chronic, relapsing-remitting, inflammatory, immune disorder. Intestinal BD is a subtype that is characterized by intestinal lesions and associated gastrointestinal (GI) symptoms. Guidelines recommend 5-aminosalicylic acid (5-ASA) for patients with mild to moderate activity; however, there is no reliable clinical evidence for this. The aims of this study were to evaluate the efficacy and safety of 5-ASA in induction and maintenance of remission in patients with intestinal BD.

Methods: This was a retrospective, observational, two-center study of 42 patients with mildly to moderate active intestinal BD who were treated with oral 5-ASA. Clinical remissions and responses were evaluated using the Crohn Disease Activity Index (CDAI) and endoscopic remissions and responses using a modified global GI endoscopic assessment score. The cumulative rates of surgical-free survival were calculated, and predictors of clinical response identified. Adverse events occurring after initiation of 5-ASA were also assessed.

Results: Of the 42 patients enrolled, 35% achieved clinical remission and 47% clinical response after taking 5-ASA for 8 weeks. The endoscopic remission rate was 30% and the response rate 65%. The probability of surgery-free survival was 84% at 60 months. Univariate analysis of factors possibly contributing significantly to clinical response failed to identify any, including severity and size of ulcer. The adverse event rate was 17% and no serious toxicities occurred.

Discussion/Conclusion: This is the first study to evaluate efficacy of 5-ASA in patients with intestinal BD: 5-ASA was well tolerated and effective in inducing and maintaining intestinal BD remission regardless of endoscopic severity.
Identification of colitis-associated bacteria in intestine of inflammatory bowel disease patients

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Introduction: Dysbiosis of intestinal microbiota in patients of inflammatory bowel disease (IBD) including ulcerative colitis (UC) and Crohn's disease (CD) is closely related to intestinal inflammation. We clarified the characteristics of IBD intestinal microbiota by metagenomic analysis and identified bacteria causing intestinal inflammation.

Methods: Whole genome shotgun sequencing (WGS) was performed on the fecal DNA of 16 UC patients, 8 CD patients, and 13 healthy donors (HD). The composition of bacterial microbiota was compared by linear discriminant analysis (LDA). Intestinal microbiota of IL10-deficient mice was sterilized using antibiotics, and the feces of the subjects were transplanted into mice. We compared body weight change, intestinal pathology score, and expression of inflammatory cytokines (TNF, IL6, IL1b, IL2b, IL17, and IL23a) of intestinal tissue using real-time PCR. We characterized the bacterial microbiota of the mice by 16SrRNA sequencing. Additionally, we isolated and cultured the specific bacteria from IBD patients and administered them to mice.

Results: Compared with the bacterial microbiota of the HD patients, there were 43 different bacterial taxonomies in the UC patients and 56 differences in the CD patients. Particularly, Enterococcus faecium in UC had the highest LDA scores. In the UC group, weight gain was less, the pathology score was higher, and the expression levels of tnf, il1b, and il17 were increased than in the HD group. In the bacterial microbiota of the UC group, Enterococcus genus were significantly more abundant than those of the HD group. Mice administered E. faecium obtained from UC patients showed less weight gain, higher pathology score, and higher expression levels of inflammatory cytokines. Finally genotype of the isolates was analyzed by WGS and compared with genotype of commercial probiotic E. faecium strain.

Discussion/Conclusion: The intestinal microbiota of UC patients induces colitis and E. faecium might be one of the bacteria causing intestinal inflammation.
Crohn’s disease patients with the anal lesion as an initial symptom undergoing our hospital treatment

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Introduction: Inflammatory bowel diseases including Crohn’s disease tend to increase. A problem is becoming of especially few surgeons specialists. While medical treatment of Crohn’s disease are advanced, it is also important to consider concurrent anal disease.

Methods: We examined the course of treatment and the presence of Crohn’s disease in patients with anal lesions who are being treated at our hospital.

Results: 166 patients with Crohn’s disease who are currently undergoing treatment as a result of outcome were found. The age of onset is 10 years old to 72 years old. The illness period before diagnosis of Crohn’s disease is from one to 53 months. Surgery was performed on 3 intestinal perforations, 2 intestinal stenosis and 1 rectal cancer. Many of them are receiving regular injections of pentasa, imuran, prednisolone, remicade, fumira and/or infliximab. Nine cases requiring hospitalization more than once, each continuing injection therapy. Twenty-seven patients were admitted to the hospital with anal symptoms as the initial symptoms. There were 20 patients who became referrals after other medical consultations and became confirmed diagnosis. Twelve cases were treated with perianal abscess or anal fistula. Other anal symptoms are anal pain and bleeding. There were 6 cases in 18 years of age or younger, 11 years old, 13 years old, 15 years old, 16 years old and 2 cases of 18 years old. Four cases were anal pain, two cases were symptoms of perianal abscess, and two cases were introduced after pediatric checkup. For anal lesions, surgical treatment (drainage or Seton’s method) and other medical treatments are mainly conducted and stable.

Discussion/Conclusion: Crohn’s disease is on an increasing trend and juvenile onset is also seen. Pediatricians and physicians are often found anal lesions during examination. On the other hand treatment should be decided based on cooperation between an anoproctologist and a physician.
Clinical usefulness of bowel ultrasonography for colonic lesion in Crohn’s disease

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Introduction: Although colonoscopy (CS) is the gold standard for the assessment of Crohn’s disease (CD), it has several limitations associated with invasiveness. Ultrasonography (US) is a non-invasive, low-cost, radiation-free tool, appropriate for monitoring the disease course in patients with CD. This study aimed to compare the precision of bowel US with CS for patients with CD.

Methods: We retrospectively analyzed the medical records of patients with CD who underwent total CS and bowel US from January 2015 to December 2015. We categorized the colon into four segments: right colon (cecum-ascending colon), transverse colon, left colon (descending-sigmoid colon) and rectum. Regarding the endoscopic assessment, we defined the presence of an ulcer (≥ 0.5 cm) as an active lesion, which was further categorized into small and longitudinal ulcers. We assessed the maximum bowel wall thickness (BWT) of the colon as the US parameter, with a thickness of ≥ 4 mm (≥ 6 mm in the rectum) defined as an active lesion, and evaluated the wall stratification. Furthermore, we evaluated the median BWT and the loss of wall stratification per endoscopic severity, followed by determining the sensitivity and specificity of bowel US to detect active lesions.

Results: We collected retrospective data from 38 patients. Endoscopic findings of inactive lesions, small ulcers and longitudinal ulcers corresponded to the median BWT of 2.6, 6.0 and 7.6 mm, respectively. US findings of partial or complete loss of wall stratification occurred in 4%, 42% and 50% of cases with respective endoscopic findings of inactive lesions, small ulcers and longitudinal ulcers. The sensitivity and specificity of bowel US for detecting active CD lesions were 71% and 86%, respectively.

Discussion/Conclusion: The BWTs and the loss of wall stratification of the colon increased as the endoscopic severity became higher. Bowel US may be a useful monitoring tool for patients with CD.
Clinical features and long-term prognosis of intestinal Behçet’s disease after surgery

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Introduction: Intestinal Behçet’s disease (BD) is a rare type of BD, which occurs in 1–4% of patients with BD.

Methods: Between 1996 and 2015, 6 patients with intestinal BD underwent intestinal surgery at Tohoku University Hospital. We collected the patients’ data from medical records and assessed the clinical features and prognosis of these patients with intestinal BD.

Results: The age at the diagnosis of BD was 17 years (14–43) (median [range]). The age at the first intestinal surgery was 27 years (14–73). The follow-up periods from the first surgery was 16 years (0.2–16 years). Six of 6 patients (100%) had oral ulcers. The site of disease was as follows; ileocecum (4 cases), ascending colon (2 cases), ileum (2 case). Surgical procedure was as follows; ileocecal resection (4 cases [laparoscopic surgery; 2]), right hemicolecctomy (1 case), small bowel segmental resection (1 case). During a median follow-up of 16 years, 4 of the 6 patients (67%) required additional surgery due to recurrent disease; 6 times (1 case), 3 times (3 cases), 1 time (2 cases). The time period from the first operation to the second operation due to recurrence was 5.7 years (4–21.6 years) and the cumulative reoperation rate was 24.7% (3 years) and 37.7% (5 years) from the first operation. The pattern and the site of recurrent disease was various; anastomosis site (8 cases), ileum (3 cases), ileocecum (1 case), colon (2 cases), stoma (1 case). There was 1 case (79 years, male) who was dead during follow-up due to panperitonitis with anastomotic perforation after 15 years from the first operation.

Discussion/Conclusion: The rate of recurrence was high in patients with intestinal BD, therefore, long-term follow-up is mandatory. The pattern of recurrence was various and we should especially take care of the site of anastomosis.
Antimicrobial peptides in different phenotypes of Crohn’s disease in children

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Introduction: Bacterial microflora and mucosal barrier plays a central role in the etiopathogenesis of Crohn’s disease (CD). Important factors providing mucosal barrier are antimicrobial peptides, modifying bacterial microflora – host interactions. The aim of our study was to assess plasma elafin, cathelicidin, alpha and beta defensins concentrations and evaluate their relationships with phenotype of CD in children.

Methods: There were 35 children with newly diagnosed CD (18 boys, 17 girls, mean age: 13.8 years, range: 6.5–18) and 18 healthy controls enrolled into the study. Alpha and beta defensins, elafin and cathelicidin concentrations in plasma were assessed at the baseline and after 2 weeks of treatment using ELISA immunoassays, the phenotype of CD was assessed according to Paris classification.

Results: We found significantly elevated plasma elafin (p < 0.05), cathelicidin (p < 0.05) and alpha (p < 0.05) but not beta defensins concentrations in inflammatory (B1) subgroup of CD as compared to stricturing (B2) and penetrating (B3) subgroup of CD and controls. Additionally, elafin and cathelicidin but not alpha and beta defensins significantly decreased during the treatment (p < 0.05). When we analysed antimicrobial peptides in different CD location we found elevated elafin and cathelicidin in colonic (L2) involvement (39.8 and 820.7 ng/ml, respectively, p < 0.05) when compared to ileal (L1) (12.4 and 464.4 ng/ml, p < 0.05), ileocolonic (L3) (15.4 and 599.7 ng/ml, p < 0.05), ileocolonic and upper (L3 and L4a) involvement (7.7 and 310.4 ng/ml, p < 0.05) and controls (9.8 and 198.2 ng/ml, p < 0.05). In contrast, alpha-defensins were elevated in ileal (L1) (561.6 ng/ml) and ileocolonic and upper (L3 and L4a) involvement (420.8 ng/ml) when compared to colonic (L2) (322.6 ng/ml, p < 0.05), ileocolonic (L3) involvement (280.8 ng/ml, p < 0.05) and controls (256.4 ng/ml, p < 0.05).

Discussion/Conclusion: Assessing plasma elafin, cathelicidin and alpha-defensins may have prognostic value in estimating of the location and behaviour of CD in children.
Therapeutic efficacy of an elemental diet for patients with Crohn’s disease and its association with amino acid metabolism

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Introduction: An elemental diet (ED) has been shown to be extremely safe for patients with CD. The balance of amino acid concentrations in blood has been found to be useful for diagnosing CD and assessing disease activity. We investigated the association between blood amino acid concentration changes caused by elemental diet (ED) and their relationship to its therapeutic effect.

Methods: Patients with active Crohn’s disease (CD) followed ED for 12 weeks. Patients not previously treated with ED were defined as new ED, and those with previous ED therapy (≥ 900 kcal/day) were defined as previous ED. Disease activity markers [Crohn’s disease activity index (CDAI) and C-reactive protein (CRP) level], blood biochemistry test results, and plasma amino acid concentrations were measured before and after the treatment.

Results: Histidine (His), tryptophan (Trp), valine (Val), and methionine (Met) increased after the treatment in the 17 patients with clinical remission, however, no increase occurred in plasma amino acid concentrations in the 8 patients without remission. The multivariate index using AminoIndex™ technology (MIAI) was correlated with the CDAI (r = 0.475, p < 0.001), and it decreased as patients’ conditions improved during the treatment. All patients in the new ED group (n = 11) exhibited increases in the nutritional indices, albumin level, and body mass index after treatment, as well as increased levels of His, Trp, Val, and phenylalanine. None of these changes were observed in the previous ED group (n = 14).

Discussion/Conclusion: Plasma amino acid concentrations and MIAI may provide useful noninvasive markers for evaluating disease activity and response to treatment. ED was effective in improving disease activity, nutritional status, and plasma amino acid levels, and thus it may be particularly effective for poorly nourished patients with CD who have not previously undergone this treatment.
Capsule enteroscopy in diagnosis of isolated small bowel Crohn’s disease

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Introduction: Capsule endoscopy have made a great contribution in diagnosis of small bowel diseases. It is the best way to assess all over the gut and to detect mucosal inflammatory changes better than any other imaging modality. The aim of this retrospective study is to estimate the importance of video capsule endoscopy (VCE) in visual diagnosis of isolated small bowel Crohn's disease (CD).

Methods: From II.2007 until V.2018 we've performed 689 VCE in 668 patients (m – 325, f – 343, mean age 41.2 ± 16.7 years, range 17–86). The indication for the small bowel examination in 189 (28.3%) patients was suspected IBD. The main clinical symptom in most (87.3%) 165/189 of patients was pain, including 100 (60.6%) patients in combination with diarrhea. Capsule endoscopy was performed using small bowel (Olympus and Given Imaging) and colon (CC2) capsules, followed by balloon-assisted enteroscopy in 86 (45.5%) cases.

Results: Endoscopic signs of enteritis were estimated in 98 (51.9%) patients, incl. 48 (49.0%) with typical endoscopic criteria of CD. The main findings were aphthous erosions and ulcers. Isolated small bowel involvement was registered in 17/48 (35.4%) patients. According to clinical, VCE and morphological results the diagnosis of CD was confirmed in 41/48 patients; however Langhans giant cells were detected just in 9/41 (22.0%) cases, including post-surgical specimens. In other 45/86 cases histology showed chronic enteritis in 10 (22.2%) patients, erosive enteritis in 17 (37.8%) patients, ulcerative enteritis in 2 (4.4%), eosinophilic enteritis in 6 (13.3%), exudative enteropathy in 4 (8.9%) patients, celiac disease in 4 (8.9%) patients, radiation enteritis in 2 (4.4%). There were 4 cases of capsule retention: Crohn's strictures (3) and stenotic post traumatic ulcerative enteritis (1), resolved endoscopically.

Discussion/Conclusion: VCE gives essential information for diagnostics of Crohn’s disease in patients with unclear diagnosis, allowing to identify isolated small bowel Crohn’s lesions in 35.4% of patients, thus radically change management of these patients.
Diagnostic gastrointestinal ultrasound examination (GIUS) in patients with inflammatory bowel disease (IBD) – The New Zealand experience

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Training for upper abdominal diagnostic ultrasound is usually achieved within 6–12 months. In contrast the quality of GIUS examinations is significantly more dependent on the skills of the operator. This highly specialized service is offered by dedicated units with a high turn-over, and managed via referral to these centers due to acceptable travel distances.

More recently in patients with Crohn’s disease GIUS publications report an overall diagnostic sensitivity of 84–87% with a specificity of 98–100%, and with respect to the exact location of the pathology a sensitivity of 93% and specificity of 97%, in case of the TI: 100% (Novak K et al. J Ultrasound Med. 2012;31:1147–52). Regular surveillance with GIUS demonstrated intestinal pathology > 1 year earlier than both, 2D imaging (MRI, CT) and endoscopy (Panes J et al. Aliment Pharmacol Ther. 2011;34:125).

In the late 1980s the author of this paper (RL) introduced diagnostic abdominal ultrasound at the Gastroenterology Department at Dunedin Public Hospital to facilitate the surveillance of patients with chronic liver disease including US-guided liver biopsies (Pan A et al. BMC Gastroenterology. 2015;15:33).

Following a sabbatical leave in 2014 (Gastroenterology Department, University D-Freiburg, Medical School, Germany) GIUS was introduced in Dunedin for selected cases (1st presentation as well as surveillance, 71 patients to date. This was facilitated by the acquisition of a ‘top line’ US machine (6-1 curved array & 10-1 linear array probes), equipped with shear-wave elastography (SWE) and ultra-fast Doppler capability (to identify inflammatory changes). Currently a ‘Chronic Liver Disease Specialist Nurse’ (MP) undergoes training in general abdominal U/S – to be extended towards GIUS.

Representative GIUS images will be presented for discussion.

In conclusion a good foundation of skills in general diagnostic abdominal ultrasound is mandatory to facilitate a steep learning curve for GIUS, provided a high end US machine with an appropriate selection of ultrasound probes (particularly high resolution, ie 10-1 MHz) is available. In Dunedin we are currently introducing shear-wave elastography (SWE) into our GIUS clinic to better characterize developing fibrotic processes in patients with Crohn’s disease.
Fecal calprotectin predicts disease activity of Crohn’s disease evaluated by balloon-assisted endoscopy

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Introduction: Fecal calprotectin (FC) is a useful marker for assessing the activity of intestinal inflammation. However, it is not clear whether FC is useful for the evaluation of small bowel Crohn’s disease (CD). This study aimed to determine the usefulness of FC for predicting intestinal inflammation evaluated by balloon-assisted endoscopy (BAE), which can visualize the deep small intestine.

Methods: This was a cross-sectional, observational study involving 75 CD patients, 41 of whom had only small bowel disease. We used the extended simplified endoscopic activity score for Crohn’s disease (eSES-CD) which was modified SES-CD. The eSES-CD was calculated based on the findings of BAE. Mucosal healing was defined as an eSES-CD of 0. FC levels were determined with EliA Calprotectin 2.

Results: In all CD patients, FC levels were correlated with the eSES-CD (r = 0.64, p < 0.001). The cut-off value to predict mucosal healing was 92 mg/kg, with a sensitivity of 94%, specificity of 89% and the area under the curve (AUC) of 0.91. Even in small bowel CD patients, FC levels were correlated with the eSES-CD (r = 0.60, p < 0.001). The cut-off value was 92 mg/kg, with a sensitivity of 88%, specificity of 89% and AUC of 0.86.

Discussion/Conclusion: FC showed a significant correlation with the intestinal inflammation evaluated with BAE even in patients with only small intestinal disease. FC is useful for the evaluation of CD including both the small and large intestine.
Treatment outcome of thiopurines in patients with ulcerative colitis who were heterozygous for NUDT15 R139C (C/T)

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Introduction: NUDT15 R139C (rs116855232) is strongly associated with thiopurine-induced adverse events. However, it remains unknown whether there are differences in therapeutic efficacy of thiopurines depending on its genotype. In this study, we investigated the therapeutic efficacy up to 1 year after thiopurine initiation in patients with UC receiving prednisolone (PSL) and carrying C/C or C/T genotype.

Methods: We recruited 72 patients with UC treated with PSL at initiation of thiopurines (azathioprine or 6-mercaptopurine [6-MP]). All were followed more than 1 year. We assessed thiopurine dose at the initiation and at one year after initiation, additional therapy within one year after thiopurine initiation, PSL free remission rate without any additional therapies at one year. The additional therapy was defined as any therapies for UC added after 2 months from thiopurine initiation. 6-MP dose was converted to AZA equivalent dose using a conversion factor of 2.08.

Results: Of the 72 patients, 53 (74%) and 19 (26%) were C/C and C/T genotype. Thiopurine dose at initiation and at 1 year after initiation in patients with C/T genotype (28.2 ± 13.9 and 43.4 ± 21.9 mg/day) was significantly lower than those in patients with C/C genotype (36.7 ± 13.5 and 59.9 ± 23.9 mg/day) (p = 0.028 and p < 0.01). Patients with C/T genotype had significantly lower cumulative additional therapy rate within 1 year than those with C/C genotype (p = 0.013). The percentage of patients with C/T and C/C genotype who had additional therapy within 1 year was 31.6% (6 of 19 patients) and 64.1% (34 of 53 patients) (p = 0.014). PSL free remission rates without any additional therapies at 1 year in patients with C/T and C/C genotype were 47.4% (9 of 19 patients) and 17.0% (9 of 53 patients) (p < 0.01).

Discussion/Conclusion: Patients with C/T genotype could be followed with lower thiopurine dose and have better therapeutic efficacy than those with C/C genotype.
Berberine improved experimental chronic colitis via regulating interferon-γ productive lamina propria CD4+ T cells through AMPK activation

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Introduction: A herbal medicine, berberine (BBR), which is used as an anti-diarrhea medicine in Japan, is known as an AMPK activator to show various kinds of action on diabetes control and anti-tumor response. Recently, BBR has been reported to elicit anti-inflammatory response of CD4+ T cells resulting in improvement of experimental chronic inflammatory diseases such as multiple sclerosis. However, little is known about the BBR effect on CD4+ T cells and AMPK of inflammatory bowel disease (IBD). IBD is characterized by a chronic inflammation of the gastrointestinal tract. It was considered lamina propria (LP) CD4+ T cells was one of the pathogenesis.

Methods: To investigate the effect of BBR on IBD, we conducted a T cell transfer model of chronic colitis in which SCID mice were injected with CD4+CD45RB^high T cells resulting in T cell mediated colitis. The excised colitic LP CD4+ T cells were used for in vitro experiments (1) and the chronic colitis model mice were used for in vivo experiments (2).

(1) In in vitro experiments, we stimulated colitic LP CD4+ T cells by PMA/Ionomycin to induce interferon (IFN)-γ. With this model, we investigated the effect of BBR connecting AMPK metabolic pathway and immune system activation. To investigate the effect of AMPK activation on immune system, AMPK agonist AICAR and antagonist Compound C were added to the culture.

(2) In in vivo experiments, the colitic mice were fed with BBR and subjected to the mechanism investigation.

Results: (1) BBR has no cytotoxic effect at concentrations of 100 uM in vitro. When colitic LP CD4+ T cells were cultured with BBR, the frequency of IFN-γ productive cells reduced. Western blotting analysis showed BBR significantly activated AMPK activities of colitic LP CD4+ T cells. Metabolic assay showed that BBR inhibited oxidative phosphorylation (OXPHOS) and ATP production in colitic LP CD4+ T cells. When colitic LP CD4+ T cells were cultured with AICAR or Compound C, AICAR significantly suppressed inductions of IFN-γ productive cells frequency, while Compound C significantly increased these cells frequency.

(2) The BBR fed mice were monitored up to 7 weeks and evaluated the severity of colitis as significantly reduced. The colitic LP CD4+ T cells of BBR fed mice exhibited reduced frequency of IFN-γ productive cells, and increased phosphorylation of AMPK as in vitro experiments.

Discussion/Conclusion: BBR elicited anti-inflammatory response in colitic LP CD4+ T cells via AMPK activation possibly induced by OXPHOS inhibition. A safe and widely used anti-diarrhea BBR might be a good candidate for the treatment of IBD.
Treatment outcome of pouch related complications after ileo-anal anastomosis in ulcerative colitis

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Introduction: Ileo-anal anastomosis (IAA) and ileo-anal canal anastomosis (IACA) are standard operation for ulcerative colitis (UC) which combine curability and QOL. However, pouch related complications sometimes become a problem, which need appropriate treatment.

Methods: From 2007 to 2016, 83 patients with UC underwent IAA (74 cases) or IACA (9 cases) in our hospital. Incidence and therapeutic outcome of pouchitis in these patients are investigated. In addition, therapeutic outcome of other pouch related complications are investigated in 90 patients which include 7 other cases who underwent IAA or IACA in another hospital besides our cases.

Results: Pouch failure rate in our 83 series was zero percent in mean observation period of 4.6 years. Pouchitis occurred in 27 cases. The cumulative incidence was 34%. Although most cases had good response to antibiotic therapy including metronidazole, it was difficult to stop antibiotic therapy in 8 cases because of easily relapsing disease. Other pouch related complications were observed in 15 cases: anastomotic fistulas in eight, anastomotic ulcers in five, prepouch ileitis in two, afferent limb syndrome in one. For anastomotic fistula, diverting ileostomy was constructed in all cases. Although excision of ileal pouch and permanent ileostomy were needed in two cases, the anal sphincter can be preserved by re-do IAA or by fistula repair in other four cases. Endoscopic findings of anastomotic ulcers showed deep ulcers with discrete margins. All anastomotic ulcers successfully healed with medical treatment including steroids. Although the cases with prepouch ileitis showed resistance to antibiotic therapy, they were successfully cured by anti-TNF antibody. The case with afferent limb syndrome who showed repeated intestinal obstruction underwent surgical operation by strictureplasty and fixation of afferent limb with good postoperative course.

Discussion/Conclusion: Pouch function can be preserved in most cases with pouch related complications by appropriate medical and surgical treatment.
The additional value of cytapheresis therapy in patients with severe ulcerative colitis treated with oral tacrolimus

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Introduction: Oral tacrolimus therapy is an effective induction therapy for the remission of severe ulcerative colitis (UC), but it usually takes about one week to reach the therapeutic window. We evaluated the additional value of cytapheresis therapy to oral tacrolimus in patients with severe UC.

Methods: We retrospectively evaluated 31 consecutive severe UC patients (Mayo score = 11,12) who received oral tacrolimus therapy from April 2010 to June 2017 and were followed up for 120 days or longer. Three out of 31 patients who received biologics (anti-tumour necrosis factor agents) 120 days before or after the initiation of tacrolimus were excluded. Patients were divided into two groups (Tac group: received tacrolimus but not cytapheresis therapy, and Tac+CAP group: tacrolimus plus cytapheresis therapy) and the remission and response rates 120 days after the initiation of tacrolimus were compared between the two groups using partial Mayo score. Clinical response was defined with a decrease from baseline of at least 3 points in partial Mayo score. Clinical remission was defined as partial Mayo score equal to or less than 2 with no individual subscore higher than 1. The overall adverse events were also compared between the two groups.

Results: Twenty patients received tacrolimus but not cytapheresis therapy (Tac group) and eight patients received tacrolimus plus cytapheresis therapy (Tac+CAP group). In the Tac+CAP group, six patients received granulocyte and monocyte adsorption apheresis (GMA) and two patients received leukocytapheresis (LCAP). There were no significant differences between the two groups with respect to subject age, sex, disease duration, disease extent, disease severity, concomitant drugs, total administered steroid dose, C-reactive protein (CRP), white-cell count, haemoglobin, platelet count, albumin levels, initial dose of tacrolimus per body weight and tacrolimus trough level on day 7. Eleven patients (55.0%) in the Tac group and eight patients (100%) in the Tac+CAP group achieved clinical response (p = 0.12). Moreover, six patients (30.0%) in the Tac group and seven patients (87.5%) in the Tac+CAP group achieved clinical remission (p = 0.020). The log-rank tests revealed that remission rate was significantly higher in the Tac+CAP group compared with those in the Tac group (p = 0.005). Epilepsy occurred in one Tac group patient and temporary renal dysfunction occurred in one Tac+CAP group patient.

Discussion/Conclusion: Cytapheresis therapy as an additional therapy to oral tacrolimus is effective in patients with severe ulcerative colitis.
Proper timing of human adipose-derived mesenchymal stem cell (hAdMSC) injection ameliorates dextran sulfate sodium (DSS)-induced colitis in mice through the induction of M2 macrophages and regulatory T cells

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**Introduction:** Inflammatory bowel diseases (IBD) are sometimes refractory to current therapy or are associated with severe adverse events during immunosuppressive therapy; thus, new therapies are urgently needed. Recently, mesenchymal stem cells (MSCs) have attracted attention based on their multitude of functions including anti-inflammatory effects. However, proper timing of MSC therapy and the mechanisms underlying the therapeutic effects of MSCs on colitis are not fully elucidated.

**Methods:** hAdMSCs; 1 x 10^6 were administrated via the tail vein on day 3 (early) or 11 (delayed) using a 7 days DSS-induced mouse model of colitis. The effects were evaluated based on colon length, disease activity index (DAI), and histological score. Cytokine-encoding mRNA levels, T-cells, and macrophages were evaluated by real time-PCR and flow cytometry.

**Results:** Regarding the timing of administration, early (day 3) injection significantly ameliorated DSS-induced colitis in terms of both DAI and histological score, compared to those parameters with delayed (day 11) injection. With early cell injection, the tissue mRNA levels of anti-inflammatory cytokine genes (Il10, Tgfb) increased, whereas those of inflammatory cytokine genes (Il6, Tnfa, and Il17a) decreased significantly. Regarding the associated mechanism, hAdMSCs suppressed T cell proliferation and activation in vitro, increased the number of regulatory T cells in vivo, and changed the polarity of macrophages (into the anti-inflammatory M2 phenotype) in vitro.

**Discussion/Conclusion:** Timing of inflammation is critical for the effective therapeutic effects of hAdMSCs. Furthermore, part of the associated mechanism includes T-cell activation and expansion and altered macrophage polarization.
Small bowel mucosal healing of Crohn’s disease treated with anti-TNF antibodies

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Introduction: Objective assessment of Crohn’s disease (CD) in patients treated with anti-tumor necrosis factor (anti-TNF) antibodies is crucial. Mucosal healing evaluated endoscopically has a strong predictive value; however, a vast majority of available evidence is based on ileocolonoscopic data, whereas the impact of small bowel (SB) is much less studied.

Methods: We analyzed prospectively collected data from CD patients who received induction and following maintenance anti-TNF antibodies therapy from January 2013 to September 2017. We compared balloon-assisted enteroscopic findings before treatment with those after maintenance therapy. Mucosal healing was defined as absence of ulceration.

Results: 27 ileal (L1) and 54 ileocolonic (L3) CD patients were enrolled into this study (female: 21%; median age: 29 years old). 35 patients were treated with Infliximab and 46 were Adalimumab. Before treatment, SB ulceration was seen in 27 (100%) patients among L1 type; and SB and colonic ulceration were seen in 50 (93%) and 21 (39%) patients among L3 type, respectively. After maintenance, SB mucosal healing was achieved in 41% (11/27) among L1 type; and SB and colonic mucosal healing were achieved in 34% (17/50) and 86% (18/21) among L3 type, respectively. In L3 type patients, the rate of SB mucosal healing were significantly lower than that of colonic mucosal healing (p < 0.01). Among all patients, delayed treatment (over 2 years after diagnosis), B2/B3 behavior, and non-naïve patients were associated with failure to achieve SB mucosal healing (p = 0.02, 0.02, < 0.01, respectively).

Discussion/Conclusion: SB inflammation was more difficult to treat with anti-TNF antibodies than colonic inflammation. It is necessary to construct a treatment strategy for patients with high risk and high severity.
The air-enema image of ultra-low dose CT colonography can be an alternative diagnostic technique for the assessment of mucosal healing in the patients with ulcerative colitis

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Introduction: A ‘treat to target’ (T2T) approach has been proposed for ulcerative colitis (UC), with a target of combined clinical and endoscopic remission. Computed tomography colonography (CTC) emerged as a noninvasive screening procedure for colorectal cancer. However, the radiation exposure could be a major concern for application in UC. The air-enema image (AI) of CTC can be gained by volume rendering technique at very low radiation as much as 5 mAs. The aim of this study is to examine the usefulness of ultra-low dose CTC (uCTC) in UC.

Methods: A hundred patients with UC underwent colonoscopy and uCTC on the same day. The AI of CTC were evaluated by a novel CTC air-enema score for UC (CTCAES-UC), in which selected valuables (loss of colonic haustra and luminal narrowing in the air enema images) were scored from 0 to 1 in 0.5 increments in the worst segment of the colon and calculated from 0 to 2 totally. The endoscopic severity was evaluated by Mayo clinic endoscopy subscore (MCS) and UC endoscopic index of severity (UCEIS). The correlation between the endoscopic and the CTC findings was assessed for the disease severity and also the disease extension.

Results: In the assessment of UC severity, CTCAES-UC showed significant correlation with MCS (r = 0.6885, p < 0.001) and UCEIS (r = 0.6878, p < 0.001). CTCAES-UC showed a significant difference between endoscopic active stages and mucosal healing (MCS 1 vs. 2 or 3, p < 0.05 and 0.001, respectively) and even between 0 and 1 in MCS (p < 0.01). The disease extension was consistent in 67% of patients between CTC and colonoscopy.

Discussion/Conclusion: The AI of uCTC could be an alternative diagnostic technique for the assessment of the severity in UC and the normalization of CTCAES-UC may be an alternative therapeutic target of endoscopic mucosal healing.
QingChangHuaShi granule contributes to the balance of Th17/Treg in TNBS-induced colitis

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Introduction: Complementary and alternative medicine is commonly used in patients with inflammatory bowel disease. The single most commonly used modality in most surveys is traditional Chinese medicine. Chinese herbal medicine is helpful in improving symptoms. QingChangHuaShi granule is used in clinics to treat UC. However, the mechanisms underlying the anti-inflammatory effect of QingChangHuaShi granule remain unclear. Th17 secretes proinflammatory cytokines IL-17. IL-10 is an immunosuppressive cytokine produced by Treg. The balance of Th17/Treg is important to immune tolerance. The aim of this study was to explore the effect of granule on the Th17/Treg in TNBS-induced colitis.

Methods: To study the effect of QingChangHuaShi granule on the balance of Th17/Treg. The project team carried out QingChangHuaShi granule intervention in vivo experiments in animal models of UC. The granule includes Rhizoma Coptidis (Huanglian), Radix Scutellariae (Huangqin), Radix Paeoniae Alba (Baishao), Radix Aucklandiae (Muxiang), Radix Sanguisorbae (Diyu), Dahuricae (Baizhi), and Radix Et Rhizoma Glycyrrhizae (Gancao). Then observed the Th17/Treg proportions, the expression levels of IL-17 and IL-10, and intestinal inflammation in TNBS-induced colitis.

Results: QingChangHuaShi granule reduced protein expression levels of IL-17 and gene expression levels of IL-17mRNA, elevated IL-10, Foxp3 mRNA levels and protein expression levels, and lowered the Th17 cell proportions in TNBS-induced mice model. Histological improvements in TNBS-induced colitis were observed in response to the granule. QingChangHuaShi granule could upregulate the secretion of anti-inflammatory cytokine IL-10, downregulate the secretion of inflammatory cytokines IL-17 and relieve intestinal inflammation, restore the balance of Th17/Treg.
**Figure 1:** Histopathology assessment in mice (HE, 200 x).

**Discussion/Conclusion:** These results indicate that QingChangHuaShi granule can treat UC effectively through regulating the balance of Th17/Treg differentiation.
Measurement of serum trough levels of infliximab is useful in patients with Crohn’s disease ineffective to infliximab treatment: A single center study

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Introduction: Serum trough levels of infliximab (TLI) correlate with efficacy. Here we aimed to clarify the adequate TLI of patients with Crohn’s disease.

Methods: We examined 64 patients (non-responders: 6 cases, loss of response (LOR): 29 cases, responders: 29 cases) which had been administered of infliximab (IFX) between 2007 and 2015 in Ryukyu University Hospital, Okinawa, Japan. Response was defined as CDAI less than 150 and CRP less than 1.0 mg/dl. LOR was defined as CRP more than 1.0 mg/dl or symptom reappeared at less than 8 weeks after IFX administration. TLI and anti-IFX antibody (ATI) were determined by immuno-assays.

Results: The CRP level at the time of administration of IFX in the non-responders was significantly higher than that of the responders (2.93 ± 2.87 mg/dl vs. 0.37 ± 0.79 mg/dl). TLI of all non-responders was less than 0.1 μg/ml. TLI of LOR was significantly lower than that of the responders (1.23 ± 1.59 μg/ml vs. 4.19 ± 4.20 μg/ml). Seven cases were positive for ATI among the cases of LOR. Four cases with ATI were administered increased IFX (10 mg/kg), however the efficacy was not obtained. Among the cases of LOR which administered increased IFX (10 mg/kg), TLI of the responders tended to be higher than that of the non-responders (3.93 ± 4.29 μg/ml vs. 2.17 ± 3.50 μg/ml). In receiver operating characteristic curve analysis, the cutoff value of TLI was 1.45 μg/ml (sensitivity 75.0%, specificity 71.1%, AUC 0.79).

Discussion/Conclusion: The effectiveness of IFX was influenced by TLI. In cases with positive ATI, the effectiveness was poor despite the increased administration of IFX (10 mg/kg). It seemed that measuring TLI and ATI of ineffective cases is useful for considering the optimal treatment.
Clinical characteristics and outcomes of 5-ASA intolerance in patients with ulcerative colitis

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Introduction: 5-aminosalicylates (5-ASA) is a key medication for induction and maintenance of remission in patients with ulcerative colitis. It is previously reported that 6.5% of patients administered 5-ASA showed adverse effects. How we should manage these patients who are intolerant to 5-ASA is not well established.

Methods: Medical record of a total of 697 patients with ulcerative colitis who had taken any 5-ASA were retrospectively reviewed. Clinical outcomes of patients who discontinued due to adverse events were investigated.

Results: Numbers of patients intolerant to 5-ASA were 43 (6.2%) (46 attempts). Of the 46 attempts, 11 were excluded because detailed information was unavailable. Pentasa®, Asacol®, Lialda® and Salazopyrin® were responsible in 10, 16, 2 and 7 patients, respectively. Fever, diarrhea, hematochezia, eruption or pancreatitis was seen in 13, 11, 8, 5 and 4 patients, respectively. In 4 patients, the same 5-ASA formulation was re-challenged and all of them were intolerant. Switch to another 5-ASA was tried in 22 patients, of which 11 patients (50%) were tolerable. There were 9 patients who did not try to resume 5-ASA.

Discussion/Conclusion: Re-administration of the same 5-ASA was not successful in patients who were diagnosed as 5-ASA intolerance, however, many patients turned out to be tolerable to another type of 5-ASA. It is likely that switching to another 5-ASA medication might be the best option if patients were suspected to have 5-ASA intolerance.
Preoperative oral antibiotic prophylaxis for the prevention of surgical site infections in patients with Crohn’s disease

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Introduction: Although oral antibiotic prophylaxis with mechanical bowel preparation has been recommended for colorectal surgery, the use of this approach remains somewhat controversial. Moreover, the efficacy of this approach for inflammatory bowel disease also remains unclear. Therefore, I investigated the efficacy of oral antimicrobial prophylaxis in patients undergoing surgery for Crohn’s disease.

Methods: This study was conducted as a randomized controlled trial at the Hyogo College of Medicine. A total of 335 patients with Crohn’s disease who were scheduled to undergo surgery were randomly assigned to either group A (with oral prophylaxis) or group B (without oral prophylaxis). All patients underwent preoperative MBP with sodium picosulfate hydrate. The primary endpoint of this study was the incidence of surgical site infection (SSI) according to an intention-to-treat analysis.

Results: Although the incidences of overall and organ/space SSI were not significantly different, the incidence of incisional SSI was significantly lower in group A (12/163; 7.4%) than in group B (27/162; 16.6%) (p = 0.01). In the multivariate analysis, the absence of oral antibiotic prophylaxis was an independent risk factor for incisional SSI (OR = 3.3; 95% CI: 1.3–8.3; p = 0.01).

Discussion/Conclusion: Oral antimicrobial prophylaxis in patients with Crohn’s disease also contributed to the prevention of SSI.
The utility as a biomarker of fecal calprotectin for predicting the clinical outcome of granulocyte and monocyte adsorptive apheresis treatment in patients with ulcerative colitis

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Introduction: The diagnosis and assessment of ulcerative colitis (UC) has been based on clinical symptoms, blood parameters and endoscopy. Recently, fecal calprotectin (FC) was developed as a non-invasive tool to detect and monitor intestinal inflammation in clinical practice. However, its value as a biomarker for predicting the clinical outcome of remission induction therapy in patients with UC is still unclear. Granulocyte and monocyte adsorptive apheresis (GMA) treatment is widely used for UC therapy in Japan. So the aim of this study was to evaluate the utility of FC as a biomarker for predicting the efficacy of GMA treatment.

Methods: This prospective study was conducted at Asahikawa Medical University Hospital from October 2015 to November 2017. FC was measured during GMA treatment at week 0, 1, 2 and 4. Colonoscopy was performed at week 0 and within 24 weeks after the end of GMA treatment. Clinical activities using the partial Mayo score were assessed at the same time as FC was monitored. Clinical remission was defined partial Mayo score of \( \leq 2 \). Mucosal healing was defined Mayo endoscopic subscore of \( \leq 1 \).

Results: Fourteen of the 20 patients enrolled in this study completed GMA treatment. Six patients achieved clinical remission (included two mucosal healing), two showed a clinical response and six showed no response. In the clinical remission group, FC was decreased earlier than the partial Mayo score, and FC at week 1 was \( \leq 40\% \) of the baseline value. In the patients achieving mucosal healing, FC after the end of GMA treatment was \( \leq 5\% \) of the baseline value. The correlation between FC and the partial Mayo score was superior to that between the CRP or WBC count and partial Mayo score.

Discussion/Conclusion: FC is considered to be a useful predictor of the efficacy of GMA treatment in UC patients.
**Dietary medium-chain triglycerides prevent chemically induced experimental colitis in rats**

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**Introduction:** It was previously reported from this laboratory that medium-chain triglycerides (MCTs) have anti-inflammatory effects by inhibition of activation of macrophages. Therefore, the specific purpose of this study was to investigate effects of dietary MCTs on experimental colitis induced by 2,4,6-trinitrobenzene sulffonic acid (TNBS) in rats.

**Methods:** Male Wistar rats were given an intracolonic injection of TNBS and were then fed liquid diets containing MCTs or corn oil (AIN93) as controls. Serum and tissue samples were collected 1 week after TNBS enema. The severity of colitis was evaluated pathologically, and tissue myeloperoxidase (MPO) activity was measured. Furthermore, mRNA and protein levels for inflammatory cytokines and a chemokine were assessed by reverse-transcription polymerase chain reaction and enzyme-linked immunosorbent assay, respectively. In another set of experiments, protein expression of Toll-like receptor (TLR)-4 in the colon was measured 1 week after feeding of liquid diets. To investigate effects of MCTs on macrophages, RAW264.7 macrophages were incubated with media containing albumin conjugated with MCT or linoleic acid, which is the major component of corn oil, and production of TNF-α was measured.

**Results:** Dietary MCT blunted significantly protein levels of TLR-4 in the colon. Furthermore, the expression of TLR-4 was significantly blunted in RAW264.7 cells incubated with MCTs compared with cells incubated with linoleic acid. Induction of IL-1β, TNF-α and MIP-2 in the colon was attenuated by dietary MCT. Furthermore, MPO activities in the colonic tissue were significantly blunted in animals fed the MCT diets compared with those fed the control diets. As a result, dietary MCTs improved significantly chemically induced colitis.

**Discussion/Conclusion:** MCTs most likely are useful for the therapy of inflammatory bowel disease as an anti-inflammatory immunomodulating nutrient.
Impact of an olive leaf extract on intestinal permeability and liver inflammation in a mouse model of diet-induced obesity

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Introduction: The incidence of obesity and related conditions, including non-alcoholic fatty liver disease (NAFLD), has dramatically increased worldwide. The altered intestinal permeability and gut dysbiosis contribute to the inflammatory pathways involved in obesity associated NAFLD. It has been proposed that this condition can be modulated by several phenolic plant extracts. In this study we evaluate the effect of an olive leaf extract (OLE) in diet-induced obesity (DIO) in mice, to investigate its impact on intestinal permeability and liver inflammation in obese mice.

Methods: Male C57BL/6J mice (7–9 weeks old) were assigned to different groups (n = 9): control, control-treated, obese (fed a high-fat diet –HFD-), and obese-treated, which were daily administered with OLE (1, 10 and 25 mg/kg p.o.) for 5 weeks. Animal body weight and food intake were controlled regularly. Once sacrificed, the inflammatory status was evaluated biochemically by determining the liver expression of mediators involved in the inflammatory response or in the intestinal epithelial barrier function.

Results: OLE administration to HFD-fed mice significantly decreased body weight gain, although no difference in energy intake was observed among groups. This effect was associated with a reduced expression of liver pro-inflammatory mediators (IL-1β, IL-6 and TNFα) and an increment of molecules involved in the maintenance of intestinal epithelial integrity (occludin, ZO-1, MUC-2 and MUC-3). In addition, OLE ameliorated gene expression of adipocyte-specific transcription factors, such as peroxisome proliferation-activity receptor (PPARs), and upregulated the mRNA expression of leptin receptor, which is critical for adipogenesis. Moreover, OLE decreased significantly TLR-4 expression in liver.

Discussion/Conclusion: OLE exerts beneficial effects in HFD-induced obesity in mice, including improvement of liver inflammatory status and amelioration of gut barrier functionality.
Clinical efficacy of GMA in elderly patients with ulcerative colitis is associated with mucosal expression of MIP-1β and IL23

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Introduction: The number of elderly patients of ulcerative colitis (UC) is growing as the population ages. Since safer therapy is desired in elderly, granulocyte and monocyte adsorptive apheresis (GMA) is one of hopeful candidate. Elderly patients with UC sometimes become refractory to treatment therefore it is important to select an appropriate induction therapy. We investigated the relationship between therapeutic efficacy of GMA, age, and mRNA expressions of inflammatory related molecules in colonic mucosa.

Methods: Thirty-two active UC patients, mean age 36.7 years, range 21–70 years, were enrolled in this study. All UC patients received 10 times of GMA, and colonoscopies were applied before the first GMA and after the last GMA. Assessment of disease activity and colonic mucosal healing were determined based on Mayo score. In this study, elderly patients were defined as those > 55 years of age. mRNA expressions were determined by quantitative RT-PCR using biopsy specimen of colonic mucosa.

Results: After the last GMA session, the ratios of mucosal healing in elderly group and non-elderly group were 2 of 6 (33.3%) and 16 of 26 (61.5%), respectively. In all patients before treatment, mucosal healing group showed significantly higher mRNA expression of inflammatory related molecules such as MIP-1β, TNFα, IL1β and IL8 in colonic mucosal than that of non-mucosal healing group (p < 0.05). In particular, in elderly group, MIP-1β and IL23 in mucosa were higher in mucosal healing group (p < 0.05). High expressions of MIP-1β and IL23 before treatment showed high sensitivity and specificity as a marker of mucosal healing after GMA in elderly group.

Discussion/Conclusion: Mucosal MIP-1β and IL23 expression before treatment in elderly were significantly higher in responder than non-responder to GMA treatment. Measuring of these molecule levels may be useful to expect therapeutic efficacy of GMA in elderly patients with UC.
Non-adherence of IBD medications and disease activity during pregnancy and pregnancy outcomes

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Introduction: Discontinuation of inflammatory bowel disease (IBD) maintenance medications during pregnancy is known to increase the risk of disease flare and adverse pregnancy outcomes. Using self-reporting adherence and clinical symptoms from both patients and physicians, this multicenter prospective study aimed to evaluate the effect of discontinuing maintenance medications on the course of IBD and pregnancy outcome among female IBD patients in Japan.

Methods: Pregnant IBD women were recruited from 17 institutions participating in the Japanese nationwide IBD registry and completed surveys. Data were collected regarding self-reported adherence to IBD medications and IBD questionnaires during the course of pregnancy (at conception, 1, 2 and 3 postpartum). Concurrently, physician assessment of maternal condition, birth outcome and medication adherence were also collected. Disease activity was evaluated by Harvey Bradshaw Index for Crohn’s disease or partial Mayo score for ulcerative colitis. Non-adherence was defined as refilling less than 80% of prescribed medication.

Results: Overall, 81 patients with 76 birth outcomes were included between 2014 and 2016. Comparison of adherence reports between patients and physicians, revealed that non-adherence was underestimated by physicians. At the first trimester, mesalamine and immunomodulators showed as low as 0–50% adherence, which was improved after physician counseling resulting in improvement of clinical symptoms. Among pregnant women with UC, clinical recurrence was observed higher in the non-adherent patients compared to good-adherent patients (40.0% vs. 17.6%, p < 0.05), however there were no difference in the risk of adverse pregnancy outcomes such as LBW or SGT (p = 0.52).

Discussion/Conclusion: The results of the multicenter prospective study suggested that physicians underestimate medication adherence in female pregnant IBD patients emphasizing the need of preconception education regarding medication safety and the risk of disease flare up during pregnancy.
Clinical features of anorectal cancer in patients with Crohn’s disease

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Introduction: Patients with long-standing Crohn’s disease (CD) are at a high risk of development of anorectal cancer (ARC), however, the clinical features of ARC complicating CD are still uncertain. The aim of the present study was to clarify the clinical features of ARC complicating CD.

Methods: From 1995 to 2017, 450 patients with CD underwent surgery in our hospital. Of the 450 patients, 13 patients (2.9%) were diagnosed with ARC. A detailed review of the medical records of the patients was undertaken.

Results: The median age at diagnosis of ARC was 38 years (25–72 yrs). The median time interval between the diagnosis of CD and ARC was 20 years (7–40 yrs). Seven patients had fecal diversion because of their perianal disease. Nine patients had cancer-related symptoms. The other 4 patients had no cancer-related symptom but serum CEA was elevated. The 4 patients were diagnosed by cancer surveillance biopsy and 3 of the 4 patients (75%) underwent R0 resection. On the other hand, only 2 of the 9 patients (22%) underwent R0 resection in patients who had cancer-related symptom at the diagnosis of ARC.

The surgical procedures were as follows: total pelvic exenteration (n = 3), abdomino-perineal resection (n = 3), total proctocolectomy (n = 1), loop ileostomy (n = 1), and exploratory laparotomy (n = 1). UICC staging was as follows: stage II;3, stage III;3, stage IV;3. After the surgery, chemoradiotherapy was performed in 3 patients, radiotherapy in 2, and chemotherapy in 2. All the patients who underwent R0 resection achieved 5-year relapse free survival, while the 1-year and 2-year overall survival rate of the patients’ group who underwent R1/R2 resection was 66.7% and 16.7%.

Discussion/Conclusion: ARC was not rare in the patients with long-standing CD. The prognosis is poor in patients’ group with R1/R2 resection, while, patients’ group who achieved R0 resection is good. There is a pressing need to develop strategies for the early detection of ARC.
Anti-inflammatory properties of oligosaccharides derived from *Cynara scolymus* in DSS-induced colitis in mice

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**Introduction:** Prebiotics have been reported to be useful in IBD treatment through different mechanisms, including their capacity to modulate the immune response and the microbiota composition. Thus, they could down-regulate inflammatory mediators and restore the colonic epithelial integrity. The aim of the present study was to test the preventative effects of oligosaccharides derived from *Cynara scolymus L.* as prebiotics in DSS-induced colitis in mice.

**Methods:** Male C57BL/6 mice were treated with oligosaccharides derived from *Cynara scolymus L.* for three weeks, when mice were sacrificed. Colitis was induced by adding 3% DSS to the drinking water for 5 days after two weeks. Non-colitic and non-treated colitic groups were included as reference. The development of colitis was evaluated by a disease activity index (DAI) and when the mice were sacrificed different inflammatory markers were analyzed by qPCR.

**Results:** The administration of oligosaccharides had a beneficial effect in colitic mice evidenced macroscopically by lower DAI values. Biochemically, it was observed a decreased expression of inflammatory markers such as IL-6 and iNOS. The treatment improved the epithelial integrity increasing the expression of mucins (MUC-2 and MUC-3) and tight junction proteins. It also enhanced the colonic expression of TLR4, affected by the development of colitis.

**Discussion/Conclusion:** Oligosaccharides with prebiotic activity derived from *Cynara scolymus L.* are able to modify the expression of different inflammatory markers supporting its immunomodulatory effects and its use in IBD. They could be considered as a complementary treatment for intestinal inflammation.
Effects of quercetin loaded silk fibroin nanoparticles in DSS experimental colitis in mice

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Introduction: Flavonoid glycosides have shown beneficial effects in experimental colitis. The use of silk fibroin nanoparticles (SFN) to deliver the aglycone quercetin could increase the bioavailability of the flavonoid in the intestine and be useful for the treatment of intestinal inflammatory conditions at lower doses.

Methods: C57BL/6J male were induced intestinal inflammation by incorporating DSS in the drinking water (3%) for 5 days. Then, they were treated p.o. with: a) unloaded SFN, b) quercetin (5 mg/kg) or c) quercetin encapsulated in SFN (5 mg/kg in 8 mg nanoparticles). A non-colitic and a non-treated colitic groups were kept as controls. Mice were sacrificed after 6 days of treatment. The inflammatory status was assessed by the disease activity index (DAI), histological evaluation and colonic gene expression of inflammatory markers.

Results: Quercetin loaded SFN administration to colitic mice resulted in an intestinal anti-inflammatory effect, greater than any of the single treatments. This effect was evidenced by a reduction in DAI values in comparison with the control group, and confirmed by the histological analysis. Biochemically, the treatment with quercetin loaded SFN ameliorated the expression of pro-inflammatory cytokines (TNFα, IL-1β and IL-6), the adhesion molecule ICAM-1, the chemokine MCP-1 and the inducible enzyme iNOS. When the colitic groups treated with unloaded SFN and quercetin were considered, not all the biochemical markers evaluated were significantly ameliorated in comparison with the control colitic group.

Discussion/Conclusion: Encapsulation of quercetin in SFN increased its biological activity, which helped to ameliorate colonic inflammation and accelerate tissue healing in a model of colitis induced by DSS in mice.
Medication adherence to thiopurines is improved by nursing intervention for IBD outpatients: A single center prospective study

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Introduction: Thiopurines are widely used immunomodulators for maintaining remission of patients with inflammatory bowel disease (IBD). However, there are few reports showing the real-world data of medication adherence to thiopurines for adult IBD patients, and the efficacy of patient education for medication adherence has not been clarified. We then conducted a single center prospective study to investigate whether nursing intervention can improve adherence to thiopurines in IBD patients.

Methods: IBD patients receiving constant doses of thiopurines for more than one year were enrolled from May 2016 to May 2017. After obtaining baseline questionnaire including 8-item Morisky Medication Adherence Scales (MMAS-8), a nurse provided each patient with the guidance about the importance of medication adherence by using the defined leaflet. The same questionnaires as baseline were obtained after 2 and 6 months. Primary endpoint was set at the alteration of MMAS-8 scores between the baseline and 6 months after the guidance.

Results: Among a total of 110 patients enrolled, 74 patients were analyzed after excluding patients who discontinued thiopurines during the study period or answered the questionnaires incompletely. Mean baseline MMAS-8 score was 6.54 out of 8, and after 6 months of nursing intervention, mean MMAS-8 score was increased to 6.75, but not significant. However, in 22 patients with low baseline MMAS-8 scores of less than 6, MMAS-8 scores were significantly improved from 4.19 to 5.20 (p = 0.0005). Interestingly, the significant increase in mean corpuscular volume (MCV), a marker for the efficacy of thiopurines, was observed in both baseline MMAS-8 low and high (6–8) patients.

Discussion/Conclusion: Nursing intervention is effective for improving adherence to thiopurines especially for patients whose baseline adherence are low. Also, MCV is increased even in patients whose baseline adherence are high, suggesting that all IBD patients receiving thiopurines can receive a significant benefit from nursing intervention.
The effect of rebamipide, sucralfate and rifaximin on radiation-induced intestinal epithelial barrier injury

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Introduction: Radiotherapy for malignant abdominopelvic disease results in radiation-induced enterocolitis, intestinal epithelial barrier injury. However, there is no well-established preventive strategy. The aim is to evaluate the suppressive effect of rebamipide, sucralfate and rifaximin on ionizing radiation (IR)-induced acute inflammation and apoptosis, intestinal epithelial barrier injury in the intestine of mouse.

Methods: Thirty ICR mice were divided into (1) a vehicle-treated control group before sham IR, (2) a vehicle-treated group before IR, and (3-5) rebamipide, sucralfate or rifaximin-treated groups before IR. The intestine was resected at 4 hours after 4 Gy IR to the abdominopelvis. Pro-/anti-inflammatory and pro-/anti-apoptotic factors were investigated.

Results: NAMPT was down-regulated after IR, which was attenuated by rebamipide, sucralfate and rifaximin (p < 0.05). Activation of NF-κB and phosphorylation of MAPKs were induced by IR, which were suppressed by rebamipide, sucralfate, and rifaximin (p < 0.05). TNF-α, IL-1β, and IL-6 were increased by IR, while attenuated by rebamipide, sucralfate, and rifaximin down to similar level of control group (p < 0.05). The iNOS, COX-2 and PGE2 were significantly induced by IR, which were attenuated by rebamipide, sucralfate, and rifaximin (p < 0.05). ICAM-1 was corresponded to above mentioned results. [Ca2+] oscillation was increased by IR, which was attenuated by rebamipide, sucralfate, and rifaximin. Proapoptotic gene (Bax, c-Myc) and antiapoptotic gene (Bcl-2, Bcl-xL) expressions were potently suppressed and induced, respectively, by rebamipide, sucralfate, and rifaximin. The release of cytochrome C was increased by IR, while it was attenuated by rebamipide, sucralfate, and rifaximin (p < 0.05). Caspase 3 and caspase 7 were also elevated by IR compared to control group, however, they showed decline by rebamipide, sucralfate, and rifaximin (p < 0.05).

Discussion/Conclusion: This study demonstrated that rebamipide, sucralfate, and rifaximin have the suppressive effects on IR-induced acute inflammation and apoptosis, intestinal epithelial barrier injury in the intestine of mouse. Rebamipide, sucralfate, and rifaximin may have beneficial effects in preventing acute radiation-induced enterocolitis, intestinal epithelial barrier injury.
Evaluation of CD11c-, CD123- and CD68-positive cells in the colonic membrane of children with Crohn’s disease

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Introduction: Crohn’s disease (CD) is a type of inflammatory bowel disease (IBD) that may affect any part of the gastrointestinal tract from mouth to anus. Manifestations often include abdominal pain, diarrhea, weight loss and fever. The cause of Crohn’s disease is still unknown. It is believed to be due to a combination of immune, environmental and bacterial factor in genetically susceptible individuals. Furthermore, the disease refers to immunodeficiency state, where the main role is assigned to the lymphocytes. The aim of this study is the evaluation of CD11c-, CD123- and CD68-positive cells in biopsy specimens of the large intestine mucosa in children with Crohn’s disease.

Methods: The study included 10 patients with Crohn’s disease aged 3–17 years of age, with the mean age of 12.30 (± 2.83), and 10 children as the control group with the mean age of 10.28 (± 4.07). The expressions of CD11c, CD123 and CD68 receptors were analyzed by immunohistochemistry. The staining reaction was assessed as% of positive cells with membrane reaction.

Results: In the assessment of differences between the group of patients with Crohn’s disease and the control group it was shown that the number of CD123-positive cells in the colon mucosa was higher in the group of patients with CD than in children from the control group (p = 0.001). The higher number of CD68-positive cells were also noticed in patients with CD in relation to individuals from the controls (p < 0.001). Thirty percent (30%) of CD123 and twenty nine percent (29%) of CD68-positive cells were found - in the mucous membrane of the large intestine in children with ulcerative colitis. The result is significantly higher than in the control group – only one percent (1%). The number of CD11c-positive cells in the colon mucosa was higher in patients with CD then in the control group (p = 0.036). Twenty percent (20%) of CD11c receptor is observed in the mucous membrane of the large intestine in control group and fifty five percent (55%) of cells in the rectum in individuals with Crohn’s disease.

Conclusion: The number of CD11c-positive cells is increased in Crohn’s disease in comparison to healthy mucosa in children. There is also a significant increase in the CD68- and CD123-positive cells that physiologically occur in a small percentage.
The change of bone mineral density in patients with inflammatory bowel disease

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Introduction: There is limited data regarding the impact of treatment for IBD on the BMD status. Therefore, this study aimed to identify the change of BMD in the patients with IBD after treatment including 5-aminosalicylic acid, thiopurine, and anti-TNF agents.

Methods: The cases were retrieved from 442 patients who were diagnosed with IBD in a single university hospital. Of those, 119 patients (CD 84, UC 35) had the follow-up BMD with at least 1-year interval. The associations between BMD, BMI and disease activity parameters including CDAI, Mayo-score, hemoglobin (Hb), C-reactive protein (CRP), serum albumin were evaluated as Pearson correlation analysis and partial correlation; BMD was measured as Z-score and low BMD was defined as less than -1.

Results: In enrolled 84 patients with inactive CD, the baseline mean of BMD Z score at the lumbar spine and femur neck were -0.44 ± 1.36, -0.13 ± 1.28; the follow-up mean of BMD Z score at the lumbar spine and femur neck were -0.47 ± 1.21 (p = 0.512), -0.18 ± 1.17 (p = 0.304). In enrolled 35 patients with inactive UC, the baseline mean of BMD Z score were -0.20 ± 1.04, -0.11 ± 1.06; the follow-up mean of BMD Z score at the lumbar spine and femur neck were -0.26 ± 1.05 (p = 0.145), -0.08 ± 1.06 (p = 0.633). The proportion of low BMD patients of CD and UC at the baseline were 30 (35.7%), 11 (30.6%); the number of low BMD patients with CD and UC at the disease controlled-status were 31 (36.9%, p = 0.873), 9 (25%, p = 0.599) respectively. Only in the low BMD group of CD, the BMD of femur neck was correlated with BMI, Hb, CRP, and albumin. (0.517: p = 0.003, 0.423: p = 0.02, -0.394: p = 0.031, 0.378; p = 0.039). However, there was no correlation with disease activity parameter and BMD status in partial correlation, using BMI as control variable.

Discussion/Conclusion: There is no correlation with improvement of disease and BMD status after treatment in patients with IBD. However, in low BMD group of CD, treatment itself could improve the status of BMD of femur neck.
Clinical assessment of cases of intestinal Behçet disease treated with anti-TNF-α antibody at our hospital

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Introduction: Although the efficacy of anti-TNF-α antibody for intestinal Behçet disease (BD) was reported, its details, such as its clinical course, remain unclear. Thus, our aim was to investigate the clinical course of cases of intestinal BD treated with anti-TNF-α antibody.

Methods: Patients from our inflammatory bowel disease database who had intestinal BD diagnosed in our hospital between 2002 and 2017 were eligible for this study. We retrospectively examined the background (age, disease type, HLA type, and complication of myelodysplastic syndrome [MDS]) and the clinical outcome of intestinal BD cases.

Results: Thirty-seven cases were diagnosed as having intestinal BD, including 9 cases (mean patient age at induction, 43 years; male-to-female ratio, 1:8) treated with anti-TNF-α antibody. Of the cases, 3 were severe and 6 were moderate according to the disease activity index for intestinal Behçet disease (DAIBD) score. Seven patients received infliximab (IFX) therapy and 3, adalimumab (ADA) therapy. In addition, 1 case was HLAB1 positive and 2 were complicated with MDS. Conventional therapy was effective in none of the cases. Of the 7 cases treated with IFX, 4 revealed brisk response, while the remaining 3 required surgical operation due to intestinal perforation. ADA therapy was successful in 2 cases. IFX was successful in the case complicated with MDS. However, the patient eventually died from sepsis. Although 6 patients were referred to other clinics and current information was not available, 2 patients remain in remission.

Discussion/Conclusion: Anti-TNF antibody therapy was effective in 66% of the cases, with DAIBD reduction. The remaining 33% of the patients had high DAIBD scores at the start of biologics and required surgical therapy. Anti-TNF antibody therapy appeared effective in most of the patients with intestinal BD.
Inflammatory infiltration CD15/CD45-positive cells and correlation of c-erbB-2 expression in IBD

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Introduction: In colitis ulcerosa and Crohn’s disease we observed response of immune system and increased of Cd45 expression. c-erB-2 protein is a member of epidermal growth factor family.

Background and aim: The aim of the study was to compare the expressions of c-erB-2 in correlation to Cd45/Cd15.

Material and method: The study group consisted of 41 patients diagnosed with ulcerative colitis and 20 with Crohn’s disease. The biopsy slices were used as the study material in which the expression of CD45 and CD15 protein was determined by immunohistochemical method. The intensity of staining reaction was evaluated in 4-point scale was assessed as absent, weak, medium and strong.

Results: We observed different level of expressions of CD15/CD45-positive cells. In colitis ulcerosa the CD15/CD45 was decreased in correlation with expressions of c-erB-2. We fund positive staining c-erB-2 in 10% patients in CU and in 30% in patients with Crohn’s Disease. At the same times we found strong expressions of c-erB-2 in 70% of dysplastic cells 7 in Crohn’ disease.

Conclusion: Our results may suggest, that expressions of c-erB-2 protein is strongly depend of CD15/CD45 inflammatory infiltration in colitis ulcerosa and Crohn’s disease.
Usefulness of measuring serum infliximab concentrations during treatment with infliximab for ulcerative colitis

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Introduction: We prospectively studied treatment outcomes including measurement of serum infliximab (IFX) concentrations and the titers of antibodies to IFX (ATI).

Methods: The study group comprised 21 patients with UC. Disease activity was evaluated according to the Seo index (remission ≤ 120, effective < 150 or a decrease of ≥ 70 as compared with the baseline value). A Mayo endoscopic score (MES) of 0 was defined as mucosal healing (MH). The following variables were evaluated: 1) the improvement (remission + effective) rate at 54 weeks and the IFX concentration according to the treatment response; 2) the mucosal healing (MH) rate and the IFX concentration at 54 weeks. This study was approved by the ethics committee of our hospital (B13-158, UMIN 000026006).

Results: 1) The Seo index at the time of starting treatment with IFX was 207.4 ± 44.8. The improvement rate at 54 weeks was 52%. The IFX concentration [median; interquartile range (IQR)] at 54 weeks was significantly higher in patients with improvement than in patients without improvement [3.9 vs. 0.4; IQR, 0.8–9.1, 0.1–1.1, p < 0.05]. 2) The MH rate at 54 weeks was 33% and the IFX concentration at that time was slightly but not significantly higher in patients with an MES of 0 than in patients with an MES of 2 or 3 [7.6 vs. 1.0; IQR, 1.6–10.9 vs. 0.4–1.8; p = 0.29].

Discussion/Conclusion: The IFX concentration at 54 weeks was higher in patients with an improvement in disease activity than in patients with no improvement in disease activity and was further higher in patients in whom mucosal healing was achieved.
Efficacy and related issues of cytopheresis in elderly patients with ulcerative colitis

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Introduction: There are special concerns that need to be addressed when devising treatment strategies for ulcerative colitis (UC) in elderly patients. Cytapheresis (CAP) are some non-pharmacological treatment options that are safe and effective mainly in intractable UC patients. We aimed to investigate the efficacy and related issues of CAP in elderly UC patients.

Methods: In this single center retrospective case-control study, we investigated 92 active UC patients who received CAP from January 2012 to December 2016 at our hospital. The patients were divided into two groups, an elderly group aged ≥ 60 years (n = 22) and a non-elderly group aged < 60 years (n = 70). Remission was defined as a Lichtiger clinical activity index (CAI) ≤ 3.

Results: The elderly group (69.1 ± 6.4 years) had significant complications, such as hypertension and diabetes, compared with the non-elderly group (37.1 ± 13.4 years) (p < 0.001). Baseline CAI of the elderly group was significantly lower than that of the non-elderly group (6.6 ± 2.0 vs. 8.5 ± 2.8, p < 0.01). After CAP treatment, CAI was significantly decreased from baseline in both groups (4.5 ± 1.9 vs. 4.7 ± 3.1, p < 0.01), however, remission rate tended to be lower in the elderly group than the non-elderly group (31.8% vs. 52.9%, p = 0.08). Multivariate analysis revealed that elderly group and higher CAI at baseline was a significant predictive factor of failing remission induction (OR = 0.28, 95% CI: 0.09–0.86; OR = 0.78, 95% CI: 0.64–0.95). Safety profiles were not different between the two groups (9.1% vs. 15.7%, p = 0.44). Insufficient blood removal or failing vascular access was significantly more prevalent in the elderly group than in the non-elderly group (72.7% vs. 41.4%, p = 0.03), therefore intensive therapy was significant lower in the elderly group in the non-elderly group (13.6% vs. 38.6%, p = 0.03). However failure rates of CAP through the whole session were not different between the two groups by additional infusion for rehydration during extracorporeal circulation (28.6% vs. 26.1%, p = 0.79).

Discussion/Conclusion: Although the elderly UC patients was difficult to achieve remission induction, CAP was safe and effective in decreasing CAI even in elderly UC patients through incorporating practical measures. The indication of CAP has to be optimized to increase the efficacy.
Evaluation of CD11c-, CD123- and CD68-positive cells in the colonic mucosal membrane of children with ulcerative colitis

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Introduction: CD68 receptor, a transmembrane glycoprotein which consists of 354 amino acids, is a selective marker for cells in monocytes and macrophages. CD11c is classified as the type I transmembrane protein and expressed on monocytes, macrophages, neutrophils and B cells. CD123 receptor, also known as interleukin 3, is a molecule included to the type I cytokine receptor family. It is present on the surface of pluripotent cells, basophils and plasmacytoid dendritic cells. The aim of this study is the evaluation of CD11c-, CD123- and CD68-positive cells in biopsy specimens of the large intestine mucosa in children with ulcerative colitis.

Methods: The study objective was the group of 18 children with ulcerative colitis, aged 3–17 years of age (mean age was 11.5 (± 4.1) and 10 children (the control group) the mean age was 10.3 ± 4.1. The expression of CD11c, CD123 and CD68 protein in tissue sections was assessed by immunohistochemical methods. The STATISTICICA 12 was used for the mathematical analysis of the results.

Results: The number of CD68-positive was statistically significantly higher in the group of patients with ulcerative colitis. In relation to individuals from the controls (p < 0.001). The higher number of CD123-positive cells were also noticed in patients with ulcerative colitis in relation to individuals from the controls (p = 0.002). Thirteen percent (13%) of CD123 receptors and twenty eight percent (28%) of CD68 receptors were found as positive cells in the mucous membrane of the large intestine in children with ulcerative colitis. The result is significantly higher than in control group compared to only one percent (1%). The higher number of CD11c-positive cells in patients with ulcerative colitis was also in relation to individuals from the controls (p < 0.001). The highest percentage of CD11c (76%) cells is observed in the rectum in individuals with ulcerative colitis. Twenty percent (20%) of CD11c-positive cells are observed in the mucous membrane of the large intestine in control group.

Conclusion: In inflammatory infiltration of ulcerative colitis in children CD11c-positive cells dominate (more than three-fold increase in comparison to healthy mucosa). In addition, there is a significant increase in CD68- and CD123-positive cells compared to the healthy intestinal mucosa.
Study of surgical cases of anal fistula associated with Crohn’s disease

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Aim: We aimed to study surgical cases of anal fistula associated with Crohn’s disease (CD) and to make the data helpful to the future medical treatment.

Materials and methods: Fifteen CD patients underwent surgery for anal fistula from April 2007 to March 2018. Eleven male and 4 female patients were included. The number of surgeries was 18. We retrospectively examined their clinical courses.

Results: The medium age was 27 years (18–43). As for the type of anal fistula, II-L cases were 18 and III-U one. As for the surgical method, 18 cases underwent seton drainage, and one coring out. Four cases underwent both surgery for anal fistula and resection of intestine. The median operative time was 43 minutes, and the median blood loss was 10 cc. The number of the primary holes was one in 8 cases, 2 in 5, 3 in one, 4 in one. The median number of the second holes was three (1–6). The median number of tubes used in seton drainage was three (1–5). The median duration of tube remaining was 13 months (3–36). The rate of tube remaining one year after the operation was 60%. The median duration of postoperative observation was 5 years and 2 months. The rate of curative cases without tube remaining was 20%. As for a concomitant drug, mesalamine had been administered to all cases preoperatively. Anti TNF-α antibody drug had been administered to 6 cases (40%). Five of the 6 patients (33%) received the drug postoperatively.

Conclusions: The anal fistula associated with CD was intractable. The curative rate was low and long-time indwelling tube was necessary even in cases with anti TNF-α antibody drug administration.

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Study of ulcerative colitis complicated by primary sclerosing cholangitis

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Introduction: Primary sclerosing cholangitis (PSC) is often associated with autoimmune diseases, and approximately 70% of PSC patients in Europe/United States and 32% in Japan also have ulcerative colitis (UC). While complications of PSC are confirmed in about 5% of UC patients, the clinical features of UC associated with PSC differ from those of UC without PSC. We investigated the clinical and colonoscopic features of colitis associated with PSC.

Methods: We retrospectively examined clinical features, including the clinical course and colonoscopic findings, in 25 colitis patients with PSC attending our hospital from 2000 to 2016.

Results: The male-female ratio was 12:13 and the age at diagnosis of PSC was 49 ± 15 years. PSC was diagnosed first in 12 patients (48%), colitis was diagnosed first in 4 patients (16%), and both diseases were found concurrently in 9 patients (36%). Among 21 patients with a diagnosis of PSC first or concurrently, 12 patients (57%) had no symptoms of PSC and it was found by screening. There were 12 patients with UC (52%) and 11 patients with nonspecific colitis (48%). Among 24 patients in whom the disease extent was assessed, 22 had pancolitis, 1 had left-sided colitis, and 1 had proctitis. Inflammation predominantly affected the right colon in 20/22 patients with pancolitis and also involved the terminal ileum in 9 patients (48%). The Mayo score for colonoscopic evaluation of UC was 1 in 16 patients (64%), 2 in 8 patients (32%), and 3 in 1 patient (4%). There were no rectal lesions in 10 patients (40%). Liver biopsy was performed in 17 patients, and Ludwig’s stage was Stage I in 1 patient (6%), Stage II in 12 patients (71%), Stage III in 3 patients (18%), Stage IV in 1 patient (6%). Ludwig’s stage was unrelated to the Mayo score. All patients with PSC and enterocolitis received oral ursodeoxycholic acid (UDCA), including 13 patients with UDCA only (52%), 2 patients with combined salazosulfapyridine (SASP) (8%), 5 patients with combined 5-aminosalicylic acid (5-ASA) (20%), 2 patients with combined prednisolone (PSL) (8%), 1 patient with combined SASP + PSL (4%), and 2 patients with combined 5-ASA + PSL (8%). The UDCA dose was 300 mg in 2 patients (8%), 600 mg in 15 (60%), and 900 mg in 8 (32%).

Discussion/Conclusion: In colitis patients with PSC, there was no obvious association between colonoscopic disease activity and the severity of PSC. There was no sex difference and the age at diagnosis of PSC showed a bimodal distribution (30s and 60s). Pancolitis was very frequent and predominantly affected the right colon, but disease activity was low. Rectal lesions were mild or absent. About half of the patients had inflammation of the terminal ileum.
The actin-bundling protein fascin-1 is overexpressed in ulcerative colitis and is associated with CEACAM 1 expression

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Introduction: Fascin-1 is an actin-bundling protein and is associated with increased cell motility in colorectal tumours but is absent in the normal colonic epithelium. Moreover, it has been observed that fascin may be involved in tissue repair in IBD that require dynamic rearrangements in the actin cytoskeleton. CEACAM 1 may take part in this process and behaves as an intercellular adhesion molecule. In normal epithelial cells CEACAM1 acts as a regulator of proliferation and apoptosis. This function may be impaired in inflammatory and cancer diseases resulting in excessive and uncontrolled cell proliferation. Therefore, the aim of our study was to evaluate the expression of fascin-1 and CEACAM 1 in patients with ulcerative colitis.

Methods: The study consisted of 30 patients with ulcerative colitis (UC). Endoscopic materials were taken from archival paraffin-embedded tissue. Sections were stained with H&E and subjected to routine histological evaluation. According to Geboes classification, an analysis of the severity changes (architectural changes, the assessment of crypt destruction, erosions and ulcers, infiltration of inflammatory cells) was performed. The expression of fascin-1 and CEACAM 1 proteins in tissue sections was assessed by immunohistochemical methods.

Results: The color reaction of fascin-1 was observed in cytoplasm of the glandular epithelium and inflammatory cells whereas CEACAM 1 reaction was observed on the surface of intraepithelial cells of villi. Patients showed no expression of fascin-1 in epithelial cells in 45% cases, weak, medium and strong in 55% (12.5%, 20% and 22.5%, respectively). In inflammatory cells of UC we observed rather positive reaction of this protein (72.5%). Statistical analysis showed a correlation between fascin-1 expression in epithelial cells and inflammatory cells (p < 0.001). Positive reaction of CEACAM 1 was found in 71% patients with UC. The expression of CECAM 1 was present in all patients without dysplasia (100%) and 80% patients with low grade of dysplasia whereas lack of positive expression of this protein was observed in patients with high grade dysplasia. These results were statistically significant (p = 0.029). Statistical analysis showed that increased expression of fascin-1 protein was associated with CEACAM 1 expression (p = 0.015).
**Discussion/Conclusion:** Overexpression of fascin-1 may play a role in the inflammatory reaction in patients with UC. Simultaneously, decrease of CEACAM 1 expression may be associated with uncontrolled proliferation and consequently development of dysplastic lesions as a result of impaired mucosal healing.
Exploration for natural medicines that stimulate the production of IL-10 in intestinal macrophages as a novel therapeutic approach in inflammatory bowel disease

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Introduction: Interleukin-10 (IL-10) is an immunoregulatory cytokine that limits excessive mucosal immune responses and regulates intestinal homeostasis. Evidences from human and animal studies suggested that the augmentation of the production of IL-10 in the intestinal mucosa especially by macrophages has a potential to be a novel therapeutic target for inflammatory bowel disease (IBD). Therefore, we screened aqueous extracts of 120 herbal medicines well-used in the Japanese pharmacopeia for their effects on the production of IL-10 in macrophages. Next, we evaluated therapeutic effects of the selected extracts in a mouse experimental colitis model.

Methods: Bone marrow-derived macrophages (BMDMs) and intestinal macrophages were treated with the extracts and stimulated with LPS for 24 h, and the concentration of IL-10 and TNF-α in the supernatant was measured by CBA. To evaluate the protective effect in intestinal inflammation, BALB/c mice were pre-administrated by the screened extracts for 7 days, and acute colitis was induced by administration of 3% DSS in their drinking water for another 7 days.

Results: Among the 120 extracts, one extract specifically increased the production of IL-10 in macrophages in vitro. In addition, an oral administration of the extract to normal mice specifically enhanced the expression of IL-10 and other anti-inflammatory cytokines in the colon. Furthermore, the extract protected mice against the development of DSS-induced colitis.

Discussion/Conclusion: The reduction of the colitis symptoms in mice following the enhancement of IL-10 expression by the extract is consistent with previous reports pointing out the critical role of IL-10 level in the treatment of colitis. Although further investigations are imperative to determine the mechanisms of action of the extract, the present study proposed a novel approach of the augmentation of IL-10 production in the intestinal macrophages for maintaining the remission of inflammatory bowel disease.
TRPV4 regulates vascular endothelial permeability during colonic inflammation in dextran sulphate sodium-induced murine colitis

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Introduction: The transient receptor potential vanilloid 4 (TRPV4) is a nonselective cation channel involved in physical sensing in various tissue types. The present study aimed to elucidate the function and expression of TRPV4 in colonic vascular endothelial cells during dextran sulphate sodium (DSS)-induced colitis.

Methods: The role of TRPV4 in the progression of colonic inflammation was examined in the 2% DSS-induced murine colitis model using immunohistochemical analysis, western blotting, and Evans blue dye extrusion assay.

Results: DSS-induced colitis was significantly attenuated in TRPV4-deficient (TRPV4 KO) mice when compared to wild-type mice. Repeated intrarectal administration of GSK1016790A, a TRPV4 agonist, exacerbated the severity of DSS-induced colitis. Bone marrow transfer experiments demonstrated a dominant role of TRPV4 in non-haematopoietic cells for DSS-induced colitis. DSS treatment upregulated TRPV4 expression in the vascular endothelia of colonic mucosa and submucosa. DSS treatment increased vascular permeability, which was abolished in TRPV4 KO mice. The DSS-induced increase in vascular permeability was further enhanced by intravenous administration of GSK1016790A, which was abrogated by TRPV4 antagonist RN1734. TRPV4 was co-localized with vascular endothelial (VE)-cadherin, and VE-cadherin expression was decreased by repeated intravenous administration of GSK1016790A during colitis. Furthermore, TRPV4 activation by GSK106790A decreased VE-cadherin expression in mouse aortic endothelial cells exposed to TNF-α.

Discussion/Conclusion: These findings indicate that TRPV4 upregulation in vascular endothelial cells contributes to the progression of colonic inflammation via the activation of vascular permeability. Thus, TRPV4 is an attractive target for the treatment of inflammatory bowel diseases.
Ustekinumab-induction and maintenance effects in patients with Crohn’s disease in a single center study

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Introduction: Ustekinumab (UST), monoclonal antibody against p40 subunit of IL-12/23 was able to be used in the treatment of Crohn’s disease (CD) in 2017 in Japan. However, the data of UST in clinical practice is still rare. The aim of this study was to evaluate the induction and maintenance effects of UST.

Methods: This single-center observational study was approved by the IRB of our hospital. Here we report the intermittent analysis of this study. CD-patients refractory or naïve to anti-TNF alpha antibody were enrolled. Remission was defined as CDAI < 150, as well as response was defined as – ΔCDAI ≥ 70 and – 25% of baseline. Primary and secondary endpoints were the remission induction and response rate at 8 week, respectively. The ratios of quiescent patients as well as steroid free patients at week 8 and 24 week were also examined.

Results: Eight active and 6 quiescent CD-patients were enrolled in this study. The mean age was 42.6 ± 13.4 years old, and disease duration was 14.2 ± 10.9 years. Disease types were 8 small intestinal types, and 6 small intestinal and colonic types. The 78.6% (11/14) of the patients were TNF-failure. The 35% (5/14) were treated with prednisolone and/or budesonide before the start of UST. Remission induction and response rates of active CD-patients at week 8 were 12.5% (1/8) and 50% (4/8), respectively. The ratio of quiescent patients at 8 and 24 week were 46.1% (6/13) and 88.9% (8/9). The 40% (2/5) patients became steroid free and the 80% dose of baseline steroid was decreased at week 24. One hundred percent (11/11) and 87.5% (7/8) of the patients could be switched from anti-TNF alpha to UST at week 8 and 24, respectively.

Discussion/Conclusion: UST were effective for the TNF-failure. However, it took 24 weeks for the expression of effectiveness.
Diagnostic and clinical role of serum proteinase 3 antineutrophil cytoplasmic antibodies in inflammatory bowel disease

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Introduction: Proteinase 3 antineutrophil cytoplasmic antibodies (PR3-ANCAs) are well-known serological markers for granulomatosis with polyangiitis, but their role as serological markers for inflammatory bowel disease (IBD) remains uncertain. The present study aimed to evaluate the diagnostic and clinical roles of PR3-ANCAs as markers for IBD.

Methods: Using a new methodology with chemiluminescence enzyme immunoassay, serum PR3-ANCA titres were assessed in 102 patients with ulcerative colitis (UC), 67 patients with Crohn’s disease (CD), 44 controls with other intestinal diseases and 66 healthy controls. Associations with clinical data were investigated. The diagnostic role of PR3-ANCAs was evaluated by receiver operating characteristic (ROC) analysis.

Results: PR3-ANCA titres were significantly higher in patients with UC than in those with CD patients, patients with intestinal diseases (intestinal controls) and healthy controls (all \( p < 0.001 \)). ROC analysis demonstrated an area under the curve of 0.85 (95% confidence interval: 0.83–0.87) and showed that the manufacturer’s cut-off value (3.5 U/ml) had a sensitivity of 39.2% and specificity of 96.6% for UC. There was a significant difference between PR3-ANCA-positive and negative patients with regard to disease duration (\( p < 0.05 \)) and disease severity (\( p < 0.01 \)).

Discussion/Conclusion: PR3-ANCAs were significantly more prevalent in patients with UC than in those with CD and controls. Our results suggested the role of PR3-ANCAs as serological markers for helping in diagnosing UC and evaluating disease severity.
Different involvement of innate versus adaptive immunity in drug resistant inflammatory bowel disease

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Introduction: Inflammatory bowel disease (IBD) is classified into two major forms, Crohn's disease (CD) and ulcerative colitis (UC), which are characterized by common and distinct clinical features. Recent studies have classified CD into auto inflammatory diseases mediated mainly by innate immune responses, but there is no direct evidence sufficient to support the new concept.

Methods: Adaptive immune responses are characterized by antigenic exposure that can induce a clonal expansion of antigen-specific T cells with identical T cell receptor (TCR). Therefore, involvement of adaptive immunity was evaluated by T cell clonality in the lamina propria of surgically resected intestines from drug-resistant IBD patients. To do so, T cell clonality was analysed using a combination of unbiased amplification of TCRα and TCRβ genes and next generation sequence.

Results: In healthy colon, polyclonal expression patterns of both TCRα and TCRβ chains were observed. This polyclonal pattern was changed to oligoclonal pattern in the colon of UC patients, but no restricted usages of specific variable or joining regions were recognized. In addition, restricted expansion of identical T cell clones was found throughout the individual colon (e.g. descending colon, ascending colon and rectum) of each UC patient. Unexpectedly, the oligoclonal expansion pattern was changed to polyclonal pattern in the reduction of enteric bacteria. Alternatively, TCR clonality varied in CD patients, some patients showed oligoclonal pattern and others showed polyclonal pattern. Of note, expanded T cell clones differed from area to area throughout an individual intestine of CD patient. Different T cell clones expanded in the colon versus small intestine, and, even in the small intestine, different T cell clones were detected in pre-stenosis versus post-stenosis regions.

Discussion/Conclusion: Oligoclonal expansion of restricted and identical T cell clones responding to enteric bacteria was seen throughout an individual colon of UC patient, whereas expanded T cell clones differ from area to area in the individual intestine of CD patients. These findings suggest a close involvement of antigen-specific adaptive immune responses in UC but not CD.
Expression of CD15/CD45-positive cells in correlation of apoptosis in IBD and CRC

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Introduction: In the lamina propria of colon mucinosum can be found expression of CD45-positive cells in different intensity. The expression of CD45 and CD15 can be correlated with expression of Fas and FasL. Therefore, the objective of the current study was to assess the expression of Fas ligand (FasL) and Fas receptor (FasR) as the proteins of post mitochondrial apoptotic pathway in ulcerative colitis, Crohn’s disease and colorectal cancer.

Material and method: Our study was performed on 35 patients with ulcerative colitis, 30 patients with Crohn’s disease and 50 patients with colorectal cancer. Standard immunohistochemical technique was adopted to detect the expression of CD45, CD15, Fas and FasL. The intensity of staining reaction was evaluated in 4-point scale was assessed as absent, weak, medium and strong.

Results: The medium Fas receptor expression was observed in epithelial cells in ulcerative colitis and Crohn’s disease, weak in 75.5% of colorectal cancer patients, as compared to normal glandular epithelium where Fas receptor expression was strong in 100% of cases. Whereas FasL expression was mostly expressed in ulcerative colitis, strong in 70% of colorectal cancers, but absent in Crohn’s disease and normal colorectal epithelium. Moreover, 70% of the CD15/CD45-positive cells present in the inflammatory infiltration accompanying the tumor showed strong FasR expression.

Conclusion: We observed correlation between strong expression of CD15/CD45 and expression of FasL but no correlation we found between FasR expression and CD15/CD45. But in colorectal cancer we observed decrease expression of CD15/CD45 dependence of FasL expression in epithelial cells. It can be known as a mechanism by which cancer cells escape death via apoptosis-inducing Fas/FasL pathway disorders.
The effects of smoking on gut ecosystem of patients with IBD

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Introduction: Gut microbiota and their metabolites have impacts on host pathophysiology. Although smoking is known to be a major risk factor for IBD, details are still incompletely understood. This study aims to investigate the effects of smoking on gut microbiota and their metabolites in IBD patients.

Methods: Feces from ulcerative colitis (UC) and Crohn’s disease (CD) patients were suspended in methanol, homogenized, and centrifuged. The supernatants were subjected to GC/MS system. Bacterial DNA was isolated from fecal pellets, colonic aspirates, and saliva, and then 16S rRNA gene sequencing of V4 region was performed using a MiSeq.

Results: Short chain fatty acids (SCFA) including butyric acid and acetic acid were significantly increased in feces from UC smokers compared to those from UC ex-smokers. Correlation network analysis of metabolites and bacterial OTUs revealed that SCFA-producing bacteria, such as Blautia, Bifidobacterium, and Butyricoccus, were significantly correlated with butyric acid and acetic acid. We also found that colonic aspirates contained enriched mucosa-associated bacteria. Based on the microbial structures of colonic aspirates, patients were clustered into four groups. Co-abundance groups analysis indicated that one group, Cluster D, had increased abundance of oral bacteria in the colonic aspirates. Compared to non-smokers and ex-smokers, smokers were significantly biased into Cluster D.

Discussion/Conclusion: Our results indicate that smoking modulates the concentrations of intestinal SCFA and the population of mucosal microbiota. These effects of smoking may affect the intestinal barrier integrity and immune functions.
Severity of stress-induced intestinal dysfunction associates with characteristic changes in behavioral pattern and gut microbiota composition in an experimental rat model

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Introduction: It has been reported that about one-third of patients with inflammatory bowel disease (IBD) in remission have abdominal symptoms like irritable bowel syndrome (IBS). In addition, it is well known that some psychological disorders such as depression or post-traumatic stress disorder (PTSD) are often accompanied by IBS. Recently, the gut microbiota composition in the patients who suffer from depression have attracted attention. However, which kind of psychological disorder is related to gut function and how the changes in the gut microbiota are involved have not been understood. We verified the relevance between them.

Methods: Rats were placed in a shuttle box and given invariable foot shocks. After 2 weeks, they were placed in the same box to perform a behavioral test. Rats were classified some groups by their behavioral patterns. Colorectal distension (CRD) was performed to evaluate visceral perception by comparing the mean threshold. Cecal contents were analyzed to evaluate changes in the gut microbiota compositions.

Results: 14% of rats showed depression-like behavior and 50% of rats showed PTSD-like behavior. The threshold value of CRD in depression-like rats was significantly lower than that in control rats. High-throughput microbial community analysis of cecal microbiota showed that the relative abundance of Clostridiales incertae sedis was significantly lower in depression-like rats than in control rats. The distribution pattern of the microbiota was clearly different between depression-like rats and control rats. Neither visceral hypersensitivity nor the composition of gut microbiota was altered in rats with PTSD-like phenotype.
Discussion/Conclusion: We demonstrated that the same stress induced different types of psychological disorders to individual rats accompanied with their characteristic patterns of the gut microbiota and gut function in the animal model. These results suggest that the close connection of microbiota-gut-brain axis stimulated by psychological stress.
Rotavirus infection in pediatric IBD patients

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Introduction: Patients with inflammatory bowel disease (IBD) in the case of rotavirus infection are at very high risk for developing a relapse regardless of the treatment used for the underlying disease.

Methods: We collected retrospective case-control data during the period from January 1, 2013 to April 1, 2018 for patients diagnosed with IBD who were treated for Rotavirus infection (RVI) at our hospital.

Results: During this period, 81 patient with inflammatory bowel disease were treated at our hospital. 9 patients (6 boys, 3 girls) were treated with RVI (1 patient with Crohn’s disease and 8 patients with ulcerative colitis). 8 of 9 patients had total pancolitis. In 3 patients, Rotavirus infection was diagnosed concurrently with an IBD diagnosis. During or immediately following RVI, 8 of 9 patients received short-term antimicrobial therapy. At the time of the onset of Rotavirus disease, 3 of 9 patients did not receive basic medical treatment. In one of the 4 patients received blood transfusions, colectomy was performed due to a fulminant colitis. 3 patients with ulcerative colitis and RVI receiving blood transfusion, had only 5-ASA as a primary treatment. Two of 8 patients with ulcerative colitis received a biological treatment combined with a 5-ASA or an immunomodulator. These patients did not require blood transfusions and a change of treatment. The duration of hospitalization for these patients was 4 and 5 days respectively, while the average duration of treatment in hospital was 34.28 days (20–99 days) for other patients with IBD and Rotavirus infection.

Discussion/Conclusion: It is more likely that patients with IBD receiving a biological treatment develop less severe Rotavirus infection and reduce the risk of relapse an underlying disease. In the future, a more detailed study is required.
Single-center experience of infliximab in Japanese children with ulcerative colitis

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Introduction: Infliximab (IFX) is effective and safe in pediatric ulcerative colitis (UC). However, evidence specific to its use in Japanese children with UC is limited.

Methods: We retrospectively reviewed medical records of 81 pediatric patients with UC from the National Center for Child Health and Development in Japan, during September 2006–March 2018.

Results: This study included 20 patients (14 girls and 6 boys) aged < 18 years at the first IFX infusion. The mean disease duration from the diagnosis to the first IFX infusion was 68.2 (range, 6.1–215.0) weeks. All patients had a Paris classification score of E4 (pancolitis), except for one boy with E3 (extensive), and 14 (70%) had severe disease activity (Pediatric Ulcerative Colitis Activity Index ≥ 65). Nineteen (95%) patients received corticosteroids (CS) prior to IFX infusion; 18 (90%) and seven (35%) patients received azathioprine and tacrolimus, respectively. Nineteen (95%) patients were administered IFX as a first biologics, and one was switched from adalimumab. At week 30 after IFX initiation, seven (35%) patients achieved clinical remission, of which six were CS-free. Seven (35%) patients exhibited primary nonresponse to IFX, of which three underwent colectomy. All five patients diagnosed under the age of 6 years (very early onset IBD) were refractory to IFX. We studied the long-term efficacy in 13 responders with a median follow-up period of 210.5 (range, 56–512) weeks. Three patients continued the IFX therapy with dose escalation and/or concomitant CS administration; three maintained long-term recurrence-free clinical remission without IFX dose escalation and/or concomitant CS. After week 30, three more patients underwent colectomy. No patients experienced serious adverse events.

Discussion/Conclusion: IFX appears safe and effective in Japanese children with UC. In this study, CS-free clinical remission rate at week 30 was 30%, and 50% patients maintained remission for longer periods. Six patients required colectomy.
Efficacy of mesalazine powder on upper gastrointestinal lesions involved in inflammatory bowel disease

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Introduction: Inflammatory bowel disease (IBD) may involve the upper gastrointestinal (GI) tract. However, no definitive data or consensus has been obtained regarding the optimal treatment of upper GI involvement of IBD. This study was performed to evaluate the efficacy and safety of oral administration of mesalazine powder on upper GI lesions in IBD.

Methods: The medical records of 3,210 patients with IBD (Crohn’s disease [CD], n = 1040; ulcerative colitis [UC], n = 2090; intestinal Behcet’s disease [BD], n = 80) treated at our center were retrospectively reviewed. Among them, 215 had active disease-specific upper GI involvement and 41 were treated with oral mesalazine powder. The endoscopic response was evaluated in 29 patients based on Simple Endoscopic Score for Crohn’s Disease or Ulcerative Colitis Endoscopic Index of Severity. The patients’ endoscopic findings and clinical characteristics were analyzed, and the factors predictive of endoscopic response were identified. Adverse effects were recorded.

Results: Active upper GI involvement occurred in the esophagus in 21% of patients, stomach in 79%, duodenal bulb in 83%, and descending duodenum in 62%. The median mesalazine powder dosage was 1000 mg/day and the median administration period was 32 months. After the treatment, the endoscopic response rate was 45% and the remission rate was 38%. No factors that significantly contributed to an endoscopic response were identified, but an apparently poorer response was observed in patients with CD (p = 0.018). The probability of remaining recurrence-free on endoscopy was 79% at 48 months. Adverse effects were reported in two patients (7%), but no serious toxicities occurred in the study.

Discussion/Conclusion: To our knowledge, this is the first examination of the endoscopic efficacy of oral mesalazine powder on IBD upper GI lesions. Mesalazine powder was well tolerated and was effective in the induction and maintenance of remission of upper GI lesions in IBD.
Breath testing for inflammatory bowel disease

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Introduction: Non invasive detection and monitoring of gastrointestinal diseases is an attractive prospect, particularly as colonoscopy is invasive, expensive and not without risk. Studies have shown that the breath volatile organic compound (VOC) profile may be distinct in inflammatory bowel disease (IBD). COBRA (Colorectal Breath Analysis) is a prospective cohort study analysing the breath of 2000 patients attending for colonoscopies across 4 centres.

Methods: Exhaled breath (500 mls) is collected using the ReCIVA™ breath sampling device, onto 4 thermal desorption tubes. Analysis by gas chromatography mass spectrometry and proton transfer reaction mass spectrometry identifies and quantifies breath compounds, at St. Mary’s Hospital London VOC laboratory. Clinical and colonoscopy data is collected.

Results: The first 406 patients were recruited between July and December 2017, where 80 samples were excluded due to inadequate colonoscopy, failure to meet QC standards or instrument faults. Of the remaining 326 patients, 31 had IBD on colonoscopy, with a wide range of other pathology in the remaining patients, including colorectal cancer and polyps (the main diagnostic focus of the COBRA study).

Discussion/Conclusion: Whilst analysis of this preliminary dataset suggests the presence of discriminatory compounds between IBD and other pathologies, analysis is on going. The true diagnostic accuracy of breath testing in this setting is expected to be revealed once all patients have been recruited to this study.
The use of vedolizumab in preventing postoperative recurrence in high-risk Crohn’s disease patients

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Introduction: Clinical and endoscopic recurrence are common after surgery in Crohn’s disease (CD). Vedolizumab has been increasingly used to treat CD, however, its effectiveness in preventing postoperative recurrence remains unknown. We aimed to investigate the use of vedolizumab in the postoperative setting and compare the risk of recurrence among patients receiving vedolizumab, anti-tumor necrosis factor (TNF)-α agents or immunomodulators.

Methods: Medical records of University of Chicago Medicine and Toho University Sakura Medical Center CD patients who underwent surgery between April 2014–June 2016 were reviewed. We analyzed how frequently vedolizumab is used to prevent postoperative recurrence and compared the patient characteristics with those being treated with other therapies. Furthermore, the rates of endoscopic remission, defined as a simple endoscopic score for CD of 0, at 6–12 months after surgery were compared with patients receiving vedolizumab, anti-tumor necrosis factor (TNF)-α agents or immunomodulators. Clinical, biological and histologic outcomes such as Harvey-Bradshaw index, C-reactive protein, and histologic inflammation were also compared between the two groups. Risks of recurrence were assessed by univariate and multivariate analyses.

Results: Among 245 patients that underwent a CD related surgery, 22 patients received vedolizumab as postoperative treatment. There were 70 and 40 patients who received anti-TNF-α agents and immunomodulators respectively, while 69 patients were monitored without any medication. Rate of endoscopic remission at 6–12 months in the anti-TNF-α agent group was 66%, which was significantly higher as compared to vedolizumab group and immunomodulators group (25% and 44%, p = 0.009). Anti-TNF-α agent use was associated with a reduced risk of endoscopic recurrence on multivariate analysis (OR = 0.17, 95% CI: 0.05–0.59, p = 0.005), while vedolizumab (OR = 5.77, 95% CI: 1.71–19.4, p = 0.005) was associated with an increased risk.

Discussion/Conclusion: Endoscopic recurrence in postoperative high-risk CD patients was higher with vedolizumab than with anti-TNF-α agents, but further investigation including controlled trials is required prior to determining the utility of vedolizumab in preventing postoperative recurrence of CD.
Fecal immunochemical test as an alternative monitoring method for colonoscopy for ulcerative colitis

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Introduction: Colonoscopy is the gold standard imaging modality for assessing inflammation in ulcerative colitis. However, it causes much stress in the patients. Recently, fecal immunochemical test (FIT) is gaining attention as a replacement for colonoscopy. To evaluate the correlation between FIT to colonoscopy findings and clinical activity.

Subject and methods: The subjects were 190 patients (257 times) [mean age: 49 ± 14 years; 145 men; mean disease duration: 12.7 ± 10.2 years; Lichtiger CAI 3.3 ± 2.4, Mayo score 2.1 ± 2.8] with ulcerative colitis who underwent a FIT 3 days immediately before undergoing colonoscopy between January 2014 and January 2018. The correlations 1) between FIT level and mucosal healing, and 2) among FIT results, colonoscopy score (Mayo endoscopic sub score [MES]; Ulcerative colitis endoscopic index of severity [UCEIS]), and clinical activity level (Lichtiger CAI [CAI]; Mayo score) were investigated.

Results: When the patients were divided into 1) MES0,1 (mucosal healing: MH group/164 times) and MES2,3 (non-mucosal healing: NMH group/93 times), the FIT levels were 45 ± 193 and 2024 ± 5776 ng/ml (p < 0.0001) in the MH and NMH groups, respectively, showing a significantly low level in the MH group. When the cutoff value was set at 100 ng/ml, the sensitivity was 53.8%, specificity was 91.5%, positivity predictive value was 78.1%, and negative predictive value was 77.7%. 2) CAI, Mayo score, MES, and UCEIS showed positive correlations (r = 0.45, 0.65, 0.63, and 0.66, respectively; p < 0.0001). In addition, FIT level positively correlated with local presence of inflammation in the affected range (r = 0.59, p < 0.0001).

Discussion/Conclusion: FIT level was found to be correlated with clinical activity level and colonoscopy score. Furthermore, a moderate correlation with the affected range was observed.
Clinical courses and correlations with ulcerative colitis in Japanese patients with primary sclerosing cholangitis

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Introduction: Comorbidity rate of primary sclerosing cholangitis (PSC) and ulcerative colitis (UC) was low in East Asia. The number of UC patients in East Asia has increased obviously past two decades; however, current clinical features of PSC and PSC with UC (PSC-UC) have not clarified in East Asia. The aim of this study was to disclose present situations of clinical course and correlations with UC in Japanese patients with PSC.

Methods: We retrospectively retrieved the medical records of both PSC and UC patients who were diagnosed in Chiba University Hospital between June 1991 and August 2017.

Results: Total of 69 patients diagnosed with PSC and 1242 patients diagnosed with UC. In the present cohort, 37 patients combined PSC with UC and cumulative risks of PSC in UC patients and UC in PSC patients were 53.6% and 3.0%, respectively. Of PSC cohort, median age of PSC-UC and PSC alone were 26 and 57 years old, respectively. During the follow up period, 11 patients were diagnosed cholangiocarcinoma and 23 patients deemed as liver transplantation (LT) candidate. However, only 3 patients have been able to receive LT. Of UC cohort, overall survival from initial diagnosis of UC was significantly shorter in PSC-UC compared with UC alone (log-rank; p < 0.001). Young-onsets tended to increase in both UC and PSC-UC.

Discussion/Conclusion: In our cohort, PSC-UC indicated poor prognosis, and incidence of PSC may increase in future as well as UC in East Asia, especially in Japan.
Enteric neurons interact with dendritic cells in the mouse colonic mucosa by releasing IL-6

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Introduction: Neuro-immune interactions play a critical role in the maintenance of homeostasis in the gastrointestinal tract. Recent studies have suggested the possibility that the enteric nervous system can modulate gut mucosal immunity. In addition, interleukin-6 (IL-6) has been shown to be a major regulator of the immune responses, and IL-6 signalling is of central importance in inflammatory bowel diseases (IBD). In this study we demonstrate that the enteric neurons (ENs) produce IL-6 to activate dendritic cells (DCs) in the mouse colon.

Methods: The localization of DCs (CD11c+ F4/80- cells), ENs and IL-6 was investigated with immunohistochemistry in the proximal colon of normal mouse. Bone marrow-derived dendritic cells (BMDCs) (CD11c+ MHCII+ F4/80- cells) were sorted by BD FACSAria, and responses of BMDCs to IL-6 were examined by calcium imaging.

Results: In the immunohistochemical studies, 50% (50.0 ± 6.7%) of DCs were found in the close proximity to nerve fibers in the colonic mucosa. Also, IL-6 immunoreactivities were observed in the neuronal cell bodies of myenteric neurons and the mucosal nerve fibers. Furthermore, IL-6 increased the intracellular calcium level in BMDCs.

Discussion/Conclusion: The immunohistochemical results provide morphological evidences of the interaction between dendritic cells and the neural network, and the expression of IL-6 in the ENs. Furthermore, IL-6 can activate dendritic cells by increasing the cytosolic calcium concentration, indicating that IL-6 in the ENs modulate the activity of dendritic cells through the neuro-immune communication in the gastrointestinal tract. Taken together, this study provides a new finding of the interaction between the enteric nervous system and the colonic immune system through the activation of dendritic cells by releasing IL-6 from ENs.
Protective role of IL-10 producing plasmablasts (Preg) in colitis

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Introduction: Although B cells are typically characterized by their ability to produce antibodies, it is becoming increasingly apparent that B cells play more complicated functions (e.g. cytokine production) than previously predicted. In T-cell receptor α knockout (TCRαKO) mouse (model of ulcerative colitis), inducible B cell population termed Breg has been identified to contribute for improving the colitis through the production of IL-10. However, the developmental pathway of Breg still remains obscured.

Methods/Results: To visualize the spontaneous production of IL-10, green fluorescent protein (GFP) / IL-10 reporter mouse system was employed. In the spleen, GFP(+) cells were mainly detected in both CD3(+) T cell population and CD19(+) B cell population. Unexpectedly, the splenic GFP(+)CD19(+ ) cells co-expressed CD138 (a marker of plasma cells) and CD93 (a marker of immature B cells). In addition, splenic IL-10 producing CD138(+)cells were characterized by intracellular accumulation of IgA rather than IgM. To identify the origin of the unique cells that are characterized by co-expression of plasma cell and immature B cell markers, GFP/IL-10 reporter mice were crossed with Blimp 1-deficient mice that lack the differentiation into plasma cells. Interestingly, IL-10-producing CD138(+) cells was rarely detected in these mice. To examine Preg in colitis, TCRα deficient GFP/ IL-10 reporter mice were generated. Interestingly, Preg was increased in the mesenteric lymph nodes (MLN), proposing the migration of Preg from spleen to MLN under intestinal inflammatory conditions. To test the role of Preg in colitis, these mice were further crossed with B cell-specific Blimp-1 deficient mice. The absence of Preg significantly exacerbated colitis.

Discussion/Conclusion: Although B cells were initially proposed to produce IL-10 under intestinal inflammatory conditions, our data suggest that plasmablasts, which undergone immunoglobulin class switch particularly to IgA, may be a major source of IL-10 and they contribute for the attenuation of colitis.
Long-term prognosis of Japanese patients with biologic-naïve Crohn’s disease treated with anti-tumor necrosis factor-α antibodies: A retrospective single-center cohort study

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Introduction: Few reports have described the long-term treatment outcomes of the anti-tumor necrosis factor-α antibody for Japanese Crohn’s disease (CD) patients. The aim of this study was to evaluate them and clarify the clinical factors that affect the long-term prognosis of the anti-tumor necrosis factor-α treatments.

Methods: This was a retrospective, observational, single-center cohort study. Japanese CD patients treated with either infliximab or adalimumab as a first-line therapy were analyzed. The cumulative retention rates of the biologics, relapse-free survival, and surgery-free survival were analyzed using Kaplan-Meier methods. The clinical factors associated with the long-term outcomes were estimated by both the log-rank test and Cox’s proportional hazard model.

Results: The cumulative retention rate was significantly higher in the group with a concomitant elemental diet of ≥ 900 kcal/day, baseline CRP levels < 2.6 mg/dl, and baseline serum albumin levels ≥ 3.5 g/dl, respectively. The baseline serum albumin levels were also associated with both relapse-free and surgery-free survival. The lack of concomitant use of an elemental diet ≥ 900 kcal/day was identified as the only independent risk factor for the withdrawal of the biologics.

Discussion/Conclusion: Baseline CRP levels and serum albumin levels could affect the long-term outcomes in CD patients. Concomitant elemental diet of ≥ 900 kcal/day could have a positive influence on clinical treatment course.
Comprehensive analysis to identify aberrant DNA methylation for predicting colitis associated cancer in ulcerative colitis patients

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Introduction: Colitis-associated cancer (CAC) is often difficult to detect endoscopically and histologically because of mucosal structure modifications by inflammation in ulcerative colitis (UC) patients. We recently reported that methylation of specific miRNAs occurred in an age- and cancer-dependent manner in UC patients, and miRNA methylation in non-neoplastic rectal mucosa successfully discriminated patients with CAC from those without in two independent patient cohorts, which indicates methylation status of a panel of miRNAs from a single rectal biopsy specimen may serve as important biomarkers for improving surveillance efficiency for patients at greatest risk for developing CAC. In this study, we conducted comprehensive methylation array to identify novel DNA methylation markers for predicting the risk of neoplasia in UC patients.

Methods:
Cohort 1: We collected 23 rectal samples from UC patients with CRC and 24 rectal samples from UC patients without CRC in Hyogo University Hospital.
Cohort 2: We also collected 8 rectal samples from UC patients with CRC and 24 rectal samples from UC patients without CRC in Mie University Hospital.

Results: We identified 486 differentially methylated regions (DMRs) with absolute delta beta-value > 0.1 in rectal mucosal tissues of UC patients with CAC compared with that without neoplasia. Next, pathway enrichment analysis was performed to select coordinately methylated DMRs, and 180 DMRs were extracted. Finally, optimal 11 DMRs were selected by the Elastic Net classification algorithm. In the ROC analysis for the training set (n = 47, Cohort 1), the AUC was 0.96 (95% CI: 0.90, 1.00). For the test set (n = 16, Cohort 2), the AUC was 0.81 (95% CI: 0.55, 1.00).

Discussion/Conclusion: We identified 11 DMRs for identifying UC patients with high risk of developing CAC. Large prospective trial may be needed to further confirm the validity of these DMRs.
Loss of response in anti-tumor necrosis factor-α biologic agents in patients with Crohn’s disease: A multicenter retrospective study

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Introduction: Anti-tumor necrosis factor-α (TNF-α) agents known as biologics are important treatment strategy in patients with Crohn’s disease (CD). However, loss of response (LOR) remains problematic. We assessed the LOR of TNF-α agent in the treatment of CD.

Methods: This was multicenter, retrospective cohort study. We enrolled 124 CD patients treated with initial TNF-α (IFX, infliximab and ADA, adalimumab) for induction and maintenance of remission therapies between May 2002 and July 2017. We excluded 6 patients of primary non-responder, and analyzed risk factors of LOR in 118 patients using the log-rank test and Cox regression analysis.

Results: The median age was 31 years old and the number of female was 30. The median of disease duration before TNF-α treatment and follow-up period after TNF-α treatment was 2.2 and 3.8 years, respectively. LOR in IFX treatment and ADA treatment was recognized in 23 (28%) of 80 patients and 7 (18%) of 38 patients, respectively. Disease location, disease behavior, past history of ileocolonic resection, kind of first TNF-α, presence of perianal disease, and concomitant treatment with azathioprine (AZA) was not associated with cumulative non-LOR ratio (p = 0.83, p = 0.76, p = 0.52, p = 0.47, p = 0.18, and p = 0.66, respectively). The cumulative non-LOR rate of patients with short disease duration (< 3 years), ADA treatment, and high C-reactive protein (CRP, > 2.25) before induction was lower than that of patients with long disease duration (p < 0.05), IFX treatment (p < 0.05), and low CRP level (p < 0.05), respectively. By multivariate analysis, disease location (ileal and ileocolonic) and CRP level before induction were detected as risk factors of LOR (p < 0.05).
Discussion/Conclusion: This study demonstrated that disease duration, type of biologics, and CRP level may influence the prognosis of initial TNF-α treatment. Further prospective studies regarding LOR are needed.
Association between gut microbiota and undercarboxylated osteocalcin which is an alternative indicator of vitamin K deficiency in patients with Crohn’s disease

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Introduction: Recent reports have suggested a possible association between gut microbiota and osteoporosis. Vitamin K (vit K), a fat-soluble vitamin, affects blood coagulation, bone fracture healing, and osteogenesis. Various gastrointestinal issues can affect vit K deficiency in patients with Crohn's disease (CD). However, there have been no reports on associations between gut microbiota and vit K in patients with CD. Our aim was to assess the relationship between gut microbiota and alternative indicators of vit K deficiency in patients with CD.

Methods: We included 26 patients in CD remission and collected their feces. We extracted 16S rRNA from the intestinal bacteria in the feces, and amplified by PCR. The generated PCR product was analyzed using a 16S metagenomic approach. Serum undercarboxylated osteocalcin (uc-OC) concentrations ≥ 4.5 ng/ml were defined as indicative of vit K deficiency.

Results: The group with a high proportion of order Bifidobacteriales and a low proportion of orders Bacterioidales and Clostridiales had high Crohn's disease activity indicator (CDAI) scores, anal lesions, and history of ileocecal resection. In the high uc-OC concentrations group, the proportion of order Bifidobacteriales was high and the proportion of order Clostridiales was low.

Discussion/Conclusion: Gut microbiota in the vit K deficient group of patients with CD showed similar trends to those in patients with highly active CD despite achieving clinical remission, in contrast to patients with osteoporosis but without inflammatory bowel disease. Thus, there is a high possibility that disease activity and dysbiosis are related to vit K deficiency.
Disorders of liver function in inflammatory bowel diseases in children

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Introduction: Enterohepatic circulation requires determining the nature and extent of liver damage in inflammatory bowel diseases (IBD) such as ulcerative colitis (UC) and Crohn’s disease (CD) in children.

Purpose: Reveal changes in biochemical markers of cholestasis and cytolysis and determine the degree of impaired liver function in children with IBD.

Materials and methods: We observed 25 children with IBD: 12 children with UC and 13 with CD. We evaluated biochemical markers of cholestasis such as gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (AP), total and direct bilirubin, cholesterol, and of cytolytic activity such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The degree of impaired liver function was determined based on the following indicators scored on a scale from 0 to 4: ALT, AST, De Ritis coefficient, ammonia, fibrinogen, prothrombin, albumin, cholesterol, urea, bilirubin, glucose, lactate, ceruloplasmin, and transferrin. The degree of liver impairment was defined by summarizing points for each indicator.

Results: The biochemical markers of cholestasis were as follows: the GGT level was 53.5 ± 25.2 [8.0 53.5* 578.0] U/l, AF 237.2 ± 56.2 [53.0 237.2* 1283, 0] U/l, cholesterol 4.9 ± 0.31 [2.9 4.9* 12.8] mmol/l, total bilirubin 30.9 ± 14.9 [3.4 30.9* 377.5] μmol/l, and direct bilirubin 10.3 ± 7.7 [0.3 10.3* 190.2] μmol/l. We observed the increased activity of cytolytic activity: the ALT level was 62.2 ± 25.5 [9.0 62.2* 582.0] U/l, and AST level 64.5 ± 21.1 [17.0 64.5* 465.0] U/l.

There were no significant differences in the level of both biochemical markers of cholestasis and indices of cytolytic activity for UC and CD: the GGT level for UC was 19.6 ± 3.0 [8.0 19.6* 36.0] U/l, and for CD - 21.8 ± 5.1 [10.0 21.8* 77.0] U/l, p = 0.7136; the level of alkali-earth metals for UC was 150.0 ± 17.6 [53.0 150.0* 254.0] U/l, for CD - 223.2 ± 66.1 [54.0 223.2* 937.0] U/l, p = 0.2962; the level of total bilirubin for UC was 13.1 ± 1.9 [3.4 13.1* 23.6] μmol/l, for CD - 46.3 ± 28.9 [7.5 46.3* 377.5] μmol/l, p = 0.263981; the level of direct bilirubin for UC was 1.8 ± 0.3 [0.3 1.8* 3.2] μmol/l, for CD - 17.1 ± 15.0 [0.9 17.1* 190.2] μmol/l, p = 0.3189. However, the level of cholesterol for UC was lower than for CD amounting to 4.2 ± 0.3 [2.9 4.2* 7.0] mmol/l and 5.2 ± 0.3 [3.2 5.2* 12.8] mmol/l, respectively, p = 0.0277. The ALT level for UC was 30.1 ± 5.3 [9.0 30.1* 60.0] U/l, for CD - 29.3 ± 7.9 [10.0 29.3* 102.0] U/l, p = 0.9337; the AST level for UC was 34.3 ± 5.1 [17.0 34.3* 66.0] U/l, for CD - 39.8 ± 8.9 [21.0 39.0* 130.0] U/l, p = 0.5972.
The liver function for IBD was impaired by 20.4 ± 1.4% [7.0% 20.4%* 39.0%], which was insignificant in 18 cases and moderate in 7 cases. At the same time, significant differences in the degree of liver function abnormalities were not detected for UC and CD (liver function was reduced by 20.8 ± 1.5% [15.0% 20.8%* 29.0%] for UC and by 20.2 ± 2.5% [7.0% 20.2%* 39.0%] for CD). The impaired liver function in IBD was found in all cases.

**Conclusion:** The research reveals liver pathology in IBD and the need for further examinations to determine the nature and extent of liver damage.
Three boys with XIAP deficiency mimicking refractory Crohn’s disease

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Introduction: X-linked inhibitor of apoptosis protein (XIAP) deficiency is a rare immunodeficiency that is characterized by recurrent hemophagocytic lymphohistiocytosis (HLH) and splenomegaly and is sometimes associated with refractory Crohn’s disease (CD).

Methods: We report on three pediatric cases with XIAP deficiency whose initial diagnosis was refractory CD.

Results: Case 1 was initially diagnosed with refractory CD at the age of eight and was then complicated by HLH twice. At age 11 genetic analysis confirmed XIAP deficiency (c.1141C>T, p.Arg381X). Case 2 was initially diagnosed with severe CD at age 11. He had developed HLH four times since infancy and relapsed CD frequently resulting in sigmoid colon stenosis. Genetic analysis revealed XIAP deficiency (c.340C>T, p.Glu114X) at age 17. Case 3 was initially diagnosed with refractory CD at eight. Subsequently, colectomy and colostomy were carried out. He developed HLH twice including EB-VAHS at one year of age. There is no mutation in the coding exon of XIAP gene, but XIAP protein expression was completely deficient by the Western blotting analysis. Approximately 2 kbps defect in exon 1 including 5’UTR was detected. All the three patients underwent hematopoietic stem cell transplantation (HSCT).

Discussion/Conclusion: Because HSCT is the only curative therapy for patients with XIAP deficiency, it is necessary for refractory CD patients complicated with HLH to perform DNA analysis and Western blotting analysis as soon as available.
Investigation of IBD unclassified with MEFV gene analysis

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Introduction: Familial Mediterranean Fever (FMF) is a hereditary disease characterized by periodic fever and serositis. In recent years, FMF with gastrointestinal lesions have been reported, and the relation with IBD unclassified (IBDU) has drawn attention. We investigated MEFV gene polymorphism in IBDU patients.

Methods: We performed MEFV gene analysis in the patients who had been diagnosed IBDU patients at Kyorin university hospital from April 2016 to March 2018.

Results: MEFV gene analysis had been performed in 8 patients with IBDU (4 males, 4 females). The average age was 32.7 ± 26.4 years old (26–76 years old). Symptoms were diarrhea (8), bloody stools (8), abdominal pain (6), fever (5). The lesion site was whole colon (6), sigmoid colon (1), and cecum (1). Rectal lesions were confirmed in 2/8 cases. Endoscopic findings were granular mucous membranes (4), rough mucous membranes with vascular permeability disappearance (2), and shallow ulcers (2). MEFV gene mutation was confirmed in 4/8 cases, and many were mutations of EXON 2. Colchicine was administered to 4/8 patients with poor improvement in 5-ASA and PSL, and then symptoms were improved in all cases.

Discussion/Conclusion: IBDU may include colitis related to MEFV mutation, and colchicine may be effective in those patients. Confirmation of MEFV gene mutation should be examined in patients with refractory IBDU.
Tenofovir and entecavir treatments decrease renal function in chronic hepatitis B patients in real clinical practice

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Introduction: Tenofovir disoproxil fumarate (TDF) is known to be associated with nephrotoxicity in patients with human immunodeficiency virus (HIV). However, its incidence has not been known to date. Therefore, we have been interested the real-life incidence of renal toxicity in chronic hepatitis B (CHB) patients treated with TDF.

Methods: From January 2012 to December 2014, medical records of patients who had been treated with TDF or entecavir (ETV) in Kosin University Gospel Hospital were reviewed retrospectively, focused on the estimated GFR (eGFR) reduction.

Results: A total of 295 patients received TDF or ETV. The following patients were excluded, 25 patients treated for 30 days or less, 45 patients without follow-up creatinine level, 9 patients with base-line eGFR < 60 (ml/min/1.73 m²) patients, one patient with polycystic kidney disease, and 17 patients with decreased eGFR by other obvious nephrotoxic factors. Among analyzed 198 patients, 99 received TDF and 99 ETV. In 18 (9.1%) patients, eGFR was reduced by more than 30% compared to baseline. Half of them treated with TDF and the other half with ETV. Baseline eGFR (p = 0.006) and age (p = 0.015) were significantly related with eGFR reduction. The presence of liver cirrhosis (p = 0.684), non-selective beta blocker (p = 0.309), type of antiviral agents (p = 0.927), and body mass index (p = 0.965) were not associated with the eGFR reduction. Subgroup analysis of chronic kidney disease stage 2 (60 < eGFR < 90) or old age (> 60 year) did not show any significant association between antiviral agents and eGFR reduction.

Discussion/Conclusion: A significant reduction of eGFR is observed in a tenth part of CHB patients receiving TDF and ETV in real clinical practice. Baseline low eGFR and old age are associated with eGFR reduction. Therefore, renal function should be closely monitored in CHB patients with old age and renal insufficiency in the treatment of TDF and ETV.
An imbalance of free amino acids in portal blood induces endoplasmic reticulum (ER) stress to hepatocytes via the suppression of lipid secretion in non-alcoholic fatty liver disease (NAFLD)

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Introduction: The aim of this study is to elucidate the association between the free amino acids (FAAs) in portal blood (PB) and the pathogenesis of hepatic steatosis in NAFLD.

Methods: We made two media that were consistent with the levels of glucose and FAAs in the PB of wt or ob/ob (NAFLD model) mice (PBM-wt, PBM-ob, respectively). Steatotic mouse hepatocytes (immortalized or primary) induced by oleate were cultured under PBM-wt and PBM-ob using a 3D perfusion culture system. Then we measured triglyceride (TG) in cells and free fatty acid (FFA) in media, and counted lipid droplets (LDs) in cells by flow cytometer. GRP78 and ATF4, apoB40/100 and MTTP were also determined by western blotting. C57BL/6 mice were fed a high-fat (HF) and L-methionine (Met) / L-tyrosine (Tyr) -deficient (HF-MTD) diet and a pathological analysis was conducted. Additionally, we quantified the area of LDs in samples of liver tissues obtained from NAFLD patients (n = 70) by image processing software. Then we analyzed the correlations between the area of LDs and FAAs in peripheral blood.

Results: The levels of TG and LDs in hepatocytes were significantly higher under PBM-ob than PBM-wt, although the concentration of FFA was lower in PBM-ob than PBM-wt. Interestingly, the lipid accumulation in hepatocytes under PBM-ob was significantly decreased by adding 7 FAAs which were significantly decreased in PB of ob/ob mice, to PBM-ob. Especially Met and Tyr had a strong reducing effect on LDs in hepatocytes. The expressions of GRP78 and apoB100 in hepatocytes were significantly higher in PBM-ob than in PBM-wt and these were also decreased by adding Met and Tyr to PBM-ob. The HF-MTD diet induced steatosis and ballooning pathologically in the liver of mice, but the HF diet did not. In NAFLD patients, there were negative correlations between the area of LDs in liver samples and 6FAAs that were significantly decreased in PB of ob/ob.

Discussion/Conclusion: The decrease of 7 FAAs, especially Met and Tyr, in PB induced TG accumulation and ER stress via the suppression of lipid secretion from hepatocytes in NAFLD.
Gut microbiota in primary sclerosing cholangitis is characterized by specific composition of fungi

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Introduction: Primary sclerosing cholangitis (PSC) is a progressive disorder of biliary tree which can lead to end-stage liver disease, liver transplantation or even death. Colitis accompanying PSC (in up to 80% of patients) is considered as a phenotype of IBD inflammatory bowel disease (IBD) distinct from ulcerative colitis (UC) and is often referred to as PSC-IBD. Gut microbiota presumably plays an important role in both PSC and IBD pathogenesis. The aim of this study was to characterize gut mycobiota composition in patients with PSC, PSC-IBD and UC.

Methods: Stool samples were prospectively collected and relevant clinical data obtained from 109 study participants: 50 PSC patients with (n = 38) or without (n = 12) IBD, 32 controls with UC and 27 healthy controls (HC). Sequencing of the ITS1 gene was performed using Illumina MiSeq platform. Acquired data were processed in QIIME employing MaAsLin for analysis of the output results.

Results: No significant shifts in regards of alpha and beta diversity were found among the study groups. However, PSC was characterized by high relative abundance of several genera as compared to healthy controls: Candida (5.2% vs 2.5%), Lysurus (20.7% vs 11.7%) and Cladosporium (1.2% vs 0.6%). Furthermore, relative abundance of genus Rhodosporidium clearly distinguished PSC-IBD from UC (14.4% vs 2.8%). Such differences were further tracked down to the species level, identifying major taxa responsible for respective shifts: Candida albicans, Lysurus cruciatus, Cladosporium herbarum and Rhodosporidium Babjevae. Subsequent multivariate analysis determined high abundance of Cladosporium herbarum sp. to be tightly associated with presence of PSC (p ≤ 0.05).

Discussion/Conclusion: PSC is characterized by specific features of gut fungal microbiota composition. Intestinal mycobiota profiles differ between IBD subphenotypes (PSC-IBD and UC). High abundance of Cladosporium herbarum sp. (exceedingly common plant pathogen) in PSC may suggest an association with certain dietary habits.

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Hepatic steatosis and fibrosis in patients with chronic hepatitis C: The effect of S-adenosylhomocysteine

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Background and aim: The mechanism that leads to steatosis is complex and multifactorial in hepatitis C virus (HCV) infection. Oxidative stress plays a key role in the pathogenesis of hepatic disease. S-adenosylmethionine (SAM) is the precursor of glutathione; it is a hepatoprotective agent that has antioxidant and antifibrotic peculiarities. The role of the relation of SAM and its methylation ability with HCV related steatosis and fibrosis was researched.

Methods: Fifty two untreated chronic hepatitis C patients were included in the study to evaluate the effects of S-adenosylhomocysteine (SAM), body mass index (BMI), age, and insulin resistance on hepatic steatosis. We used HPLC method to measure the serum homocysteine, SAH and SAM levels. We used SAM/SAH ratio (4/1) to evaluate the methylation ability. The liver biopsies were evaluated according to Knodell’s. Hepatic steatosis was evaluated as; 0: there is steatosis in < 5% of hepatocytes, 1: there is steatosis in 5–34% of hepatocytes, 2: there is steatosis in 35–69% of hepatocytes, 3: there is steatosis in > 69% of hepatocytes.

Results: In 45 (86.5%) of 52 patients, steatosis was found. This ratio is rather high. No correlation was found between any factors with the grade and steatosis. When we separated SAH, SAM and SAH/SAM to steatosis and grade degrees, the value of any group was not found different from the other group. Respectively, p value was found as: p = 0,774, p = 0,783, p = 0,801. When steatosis was separated as present – absent and grade 0, low grade (1–2), and high grade (3–4), a significant difference was found. Respectively, p value was found as: p ≤ 0,0001, p ≤ 0,001, p ≤ 0,001, p ≤ 0,001, p ≤ 0,009.

Conclusion: In patients with chronic HCV, there is a meaningful relation between hepatic steatosis with SAH and SAM levels, and SAH/SAM ratio. The relation of the complete blood level of SAM with steatosis in patients with HCV can show that, in these populations, the level of SAM could aggravate the progression of steatosis and hepatic damage.
Effect of a *Morus alba* leaf extract in an experimental model of obesity: Impact on liver steatosis and inflammation

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**Introduction:** In obesity there is a chronic low-grade inflammation caused by an increase in intestinal permeability and serum lipopolysaccharide levels. It leads to activation of Kupffer cells and hepatic TLR-4 causing inflammation and non-alcoholic fatty liver disease. Phenolic compounds present in fruit and vegetables have been shown to ameliorate the subclinical inflammatory status in obesity. The aim of this study was to evaluate the effect of a polyphenolic extract from *Morus alba* leaves in an experimental model of obesity, focusing on changes in intestinal permeability and liver disorders.

**Methods:** Male C57BL/6 mice were divided in groups (n = 10): control, obese and obese daily treated with the mulberry leaf extract (10 mg/kg, p.o.). Control mice were fed with a standard diet, whereas obese mice received a high-fat diet. The treatment was followed for 6 weeks, and animal body weight and food intake were controlled regularly. After sacrifice, liver samples were taken for histological evaluation and to assess the expression of pro-inflammatory mediators. Moreover, the expression of markers of intestinal epithelial barrier were analyzed.

**Results:** Obese mice treated with the extract showed a decrease in weight gain in comparison with untreated obese mice. The expression of hepatic cytokines, such as IL-1β, TNFα, IL-6, and TLR-4 was downregulated by the treatment. The loss of intestinal barrier integrity was reversed by the treatment, which elevated the expression of MUC-3. In addition, treated mice showed less lipid accumulation in the liver, assessed in the histological sections, than the non-treated obese mice.

**Discussion/Conclusion:** The mulberry leaf extract exerted beneficial effects against obesity by ameliorating the liver inflammation and fat accumulation. These effects were associated with an improvement of the intestinal barrier function and a downregulation of liver inflammation. Therefore, the mulberry extract could be developed for ameliorating liver-associated damage in obesity.
Combining probiotics and an angiotensin-II type 1 receptor blocker has beneficial effects on hepatic fibrogenesis in a rat model of non-alcoholic steatohepatitis

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Introduction: Intestinal endotoxin is important for the progression of non-alcoholic steatohepatitis (NASH). Circulating endotoxin levels are elevated in animal models of non-alcoholic fatty liver disease (NAFLD) and NASH. Plasma endotoxin levels are also higher in NAFLD patients, which is associated with small intestinal bacterial overgrowth and increased intestinal permeability. By improving the gut microbiota environment and restoring gut-barrier functions, probiotics are effective for NASH treatment in animal models. It is also widely known that hepatic fibrosis and suppression of activated hepatic stellate cells (Ac-HSCs) can be attenuated using an angiotensin-II (AT-II) type 1 receptor blocker (ARB). We thus evaluated the effect of combination probiotics and ARB treatment on liver fibrosis using a rat NASH model.

Methods: Fisher 344 rats were fed a choline-deficient/L-amino acid-defined (CDAA) diet for 8 weeks to generate the NASH model. Animals were divided into ARB, pro-biotics, and ARB plus probiotics groups. Therapeutic efficacy was assessed by evaluating liver fibrosis, the lipopolysaccharide (LPS) Toll-like receptor (TLR)4 regulatory cascade, intestinal barrier function, and microbiome of the stool by NGS.

Results: Probiotics and ARB are effective in suppressing liver fibrosis via different mechanisms. The combination treatment exerted a greater inhibitory effect than mono-therapy. Both probiotics and ARB inhibited liver fibrosis, with concomitant HSC activation and suppression of liver-specific TGF-β and TLR4 expression. Probiotics reduced intestinal permeability by rescuing zonula occludens-1 (ZO-1) disruption induced by the CDAA diet. ARB directly suppress regulation of Ac-HSC. And probiotics administration improved the microbiome disrupted by the CDAA diet.

Discussion/Conclusion: Combination of probiotics and ARB are effective in suppressing liver fibrosis via different mechanisms. Currently both drugs are in clinical use; therefore, the combination of probiotics and ARB is a promising new therapy for NASH.
HBV patients with the HBeAg negative/low HBsAg/high HBcrAg have a high risk of HBV-related HCC

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Introduction: Although the viral markers HBsAg and HBcrAg may reflect intrahepatic hepatitis B virus (HBV) replication activity and may constitute important biomarkers for hepatocellular carcinoma (HCC), the value of using these two markers in combination for assessing the HCC risk has not been clarified in detail until now.

Methods: Three hundred and sixty-eight consecutive patients with chronic HBV infection and no history of HCC were included in the study and the association of HBeAg, HBsAg and HBcrAg with the HCC risk was investigated longitudinally. 143 patients received nucleos(t)ide analogue (NA) therapy.

Results: On average 4.6 years of observation, 16 patients developed into HCC. When the cutoffs of HBsAg and HBcrAg were respectively defined as 3.0 logIU/ml and 3.0 logU/ml, HCC patients were found frequently in the elder, male, low albumin, low platelets, and HBeAg negative/low HBsAg/high HBcrAg. In multivariate analysis using Cox proportional hazard model, the low albumin (HR = 3.20, p = 0.033) and HBeAg negative/low HBsAg/high HBcrAg (HR = 3.57, p = 0.031) were risks with significant differences in HCC development. In the NA treatment group, the elder (HR = 4.54, p = 0.033) and HBeAg negative/low HBsAg/high HBcrAg (HR = 4.42, p = 0.040) were risks of HCC development. On the other hand, in the no treatment group, HCC risk were male, low platelets, low HBsAg (p < 0.05).

Discussion/Conclusion: The HBeAg negative/low HBsAg/high HBcrAg group is at high risk of developing HBV-related HCC. Especially demonstrating that the combination of HBsAg and HBcrAg values is an excellent biomarker for assessing the HCC risk in the era of NA therapy.
Treatment of hepatocellular carcinoma using 2-deoxy-D-glucose encapsulated in PLGA nanoparticles in mice

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Introduction: The glucose derivative, 2-deoxy-D-glucose (2DG) has been shown to exert antitumor effect through inhibition of anaerobic glycolysis, but simultaneously induces adverse effects, which makes it difficult to apply this agent to clinical use.

Aim and methods: We encapsulated 2DG in polymer poly lactic acid-co-glycolic-acid (PLGA) nanoparticles and evaluated its antitumor effect and safety using hepatoma cell lines and xenograft tumors in nude mice.

Results: In vitro 2DG suppressed production of lactate and ATP, inhibited cell proliferation, and induced apoptosis in dose dependent manner through ROS production and activation of endoplasmic reticulum stress. In vivo 2DG suppressed the growth of xenograft tumors in nude mice in dose dependent manner as well, but induced marked hyperglycemia and liver dysfunction. Weekly intravenous administration of 2DG-PLGA more significantly suppressed the growth of xenograft tumors through induction of apoptosis and inhibition of cell proliferation in dose dependent manner than weekly intravenous administration of 2DG or daily intraperitoneal administration of 2DG. It should be noted that 2DG-PLGA did not show any adverse effects. In addition, combined administration of 2DG-PLGA and sorafenib markedly suppressed the growth of xenograft tumors. We also assessed the antitumor effect of 2DG-PLGA in STAM mice. 2DG-PLGA showed significant antitumor effect in STAM mice as well. We also intravenously injected indocyanine green encapsulated PLGA nanoparticles and confirmed their specific accumulation in xenograft tumors until 10 days after injection, using in vivo imaging system.

Discussion/Conclusion: These results suggest that 2DG-PLGA shed light on the novel approach for the treatment of hepatocellular carcinoma.
Occurrence/recurrence of hepatocellular carcinoma after eradication of hepatitis C virus with IFN-free DAA therapy

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Introduction: IFN-free DAA therapies enabled HCV elimination (sustained viral clearance, SVR) in patients with high HCC risks (elderly, advanced fibrosis, and past HCC histories). It is unclear how viral clearance achieved by IFN-free DAA therapy affects the future development of HCC.

Methods: In 433 SVR12-patients (115 with DCV/ASV, 214 with SOF/LDV, 17 with OMV/PTV/r, 21 with EBR/GZR, 64 with SOF/RBV, and 2 with OMV/PTV/r), the risk factors of HCC occurrence/recurrence and recurrence after SVR were examined.

Results: In the patients without HCC history, only 1.7% newly developed HCC after SVR, while HCC recurrence was observed in 45.8% in the patients with HCC history. In DAA-SVR patients, HCC occurrence was observed in 0.3%/1.8% (1-year/3-year) while HCC recurrence was observed in 6%/42% (1-year/3-year). In the analysis of patients with HCC history, HCC recurrence was associated with number of HCC therapy (p = 0.002) in multivariate analysis. In the analysis of patients without HCC history, new HCC occurrence was associated with cirrhosis (p = 0.045), ALT (p = 0.027) in multivariate analysis. In the comparison of cumulative HCC recurrence rate after 1st HCC treatment between DAA-SVR patients and DAA-untreated patients, recurrence rate was lower in DAA-SVR patients (4% in SVR vs. 28% in untreated as to 1-year recurrence, p < 0.001). Recurrence after 1st HCC treatment was significantly reduced in DAA-induced SVR patients irrespective of treatment modalities (surgery vs. TACE/RFA) or the stages of HCC.

Discussion/Conclusion: It was clarified that DAA-induced SVR significantly reduced HCC incidence, however, HCC recurrence rate is still high especially in patients with HCC history. In the era of DAA therapy targeting high-HCC risk patients, it is needed to clarify the features of SVR-HCCs further.
The influence of MTHFR gene polymorphism on the long-term course in G1 hepatitis C patients who are achieved sustained viral response treated with pegylated interferon plus ribavirin

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Background: A genetic history, such as the MTHFR polymorphism responsible for hyperhomocysteinemia, plays a role in the development of high-grade steatosis and accelerates the progression of liver fibrosis in chronic hepatitis c. The aim of this study was to investigate the long-term effects of MTHFR gene polymorphism in genotype 1 (G1) hepatitis C patients who achieved sustained viral response (SVR) with pegylated interferon plus ribavirin (PIR) therapy.

Methods: 58 G1 hepatitis C patients who had SVR with PIR therapy and who had been followed since 2008 were included in the study. MTHFR gene polymorphisms (C677T and A1298C) were tested by PCR-RFLP. Medical records of patients were screened between 2008 and 2017 and cases of cirrhosis and hepatocellular carcinoma (HCC) were recorded. Fibrosis status was assessed by APRI, a non-invasive method. The difference between the values between 2008 and 2017 was recorded as ∆APRI.

Results: One patient had HCC and 7 patients had cirrhosis. Because there were few patients, cirrhosis and HCC were taken as event group. For MTHFR C677T, 34 patients had wild type and 24 had mutant alleles. For MTHFR A1298C, 32 patients were wild type and 25 patients had mutant alleles. There was no statistically significant relationship between MTHFR C677T and A1298C and events occurring in the course (p = 0.859, p = 0.273, respectively). There was no interaction for both C677T and A1298C in the change of APRI over years (p > 0.05). Those of the MTHFR C677T wild type; the mean value of the APRI parameter was decreased from 0.73 ± 0.49 to 0.29 ± 0.09 and there was a significant difference (p < 0.001). Those with MTHFR C677T mutant alleles; the mean value of the APRI parameter was increased from 0.87 ± 0.68 to 1.75 ± 4.80 and this change was not significant (p = 0.551). The ∆APRI difference between the two groups was significant (p = 0.022). There was no significant difference in ∆ values for MTHFR A1298C.

Conclusion: An improvement in fibrosis appears to be adversely affected in patients with the MTHFR C677T allele.
A study on the usefulness of serine palmitoyltransferase long chain subunit3 (SPTLC3) in NAFLD-related hepatocarcinogenesis

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Introduction: The proportion of hepatocellular carcinoma (HCC) patients with background of NAFLD is increasing. However, a useful biomarker finding out patients with HCC among NAFLD patients has not been established yet. We previously reported that the hepatic expression of serine palmitoyltransferase long chain subunit3 (SPTLC3) mRNA in NASH mouse models was potentially associated with NASH progression and carcinogenesis of the liver. The aim of this study was to elucidate the role of SPTLC3 in patients with NAFLD.

Methods: We analyzed 61 patients with biopsy-proven NAFLD including 19 with hepatocellular carcinoma and 6 healthy volunteers (HV), and investigated the association between serum SPTLC3 levels measured by ELISA test and the clinical features. Additionally, we compared serum SPTLC3 levels before and after treatments of 18 NAFLD patients with HCC receiving HCC treatments.

Results: In NAFLD patients with HCC age, HbA1c, hyaluronic acid, AFP, DCP were higher than in those without HCC. SPTLC3 levels were significantly higher in the HCC group than in the non-HCC group (HV: 0.6 vs. NAFLD group: 1.0 vs. HCC group: 1.6 [ng/ml], respectively; p < 0.01). Receiver operating characteristic analysis revealed that the SPTLC3 cut-off value of 1.42 discriminated between HCC and non-HCC (AUC-ROC, 0.745) better than AFP (0.665). SPTLC3 and DCP (0.777) had equivalent diagnostic capabilities. The diagnostic rate of HCC using SPTLC3 was 63.2%. The rate using 3 parameters: AFP, DCP, and SPTLC3 (if one or more of them exceed cut off value, we regard as “positive”) was 84.2%. SPTLC3 levels of patients which received HCC treatments decreased compared with levels of before treatments (before: 1.412, after: 1.094, p < 0.001).

Discussion/Conclusion: Serum SPTLC3 levels were high in the NAFLD patients with HCC. Further, SPTLC3 might be the novel biomarker finding out patients with HCC from great numbers of NAFLD patients in conjunction with existing biomarkers.
The relationship between serum albumin and plasma-branched chain amino acids (pBCAAs) in recent chronic liver disease: A single-center retrospective study

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Introduction: It is poorly understood how an imbalance of plasma-free amino acids (PFAAs) occurs and how the imbalance shows an association with the serum albumin (sAlb) level during the progression of chronic liver disease (CLDs). The aim of this study is to elucidate the profiles of PFAAs and the relationship between sAlb and PFAAs in recent patients with CLDs during the progression.

Methods: We retrospectively evaluated the 1569 data of PFAAs data obtained from 908 patients with various CLDs (CHC, CHB, alcoholic, NAFLD/NASH, PBC, AIH, PSC, and cryptogenic). In total, 1140 data of PFAAs could be analyzed in patients with CLDs dependent of their Child-Pugh (CP) score.

Results: Various imbalances in PFAAs were observed in each CLDs during the progression. Univariate and multivariate analysis revealed that among 24 PFAAs, the level of plasma-branched chain amino acids (pBCAAs) was significantly associated with the CP score, especially the sAlb score, in patients with chronic hepatitis C virus (CHC), NAFLD/NASH and PBC. The correlation coefficient values between sAlb and pBCAAs-to-Tyrosine ratio (BTR) in these patients were 0.53, 0.53 and 0.79, respectively. Interestingly, although the pBCAAs in NAFLD/NASH patients varied even when the sAlb was within the normal range, the pBCAAs tended to be low when the sAlb was below the normal range.

Discussion/Conclusion: Although a decrease in the level of pBCAAs was observed during the progression regardless of the CLD etiology, the level of total pBCAAs was independently associated with the sAlb level in the PFAAs of CHC, PBC and NAFLD/NASH. The correlation between sAlb and BTR showed the highest value in PBC patients among the patients with CLDs. A decrease in pBCAAs often occurred in NASH even when the sAlb level was kept in the normal range.
Chitinase 3-like 1 exacerbates liver fibrosis by enhancing intrahepatic accumulation and activation of macrophages in mice

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Introduction: Chitinase 3-like 1 (CHI3L1) is a chitinase-like protein that is related to 18-glycosylhydrodolase, but lacks enzyme activity. Serum level of CHI3L1 has been reportedly associated with liver fibrosis progression in several liver diseases and suggested to be a serum marker of liver fibrosis, but its role has not yet been clarified. We aimed to clarify the role of CHI3L1 in the pathophysiology of liver fibrosis using CHI3L1-deficient mice.

Methods: Liver fibrosis was induced in male wild-type and CHI3L1-deficient mice using corn oil or carbon tetrachloride (CCl4) for 4 weeks. These mice were also fed a control diet or a methionine- and choline-deficient diet for 12 weeks to generate another liver fibrosis model. To investigate the involvement of hepatic macrophages in the pathophysiology, liposomal clodronate was administered to these mice to deplete hepatic macrophages in the mouse models of liver fibrosis.

Results: Serum levels of CHI3L1 were significantly elevated in the liver fibrosis models. Hepatic CHI3L1 mRNA expression levels were also significantly increased compared to control mice; CHI3L1 in the liver was derived mainly from hepatic macrophages. Serum ALT levels increased in the mouse models of liver fibrosis; they were significantly lower in CHI3L1-deficient mice than in wild-type mice. Hepatic mRNA levels of TNFα, F4/80, and CD68 were increased in the mouse liver fibrosis models; they were significantly lower in CHI3L1-deficient mice than in wild-type mice. In the mouse models, liver fibrosis progression and hepatic stellate cell activation were significantly suppressed in CHI3L1-deficient mice compared to wild-type mice. When hepatic macrophages were depleted by liposomal clodronate treatment, no significant differences in the pathophysiology of liver fibrosis were noted between wild-type and CHI3L1-deficient mice.

Discussion/Conclusion: CHI3L1 exacerbated liver fibrosis progression by enhancing intrahepatic accumulation and activation of macrophages. Therefore, it may be a promising therapeutic target for liver fibrosis.
Oral administration of fructose exacerbates liver fibrosis and hepatocarcinogenesis via increased intestinal permeability in a rat steatohepatitis model

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Introduction: Recent reports have revealed the impact of a western diet containing large amounts of fructose on the pathogenesis of non-alcoholic steatohepatitis (NASH). Fructose exacerbates hepatic inflammation in NASH by inducing increasing intestinal permeability. However, it is not clear whether fructose contributes to the progression of liver fibrosis and hepatocarcinogenesis in NASH. The aim of this study was to investigate the effect of fructose intake on NASH in a rat model.

Methods: A choline-deficient/L-amino acid diet was fed to F344 rats to induce NASH. Fructose was administered to one group in the drinking water. The development of liver fibrosis and hepatocarcinogenesis were evaluated histologically.

Results: Oral fructose administration exacerbated liver fibrosis and increased the number of preneoplastic lesions positive for the placental form of glutathione S-transferase. Fructose-treated rats had significantly higher expression of hepatic genes related to toll-like receptor-signaling, suggesting that fructose consumption increased signaling in this pathway, leading to the progression of NASH. We confirmed that intestinal permeability was significantly higher in fructose-treated rats, as evidenced by a loss of intestinal tight junction proteins.

Discussion/Conclusion: Fructose exacerbated both liver fibrosis and hepatocarcinogenesis by increasing intestinal permeability. This observation strongly supports the role of endotoxin in the progression of NASH.
Is gut dysbiosis associated with the poor reactivity to urso-deoxycholic acid as well as the long-term prognosis in patients with primary biliary cholangitis?

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Introduction: Although some relationships between gut microbiota and digestive diseases have been reported, it is still uncertain whether similar results could be obtained in regard to the changes of gut microbiota because of their differences in race, food, and living environment. We have previously advocated the Nara criteria (Hepatol Res. 2017) in which the reactivity to ursodeoxycholic acid (UDCA) might predict the long-term prognosis of patients with primary biliary cholangitis (PBC), whereas little is known about a significance of gut microbiome in patients with PBC. The aim of this comparative study is to elucidate the relations between clinical profiles, biochemical reactivity to UDCA, and gut microbiota composition in Japanese PBC patients.

Methods: Fecal samples from 73 PBC patients treated with UDCA in our hospital were compared with those from 23 healthy individuals; patients whose UDCA intake was less than one year were excluded in this study. The gut microbiota community was analyzed using 16S ribosomal RNA gene sequencing.

Results: Compared with healthy individuals, bacterial diversity was lower in PBC patients, with a decrease in the order Clostridia and an increase in Lactobacillales. There was no association between gut dysbiosis and the clinical symptoms such as systemic itchiness, hepatobiliary enzyme disorder, and hepatic functional reserve. Compared with the PBC patients divided into the UDCA responder group according to the NARA criteria (reduction rate of gGTP > 69% at one year after), the UDCA non-responder group (reduction rate of gGTP < 69%) had significantly less population of a bacterial species belonged to the Firmicutes phylum (p < 0.05), though there were no significant differences of gender, BMI, PPI/probiotics user rate, and other serological data between these two groups.

Discussion/Conclusion: Gut dysbiosis is associated with the UDCA reactivity and might affect the long-term prognosis of patients with PBC.
Trans-fatty acid-rich diet promotes liver tumorigenesis in HCV core protein-expressing transgenic mice

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Introduction: Previous studies showed that consumption of trans-fatty acids (TFA), unsaturated fatty acids containing trans double bonds, is a risk factor for breast cancer, colorectal cancer, and non-alcoholic steatohepatitis while little attention has been paid to hepatocellular carcinoma (HCC). Hepatosteatosis and HCC developed in hepatitis C virus core protein transgenic (HCVcpTg) mice with aging, demonstrating a close link between HCV, altered intrahepatic lipid metabolism, and HCC. We examined the effect of dietary TFA on hepatic tumorigenesis in this transgenic mouse line.

Methods: Eight-to-ten week-old male HCVcpTg mice were treated with a control diet or isocaloric one that replaced soybean oil with shortening, abundant in TFA, for 20 months. The expression of tumorigenesis-related genes was assessed by quantitative RT-PCR or Western blot analysis.

Results: Liver tumor prevalence was significantly higher in HCVcpTg mice fed a TFA-rich diet compared with control diet-fed mice (100% vs. 39%, p < 0.001). Serum alanine aminotransferase levels were also significantly higher in TFA diet-fed mice compared with control diet-fed mice (33 ± 4 U/l vs. 13 ± 1 U/l, p < 0.01). The expression levels of genes related to cell proliferation, such as c-Myc and Pcna, were significantly elevated in TFA diet-fed HCVcpTg mouse livers. Additionally, the expression of Toll-like receptors 2 and 4 and its downstream pro-inflammatory genes, such as Tnf and Ccl2, was up-regulated in these mice, likely promoting tumor development.

Discussion/Conclusion: TFA-rich diet can aggravate HCV core protein-induced tumorigenesis in mice, presumably due to enhancing inflammatory signalling. Because of wide spread of direct-acting anti-viral agents, HCC development after HCV eradication and from non-viral non-alcoholic livers is becoming a serious issue. These results suggest the importance of dietary fat composition for HCC development in HCV-infected patients, and restricting TFA consumption might be beneficial to prevent HCC.
Upregulated expression of PD-1/PD-L1 on peripheral T and B cells correlates with the severity of alcoholic liver disease in females

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Introduction: Exposure to excessive alcohol consumption, its breakdown metabolites and gut-derived endotoxins dysregulates immune signaling. As a result the non-resolving inflammatory response and damage within the gastrointestinal tract and other organs may occur. The programed cell death 1 (PD-1) receptor and its ligand PD-L1 play a critical role in inhibition of self-reactive and inflammatory effector cells and the protection against immune-mediated tissue damage. We aimed to evaluate of the PD-1/PD-L1 expression on peripheral T and B lymphocytes, its correlation with markers of inflammation and the severity of liver dysfunction in the course of alcoholic liver disease (ALD).

Methods: Fifty six inpatients with ALD (38 males, 18 females, aged 49.23 ± 10.66) were prospectively enrolled and assigned to subgroups based on their: 1) gender, 2) severity of liver dysfunction (Child-Pugh, MELD scores, mDF), 3) presence of ALD complications, and followed for 30 days. Twenty five age- and gender-matched healthy volunteers, who consumed no more than 10 g alcohol per day, served as the control group. Flow cytometric analysis of the PD-1/PD-L1 expression on peripheral lymphocyte subsets were performed.

Results: The general expression of PD-1 and PDL-1 on T and B cells did not differ between the ALD and control group. Although, when the groups were analyzed based on their gender, significantly higher expression of PD1 and PD-L1 on CD19+ B cells in ALD females comparing to controls was observed. ALD females with severe alcoholic hepatitis (AH) and mDF > 32 or MELD > 20 showed significantly higher expression of PD-1 on CD19+ B cells and PD-L1 on all studied T and B subsets. The same pattern of the PD-1/PD-L1 expression was found when ALD females were compared with ALD males including the subgroups with mDF > 32 and MELD > 20. No correlations of PD-1+/PD-L1+ expression with mDF, CTP and MELD scores, nor with complications of ALD were observed, but significant correlations of CD19+ PD-L1+ frequencies with all conventional markers of inflammation (i.e. white blood cell and neutrophil counts, C-reactive protein and neutrophil-to-lymphocyte ratio) were found.
Discussion/Conclusion: Gender-related differences in the PD-1/PD-L1 expression on peripheral T and B cells may account for the different susceptibility to ethanol-related liver damage in males and females. Upregulation of PD-1/PD-L1 expression paralleled the severity of AH and liver dysfunction in females with ALD.
Novel biomarkers for predicting the occurrence of de novo non-alcoholic fatty liver disease in liver transplant recipients

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Introduction: Liver transplant (LT) recipients are at increased risk for developing metabolic syndrome, partly due to immunosuppressive therapy, but also to changes in lifestyle and diet which suddenly becomes less restrictive in the post-transplant setting.

Methods: We assessed 60 liver transplant recipients for clinical and biological features, performed abdominal ultrasound and transient elastography (TE) Fibroscan® with controlled attenuation parameter (CAP), calculated non-invasive scoring systems for advanced fibrosis and NAFLD (APRI, FIB-4, NAFLD score), for cardiovascular risk (Framingham risk score) and for the presence of metabolic syndrome. 5 new biomarkers were performed in all patients: Beta 7 integrin, CXCL-10, CXCL-12, Hepatocyte growth factor (HGF), and carbonic anhydrase IX (CA IX).

Results: The median age was 56.5 years and the median time from transplantation 35 months. The univariate analysis showed significant association between both beta 7 integrin and CA IX and liver fibrosis assessed with a cut-off value of advanced fibrosis of 8.7 kPa. The Spearman correlation coefficient of beta 7 integrin and the liver stiffness measurement values showed moderate correlation (r = 0.31), but significant association (p = 0.01). The carbonic anhydrase IX showed a better correlation when compared to the liver stiffness with a correlation coefficient of 0.43, p = 0.0007 and a moderate correlation when compared to both FIB-4 (r = 0.27) and APRI (r = 0.27) score but with significant p values, 0.04, respectively 0.03.

Discussion/Conclusion: The burden of NAFLD and metabolic syndrome in liver transplant recipients is very high, impacting on lifestyle and severely affecting morbidity and mortality in these patients. We consider very important for our patients the development of new non-invasive biomarkers for early diagnosis of NAFLD and NASH, as the “gold-standard” of liver biopsy is not easily accepted in clinical practice. Also NAFLD and NASH are dynamic processes that need prospective and repeated assessments, a need that cannot be met by the classical liver biopsy.
Real-life improvement of HCV-GT1 decompensated liver cirrhosis following therapy with sofosbuvir/ledipasvir

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Background: Direct-acting antiviral (DAA) regimens have shown high efficacy and tolerability for patients with HCV genotype 1/1b (GT1/1b) in clinical trials. However, real-world evidence of sofosbuvir/ledipasvir (SOF/LDV) treatment for HCV GT1-related cirrhosis patients in Romania is still lacking.

Aim: To analyze the effectiveness of SOF/LDV ± ribavirin therapy for HCV GT1 cirrhosis (actual Child B/C cirrhosis or with a previous decompensation).

Methods: We included 193 patients with HCV GT1 cirrhosis in routine clinical practice in Romania and reported SVR after 12/24 weeks end of treatment.

Results: In our cohort were 40.1% males, with a median age of 59 years, a median MELD score of 12.5 and a median viral load of 257080.5 IU/ml; at treatment initiation, 12.8% had Child A class cirrhosis, 81.4% Child B and 5.8% Child C. EOT is available in 138 patients and 137 patients (99.2%) achieved virological response; SVR is available in 68 patients and 89.7% achieved virological response. There was a significantly lower SVR rate in patients that received SOF/LDV without ribavirin (p = 0.0009). At EOT, patients with Child B/C cirrhosis had a significant decrease of MELD score (12.9 ± 2.8 vs. 11.5 ± 3.6, p = 0.0009), of AST, ALT and GGT (p < 0.0001 for each), a significant increase of sodium (p = 0.02) and cholesterol (p < 0.0001). Ascites improved significantly (42.4% of patients had disappearance of ascites at EOT, p < 0.0001), as well as episodes of hepatic encephalopathy (HE) did not occur in a significant proportion (82.8% of patients with previous HE had no recurrent episodes, p = 0.001). There was no statistically significant improvement of serum albumin, total bilirubin or platelets.

Conclusion: Treatment with ledipasvir and sofosbuvir proved to be effective and improved liver function and portal hypertension in patients with GT1 HCV infection and cirrhosis in Romanian clinical practice. Patients with decompensated cirrhosis have a clear benefit from addition of ribavirin in our cohort.
Effects of a DPP4 inhibitor on progression of NASH-related HCC with alterations in p62/Keap1/Nrf2 pathway in a mouse model

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Introduction: Diabetes mellitus is a risk factor for hepatocellular carcinoma (HCC) in patients with non-alcoholic steatohepatitis (NASH). Dipeptidyl peptidase-4 inhibitor (DPP4i), an anti-diabetic agent, is reported to affect cell proliferation. We aimed to investigate the effects of DPP4i on the progression of NASH-related HCC and its metabolic pathway in a mouse model.

Methods: A mouse model of NASH-related HCC was used in this study. Eight-week-old mice were administered with either DPP4i (sitagliptin 30 mg/kg/day: DPP4i group; n = 8) or distilled water (control group; n = 8) for 10 weeks. Then, HCC progression was evaluated by computed tomography. Changes in metabolites of HCC tissue were analyzed by metabolomic analysis. The localization and expression of p62, Keap1, and Nrf2 were evaluated by immunostaining and immunoblotting, respectively.

Results: The number and volume of HCC were significantly lower in the DPP4i group than those in the control group (1.8 ± 1.2 vs. 4.5 ± 1.7/liver, p < 0.01, 11.2 ± 20.8 vs. 37.5 ± 72.5 mm³/tumor, p < 0.05). Metabolome analysis revealed that DPP4i significantly increased 6-phosphogluconic acid and ribose-5-phosphate levels and decreased AMP-to-adenine and GMP-to-guanine ratios (AMP-to-adenine 0.7 ± 0.2 vs. 2.0 ± 1.2; p < 0.01, GMP-to-guanine 0.6 ± 0.3 vs. 1.5 ± 0.7; p < 0.01). Immunostaining showed that p62 was localized in the cytoplasm of tumor cell in the DPP4i group, while p62 was localized in the nucleus of tumor cell in the control group. Keap1 and Nrf2 expressions decreased significantly in the DPP4i group compared to those in the control group.

Discussion/Conclusion: We demonstrated that a DPP4i prevented the progression of NASH-related HCC in a mouse model. Furthermore, metabolome analysis revealed that DPP4i downregulated the pentose-phosphate pathway with the suppression of p62/Keap1/Nrf2 pathway. Thus, DPP4i may prevent tumor progression through the inhibition of metabolic reprogramming in NASH-related HCC.
Differential diagnosis of unclear cases of primary sclerosing cholangitis: Contribution of next-generation video-digital cholangioscopy

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Introduction: Differential diagnosis of strictures of the biliary tract related to primary sclerosing cholangitis (PSC) is one of the most difficult problems of pancreatobiliary endoscopy/surgery.

Aim: to assess the possibility of using new single-operated cholangioscopy (SOC) in the diagnosis of biliary strictures associated with PSC.

Methods: From December 1, 2017 to May 1, 2018 in the University Hospital No. 31 we performed 246 transpapillary retrograde endoscopic interventions, including 15 (6.1%) SOCs utilizing next-generation video-digital cholangioscope SpyGlass DS (Boston Scientific, USA). The indications for therapeutic ERCP+SOC were post-operative strictures of biliary tree (2) and difficult choledocholithiasis (1); while for diagnostic ERCP+SOC - the stricture of the main pancreatic duct in a patient with chronic pancreatitis (1) and indeterminate biliary strictures in most (11) cases. In 5/11 (45.5%) patients (3 women, 2 men; age varied from 36 to 49 years, mean 43.2 ± 7.3 years) clinical picture and preoperative findings were suspicious for PSC.

Results: Observational SOC was successful in all cases, as well as intraductal mucosal biopsy under direct cholangioscopic vision via the instrumental channel of cholangioscope. The material was taken from 2–3 loci, the number of biopsy specimens ranged from 2 to 5. In 3 cases the preliminary diagnosis of PSC was confirmed. The rest 2 patients had cancer strictures, which was revealed during endoscopic visualization and was confirmed by histological study. There were no complications and deaths in the group of patients with suspected PSC, who underwent peroral endoscopic interventions.

Discussion/Conclusion: The complex diagnostic program of PSC, especially associated with biliary strictures, in our opinion, should include endoscopic peroral cholangioscopy with a visual evaluation of the stricture and mucosal biopsy for morphological examination. SOC utilizing next-generation video-digital cholangioscope significantly improves and facilitates the process of cholangioscopy, making possible difficult differential diagnosis of biliary strictures related to PSC.
Western diet during conception and lactation development of murine non-alcoholic steatohepatitis

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Introduction: Pediatric NASH has some histological features distinct from its adult. However, a murine dietary model of early onset NASH has not been well established. In this study, we investigate the impact of a western diet rich in fat, fructose and cholesterol (FFC) on liver injury in mice before the age of maturity.

Methods: Mouse breeding pairs were employed; the first set of breeding pairs was fed the FFC diets during the conception and lactation periods, their offspring were weaned to the FFC diet. The second set of breeding pair was maintained on chow diet throughout the conception and lactation, and their offspring were maintained on the FFC diet after the weaning age. The third set of breeding pair was maintained on chow diet throughout the conception and lactation, their offspring were weaned to chow diet. Mice were sacrificed at 10 weeks of age. Liver injury, inflammation, and fibrosis were evaluated histologically and biochemically.

Results: FFC born FFC-fed mice have significantly increased body weight when compared to chow-fed mice. The hepatic inflammatory infiltrates and steatosis was increased in FFC born FFC-fed mice by HE and liver triglyceride level. Immunohistochemistry for Mac-2 (macrophage marker), showed increased stained in the FFC born FFC-fed mice. Likewise, the mRNA expression of macrophage markers (F4/80, CCR2 and TNF-\textgreek{a}) were all significantly increased in FFC born FFC-fed. Liver injury, by serum ALT and TUNEL positive hepatocytes was significantly increased in FFC born FFC-fed. Furthermore, liver fibrosis was increased in the FFC born FFC-fed, by the Sirius red stain and mRNA expression of collagen 1a1 and \textgreek{a} smooth muscle actin.

Discussion/Conclusion: Early exposure to nutrient excess diet induces liver injury and subsequent development of early onset liver fibrosis.
Lipoprotein lipase in hepatic stellate cells exaggerates liver fibrosis in non-alcoholic steatohepatitis in mice

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Introduction: Lipoprotein lipase (LPL) is the key enzyme for lipid uptake, which plays a role in lipoprotein metabolism and binding of lipoproteins to lipoprotein receptors, and is involved in the pathophysiological mechanisms of many diseases such as diabetes and atherosclerotic diseases. However, the role of hepatic LPL in the pathophysiology of non-alcoholic steatohepatitis (NASH) has yet to be elucidated. In the present study, we aimed to clarify how LPL in hepatic stellate cells (HSCs) mediates the progression of NASH, using HSC-specific LPL-knockout (LplHSC-KO) mice.

Methods: Male control (Lplfl/fl) and LplHSC-KO mice were fed a control or a high fat/high cholesterol diet for 24 weeks to induce NASH.

Results: Mice fed a high fat/high cholesterol diet for 24 weeks developed NASH with liver fibrosis; liver fibrosis progression was significantly suppressed in LplHSC-KO mice, compared to Lplfl/fl mice. In the murine model of NASH, HSC activation was also significantly suppressed in LplHSC-KO mice, compared to Lplfl/fl mice. In conformity with these results, hepatic mRNA levels of collagen1α1, collagen1α2, and αSMA were significantly lower in LplHSC-KO mice, compared to Lplfl/fl mice. In the murine model of NASH, serum levels of ALT and hepatic TG levels were significantly increased, compared to a control group fed a normal diet; no significant differences were observed between Lplfl/fl and LplHSC-KO mice. Intrahepatic recruitment and activation of macrophages were also significantly enhanced in the murine model of NASH, compared to the control group; there were no significant differences between Lplfl/fl and LplHSC-KO mice.

Discussion/Conclusion: LPL in HSCs exaggerated liver fibrosis in NASH through activation of HSCs. Therefore, the results of this study provide a promising target for the treatment of NASH.
Porphyran, a functional ingredient of Japanese “Nori”, improves non-alcoholic fatty liver via modified ceramide synthesis pathways

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Introduction: Porphyran (PP) is a major component of “Nori”, the typical Japanese food made from red algae. Previous studies have been shown that the PP protected from fat accumulation and progression of insulin resistance. To investigate the detailed mechanism of these effects of PP, we focused on bile acids signaling pathway and ceramide synthesis.

Methods: C57BL/6J mice were fed on NASH induced diet mixed with 2% w/w PP for 18 weeks. Liver, ileum and colon were collected. Total RNA was extracted from each tissues and the qPCR was performed. To clarify the effects of PP on NASH, liver histology was assessed by staining with PicrorosiusRed (for fibrosis) and Masson trichrome staining (for fibrosis) or by staining cryosections with Oil Red O (for steatosis).

Results: After 18 weeks, plasma ALT was significantly increased in NASH induced diet model. In the PP group, plasma ALT, total cholesterol and TG was significantly reduced. Oil red O staining and Masson trichrome staining revealed that PP group decreased steatosis and fibrosis in liver. Analysis of qPCR suggested that the signal pathway of ceramide synthesis was inhibited in intestine and C16-ceramide contents were lower in PP group by the LC/MS/MS methods. Increase of T-β-MCA, acts as an intestinal FXR antagonist may cause the inhibition of the ceramide synthesis related genes expression.

Discussion/Conclusion: In summary, we have shown that PP changed the ceramide synthesis pathway through changing BA composition and improved intestine environment. Effects of PP on obesity, fatty liver and insulin resistance partially be explained by bile acids and intestine interaction pathway.
Increased frequency of myeloid-derived suppressor cells in patients with non-alcoholic fatty liver disease

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Introduction: Myeloid-derived suppressor cells (MDSCs) are recognized as suppressors of T-cell functions. We recently reported an increased number of MDSCs in the liver of a murine model of non-alcoholic liver disease (NAFLD). In this study, we aimed to evaluate human MDSCs in patients with NAFLD.

Methods: Sixty patients with histologically proven NAFLD (15 NAFL and 45 NASH) were enrolled in this study. PBMCs were obtained at the time of liver biopsy. CD14(+)HLA-DR(−/low)S100A9(hi) cells were defined as MDSCs. The frequency of MDSCs in PBMC was evaluated using flowcytometry.

Results: The frequency of MDSCs significantly increased in the patients with NASH in comparison with those with NAFL (p = 0.021). In addition, the frequency of MDSCs increased with fibrosis progression, and the number in the patients with severe fibrosis (stage 3/4) was significantly higher in those with milder fibrosis (stage 0–2; 1.63% ± 0.91% vs. 1.02% ± 0.91%; p = 0.039). Furthermore, the frequency of MDSCs was significantly increased in the patients with higher activity (grade 2/3) than in those with milder activity (grade 0/1; 1.82% ± 1.31% vs. 1.01% ± 0.96%; p = 0.009). The frequency in the patients with higher NAS scores (5–8) was significantly higher than in those with lower NAS scores (0–4) (1.91% ± 1.25% vs. 0.73% ± 0.69%; p = 0.001).

Discussion/Conclusion: The frequency of MDSCs was increased in the patients with NASH associated with disease progression. Their numbers were associated with the degree of inflammation. These cells might have a role in the progression of NAFLD.
The novel cutoff points for the FIB4 index categorized by age increase the diagnostic accuracy in NAFLD: A multicenter study

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Introduction: The FIB4 index is clinically useful, but because its formula includes age, the appropriate cutoff point may differ by age group. Here, new FIB4 index cutoff points were validated using cohort data from 14 hepatology centers in Japan.

Methods: The FIB4 index was determined in biopsy-confirmed NAFLD patients (n = 1050) who were divided into four groups: ≤ 49, 50–59, 60–69, and ≥ 70 years. ROC analysis predicted advanced fibrosis in each age group; low and high cutoff points were defined by a sensitivity and specificity of 90%. The new and conventional cutoffs were compared for detecting advanced fibrosis.

Results: The modified low and high cutoff points were 1.05 and 1.21 in ≤ 49 years, 1.24 and 1.96 in 50–59 years, 1.88 and 3.24 in 60–69 years, and 1.95 and 4.56 in ≥ 70 years. In ≥ 60 years, the false-negative rate was increased using the modified high cutoff point, and the high cutoff point was better with the conventional cutoff point. The new proposed low and high cutoff points are 1.05 and 1.21 in ≤ 49 years, 1.24 and 1.96 in 50–59 years, 1.88 and 2.67 in 60–69 years, and 1.95 and 2.67 in ≥ 70 years; these cutoff points improved the accuracy of advanced fibrosis diagnosis.

Discussion/Conclusion: FIB4 index cutoff points for predicting advanced fibrosis in NAFLD increased with age. Cutoff points modified by age improved the diagnostic accuracy of estimations of advanced liver fibrosis using the FIB4 index.
STAT3 – A novel PDC-E2 interacting partner in human cholangiocytes

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Introduction: E2 component of mitochondrial pyruvate dehydrogenase complex (PDC) is the key autoantigen in PBC. Signal transducer and activator of transcription factor 3 (STAT3) plays a critical role in cellular response to a variety of cytokines and growth factors by regulating specific nuclear genes. Whereas STAT3 typically localizes in cytosol and nucleus, PDC-E2 normally resides within the mitochondrial matrix where it converts pyruvate to acetyl-CoA. Recently it has been shown that small pool of STAT3 (10–15%) is presence in mitochondria and can modulate the activity of the electron transport chain.

Methods: Normal human cholangiocytes (NHC) were treated with 100 µM of bile salt glycochenodeoxycholate (GCDC) to induce cholestasis and then cellular fractionation was performed. To determine whether STAT3 interacted with PDC-E2 co-immunoprecipitation (IP) and proximity ligation assay (PLA) were used. To check the level of PDC-E2 and STAT3 in control tissues (n = 13) and cirrhotic tissues derived from patients with PBC (n = 15) Western blot analysis was employed.

Results: Subcellular fractionation analysis revealed that a substantial amount of PDC-E2 and P-STAT3 were present in the nucleus of cholangiocytes. Moreover GCDC acid significantly elevated level of PDC-E2 (4.2-fold, p < 0.01) and P-STAT3 (3.3-fold, p < 0.03) in cytoplasm while did not change the level of both proteins in nucleus. Furthermore we identified STAT3 as novel PDC-E2 interacting partner in human cholangiocytes. This interaction took place mostly in cytoplasm under physiological conditions. Finally, in PBC tissues, PDC-E2 was shown as single band, which associated with the presence of P-STAT3 in contrast to control once, in which PDC-E2 showed up as double-band, which associated with the absence of P-STAT3.

Discussion/Conclusion: Altogether, our results suggest that PDC-E2 during direct interaction with STAT3 can be modified and subsequently exhibits other physiological functions in human cholangiocytes. This finding may help us to explain the precise nature of PDC-E2 as autoantigen, which still remains unclear.

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Clinical features and stability of hepatitis B surface antigen seroclearance in untreated and nucleos(t)ide analogue treated patients

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Introduction: The durability of nucleos(t)ide analogue (NA)-induced hepatitis B surface antigen (HBsAg) seroclearance is uncertain compared with HBsAg seroclearance of non-treated patients. We investigated to reaffirm that the quality of HBsAg seroclearance in NA is as good as that occurring spontaneously. Also, the occurrence of hepatocellular carcinoma (HCC) was investigated.

Methods: A cohort study was conducted using data from Gangnam Severance Hospital. We identified subjects with positive HBsAg between January 2001 and March 2018. NA use, liver biochemistries, serial HBsAg and anti-HBs results were retrieved. The primary endpoint was confirmed HBsAg seroclearance, defined least two negative HBsAg results, with the last HBsAg test being negative in patients with chronic hepatitis B (CHB). The secondary endpoint was to evaluate the incidence of HCC after HBsAg seroclearance in untreated and NA-treated patients.

Results: 145 CHB patients with HBsAg seroclearance were included. In patients with spontaneous HBsAg seroclearance (n = 132), 105 patients (79.5%) had confirmed HBsAg seroclearance and 2 patients (1.5%) had HBsAg seroreversion. In patients with NA-induced HBsAg seroclearance (n = 13), 10 patients (76.9%) had confirmed HBsAg seroclearance and HBsAg seroreversion was not observed. 1 patient (7.7%) received consolidation therapy for < 6 months, 1 patient (7.7%) received it for 6–12 months and 11 patients (84.6%) received it for ≥ 12 months. The incidence rate of HCC with HBsAg seroclearance was 6.21%. At a median follow-up of 12 years, 5 untreated patients developed HCC. 4 NA-treated patients developed HCC. 6 patients were male aged > 50 years (NA-treated:2, untreated:4), and 3 patients were male aged 50 years (NA-treated: 2 [age 47,39], untreated: 1 [age 37]). There was no female patient who developed HCC with HBsAg seroclearance. Similar findings were observed in patients with spontaneous and antiviral treatment-induced HBsAg seroclearance.

Discussion/Conclusion: NA-induced HBsAg seroclearance is as durable as spontaneous HBsAg seroclearance. All male patients are still at risk of HCC even if the incidence of HCC is very low.
The occurrence rate of clinical events in Japanese PSC cohort – A potential role as surrogate endpoints for clinical trials

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Backgrounds and aims: Patients with primary biliary cholangitis (PBC) frequently suffer from pruritus, which can severely impair their health-related quality of life (HRQOL). Nalfurafine hydrochloride, a selective κ-opioid receptor agonist, was recently approved in Japan for refractory pruritus in patients with chronic liver diseases, but it still remains unclear whether this treatment improves the patient-reported outcome (PRO) in PBC patients with refractory pruritus. Herein, we conducted a multi-center, post-marketing, single-arm prospective study to investigate the efficacy of nalfurafine in terms of PRO, and the associations of the efficacy with any clinical characteristics.

Patients and methods: After screening for pruritus in 496 patients with PBC using PBC-40 and the visual analogue scale (VAS), we identified 141 patients with moderate to severe pruritus; these were invited to participate in the study. The participants received 2.5 µg nalfurafine once daily for 12 weeks, and pruritus and HRQOL were assessed in week 12 of this treatment. Generic HRQOL, short form-36, blood chemistries, and serum autotaxin levels were also measured at baseline and at week 12.

Results: Forty-four patients participated in this study. The mean PBC-40 itch domain scores and VAS declined during the study period, from 8.56 to 7.63 (p = 0.041) and from 42.9 to 29.3 (p = 0.001) at baseline and at week 12, respectively, indicating a significant effect of nalfurafine. The other domains of PBC-40 and all domains of SF-36 were not significantly altered by this treatment. We failed to find any association between the change in VAS and PBC-40 itch scores and any clinical variable. Serum autotaxin levels were also significantly increased during the study period.

Conclusion: This study demonstrated that nalfurafine improved pruritus in patients with PBC, independent of their clinical characteristics, but had a limited effect on the PRO.
Elevated hepatic iron overload obtained by magnetic resonance imaging reflects hepatic inflammation and ballooning in patients with non-alcoholic fatty liver disease

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Introduction: Several studies have shown that hepatic iron accumulation, which causes oxidative stress and reactive oxygen species (ROS), might be responsible for the pathological conditions of chronic liver diseases such as chronic hepatitis C. However, whether hepatic iron accumulation contributes to the disease progression in non-alcoholic fatty liver disease (NAFLD) remains controversial. The aim of this study was to clarify the role of hepatic iron overload in pathogenesis of NAFLD using magnetic resonance imaging (MRI).

Methods: We performed a cross-sectional study of 323 patients with NAFLD (identified by liver biopsy; mean body mass index, 28.3 kg/m²). Hepatic iron accumulation was measured using a modified Dixon method with advanced processing for MRI (gradient-echo-based T2* technique, R2* value, IDEAL IQ, GE Healthcare). To confirm whether R2* value has positive correlation with hepatic iron concentration, atomic absorption analysis was performed using liver tissue obtained by biopsy in 122 patients with NAFLD.

Results: R2* value was correlated moderately with the hepatic iron concentration (r = 0.62) and serum ferritin levels (r = 0.53). R2* value was positively correlated with the grade of hepatic inflammation and ballooning, but not the grade of steatosis and the stage of fibrosis. However, R2* value was significantly decreased in patients with fibrosis stage 4. In contrast, the serum ferritin levels were positively correlated with serum ALT levels, the grade of steatosis and hepatic ballooning. In addition, R2* value reflected high mRNA levels of hepatic hepcidine which is iron regulatory hormone.

Discussion/Conclusion: While high levels of R2* value (hepatic iron overload) were associated with hepatic inflammation and ballooning and disordered iron regulatory hormone, high levels of serum ferritin were associated with steatosis and hepatic ballooning. These findings highlighted the role of hepatic iron overload evaluated by MRI in the pathogenesis of NAFLD, especially hepatic inflammation, suggesting that therapies aimed at correcting iron metabolism may be beneficial.
Exercise training mediates lipid infiltration of skeletal muscle and improves non-alcoholic fatty liver disease

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Introduction: Physical exercise has beneficial effects on metabolic homeostasis. Exercise training increases the skeletal muscle mass, decreases lipid infiltration in skeletal muscle and contributes to improve insulin resistance and non-alcoholic fatty liver disease, NAFLD. We aimed to analyze the actual impact of changes of the skeletal muscle after exercise training on NAFLD.

Methods: Longitudinal study was performed in 60 patients (30 males) who were diagnosed as fatty liver disease by abdominal ultrasound at Saga University Hospital and affiliate facilities. Patients with a drinking history (≥20 g/day of alcohol), viral hepatitis, autoimmune liver diseases or malignant disease were excluded. Patients performed exercise as 27 metabolic equivalent tasks (METs)/week for 6 months. Food intake was adjusted as 25 kcal/kg bodyweight. We evaluated the abdominal lumbar muscle area by CT imaging and obtained skeletal muscle area index (SAI; lumbar muscle area [cm²]/height [cm²]). Lipid infiltration of the skeletal muscle (intramuscular adipose tissue content, IMAC) and of liver (liver-spleen ratio, L/S ratio) was also measured by CT imaging-based technique. Factors which contributed to improve L/S ratio and ALT were identified by multiple regression model.

Results: Exercise training decreased IMAC in 46 patients (76.7%) with decreased BMI, decreased fasting plasma glucose, decreased ALT and improved L/S ratio (p < 0.001, respectively) while 14 patients without decrease of IMAC failed to improve these parameters. Exercise training increased SAI in 16 patients with decreased BMI, decreased ALT and improved L/S ratio; however, interestingly, 46 non-responders of SAI also showed significant improvement of these parameters. Decrease of IMAC (t value: 3.92, p < 0.001) and increase of SAI (t value: 2.52, p = 0.014) significantly correlated to improvement of ALT level. Decrease of IMAC was independent factor to improve L/S ratio (t value: 4.83, p < 0.001) while there was no significant correlation between increase of SAI and improvement of L/S ratio.

Discussion/Conclusion: Although increased skeletal muscle mass was not a factor to improve liver steatosis in NAFLD, decreased lipid infiltration in skeletal muscle contributes to improve liver steatosis as well as ALT level in our exercise training program for NAFLD. Lipid infiltration of skeletal muscle could be an indicator of the exercise training effect on NAFLD and possible therapeutic target of NAFLD.
Technetium-99m-GSA scintigraphy obtained within three days of admission as an early predictor of outcome in acute liver failure and severe acute hepatitis

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Introduction: Acute liver failure (ALF) is associated with a high mortality, which can be substantially reduced with liver transplantation. Therefore, prediction of ALF prognosis is required for determining the indication for liver transplantation. The aim of this study was to determine whether technetium-99m-diethylenetriaminepentaacetic acid galactosyl human serum albumin (99mTc-GSA) scintigraphy performed within three days of admission could predict the prognosis of ALF or severe acute hepatitis with an INR of > 1.5.

Methods: This was a prospective observational study. From January 2011 to October 2016, a total of 64 patients were enrolled in the present study. For the procedure, 185 MBq of 99mTc-GSA was injected into a cephalic vein, then images were obtained in 15-s frames for 20 min with a dual-head gamma camera. Time-activity curves were generated from regions of interest (ROI) for the whole liver and the heart. The hepatic accumulation index was calculated by dividing the radioactivity of the liver ROI by that of the liver-plus-heart ROI at 15 min (i.e., LHL15).

Results: Sixteen (25.0%) patients died or underwent liver transplantation (poor outcome), and LHL15 was significantly lower in these patients (0.673 ± 0.057) than in those who survived (0.842 ± 0.061) (p < 0.0001). The optimal cut-off point of LHL15 for distinguishing poor outcome and survival was 0.737, with a sensitivity of 93.8%, specificity of 93.8%, and AUC of 0.971 (95% confidence interval [CI], 0.908–0.991). Bilirubin, INR, HGF, MELD-score, presence of hepatic coma at the time of admission, and LHL15 were adopted as confounders in the logistic regression model for the multivariate analysis. The analysis revealed that only LHL15 was an independent predictor of poor outcome (95% CI: 15.6–78.7, p = 0.0112).

Discussion/Conclusion: 99mTc-GSA scintigraphy might be clinically useful for predicting the prognosis of patients with ALF or severe acute hepatitis with INR of > 1.5.
Immune response to tumor-associated antigens and immune cell profiles in NASH-related hepatocellular carcinoma

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Introduction: Host antitumor immune responses may be different between hepatocellular carcinoma (HCC) caused by metabolic disorders and HCC associated with viral hepatitis infection.

Methods: Peripheral blood mononuclear cells (PBMCs), collected from 92 HLA-A24 positive HCC patients [Hepatitis B virus (HBV), 32; Hepatitis C virus (HCV), 42; NASH, 17], were evaluated for immune responses to HLA-A24-restricted cytotoxic T-lymphocyte epitopes from 16 tumor-associated antigens (TAAs) by IFN-γ ELISpot assay. Among 92 patients, peripheral immune cell profiles of 51 PBMC samples were analyzed using multi-color fluorescence-activated cell sorting. The frequencies of TAA-specific T cells, the expression levels of surface markers on each immune cell, and the correlation between the number of peptides with positive response and the frequencies of immune cells were evaluated.

Results: The immune response to TAA was markedly different among the 3 groups. The frequency of positive immune response to TAA in the NASH-related HCC group was lowest among the 3 groups. The profiles of the peripheral blood immune cells investigated were markedly different among the 3 groups. In particular, the frequencies of effector regulatory T cells (eTregs) and CD8+ T cells, which strongly express cytotoxic T-lymphocyte antigen (CTLA)-4, were high in NASH-related HCC patients. The frequencies of C-X-C motif chemokine receptor (CXCR)3+ eTregs, CTLA-4+ CD8+ T cells, CXCR3+ CD8+ T cells, and OX40+ CD8+ T cells were inversely correlated with the strength of the TAA-specific T cell immune response. The anti-CTLA-4 antibody could restore TAA-specific T cell responses. In addition, the frequencies of CTLA4+CD8+T cell and OX40+CD8 T cell decreased by adding palmitic acid in vitro.

Discussion/Conclusion: The immune response to TAA and immune cell profiles were markedly different in NASH-related HCC patients compared with those in virus-related HCC patients, and fatty acid may be one of the factors affecting the immune response of NASH-related HCC patients.
Next-generation sequencing in whole-genome characteristics of hepatitis b virus in Korean

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Introduction: Until recently, genetic testing of the hepatitis B virus is currently limited to a small fraction of the entire viral sequence. In this study, we aimed to find all variants of HBV by analyzing the whole genome sequence of it.

Methods: Twenty-three newly diagnosed, untreated HBV infected patients participated in this study. DNA was extracted from 200 μl of serum samples of enrolled patients. The amplified products were obtained by PCR. The PCR products were sheared into 300- to 1000-bp fragments using the kit, and then analyzed on a MiSeq sequencer (Illumina, USA) for paired-end 150-bp sequencing. After a quality check and data trimming, BWA-MEM v0.6 was applied to map the sequences against the reference HBV genome (DQ683578.1). The sequences whose quality was estimated to be greater than QD30 (quality score normalized by depth 30) were selected. GATK v3.7 Mutect 2 was used to call SNPs and indels in the sequences.

Results: We sequenced and analyzed the whole genome of HBV including DNA polymerase, preS1/preS2/S, protein X, and precore/core region. The average depth of coverage spanning 3215 nucleotides was 1359 (min–max; 389–2484). Total 1546 variants (1516 snp and 30 indels) were detected in 23 samples. Among them, 605 variants in polymerase, 360 variants in preS1/preS2/S, 94 variants in X, 165 variants in precore/core region were non-synonymous variants, which make changes in amino acid and protein sequence. The most variable region was g.1950 encoding the core protein, which showed 4 types of variants.

Discussion/Conclusion: In this study, we could read WGS of HBV using NGS technology, and found various variants. It is expected that the next-generation sequencing technology will provide important clues to the identification of the drug resistance mechanism of HBV because it can read the entire nucleotide sequence of the HBV.
Multidisciplinary team approach to hepatocellular carcinoma management: Three year-study in a liver transplant center from Romania

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Introduction: Optimal care of the patient with hepatocellular carcinoma (HCC) requires the involvement of multiple specialists. The aim of our study is to evaluate the outcome following the decision taken in a Multidisciplinary Liver Tumour Board for the treatment of HCC.

Methods: In this prospective study from January 2015 to October 2017 we included 255 treatment-naive patients, diagnosed with HCC. All patients were discussed in the Tumour Board and the therapeutic decisions were: resection 11 patients (4.3%), liver transplantation: 48 patients (18.8%); intraoperative radiofrequency ablation (RFA): 14 patients (5.4%), percutaneous RFA: 10 patients (3.9%), transarterial chemoembolization (TACE): 90 patients (39.2%), Sorafenib treatment: 45 patients (17.6%) and best supportive care (BSC): 50 patients (19.6%).

Results: All patients (M = 160, F = 95) with an average age of 62 years (± 8.1) that were included had cirrhosis (HCV-related in 55.9%). Short-term mortality rate (3 months) was 17.6% (45 patients), 77.8% of those patients being in the BSC group and the rest (10 patients) died on the transplant list due to complications of cirrhosis. The success rate was nearly equal in RFA (79.3%) and surgery (81.9%), whereas the success rate was 63.9% in TACE. In 62 patients (68%) a classic lipiodol TACE was performed, while 31% of patients had a drug eluting microsphere procedure. 34 patients had complete tumor response after one TACE. There was a significant transient decrease in the mean platelet level after TACE (p = 0.006). Liver transplantation was performed in 20 patients (7.8%). The median follow-up period was 18 months. Overall patient survival at 1 year for surgery group, TACE and BSC was 96.1, 95.7% and 6% respectively.

Discussion/Conclusion: Therapeutic interventions should be decided on a case by case analysis. Surgery has comparable outcome to RFA but is more invasive. TACE was the most frequent procedure performed on HCC patients in our center, and is was shown to be a safe and effective therapy.
Exploring viral factors associated with HCC occurrence/recurrence after antiviral therapy through HCV full-open reading frame analysis

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Introduction: Due to the recent advances in antiviral therapy, sustained viral response (SVR) became possible in most of CH-C patients. However, since the occurrence of hepatocellular carcinoma is observed at a certain frequency, it is an urgent to elucidate HCC risks in those patients.

Methods:
(1) In 129 genotype-1b HCV patients receiving peginterferon (PEG-IFN) plus ribavirin (RBV) therapy, viral region associated with post-treatment HCC occurrence was examined by direct sequencing of an HCV whole open reading frame (ORF) obtained prior to the therapy.
(2) In 277 interferon-free SVR HCV-1b patients, the association between HCC occurrence (5/277, 1.8%)/recurrence (9%) after SVR and the pretreatment HCV core 70/NS5A sequences were investigated.

Results:
(1) SVR was achieved in 51% (66/129), and 14 patients developed HCC (14/129, 11%) after the therapy overall while three patients developed HCC after achieving SVR (3/66, 4.5%). In the HCV-ORF analysis, the strongest association was noted in core 70 amino acids (p = 4.0 E-5). In subclass analysis, core 70 amino acid was associated with HCC occurrence in SVR patients (p = 0.007) as well as non-SVR patients (p = 0.04).
(2) Core 70Q was observed in patients with HCC occurrence (2/4), HCC recurrence (13/22) and no-HCC (94/229) (HCC occurrence/recurrence vs. no HCC, p = 0.12). Likewise, NS5A-Y93H was observed in patients with HCC occurrence (0/5), HCC recurrence (0/25) and no-HCC (38/242) (HCC occurrence/recurrence vs. no HCC, p = 0.016). In addition, mutations in NS5A-ISDR also had a tendency to associate with HCC occurrence/recurrence.

Discussion/Conclusion: In PEG-IRN/RBV therapy, core 70 amino acid is involved in HCC development even after virus disappearance. In IFN-free DAA therapy, in addition to core 70, NS5A was also suggested to affect the future HCC development.
Are the Globe and UK-PBC scores also effective for predicting risk in patients treated with bezafibrate in addition to ursodeoxycholic acid? A validation study in Japan

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Introduction: It is crucial to stratify the risk for progression in patients with primary biliary cholangitis (PBC). Although the Globe and UK-PBC scores have been established for this purpose, these are not validated in patients treated with other drugs in addition to ursodeoxycholic acid (UDCA). We have used bezafibrate (BF) for patients who were refractory to UDCA in Japan, and herein performed a validation study of these scores in Japanese patients treated with UDCA and/or BF.
**Methods:** We took advantage of a large-scale retrospective database in Japan consisting of 9919 patients with PBC diagnosed between 1985 and 2014. We selected patients for the current study according the following criteria: 1) treated with UDCA and/or BF, 2) followed up at least 2 years after initiating treatment, and 3) biochemical treatment responses at 1 year of treatment and outcomes were clearly recorded. When patients were consecutively treated with both UDCA and BF, the treatment response at baseline and at 1 year of the secondary added drug was used for calculating the scores.

**Results:** We identified 727 patients who met the above criteria (M/F = 109/618, 58.4 ± 11.3 yo at diagnosis). Among them, 542, 183 and 3 patients were treated with UDCA only (74.5%), UDCA + BF (25.2%), and BF only (0.4%). Observation period was 8.3 ± 5.5 years. Liver transplantation (LT)-free survival rates at 5-, 10- and 15-years was 98.0%, 95.5% and 89.3%, respectively. The average estimated LT-free survival rates at 5-, 10- and 15-years using the Globe score were 81.9%, 62.9% and 47.5%, respectively, which were significantly lower than the real outcomes at each point (p < 0.001). AUROC of the Globe score was 0.811 for LT-free survival. Meanwhile, the estimated LT-free survival rates using the UK-PBC score were 96.9%, 91.8% and 86.6%, respectively, which were significantly lower as well (p < 0.05). AUROC of the UK-PBC score for LT-free survival was 0.899.

**Conclusion:** The risk predictive values of the Globe and UK-PBC scores were diminished when BF was additionally used for patients who were refractory to UDCA, while performance of the UK-PBC score was better. It is warranted to develop a new scoring system for predicting outcomes in patients treated with a new drug such as obeticholic acid in addition to UDCA.
Discovery of a novel therapeutic agent targeting HBx-DDB1 interaction for HBV cure

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Introduction: To achieve functional cure (elimination of HBs antigen) is a major goal of HBV treatment. Recently, HBV regulatory protein X (HBx) was found to promote transcription from cccDNA through the degradation of Smc5/6 by hijacking DDB1-E3 ligase. Here, we aimed to find out an inhibitor of HBx-DDB1 interaction, which possibly inhibits HBV transcription.

Methods: 1) Using split luciferase, we established a high throughput screening assay for HBx-DDB1 interaction, and examined 817 FDA approved drugs. 2) To confirm inhibiting the HBx-DDB1 interaction by candidate compounds, we performed an immunoprecipitation-western blot analysis. We also examined the binding inhibitory effect in vitro. 3) We tested whether the candidate compound inhibits the degradation of Smc5/6 using HBx expressing cells. 4) We examined whether the candidate compound decreased virus RNAs using minicircle DNA, which mimics the cccDNA. 5) Changes in viral protein levels by the compound were determined. 6) Using primary human hepatocyte, the anti HBV effect of the candidate compound was examined.

Results: 1) Five compounds showed the binding inhibitory effect. Among them, nitazoxanide (NTZ) represented the highest activity in a dose dependent manner. 2) NTZ decreased the DDB1 binding to HBx. The same was observed in vitro. 3) Smc5/6 was degraded in HBx expressing cells, but NTZ rescued the expression of Smc5/6. 4) NTZ downregulated the HBV RNAs. 5) Viral protein levels were also decreased by NTZ. 6) NTZ showed anti HBV effect in the primary human hepatocyte.

Discussion/Conclusion: NTZ can be a novel therapeutic agent for HBV functional cure.
Indirect indices of liver fibrosis in the course of alcoholic liver disease, non-alcoholic fatty liver disease and primary biliary cholangitis

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Introduction: The aim of our survey was to determine the usefulness of indirect parameters of liver fibrosis in alcoholic liver cirrhosis (ALC), non-alcoholic fatty liver disease (NAFLD) and primary biliary cholangitis (PBC).

Methods: We enrolled 70 participants in the study: 22 with ALC, 7 with NAFLD, 8 with PBC and 33 persons in control group. Several indirect parameters of liver fibrosis were obtained from their serum: aspartate aminotransferase (AST) to alanine aminotransferase ratio (AAR), AST to platelet (PLT) ratio index (APRI), fibrosis-4 (FIB-4) score and red cell volume distribution width (RDW) to PLT ratio (RPR). To evaluate a clinical outcome of patients, we assessed Model for End-Stage Liver Disease (MELD) score and neutrophil to lymphocyte ratio (NLR). PLT indices were also measured: mean PLT volume (MPV), PLT distribution width (PDW) and plateletcrit (PCT).

Results: We observed significantly higher values of AAR, APRI, FIB-4, RPR, NLR, MPV and PDW in ALC group in comparison to the controls. Their PCT value was significantly lower and correlated negatively with APRI, FIB-4 and RPR (p < 0.01). APRI correlated positively with both FIB-4 and RPR (p < 0.01). Another positive correlation in ALC group was observed between NLR and AAR (p < 0.01). NAFLD patients presented significantly higher levels of APRI, FIB-4 and AAR. PBC group had significantly higher values of RPR (p = 0.02), FIB-4 and APRI (p < 0.01) and lower level of PCT (p < 0.01). FIB-4 and AAR were significantly higher in ALC patients in comparison to NAFLD group (p = 0.02 and p < 0.01, respectively).

Discussion/Conclusion: Our data show that indirect parameters of liver fibrosis are valuable tools in monitoring ALC, NAFLD and PBC. The coexistence of enzymatic liver failure, abnormalities in PLT indices and blood cells parameters (RPR, NLR) is especially prominent in ALC patients.
Effects of *Lippia citriodora* in high-fat diet-fed mice: NAFLD and altered intestinal permeability

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**Introduction:** The prevalence of non-alcoholic fatty liver disease (NAFLD) is increasing in the general population as a consequence of a rise in diseases like metabolic syndrome, which has been lately suggested to be related to intestinal dysbiosis and altered intestinal barrier function associated with intestinal inflammation. Different plant extracts have been previously reported beneficial effects in experimental models of metabolic syndrome. The aim of the present study was to evaluate a well-characterized extract from *Lippia citriodora* in diet-induced obesity in mice, by testing its impact on liver inflammatory status and intestinal epithelial function.

**Methods:** Male C57BL/6 mice were fed a high-fat diet (HFD) and daily treated orally with an extract of *L. citriodora* (1, 10 and 25 mg/kg/day) or metformin (250 mg/kg/day) for 6 weeks. Control and untreated obese mice were included and received normal chow diet and HFD respectively. Mice weight and food consumption were measured periodically. After sacrifice, mRNA expression of markers of intestinal epithelial barrier function, liver inflammation and adipogenic metabolism was evaluated.

**Results:** *L. citriodora* treatment resulted in a reduction of body weight gain, associated with an improvement in the expression of genes involved in the maintenance of intestinal permeability (MUC-2, MUC-3, occludin, TFF-3 and ZO-1). The altered expression of key adipogenic genes like PPARs, inflammatory markers (TNFα, IL-1β or IL-6), and expression of kinases JNK1 and JNK2 were enhanced in obese treated mice. Liver TLR4 and GLUT4 expressions were also ameliorated in treated obese mice in comparison with non-treated ones.

**Discussion/Conclusion:** Treatment with *L. citriodora* extract showed beneficial effects in HFD-induced obesity improving the liver condition of the mice, ameliorating its inflammatory status, maybe through an enhancement of the altered intestinal permeability.
Risk factors associated with hypophosphatemia in chronic hepatitis B patients treated with tenofovir disoproxil fumarate

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Introduction: Tenofovir disoproxil fumarate (TDF) has been considered causing hypophosphatemia. Clinically, many patients treated with TDF experienced various degree of hypophosphatemia, and some of them should be stopped due to severe hypophosphatemia. Therefore, we investigated which factors induced moderate hypophosphatemia among patients treated with TDF.

Methods: We conducted a retrospective study of chronic hepatitis B patients who were initially prescribed TDF at Kosin University Gospel Hospital from January 2012 to January 2017. Baseline serum phosphorus and follow-up phosphorus levels were compared.

Results: Total 206 patients were treated with TDF. 128 patients were excluded for the following reasons; 59 had malignancy including HCC, 36 had no baseline Phosphorus level within 2 months of the first day of TDF administration, 14 were co-treated with other anti-viral agents, 14 were not followed up, and 5 had other reasons. Consequently, 78 patients were analysed in this study. Median duration of follow up was 350.5 days. A total of 50 (64.1%) patients developed hypophosphatemia. There were 28 patients (35.8%) less than 3 mg/dl, 16 (20.5%) less than 2.5 mg/dl and 6 (7.6%) less than 2.0 mg/dl. Using univariate analysis, male (HR = 3.397, p = 0.022), diuretics (HR = 12, p = 0.021) and liver cirrhosis (HR = 3.375, p = 0.041) were significantly associated with hypophosphatemia. Using multivariate analysis, male (HR= 3.836, p = 0.024) and liver cirrhosis (HR = 6.062, p = 0.002) were significantly associated with hypophosphatemia. The treatments for hypophosphatemia were performed with nuts, dairy protein intake (34 patients) or intravenous phosphorus supply (3 patient), or recommended stopping of drinking (3 patient). Fifty percent of them, serum phosphorus levels were normalized without stopping TDF.

Discussion/Conclusion: Administering TDF in chronic hepatitis B patients, hypophosphatemia may be more likely to occur in men and liver cirrhosis. Therefore, serum phosphorus levels should be closely monitored in these groups.
Is patient-reported outcome improved by nalfurafine hydrochloride in patients with primary biliary cholangitis and refractory pruritus? A post-market, single-arm, prospective study

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Background and aims: Patients with primary biliary cholangitis (PBC) frequently suffer from pruritus, which can severely impair their health-related quality of life (HRQOL). Nalfurafine hydrochloride, a selective κ-opioid receptor agonist, was recently approved in Japan for refractory pruritus in patients with chronic liver diseases, but it still remains unclear whether this treatment improves the patient-reported outcome (PRO) in PBC patients with refractory pruritus. Herein, we conducted a multi-center, post-marketing, single-arm prospective study to investigate the efficacy of nalfurafine in terms of PRO, and the associations of the efficacy with any clinical characteristics.

Methods: After screening for pruritus in 496 patients with PBC using PBC-40 and the visual analogue scale (VAS), we identified 141 patients with moderate to severe pruritus; these were invited to participate in the study. The participants received 2.5 µg nalfurafine once daily for 12 weeks, and pruritus and HRQOL were assessed in week 12 of this treatment. Generic HRQOL, short form-36, blood chemistries, and serum autotaxin levels were also measured at baseline and at week 12.

Results: Forty-four patients participated in this study. The mean PBC-40 itch domain scores and VAS declined during the study period, from 8.56 to 7.63 (p = 0.041) and from 42.9 to 29.3 (p = 0.001) at baseline and at week 12, respectively, indicating a significant effect of nalfurafine. The other domains of PBC-40 and all domains of SF-36 were not significantly altered by this treatment. We failed to find any association between the change in VAS and PBC-40 itch scores and any clinical variable. Serum autotaxin levels were also measured at baseline and at week 12.

Conclusion: This study demonstrated that nalfurafine improved pruritus in patients with PBC, independent of their clinical characteristics, but had a limited effect on the PRO.
Alcohol consumption is associated with the remission of fatty liver in Japanese men: A prospective study

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Introduction: Several cross-sectional and longitudinal studies have demonstrated an inverse association between light to moderate alcohol consumption and prevalence of fatty liver. We aimed to analyse the influence of ongoing alcohol consumption on fatty liver.

Methods: We obtained clinical and laboratory data from 10,054 Japanese subjects who voluntarily underwent a baseline health check-up and once or more of follow-up studies from 2006 to 2011. The presence of fatty liver was assessed by ultrasonography. Using Cox proportional hazard model, we performed multivariable analyses adjusting for obesity, dyslipidaemia, hypertension, glucose intolerance, hyperuricemia, smoking, exercise, and age.

Results: After excluding cases with concurrent liver diseases, missing component of data, or those who changed drinking pattern during the observation period, we analyzed 6608 cases (median age, 46 years old). The total follow-up period was 12,524 person-years. At baseline, 1655 of 3848 men (43%) and 389 of 2760 women (14%) had fatty liver. 2760 men (67%) and 719 women (26%) had a drinking habit. During the follow-up period, 307 men and 89 women remitted fatty liver and 332 men and 212 women developed fatty liver. The remission of fatty liver was directly associated with drinking on 1–3 days/week (hazard ratio = 1.47; 95% confidence intervals: 1.09–1.97), drinking on 4–6 days/week (1.61, 1.12–2.30), daily drinking (1.37, 1.01–1.86), drinking < 140 g/week (1.42, 1.07–1.88), drinking 140–280 g/week (1.49, 1.05–2.11), and drinking > 280 g/week (1.50, 1.07–2.09) in men. The development of fatty liver was inversely associated with drinking 140–280 g/week (0.67, 0.47–0.96) in men, but directly associated with daily drinking (2.17, 1.35–3.49), drinking 140–280 g/week (2.05, 1.04–4.06), and drinking > 280 g/week (2.72, 1.40–5.29) in women.

Discussion/Conclusion: Any pattern of alcohol consumption in men appears to reduce fatty liver, while moderate to heavy or frequent alcohol consumption in women may promote an incident of fatty liver.
Posaconazole-associated hyperbilirubinemia in a patient with acute myeloid leukemia following chemotherapy: A toxicity worth considering

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Introduction: Posaconazole is an azole antifungal and the incidence of posaconazole-associated hyperbilirubinemia is very rare in clinical practice. So far, it is only observed in clinical trials and cohort studies, but no case has been reported worldwide. We aim to analyze the correlation of suspected drugs and the adverse event, the mechanism of posaconazole-associated hepatic toxicity and risk factors of worsening it.

Methods: We report a complicated case of 57-year-old male with acute myeloid leukemia (AML) following chemotherapy who developed severe hyperbilirubinemia and jaundice after administration of posaconazole oral suspension, with total serum bilirubin (T-BIL) peak level of 170 umol/l, alkaline phosphatase (ALP) level of 739 U/l and gamma-glutamyl transpeptidase (GGT) level of 638 U/l.

Results: After posaconazole withdrawal and symptomatic treatment with liver-protective agents, the level of T-BIL and other laboratory data decreased to normal range respectively and related symptoms disappeared gradually.

Discussion/Conclusion: After analysis of both medication process and literatures regarding posaconazole and hyperbilirubinemia, other factors that could cause liver injury were excluded and posaconazole was suspected to be the causative agent. In consideration of both the mechanism and the laboratory results, the drug-induced liver injury (DILI) in the case can be classified as mixed types of hepatocellular and cholestatic drug-induced liver injury (DILI).

The case demonstrates that, besides for drug factors, DILI of posaconazole is closely related with patients’ age, past medical history, renal dysfunction, concomitant diseases, combined medication and other risk factors. To our knowledge, it’s the first case report associating hyperbilirubinemia with posaconazole worldwide and it provides 3 instructive points for colleagues. Firstly, liver and renal function should be monitored closely before and during posaconazole administration, and appropriate measures should be taken when necessary. Secondly, for hematological malignancy patients with myelosuppression and febrile neutropenia following chemotherapy, liver and renal function monitoring should be performed more frequently. At last, once pathogen was clarified, antibacterial combination should be avoided as possible. And liver and renal function should be monitored more closely by necessity.
Development of hepatocellular carcinoma in patients infected with hepatitis B virus genotype B or C in Japan

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Introduction: HBV/C infection is associated with a more severe disease progression, manifesting as liver diseases such as liver cirrhosis and hepatocellular carcinoma (HCC), than HBV/B infection, which is associated with a milder disease progression. However, no long-term studies have examined the development of HCC in HBV/B-infected patients in Japan. The aims of our long-term study were to assess the patients' backgrounds at the time of liver carcinogenesis.

Methods: Total of 295 HBsAg-positive patients with known viral genotypes were treated and followed up. In addition, we collected the relevant clinical data for each patient during the follow-up period. These patients were followed up for a median of 10 years (range, 1–36 years).

Results: Genotypes of HBV were A in 1% (4/295), B in 61% (179/295), C in 37% (110/295), and D in 1% (2/295) patients. The mean age at HCC diagnosis was significantly higher in HBV/B-infected patients than in HBV/C-infected patients (67.0 ± 10.0 vs. 57.7 ± 8.0, p < 0.001). Furthermore, significantly more carcinogenesis was found in HBV/B-infected than in HBV/C-infected patients with liver cirrhosis (p < 0.05). The value of fibrosis-4 index was significantly higher in HBV/B-infected patients than in HBV/C-infected ones (p < 0.01). The rate of HCC was higher in HBV/C-infected patients than in HBV/B-infected ones, and a significant difference was observed until the 20-year observation period (p = 0.048). However, thereafter, HCC associated with HBV/B increased, and no significant difference was observed between HBV/B- and HBV/C-infected patients after the observation period.

Discussion/Conclusion: HCC development was consistently observed even in HBV/B infection, especially among elderly patients and patients with advanced fibrosis compared with HBV/C. HBV/B-infected patients developed HCC later in life, and in the long term, we found no differences in HCC development rates between patients infected with HBV/C and HBV/-B.
Is zinc effective for the prediction and the treatment of hepatic fibrosis in patients with autoimmune hepatitis?

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Introduction: It is generally known that zinc has a protective effect on wound healing and immune reaction. Some reports revealed the positive relation between the zinc supplementation and the improvement of viral hepatitis. However, there are no reports in which the efficacy of zinc has been shown in patients with autoimmune hepatitis.

Methods: Of the 210 autoimmune hepatitis patients who had been treated in our hospital since 1990, forty-nine patients (seven males and 42 females) enrolled in this prospective study with written informed consents. Patients were prescribed with polaprezinc tablets which included 34 mg of zinc per day, and their serum levels of zinc, ferritin, fibrotic markers, and other biochemical data associated with hepatic functional reserve as well as the scores of Share Wave elastography were evaluated before and one year after its intake. A p value less than 0.05 was considered statistically significant in this study. This study (U-MIN: 000022959) was approved by our ethics committee.

Results: Autoimmune hepatitis patients with advanced hepatic fibrosis (F ≥ 3) had a significantly poor prognosis on the 10 year accumulated occurring rate of hepatic cancer and the mortality rate associated with hepatic disease. These advanced fibrotic patients were efficiently picked up by the ratio of serum ferritin to zinc as well as the well-established fibrosis predicting marker such as Fib-4 index, APRI, and Forn’s index. In the patients whose serum levels of zinc were well increased (Δ ≥ 23 μg/dl), the serum levels of both M2BPGi and type III procollagen were also significantly decreased and the average score of liver stiffness measured by ultrasound based transient elastography was improved (before 1.7 kPa, after 1.5 kPa).

Discussion/Conclusion: Ferritin/zinc ratio could efficiently pick up the advanced fibrotic patients (F ≥ 3) with autoimmune hepatitis and a long term zinc supplementation might suppress their progressions of hepatic fibrosis.
Carbon monoxide-enriched red blood cells improve murine models of non-alcoholic steatohepatitis through its multifaceted hepatoprotective actions

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Introduction: Non-alcoholic steatohepatitis (NASH) often progresses to liver cirrhosis and hepatocellular carcinoma. Since its pathologic process is complex and diverse, effective therapeutic agents have not yet been developed. Low dose of carbon monoxide (CO) was produced when catabolising heme by heme oxygenase-1 (HO-1) and it exerts multifaceted cytoprotective actions (HO-1/CO system). In this study, CO-enriched RBCs (CO-RBCs) was prepared as a CO donor and its therapeutic efficacy on NASH model mice was evaluated.

Methods: Murine NASH model was established by 4 weeks feeding of methionine choline deficient diet (MCDD) or high fat diet (HFD). CO-RBCs were injected intravenously at 2 weeks after the beginning of MCDD or HFD feeding.

Results: First, we analyzed the expression of HO-1 on murine liver sections and human liver biopsy samples. HO-1 levels in NASH condition were higher than that in non-NASH condition. Correspondingly, CO level in liver of NASH mice was higher than that of normal mice. It suggested that HO-1/CO system is activated in the NASH liver and functions as an endogenous hepatoprotective system. To confirm this, the therapeutic efficacies of CO-RBCs on MCDD induced NASH model mice was evaluated. CO-RBCs significantly suppressed MCDD induced increase in plasma AST and ALT levels, hepatic triglyceride and fibrosis markers such as hydroxyproline and α-SMA protein expression. Intriguingly, CO-RBCs significantly increased AMPK phosphorylation, and its downstream fatty acid oxidation (FAO)-associated gene expression. Consequently, ketone body byproduct of FAO in the liver was also increased. Such an activation of AMPK signalling by CO-RBCs could contribute the inhibition of hepatic fat accumulation. CO-RBCs suppressed MCDD induced TLR4 mediating inflammation and oxidative stress in the liver. These anti-oxidative and anti-inflammatory actions of CO-RBCs also play an important role for the inhibition of disease progression.

Discussion/Conclusion: The present results indicate that CO-RBCs has a potential as the novel therapeutics for NASH by multifaceted hepatoprotective actions of CO.
Isoliqiritigenin, natural flavonoid used oriental herbal medicine, regulate improved non-alcoholic fatty liver by changing macrophage

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Introduction: Isoliqiritigenin (ISL) is a natural flavonoid extracted from licorice which is one of the most used ingredients of oriental herbal medicine. Although this widespread human exposure, biological effects of ISL is poorly understood. To clarify ISL effects on non-alcoholic fatty liver (NASH), we conducted study using diet-induced non-alcoholic fatty liver disease model and explored mechanisms.

Methods: C57BL/6J mice were fed on NASH induced diet mixed with 0.25%w/w ISL for 22 weeks. Liver, ileum and colon were collected. Total RNA was extracted from each tissues and the qPCR was performed. To clarify the effects of ISL on NASH, liver histology was assessed by staining with Masson trichrome staining (for fibrosis) or by H & E staining (for steatosis). Liver lipid metabolism were also explored mice fed on diet induced obesity model.

Results: After 22 weeks, mice fed on NASH induced diet induced significantly high AST and liver total cholesterol and TG. Compared with NASH induced model, ISL significantly reduced AST. Fat accumulation was prevented in ISL especially in liver and white adipose tissue. Liver inflammation and fibrosis were also reduced in ISL fed mice. The analysis of gene expression using qPCR has shown that expression of chemokine receptor CCR2 and chemokines MCP-1 mRNA were significantly reduced in liver on ISL fed mice. In addition, the expression levels of TGR5, Dio2, UCP1 and PGC-1α in brown adipose tissue were significantly increased in mice fed ISL. BA composition analysis in plasma, ratio of TLCA which is the strongest agonist of TGR5 was increased in ISL.

Discussion/Conclusion: Our findings suggested that in NASH model, ISL improved macrophage induced inflammation in liver. Also ISL activated TGR5 signaling by changing BA composition in brown adipose tissue. Since TGR5 is express in macrophage, ISL could improve NASH through TGR5 signaling pathway.
Asperuloside, the extraction of Tochu-tea prevents fatty liver and non-alcoholic steatohepatitis disease through improving liver function

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Introduction: Non-alcoholic steatohepatitis (NASH) is characterized by increased hepatic triglyceride and inflammation. There is a risk of developing into liver cancer as NASH progress. Tochu has been used as herbal medicine. In Japan, the leaves of Tochu have been widely used as Tochu-tea, which may have health benefits. “Asperuloside (ASP)”, which is one of the glycoside contained in Tochu-tea, have known to has decrease fat mass especially in the liver. But its mechanism is unclear. In this study, we investigated its mechanism by using obesity and NASH model mice.

Methods: Obesity experiment: C57BL/6J mice into three groups which fed control diet (Control), high-fat diet (HFD) and High-fat diet with 0.25% w/w ASP, We performed animal studies including body weight gain, OGTT, IPITT, oxysterols analysis, and gene expression analysis were conducted. NASH experiment: C57BL/6J mice into three groups which fed control diet (Control), high-cholesterol diet (HCD) and high cholesterol diet with 0.125% w/w ASP. We performed animal studies including body weight gain, histological analysis, ALT/AST analysis, and gene expression were conducted.

Results: Obesity experiment: ASP treated group was suppressed body weight gain, body fat accumulation significantly. In liver, ASP suppressed gene expression of cholesterol synthesis. And oxysterols analysis, we observed decreasing of LXR ligand 22R-HC. This lead to suppressing the expression of fatty acid synthesis genes (SREBP1c, FAS, ACC, SCD-1) in the liver administered ASP. And resulted in fat accumulation in the liver. NASH experiment: ASP treated group was decreased serum ALT/AST level. In the liver histological analysis, ASP suppressed fibrosis. This induced by the suppression of inflammation and fibrosis genes (Col1a1, TGFβ, IL1β, TNFα) in ASP treated liver.

Discussion/Conclusion: ASP improves liver lipid metabolism in obese mice. Furthermore, ASP improves fibrosis and inflammation in NASH model mice. ASP may have an effective substance for metabolic disease.
L-carnitine reduces muscle mass loss in patients with liver cirrhosis

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Introduction: The effect of L-carnitine on hyperammonemia has been reported in patients with liver cirrhosis (LC). However, its effect on sarcopenia remains to be elucidated. We assessed the effects of L-carnitine on sarcopenia in patients with LC.

Methods: We retrospectively assessed LC patients treated with L-carnitine between 2013 and 2018. CT was used to measure the cross-sectional area of the skeletal muscles at the level of the third lumbar vertebra. The relative change in skeletal muscle index (SMI) per year (ΔSMI/yr) was computed in each patient. We evaluated the relationship between ΔSMI/yr and various parameters, such as age, gender, liver functional reserve, and dose of L-carnitine.

Results: Administration of L-carnitine resulted in a significant and progressive decrease in serum ammonia levels. The ΔSMI/yr values in Child-Pugh classes A, B, and C were not significantly different among the three groups. There was no significant relationship between ΔSMI/yr and each of gender, age, BMI, and sarcopenia. Multivariate analysis showed that only high dose of L-carnitine was associated with increase in muscle mass. The L-carnitine high-dose group included a significantly larger number of patients with increased muscle mass, compared with the low-dose group.

Discussion/Conclusion: L-carnitine seems to reduce muscle mass loss dose-dependently through improvement of hyperammonemia in patients with LC.
Linker phosphorylation of Smad3 promotes fibro-carcinogenesis in non-alcoholic steatohepatitis of hepatocellular carcinoma

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Introduction: Recently, a growing number of case reports demonstrate that hepatocellular carcinoma (HCC) sometimes develop in non-cirrhotic liver, although cirrhosis is a major risk factor for HCC development. Useful biomarkers to predict HCC development in patients with non-alcoholic steatohepatitis (NASH) are needed. Transforming growth factor (TGF)-β type I receptor (TβRI) and c-Jun N-terminal kinases (JNK) phosphorylate Smad3 differentially to create 2 isoforms phosphorylated (p) at the COOH-terminus (C) or at the linker region (L) and regulate hepatocytic fibro-carcinogenesis. This study aimed to elucidate how phospho-Smad signaling affected hepatic fibro-carcinogenesis in NASH.

Methods: The subject are 33 patients who were histologically diagnosed as NASH and were followed until more than 10 years or until onset of liver carcinogenesis. We divided patients into two groups: 17 patients not developing HCC at least for 10 years after a diagnosis of NASH (non-carcinogenesis group; stage I: 10 cases, II: 2 cases, III: 5 cases, IV: 0 cases) and 13 patients who developed HCC concurrently or after diagnosis of NASH (carcinogenesis group; stage I: 2 cases, II: 2 cases, III: 1 case, IV: 8 cases). Immunohistochemistry was performed to investigate the phosphorylation state of Smad3 in the hepatocytes. We also studied 5 random patients in each stage of HCV-related fibrotic liver disease (F1–F4) and also 5 patients with HCV-associated HCC.

Results: In NASH or HCV-related liver diseases, hepatocytic tumor-suppressive pSmad3C signaling shifted to fibro-carcinogenic pSmad3L signaling as liver diseases progressed. Compared with phosphorylation states of Smad3 in hepatocytic nuclei in the non-carcinogenesis group, hepatocytes in carcinogenesis group showed high phosphorylation of Smad3L. All patients with cirrhotic liver (8 cases) developed HCC. In cirrhotic liver with NASH and HCC, hepatocytic positivity for pSmad3L rather increased, and pSmad3C positivity decreased.

Discussion/Conclusion: Phospho-Smad3 profiles should represent useful predictive biomarkers able to measure risk of HCC development.
Nicotinamide ameliorates hepatic steatosis via sirtuin activation

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Introduction: Sirtuins (Sirts), the so-called NAD+-dependent deacetylases, are involved in protection from hepatic steatosis as well as longevity. Nicotinamide (NAM) is a metabolite from NAD catalyzed by Sirts, and is reutilized for NAD synthesis through nicotinamide mononucleotide (NMN) produced by nicotinamide phosphoribosyltransferase (Nampt). NAM is also converted to N¹ methyl nicotinamide (MNAM) by nicotinamide N-methyltransferase (Nnmt), and MNAM was also reported to improve hepatic steatosis. We first investigated whether NAM has a protective effect on hepatic steatosis, and then whether NAM works through the NMN~NAD or MNAM pathway.

Methods: C57BL/6J mice (n = 20) were divided into four groups and fed with normal diet (ND), ND+NAM (NAM mixed with ND to 0.1% wt/wt), high fat diet containing fat of 40% (HFD), or HFD+NAM for 8 weeks. The contents of NAM, MNAM, NMN, and NAD in the liver was measured by LC/MS. The expression of NAD metabolism-related genes was evaluated by real-time RT-PCR or Western blot analysis.

Results: NAM prevented HFD-induced weight gain, and it also prevented fat tissue increase and hepatic steatosis. In ND+NAM, MNAM was increased but with no change of NAD; by contrast, in HFD+NAM, MNAM was unchanged but NAD was decreased. The gene expression of Nnmt was significantly enhanced in ND+NAM, and that of Nampt was increased only in HFD+NAM. These results indicated that the given NAM was predominantly utilized to fill the consumed NAD probably by Sirts activation. NAM enhanced the expression of Sirt 3 known to be involved in lipid metabolism in protein levels but not in mRNA levels. NAM also enhanced the expression of fatty acid synthesis-related genes but not that of beta oxidation-related genes.

Discussion/Conclusion: NAM ameliorated hepatic steatosis through the activation of Sirts, leading to suppressed fatty acid oxidation. Our results suggest that NAM might have a novel therapeutic application for NAFLD/NASH.
Effects of a SGLT2 inhibitor on growth and metabolisms on hepatocellular carcinoma

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Introduction: Various metabolic disorders including glucose and lipid disorders are associated with growth of hepatocellular carcinoma (HCC). Sodium-glucose cotransporter 2 (SGLT2), an anti-diabetic agent, is recently reported to affect lipid metabolism. The aims of this study are to investigate expression of SGLT2 in hepatoma cell lines, and effects of SGLT2 inhibitor (SGLT2i) on growth of hepatoma cells by using a global metabolomic analysis.

Methods: We examined the expression of SGLT2 protein in human hepatocyte cell line (OUMS) and hepatoma cell lines (Huh7 and Hep3B) by Western blotting and immunofluorescence staining. Huh7 (n = 5) and Hep3B (n = 5) were incubated either SGLT2i (canagliflozin 30 μM; SGLT2i group) or DMSO (Control group) for 72 hrs and cell number was evaluated. Glycolysis activity was analyzed by measuring medium lactate level using Glycolysis Cell-Based Assay Kit (BioAssay Systems, Hayward, CA) in Hep3B cells after 48 hrs of the treatment (n = 5). Intracellular metabolites levels were evaluated by a metabolomic analysis in Hep3B cells after 48 hrs of the treatment (n = 5). The difference in glycolysis activity and metabolites levels between the SGLT2i and Control groups were analyzed by Wilcoxon signed-rank test.

Results: SGLT2 expression was not seen in OUMS cells. While, SGLT2 expression was seen in Hep3B and Huh7 cells in both immunoblotting and immunofluorescence. Cell number was significantly decreased in the SGLT2i group compared to the Control group in both Hep3B and Huh7 cells (Huh7 6.98 x 10⁵ vs. 3.55 x 10⁵ cells/100 mm dish; p < 0.01, Hep3B 22.07 x 10⁵ vs. 3.79 x 10⁵ cells/100 mm dish; p < 0.01). There was no significant difference in medium lactate level between the 2 groups in Hep3B cells. In a metabolomic analysis, no significant difference was seen in intracellular glucose and glucose-6-phosphate levels between the 2 groups in Hep3B cells. On the other hand, intracellular butyrylcarnitine level, an indicator for beta-oxidation activity, was significantly higher in the SGLT2i group than the Control group in Hep3B cells (17,533 ± 5312 vs. 28,134 ± 2134 arbitrary unit).

Discussion/Conclusion: We demonstrated that SGLT2 occurred in human hepatoma cell lines. Furthermore, we revealed that SGLT2i suppressed growth of hepatoma cells and up-regulated beta-oxidation activity in hepatoma cells with no alteration in glycolysis activity. Thus, our findings suggested that SGLT2i may suppress hepatoma cell growth via regulation of lipid metabolism rather than glucose metabolism.
ALA improves mitochondrial function and prevents lipid accumulation, oxidative stress, and diet-induced steatohepatitis

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Introduction: Non-alcoholic steatohepatitis (NASH) could be caused by excessive hepatic lipid accumulation and peroxidation. Previous studies have suggested that when reactive oxygen species (ROS) increase due to mitochondrial dysfunction, it reacts with abundant lipids in the liver to produce lipid peroxide, which may contribute to the advance of NASH. Therefore, mitochondrial dysfunction may be a central feature of the transition from simple steatosis to NASH. Furthermore, hepatic steatosis, which is a preliminary stage of NASH, could be prevented by activating the mitochondrial function and inducing energy expenditure. Therefore, we hypothesized that administration of 5-aminolevulinic acid (ALA), a precursor of heme, which is a component of mitochondria, could improve mitochondrial function and prevent hepatic steatosis and NASH. In this study, we examined whether administration of ALA could be effective in both hepatic steatosis and NASH model mice.

Methods: Six-week-old C57BL/6J mice were fed on a high-fat diet (HF) or high-cholesterol and high-fat diet (HC), and each diet mixed ALA + sodium ferrous citrate (SFC). After 15 or 22 weeks, we took blood and liver to measure serum ALT, AST, liver lipid accumulation, peroxidation, fibrosis levels, and mRNA expression levels using qPCR.

Results: In mice fed HF diet, lipid accumulation was increased. In mice fed HC diet, plasma ALT and AST were elevated and liver fibrosis was observed. However, administration of ALA + SFC improved all of them. Moreover, lipid peroxidation was significantly suppressed. Gene expressions involved in antioxidants, mitochondrial respiratory chain complex, and synthesis of heme were increased.

Discussion/Conclusion: Our data suggested that ALA + SFC increased heme, which is a component of mitochondria, improved mitochondrial function and prevented hepatic steatosis and NASH by suppressing lipid accumulation and oxidative stress.
Acute liver failure due to HBV reactivation associated with immunosuppressive or anticancer therapies in Japan (2010–2016)

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Introduction: To clarify the recent status of acute liver failure (ALF) and late onset hepatic failure (LOHF) due to HBV reactivation during and after immunosuppressive and/or anticancer therapies, we analyzed data from nationwide survey of ALF in Japan.

Methods: A total of 1898 patients with ALF and LOHF, seen between 2010 and 2016, were enrolled from 745 hospitals. All patients showed a PT-INR of 1.5 or more within 8 weeks after the onset of disease symptoms.

Results: Among the total of 351 patients (18.5%) who were diagnosed as having ALF or LOHF due to HBV infection, 138 patients (39.3%) were HBV carriers. HBV reactivation due to immunosuppressive and/or anticancer therapies was responsible for the development of ALF in 75 carriers (54.3%); they consisted of 40 patients positive for HBs-antigen and 35 patients negative for HBs-antigen (positive for anti-HBc and/or anti-HBs, before the onset of liver injuries). The outcome of carriers with ALF or LOHF showing HBV reactivation during and after immunosuppressive and/or anticancer therapies was unfavorable especially in those with resolved HBV infection (de novo hepatitis B); 32 (88.9%) died without liver transplantation, while 3 survived without liver transplantation and 1 received liver transplantation. Although HBV reactivation due to rituximab was seen in 28.2% of patients between 2010 and 2015, it was increased to 54.5% in 2016.

Discussion/Conclusion: HBV reactivation due to immunosuppressive and/or anticancer therapies was responsible for the development of liver injuries in more than half of HBV carriers with ALF and LOHF in Japan. Educational activities should be still continued for the prevention of HBV reactivation in patients receiving such therapies to reduce the number of patients developing ALF and LOHF.
Accuracy of selected CCL and CXCL chemokines in the assessment of patients with alcoholic liver disease

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Introduction: Excessive inflammatory response in the course of alcoholic liver disease (ALD) may induce a massive loss of hepatocytes leading to irreversible liver damage and progressive fibrosis. Chemokines are immune messengers implicated in pro-inflammatory signaling by recruiting selected subsets of leukocytes to the site of inflammation. We aimed to explore of the systemic blood expression of selected CCL and CXCL chemokines in patients with ethanol-related liver dysfunction and their accuracy in the noninvasive assessment of ALD liver failure and outcome.

Methods: 63 inpatients with ALD (45 males, 18 females, aged 48.63 ± 11.38) were prospectively recruited and followed for 30 days. 25 age- and sex- matched healthy volunteers served as the control group. Selected CCL (CCL2/MCP1; CCL17/TARC; CCL20/MIP-3α) and CXCL (CXCL9/MIG, CXCL10/IP-10, CXCL16) chemokine concentrations were quantified in blood samples using immunoenzymatic ELISAs. Correlation coefficients between plasma chemokine levels and (i) indicators of systemic inflammation (neutrophil-to-lymphocyte ratio, C-reactive protein, white blood cell and neutrophile counts), (ii) liver dysfunction severity scores (Child-Turcotte-Pugh, MELD scores, mDF) and (iii) complications of liver disease were calculated. The receiver operating curves (ROC) for studied chemokines were constructed, their areas under the curve (AUCs) checked and multivariable logistic regression applied in order to assess the accuracy in predicting the degree of liver failure and the development of ALD complications.

Results: Significant systemic upregulation of both CCL and CXCL chemokines was observed in patients with ALD. Only CCL17 (TARC) concentrations were markedly decreased indicating that Th2-type immune reactions are attenuated in ALD. None of studied chemokines correlated with aminotransferase activity, but CCL20, CXCL9, CXCL10, CXCL16 showed positive correlations with alkaline phosphatase level. CCL20 and CXCL16 correlated with standard indicators of inflammation. Patients with advanced liver dysfunction (MELD > 20, mDF > 32, Child B and C class) presented with significantly higher CCL20 and 6 non-survivors with significantly higher CXCL10 concentrations.
Discussion/Conclusion: Both major chemokine subfamilies are upregulated in the course of ALD. The high blood CCL20 concentration seems to be the disease severity indicator, while CXCL10 the predictor of the poor patient’s prognosis in ALD.
Combination effect of canagliflozin and exercise training in non-alcoholic fatty liver disease

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Introduction: Sodium-glucose cotransporter 2 inhibitors (SGLT2i) is a possible and novel pharmacological therapy for non-alcoholic fatty liver disease (NAFLD). Exercise is a robust treatment in obesity and NAFLD; however, effect of SGLT2i on exercise therapy is unclear. We investigated the effect of canagliflozin (CAN), exercise training and combination therapy on NAFLD and obesity in mice.

Methods: Male mice loaded with a high-fat diet for 4 weeks were housed in a normal cage (sedentary; sed) or wheel cage (WCR). A total of 0.03%w/w CAN was administrated for 4 weeks. Volume of oxygen consumption (VO2) and respiratory quotients (RQ) was analyzed in the metabolic cages. Liver triglyceride contents and gene expression related to glucose and lipid metabolism were compared among 4 groups (control/sed, control/WCR, CAN/sed, and CAN/WCR).

Results: Body weight in control/WCR and CAN/sed was significantly lower than control/sed, and further weight loss was observed in CAN/WCR. In the glucose tolerance test, glucose level was most significantly improved in CAN/WCR. CAN and/or WCR increased VO2. RQ was significantly different among the groups (control/sed 0.80, control/WCR 0.83, CAN/sed 0.75, CAN/WCR 0.77, p < 0.0001). Triglyceride contents in the liver was lower in control/WCR, CAN/sed and CAN/WCR than control/sed. In CAN/sed and CAN/WCR, hepatic CPT1a and PGC1α expression was significantly increased and FAS and SCD1 were significantly decreased comparing to control/sed (p < 0.05), and these differences were more significant in CAN/WCR than CAN/sed.

Discussion/Conclusion: Exercise might enhance the effect of SGLT2i on decreasing lipogenesis and increasing beta-oxidation in the liver. SGLT2i and exercise individually improves obesity, glucose tolerance, and liver steatosis. Concomitant exercise with SGLT2i enhances improvement of systemic metabolic phenotype in diet induced obese mice. SGLT2i extremely promotes the transition from glucose to lipid-dependent energy expenditure whereas exercise increases glucose consumption. Combination therapy of SGLT2i and exercise more strongly and physiologically improves NAFLD and metabolic disorder than SGLT2i solo therapy.
The optimal exercise regimen for patients with NAFLD: Aerobic or resistance exercise?

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Introduction: Exercise is a first-line therapy for patients with non-alcoholic fatty liver disease (NAFLD). Both aerobic and resistance exercise improve NAFLD, however, the most effective exercise protocol remains unclear. Moreover, given the high prevalence of cardiovascular diseases in NAFLD, the choice of exercise type in relation to exercise energy consumption has not been compared. We sought to assess the required frequency, intensity, and duration of aerobic and resistance exercise required for improvement of hepatic steatosis and to compare the exercise regimens with regard to energy consumption in patients with NAFLD.

Methods: A literature search was performed using PubMed, Web of Science, and Scopas to January 28, 2016 for articles assessing the effect of aerobic or resistance on hepatic steatosis. From a total of 95 articles, 24 studies including 25 aerobic and 7 resistance exercise protocols were selected for systematic review.

Results: For aerobic exercise, a decrease in hepatic steatosis was seen in 92.0% (23/25) of protocols (n = 1695). The median effective protocol was 4.8 metabolic equivalents (METs) for 40 min/session, 3 times/week for 12 weeks. For resistance exercise, a reduction of hepatic steatosis was seen in 85.7% (6/7) of protocols (n = 116). The median effective protocol was 3.5 METs for 45 min/session, 3 times/week for 12 weeks. Aerobic and resistance exercise reduced 2.4% [0–21%] and 12% [2–13%] of intrahepatic lipid, respectively. No significant difference was seen in the duration, frequency, or period of exercise between the two exercise regimens. Energy consumption was significantly lower in the resistance than in the aerobic exercise group (11,064 [6394–21,087] vs. 6470 [4104–12,310] kcal/total period, p = 0.0475).

Discussion/Conclusion: Resistance exercise improves NAFLD with less energy consumption. Resistance exercise may be more feasible and beneficial than aerobic regimens for NAFLD patients with poor cardiorespiratory fitness or for those who cannot tolerate or participate in aerobic exercise. These data also suggest a possible link between mode of exercise and hepatic lipid metabolism.
DPP-4 inhibitor suppresses the progression of hepatocellular carcinoma through activation of chemotaxis of NK cells in mice

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Introduction: We previously found that CD26 was expressed to various degree in resected specimens of hepatocellular carcinoma (HCC). CD26 functions as dipeptidyl peptidase 4 (DPP-4). It is unclear whether DPP-4 inhibitor affects HCC progression. We investigated the role of DPP-4 inhibitor, anagliptin in the therapy for HCC.

Methods: Nude mice (BALBc-nu/nu) were subcutaneously injected Huh7 or Li7 cells and then fed the control diet, DPP-4 inhibitor, anagliptin containing diet for 21 days. To clarify whether DPP-4 inhibitor exhibits antitumor effect through NK cells, we assessed the effect of NK cell depletion on antitumor effect by anagliptin. NK cells were examined for their real-time chemotaxis using EZ-TAXIScan in the presence of chemokine CXCL10. CXCL10 (1-77 aa) is truncated at its N-terminus through DPP-4 activity and a N-terminal truncated CXCL10 (3-77aa) acts as chemokine antagonist. We quantified the concentration of intact CXCL10 (1-77 aa) and truncated CXCL10 (3-77 aa), using immunoprecipitation, Edman degradation, and high performance liquid chromatography.

Results: Anagliptin significantly suppressed the growth of xenograft tumors in vivo. Anagliptin also induced NK cells infiltrations to necrotic lesion in tumor more vigorously. The reduction in growth of xenograft tumor by anagliptin was completely canceled by depleting NK cells with anti-ASGM1. Anagliptin significantly enhanced the mobility of NK cells in the presence of CXCL10. DPP4 inhibitors almost completely suppressed CXCL10 to be truncated, indicating that NK cell trafficking is enhanced through the prevention of CXCL10 from being truncated by DPP-4 inhibitors.

Discussion/Conclusion: DPP-4 inhibitor suppressed HCC progression through activation of NK cell chemotaxis.
Evaluation of ballooned hepatocytes as a risk factor for future progression of fibrosis in patients with non-alcoholic fatty liver disease

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Introduction: The prevalence of non-alcoholic fatty liver disease (NAFLD) has increased. Non-alcoholic steatohepatitis (NASH) shows progression of liver fibrosis in NAFLD. It remains unclear which patients with NAFLD will show progression of liver fibrosis. Therefore, we aimed to investigate the risk factor associated with the progression of liver fibrosis among patients with NAFLD.

Methods: This observational study enrolled 157 patients with biopsy-proven NAFLD. Thirty-two patients were excluded because of lack of data. The accuracy of the formulae for estimating liver fibrosis, i.e., the FIB-4 index, APRI, and Forns index, was compared. Using serial changes of the best formula for liver fibrosis, we identified factors associated with the progression of liver fibrosis. Histological liver fibrosis was quantified using the Brunt stage.

Results: Sixty-three patients were diagnosed as having NASH. The FIB-4 index provided the best diagnostic accuracy for liver fibrosis (Brunt stage 0 versus 1–4, areas under the curve [AUC] 0.74; 0–1 versus 2–4, AUC 0.77; 0–2 versus 3–4, AUC 0.78; and 1–3 versus 4, AUC 0.87). The association between body mass index, sex, observation period, and histological findings (liver fat content, bridging fibrosis, and hepatocyte ballooning) with the change in the FIB-4 index was evaluated among patients with NASH, using multivariate analysis. Then only hepatocyte ballooning was determined as a significant marker for fibrosis progression expressed by FIB-4 index increase.

Discussion/Conclusion: The FIB-4 index was the best formula for estimating liver fibrosis in patients with biopsy-proven NAFLD, and the presence of ballooned hepatocytes was a risk factor for the progression of liver fibrosis.
Allogenic transplantation of MUSE cell administration ameliorates liver function in pig models of chronic liver injury

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Introduction: Multilineage-differentiating Stress Enduring (MUSE) cells are identified in mesenchymal stem cells. They migrate into damaged tissues and spontaneously differentiate into cells compatible with the homed-into various tissues. We examined the safety and efficacy of allogenic MUSE cells transplantation in pig model of chronic liver injury.

Methods: Female Göttingen mini-pigs (age: approximately 12 months, BW 22–27 kg) were used as the allogenic recipient (n = 18). Male mini-pigs (12 months, BW 25–27 kg) were used as the donors of either MUSE cells or control MSC. Chronic liver injury model was induced by peritoneal injection of CCl₄ (1.2–1.5 ml/kg/week) for 12 weeks. 1 x 10⁷ cells of either GFP labeled MUSE cells or control MSC were transplanted into two groups (MUSE cells transplantation group and control MSC transplantation group, n = 6, each).

Results: After 12 weeks of peritoneal injection of CCl₄, Histopathological assessment of liver demonstrated hepatic fibrosis and increase of positive area of alpha-SMA staining. After transplantation, there was a significant difference of the average Alb levels between MUSE group and MSC group at 4 weeks (4.66 ± 0.19 vs. 4.33 ± 0.28, p = 0.04) and. Histopathological assessment of liver demonstrated the improvement of hepatic fibrosis and decreased positive area of alpha-SMA staining and increased number of positive PCNA staining cells in MUSE group.

Discussion/Conclusion: Allogenic transplantation of MUSE cells administration was both safe and effective for liver regeneration in chronic liver injury model. Moreover, MUSE cells seemed to lead earlier liver regeneration than MSC.
Clinical management of primary sclerosing cholangitis in Japan 2017

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Introduction: Primary sclerosing cholangitis (PSC) is a chronic cholestatic hepatobiliary disease of unknown origin, characterized by the progressive destruction of bile ducts caused by diffuse inflammation and fibrosis that eventually leads to liver cirrhosis. There are three categories for sclerosing cholangitis, PSC, IgG4-related sclerosing cholangitis (IgG4-SC), secondary sclerosing cholangitis (SSC), and thus PSC should be differentially diagnosed by discriminating other two categories, and thereafter treated adequately. Recently, the Japanese study group for intractable hepatobiliary diseases has performed a nationwide survey for PSC and IgG4-SC, and proposed the data-based diagnostic criteria of PSC (J Gastroenterol. 2017;52:838–844). Thereafter, the clinical management of PSC in Japan has been proposed as “Guidelines for PSC in Japan 2017 (unpublished)”, especially focusing on the following points;
1. Diagnostic algorithm through the stepwise imaging modalities
2. Availability and usefulness of liver biopsy
3. Therapeutic algorithm of nonsurgical and surgical strategies including managements of complications

Methods: Modified Delphi method was employed for guideline preparation. The production committee decided guidelines, strength of recommendations and evidence level after reviewed literatures systematically, and guidelines were evaluated by The Expert Panel. The Scientific Committee of the Japan Biliary Association (JBA) evaluated revised guidelines, and Public comments were collected on web site of JBA.

Results: Sixteen CQs were listed for Epidemiology/Pathophysiology, Diagnostics, Therapy and Prognosis. In addition, both diagnostic and therapeutic flowcharts were figured.

Discussion/Conclusion: Guidelines for PSC in Japan 2017 preferentially describe the algorithm for diagnosis and treatments including complications, to contribute clinical managements of PSC.

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A multicentre prospective study: Ballooning biomarker in patients with non-alcoholic fatty liver disease

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Introduction: Non-alcoholic steatohepatitis (NASH) is characterized by the presence of hepatic steatosis, inflammation, and hepatocellular injury with or without accompanying fibrosis. However, there are few reports about noninvasive diagnosis of ballooning. This study aimed to identify novel blood markers for ballooning.

Methods: We performed prospective study of 176 non-alcoholic fatty liver disease (NAFLD) subjects in multicentre in JAPAN. Finally 132 subjects were classified as non-alcoholic fatty liver (NAFL) (n = 85) and NASH (n = 47) based on the fatty liver inhibition of progression (FLIP) algorithm. Histopathology were reviewed again by two hepatopathologists who were blinded to the clinical data. Metabolomics (LC/MS, HPLC) and lipdomics were carried out in plasma samples. In transcriptome analysis, RNA was extracted from the liver biopsy specimen and RNA sequence was performed using the next generation sequencer.

Results: For the diagnosis of ballooning, type 4 collagen 7S showed the highest with an area under the receiver operating characteristics curve (AUCROC) of 0.780 in the clinical parameters and liso-phosphatidylcholine (LPC) (e-18:0) correlated most strongly (r = -0.37, p < 0.001) in lipidomics. We also analyzed the potential secreted factors gene by RNA sequencing analysis, and we extracted CCL20. CCL20 identified the patients with ballooning grade 1 or 2 with an AUROC of 0.768 and the patients with fibrosis stage ≥ 3 with an AUCROC of 0.638, which mean that CCL20 is a specific marker for ballooning. A multiple regression analysis with steatosis, inflammation, ballooning and fibrosis showed that CCL20 was significantly and independently associated with ballooning (t = -4.27, p < 0.0001).

Discussion/Conclusion: A diagnostic marker of ballooning is needed, since "disappearance of ballooning" is important for NASH treatment. Our multicentre prospective study suggested that the serum CCL20 is a diagnostic marker for ballooning.
Systemic profile of neutrophil-derived mediators and its association with the severity of alcoholic liver disease

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Introduction: Neutrophils are the first line effectors of human innate immune system. Inflammatory dysregulation and neutrophil infiltration are hallmarks of alcoholic liver disease (ALD). Given their destructive potential, extracellularly released neutrophil enzymes should be carefully controlled to avoid damage to host tissues. We aimed to assess the systemic profile of neutrophil-derived mediators i.e. neutrophil elastase (NE), myeloperoxidase (MPO), as well as alpha1-antitrypsin (A1AT) – a potent inhibitor of neutrophil proteases, with emphasis on their potential relevance in the course of ALD.

Methods: 62 patients with ALD (47 males, 15 females, aged 49.2 ± 9.9) were prospectively recruited and assigned to subgroups based on their 1) gender, 2) severity of liver dysfunction (Child-Pugh, MELD scores, mDF) 3) presence of ALD complications, and followed for 30 days. 24 age- and sex-matched healthy volunteers served as the control group. Selected plasma markers of neutrophil activation were quantified using immunoenzymatic ELISAs. Correlation coefficients between their blood concentrations and (i) indicators of systemic inflammation (the neutrophil-to-lymphocyte ratio, C-reactive protein, white blood cell and neutrophil counts), (ii) liver dysfunction severity scores (Child-Pugh, MELD, mDF), and (iii) ALD complications were calculated. The receiver operating curves (ROC) and their areas under the curve (AUCs) were checked in order to assess their accuracy in predicting the degree of liver failure and the development of ALD complications.

Results: Concentrations of MPO and NE were significantly increased in the blood of patients with ALD in comparison with controls, but the A1AT level was not different. ALD females presented with higher MPO levels in comparison with ALD males. There were no gender-related differences in NE levels in ALD group. NE, but not MPO, correlated with MELD and mDF scores. MPO, but not NE, correlated with standard markers of inflammation. ALD subgroups with mDF>32, Child class C and hepatic encephalopathy presented with significantly higher NE, but not MPO levels.
Discussion/Conclusion: Our results support the value of MPO and NE in the ALD assessment. MPO seems to be an inflammatory marker, while NE the disease severity indicator. The higher systemic NE/A1AT ratio in the course of ALD may facilitate the expansion of the inflammatory cascade. Gender-related differences in neutrophils’ activation in ALD may impact the different susceptibility to toxic liver injury in males and females.
Characteristics and risk factors of fatty liver disease development and non-alcoholic steatohepatitis recurrence following liver transplantation

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Introduction: Non-alcoholic steatohepatitis (NASH), which is a common and increasing indication for liver transplantation (LT), is known to recur after LT. Since the recurrence of NASH can lead to graft failure, the identification of predictive factors and preventive strategies need to be implemented.

Methods: We identified 627 consecutive adult patients who underwent LT at Karolinska University Hospital between June 2007 and February 2017. Of these, 95 patients who received LT for NASH or alcoholic liver disease (ALD) as a primary indication were included. Peritransplant characteristics and histological findings 1 year post-LT among NASH patients were evaluated in comparison with ALD patients. Risk factors of post-LT fatty liver disease development and NASH recurrence were assessed.

Results: Among 27 NASH patients, pre-LT body mass index (BMI) was higher (31.3 vs. 28.6, p < 0.001) and pre-LT diabetes was more prevalent (non-insulin-dependent, 26% vs. 3%; insulin dependent, 56% vs. 21%, p < 0.001) than ALD patients. The difference of BMI persisted at 3 months and 1 year after LT. There were no differences between the groups regarding histopathological findings including the degree of steatosis and fibrosis in 1-year biopsy. In multivariate analysis, recipient age and 1-year BMI were independent risk factors for post-LT fatty liver disease development. Regarding predictive factors of NASH recurrence, the prevalence of pre-LT insulin dependent diabetes was significantly higher in patients who developed NASH recurrence than those who did not. The increase of HbA1c until 1 year post-LT was higher in patients who developed recurrence than those who did not (15 vs. 5 mmol/mol, p = 0.051), although the differences did not reach statistical significance.

Discussion/Conclusion: The results of this study suggests that insulin dependent diabetes has detrimental effects on NASH recurrence following LT. Optimal glycemic control should be recommended, but studies are needed to prove its preventive effect on NASH recurrence.
Promising antiviral combination therapy targeting HBV eradication with a novel compound derived from spice

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\textbf{Introduction:} Nucleos(t)ide analogues efficiently suppress HBV replication. However, no existing nucleos(t)ide analogue could eradicate HBV, chiefly because of the residue of cccDNA. Consequently, novel therapies targeting HBV eradication are necessary. We found a promising compound harboring anti-HBV effects using HBV-replicating cells. In this study, we examined the efficacy of a combination therapy with this novel compound (called F, applying for patent) using HBV-infected humanized mice.

\textbf{Methods:} Fifteen-week-old male mice possessing transplanted human hepatocytes were inoculated with HBV genotype C (1.0 \times 10^7 HBV-DNA copies/mouse). Seven weeks after inoculation, mice were separated into 3 groups. In group A (n = 4), mice were inoculated subcutaneously with pegylated interferon (Peg-IFN)-2\(\alpha\) (100 \(\mu\)g/kg) twice a week, with daily oral administration of entecavir (30 \(\mu\)g/kg), fluvastatin (10 mg/kg), and F (100 \(\mu\)g/kg) for 8 weeks. In group B (n = 3), mice were treated with Peg-IFN-2\(\alpha\) (30 \(\mu\)g/kg) similarly with group A, together with daily fluvastatin (10 mg/kg) and lamivudine (15 mg/kg). In group C (n = 3), no treatment was performed.

\textbf{Results:} In group A, cccDNA in the liver significantly decreased to 198 copies/100 ng DNA (mean, \(p < 0.05\) compared with both group B and group C). HBV-DNA and HBsAg continuously decreased and reached to 1362.5 copies/ml and 177.0 IU/ml at day 56, respectively (\(p < 0.05\) compared with group C). No major complication and death were observed in all groups, but transient elevations of liver enzymes were observed in group A from day 14 to 28.

\textbf{Discussion/Conclusion:} The additional administration of F presented remarkable antiviral efficacy, especially on intrahepatic cccDNA, compared with an existing anti-viral treatment. F is a derivative of spice and its safety to human is secured, although the initial elevation of transaminases should be monitored. We expect the combination therapy with F should be a candidate for novel therapeutic strategies toward the HBV eradication.
Relevance of FXR-p62/SQSTM1 pathway for survival and protection of mouse hepatocytes and liver with steatosis

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Introduction: Liver injury and regeneration involve complicated processes and are affected by various physio-pathological conditions. Surgically, severe liver injury after surgical resection often leads to fatal liver failure, especially with moderate to severe steatosis. Therefore, protection from the injury of hepatocytes and liver is a critical concern in various clinical settings.

Methods: We studied the effects of FXR on cell survival and steatosis in mouse hepatocytes and investigated their molecular mechanisms. We also studied whether FXR improves liver injury, regeneration and steatosis in a mouse model of partial hepatectomy (PH) with liver steatosis.

Results: An FXR-specific agonist, GW4064, induced expressions of the p62/SQSTM1 gene and protein in AML12 mouse liver cells. Because we previously reported p62/SQSTM1 as a key molecule for antioxidation and cell survival in hepatocytes (Antioxid Redox Signal 21: 2515. 2014), we examined the activation of Nrf2 and induction of the antioxidant molecules by GW4064. GW4064 activated Nrf2 and subsequently induced antioxidant molecules. GW4064 also induced phosphorylation of Akt, expression of the anti-apoptotic molecules and reduced harmful hepatic molecules (Fas-ligand/Fas). GW4064 promoted hepatocyte survival via p62/SQSTM1. These findings suggest the potential relevance of the FXR-p62/SQSTM1 pathway for the survival and protection of hepatocytes. Furthermore, GW4064 induced the expression of small heterodimer partners (SHP) and suppressed LXR-induced steatosis in hepatocytes. GW4064 significantly reduced post-PH liver injury and improved steatosis in the hepatectomy model of db/db mice with fatty liver.

Discussion/Conclusion: The present study is the first to demonstrate the relevance of FXR-p62/SQSTM1 and -SHP in the protection against injury of hepatocytes and post-PH liver, especially with steatosis.
Effective biomarkers for advance fibrosis NASH and reflect biomarkers as changes of liver fibrosis with NASH

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Background and Aim: Recently there have been examines of non-invasive diagnostic methods such as biomarkers for NASH diagnosis and advance fibrosis NASH. However none of these have been definitive. In particular, as for reports on biomarkers reflected in the histological changes, there are only single marker for cytokeratin 18 (CK18). Currently, we performed multiple verifications of biomarkers for diagnosis of advance fibrosis NASH, and reflecting the histological changes of NASH, and studied markers useful for disease staging.

Methods: (1) We assessed useful markers in the diagnosis of advance fibrosis NASH in 247 NAFLD patients at General Medical Center, Kawasaki Medical School who had undergone liver biopsies from 1996 until August 2015. The testing makers were the sugar chain marker: WFA+M2BP, macrophage activation marker: soluble CD163 (sCD163), apoptosis marker: Cytokeratin (CK18), and liver fibrosis markers: type-IV collagen 7S, P-III-P, hyaluronic acid, and the FIB4 Index. (2) 100 NAFLD which underwent the repetition liver biopsy (5.4 ± 3.0 y) were measured WFA+M2BP levels, sCD163, CK18, type-IV collagen 7S, hyaluronic acid and FIB4 Index of and histology change and the relation of them were considered.

Result: (1) WFA+M2BP, sCD163, and type-IV collagen 7S, hyaluronic acid, and the FIB4 Index were useful in the diagnosis of stage 4 NASH. sCD163 and type-IV collagen 7S were useful in catching stage 3–4 fibrosis progression cases. (2) The period between the first liver biopsy and the 2nd liver biopsy was 5.3 ± 2.9 years. There were 29 cases of progress fibrosis (29%), 40 cases of no change (40%), and 31 cases of improvement (31%). Type-IV collagen 7S and sCD163, and CK18 were useful biomarker that reflect liver fibrosis progression. The result was the same whether the initial liver biopsy was limited to stages 0–2 or limited to stage 3. CK18 was the most useful in progression from stages 0–2 and sCD163 in progression from stage 3. In cases of liver fibrosis improvement, type-IV collagen 7S, CK18, and sCD163 were useful markers. However, their utility dropped when the initial liver biopsy was limited to stages 1–2.

Conclusions: Assessment Type-IV collagen 7S, CK18, and sCD163 in NAFLD was considered useful in predicting progression and improvement of fibrosis and histological changes in NASH, as well as therapeutic effects.
Beneficial effects of agomelatine on obesity associated liver inflammation in mice

CIBER-EHD. ibs.GRANADA, CIBM, University of Granada, Granada, Spain

Introduction: Previous studies have revealed the beneficial effects exerted by melatonin in non-alcoholic fatty liver disease (NAFLD), both in humans and in experimental models, most probably related to its antioxidant properties. Obesity has been associated with systemic inflammation that can promote liver damage and NAFLD. The aim of the present study was to evaluate the impact of agomelatine, an agonist of the melatonin receptors, in diet-induced obesity in mice, and to investigate its impact on the liver inflammatory status and intestinal epithelial function.

Methods: Male C57BL/6J were divided into different groups: control, obese and obese treated with agomelatine (10, 25 and 50 mg/kg/day p.o.) for 6 weeks. Control and control-treated mice were fed with normal chow diet, whereas obese mice received a high-fat diet. Animal weight and food consumption were periodically measured. At the end of the experiment, the liver inflammatory status was evaluated, as well as different markers of intestinal epithelial barrier function, by RT-qPCR.

Results: The administration of the different doses of agomelatine resulted in a reduction of body weight gain, as well as visceral and epididimal fat deposition. This effect was associated with a significant improvement in the altered expression of key adipogenic genes in the inflamed liver from obese mice, like inflammatory cytokines (TNFα, IL-1β, IL-6), the macrophage chemokine MCP-1, the protein kinases JNK1 and JNK2, and TLR4, thus evidencing a reduced impact of the systemic endotoxemia in the liver of the agomelatine-treated obese mice.

Discussion/Conclusion: Oral administration of agomelatine, an agonist for melatonin receptors, to obese mice resulted ameliorated the inflammatory status of the liver of obese mice, thus protecting this organ from the risk to develop NAFLD, similarly to that previously reported with melatonin.
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