Research @-Bulletin

The Role of Human Gut Microbiome in Advancing Human Health: Early Detection of HCC in Chronic Liver Disease

In our third Research Grand Rounds (RGR) for 2023 held on 17 May, participants gained insights on how the human gut microbiome may be an early diagnostic biomarker of hepatocellular carcinoma (HCC) in chronic liver disease. Our speakers, Assoc Prof Jeremy Lim Fung Yen (CEO and Co-Founder, Asian Microbiome Library) and Prof Piece Chow Kah Hoe (Senior Consultant, Division of Surgery and Surgical Oncology, National Cancer Centre Singapore and Singapore General Hospital) also discussed the preliminary findings of the ELEGANCE study, a high-risk prospective cohort study of 2,000 at-risk patients enrolled from 6 hospitals and 8 polyclinics in Singapore and followed up over 3 years, which aims to identify biomarkers for early diagnosis of HCC.

There is a worldwide increase in the prevalence of metabolic liver disease. The biology and clinical trajectories of metabolic liver diseases and cancer remain unclear but emerging data have shown that human gut microbiome plays a crucial role in maintaining human health.



Assoc Prof Lim began by discussing the gut microbiome and why it has been described as "one of the most significant scientific discoveries in recent decades".

He pointed out that urbanisation brings about numerous harmful artificial substances, leading to a depletion in the microbiome. While technological and medical advancements have helped reduce our exposure to pathogenic microbes, facilitated the cost-effective management of large populations, and decreased acute medical incidents; these developments often overlooked the impact on our resident microbes and their crucial role in our overall health.

When infants are exposed to antibiotics, it negatively impacts their microbiomes, leading to further issues. However, the historical use of antibiotics in Western medicine suggests a recognition of the presence of pathogens and the establishment of a symbiotic relationship. Assoc Prof Lim then provided a summary of the paper's findings, which demonstrated a correlation between infant exposure to antibiotics and childhood health outcomes, specifically related to food allergies. The evidence established that microbiomes are closely interconnected with the immune system, implying that by influencing microbiomes, we can also impact our immune system.

Through an overview of the composition of the gut microbiome, Assoc Prof Lim reiterated the importance of considering ecological principles when examining the composition of the microbiome. While focusing on the presence of specific strains, it is also crucial to understand the interactions among these strains. Studies have shown that there is a link between childhood antibiotic exposure to many conditions such as autism and eczema, as well as microbiome link to immune or metabolic related conditions such as inflammatory bowel disease and irritable bowel syndrome. It is, therefore, important for clinicians to prescribe antibiotics prudently.

Concluding his session, Assoc Prof Lim shared that short-chain fatty acid (SCFA)-producing microbes have emerged as one of the most potent biomarkers for patients with cirrhosis. He added that gut dysbiosis, resulting from changes in intestinal permeability and impaired bile acid metabolism, can contribute to systemic inflammation, with the potential for the liver to be affected. This underscores the significant role of the gut microbiome in the progression of hepatitis and hepatocellular carcinoma (HCC).



Prof Pierce Chow went on to present the initial findings from the A*STAR IAF-ICP funded programme titled EarLy DEtection of HCC: miRNA, microbiome, and imaGing biomArkers in the evolution of chroNiC livEr Disease in a high-risk prospective cohort (ELEGANCE) which recruits a prospective cohort of 2,000 patients at high-risk of developing HCC and a parallel PROSECT cohort, with another100 HCC patients recruited and scheduled for surgical resection. The two studies together recruited patients from 6 local hospitals (CGH, NCCS, NUH, SGH, SKH and TTSH) and 8 SingHealth Polyclinics (for ELEGANCE only).

In Track 3 of this funded study, the team's objective is to discover microbiome and metabolome biomarkers that can be targeted to prevent the development and slow the progression of HCC. To achieve this, the team collaborated with AMiLi to analyse the variations in bacterial communities. They also partnered with the Singapore Phenome Centre (SPC) to monitor changes in metabolites in the choline, bile acid, and tryptophan pathways during the development of HCC.

Prof Chow provided further details on the study design for their pilot conducted with AMiLi, which aimed to characterise the microbiome in HCC patients and individuals at high risk of developing HCC. In the pilot study, they focused on three distinct groups: converted cases (patients who developed HCC during the course of the study), matched controls (patients who did not develop HCC during the course of the study), and HCC cases (patients who had HCC at baseline), to examine changes in the bacterial communities present. He provided a breakdown of matched cases for each groups selected based on age, etiology and gender (amongst other parameters).

For the sample collection, Prof Chow outlined a step-by-step process, starting with the collection of

stool samples from participants within 7 days prior to their visit. The samples were collected using DNA/RNA Shield Fecal Collection Tubes and sent to AMiLi laboratory at the end of the month. Additionally, participants were instructed to complete an online food frequency questionnaire, providing detailed information about their lifestyle and dietary habits.

Subsequently, the collected stool samples underwent DNA extraction and quantification, followed by shotgun sequencing. The raw reads obtained from the sequencing were then subjected to analysis through bioprocessing pipelines, which involved computational methods such as bioBakery3, MelonPan, and ShotBred. Prof Chow provided an explanation of the shotgun whole genome sequencing process and justified its adoption for extracting microbial DNA. This method was chosen to enhance the understanding of microbial composition with greater granularity compared to the conventional 16s rRNA sequencing method. Additionally, the research team analysed the participants' diet, nutrition, lifestyle patterns, and dietary habits to gain further insights into their demographics.

In his presentation of the key findings of the pilot study, Prof Chow presented evidence of a distinct clustering differences of microbial signatures among patients with HCC and individuals at risk of developing HCC. By analysing the bacterial species and their abundance in each sample, the study revealed notable differences in the microbial communities between HCC patients, patients who did not develop HCC, and patients who progressed to HCC during the study period.

Prof Chow also presented the top 20 microbial species that contributed to the differences observed in clustering. Among these species, they identified some that aligned with findings from previous literatures investigating the stool microbiome in HCC. The differences in microbial profiles observed in HCC across various studies can be attributed to several factors, including differences in sequencing methods, etiologies, geographical locations and variations in nutritional intake.

The Q&A segment attracted active and vibrant participation from the attendees, with a variety of questions raised ranging from choice of probiotics and its role in gut health, to the longitudinal design of the microbiome study, and inquiries about the specific mechanisms by which the gut microbiota either accelerates or prevents liver cancer.



Prof Sven Pettersson facilitated the Q&A session, with several key takeaways:

- The science of microbiome interpretation is still evolving, and there are about 5000 more functions to be discovered. Therefore, collecting data and samples is crucial to establish a comprehensive understanding of gut microbe-host communications.
- Initial data and published results demonstrate the significance of gut permeability. However, to provide definitive evidence, longitudinal cohort studies are a prerequisite. Among patients with HCC, there are notable variations, particularly in high-risk patients who undergo stool changes as they progress toward HCC. One ultimate goal of the study is to establish, through human cohort studies or interventional research, whether and to what extent gut microbiota and their metabolites are part of disease progression, be it slow down or aggravating. Thus, it is essential to acknowledge that significant scientific research is still required.
- The evidence supporting the impact of the microbiome on a patient's response to immunotherapy is growing. The prospective randomized controlled trial aims to provide further information about possible proof of the link between gut microbes and health

outcomes among HCC patients. The gut microbiome data is particularly compelling because gut microbes are malleable and respond to diet and lifestyle more significance than an individual's genetic profile.

We would like to thank Assoc Prof Lim, Prof Chow and Prof Sven for sharing their perspectives on the role of human gut microbiome in advancing human health and the early detection of HCC in chronic liver disease. If you have further enquiries or are interested to collaborate with our presenters, feel free to write to:

- Assoc Prof Jeremy Lim Fung Yen (jeremy.lim@amili.asia), or
- Prof Pierce Chow Kah Hoe (<u>pierce.chow@duke-nus.com.sg</u>), or
- Prof Sven Pettersson (<u>sven.pettersson@ki.se</u>).

About Research Grand Rounds (RGR)

Held every two months over lunchtime, RGR showcases the achievements of researchers from the SingHealth Duke-NUS Academic Medical Centre (AMC), serving as a knowledge exchange and community engagement platform.

About Academic Medicine Research Institute (AMRI)

The SingHealth Duke-NUS Academic Medical Centre (AMC) is driven by 3 key pillars: clinical delivery, education and research; with the aim to discover new treatments and enhanced diagnostic tools to improve care for our patients. As one of the largest academic healthcare duster in Singapore, basic scientists and clinical researchers within the AMC work together to address disease areas that most affect our population. Academic Medicine Research Institute (AMRI) is the AMC's one-stop research enabler that provides support in administration and scientific techniques to the research community in the AMC. These research support functions reside within SingHealth and its member institutions, and Duke-NUS Medical School.



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