Parallel Session 2: Implementing Research Findings in Clinical Practice

T2a - Role of Gastrointestinal Tract and Gut Microbiota in Pathogenesis of Coronavirus Disease 2019 (COVID-19): A Missing Site for Viral Replication & Transmission

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The role of gut microbiota in pathogenesis of COVID-19 is largely unknown. We evaluated gut bacterial and viral microbiota in COVID-19 patients and its association with disease severity and outcomes and determined the effect of SARS-CoV-2 on gut inflammation and ACE2 expression by a prospective case control studies including 50 hospitalised patients with laboratory-confirmed SARS-CoV-2 infection, 30 patients hospitalized with community-acquired pneumonia, and 30 healthy individuals. 73.3% of COVID-19 patients had SARS-CoV-2 nucleic acid detected in faeces during hospitalization (median 3.86×103 copies per mL inoculum). 46.7% showed active SARS-CoV-2 infection with strikingly higher coverage the 3' vs 5' end of SARS-CoV-2 genome in faecal viral metagenome profile, even after disease resolution. Patients with COVID-19 had altered bacterial and viral microbiota, compared with healthy controls ($P < 0.05$), which persisted up to 6 months after recovery. Several gut commensal bacteria with known immunomodulatory potential e.g. Faecalibacterium prausnitzii, Eubacterium rectale and bifidobacteria and two Pepper-derived RNA virus species (RNA virus) were underrepresented in COVID-19 patients. Depletion of these bacterial and viral taxa was associated with more severe disease as well as elevated concentrations of inflammatory cytokines and blood markers ($P < 0.05$).

Our study showed that there was prolonged and active SARS-CoV-2 virus in the faeces of COVID-19 patients, even after recovery, which highlights the threat of potential fecal-oral viral transmission. We, for the first time, identified several biomarkers of gut bacterial and viral microbiota specific to COVID-19, and elucidate their associations with disease severity and host immune response. This will allow potential therapeutics to modulate the gut microbiota to reduce severity and complication of COVID-19.

Project Number: COVID190111

Parallel Session 2: Implementing Research Findings in Clinical Practice

T2a - Novel Strategies to Facilitate Early Detection, Prevention and Intervention for Long-term Health Problems Related to COVID-19 (NovITor-COVID Study)

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In order to study the effect of COVID-19 on the development of co-morbidities (**Programs 1-2**) and to examine the impact of a novel digital mental health (DMH) platform on neuropsychiatric disorders (**Program 3**); and evaluate the role of a novel oral microbiome replacement therapy on reducing chronic comorbidities in COVID-19 survivors; and the impact of gut microbiota on immunity to COVID-19 vaccination (**Program 4**), we performed a total of 4 studies: prospective cohort studies (**Programs 1-2**); (ii).Prospective cohort and pre-post observational study (**Program 3**); (iii).a mixed randomized, placebo-controlled (**Program 4a**) and prospective cohort design (**Program 4b**) including COVID-19 survivors, healthy controls and subjects going to receive COVID-19 vaccines. We hoped to evaluate the incidence and trajectory of various COVID-19 complications and neuropsychiatric disorders, the effect of modulation of gut microbiota on long-term complications associated with COVID-19 and the seroprevalence of SARS-CoV-2 specific antibodies after COVID-19 vaccines.

Project Number: COVID1903002

Parallel Session 2: Implementing Research Findings in Clinical Practice

T2a - Modulation of Gut Microbiota to Enhance Health and Immunity in Vulnerable Individuals During COVID-19 Pandemic

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Elderlies and patients with type 2 diabetes mellitus (DM) have a higher risk of developing severe COVID-19 infection and mortality. Gut microbiota has been linked to the pathogenesis of COVID-19 and to our immune function. We aimed to evaluate the efficacy of modulating gut microbiota with a microbiome immunity formula in vulnerable subjects (patients with underlying type 2 DM and elderlies) in improving immune functions, reducing adverse events associated with COVID-19 vaccines, and reducing hospitalisation in susceptible individuals during the COVID-19 pandemic. A 12-month double-blinded, randomised controlled trial on the use of a microbiome immunity formula vs. Placebo in enhancing health and immunity in patients with Type 2 DM and a 12-month, open-labelled, randomised controlled comparing 3-month vs. 6-month regimen of microbiome immunity formula in elderly individuals will be performed to assess the proportion of subjects achieving restoration of gut dysbiosis at 6 months, adverse events associated with COVID-19 vaccines and number of unplanned hospitalisation and clinic visits.

Project Number: COVID19F07

Parallel Session 2: Implementing Research Findings in Clinical Practice

T2b - Comprehensive Clinical, Virological, Microbiological, Immunological and Laboratory Monitoring of Patients Hospitalized with Coronavirus Diseases (COVID-19)

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Introduction and Project Objectives: The SARS-CoV-2 emerged in late 2019 and became a pandemic of devastating disease (COVID-19). Our project aimed at: (i) Evaluating the diagnostic performance of various specimen types; (ii) Delineating the profiles of virological and immunological markers; and (iii) Exploring alternative detection methods targeting different gene regions.

Methods: Prospective studies were performed on hospitalized COVID-19 patients. Diagnostic performance of self-collected samples was evlauted. Cytokine profile in association with clinical outcome was delineated. Clinical value of subgenomic viral RNA profiling from serial respiratory and stool specimens was examined.

Diagnostic value of self-collect samples: Deep throat saliva (DTS) had the lowest PCR positive rate (68.7% vs 89.4% [sputum] and 80.9% [pooled nasopharyngeal and throat swabs, NPSTS]), and the lowest viral RNA concentration (mean log copy/mL 3.54 vs 5.03 [sputum] and 4.63 [NPSTS]).

Mouth gargle (MG) was not different from DTS in the positive rates across test platforms (ranged from 89.9% to 96.3%, p=0.46 to 1.00). A positive correlation between the paired MG and DTS was observed (Spearman's correlation: 0.662-0.727).

Nasal strip showed significant correlation with NPSTS (p=0.0003) and DTS (p=0.01). Nasal strip and NPSTS showed 94% and 100% agreement for NPSTS-positive and -negative samples, respectively.

Cytokine/chemokine immune response: IL-38 showed a regulatory and protective role in SARS-CoV-2 infection. Proinflammatory Th1 helper (IL-18, IP-10, MIG, IL-10) and ARDS-associated cytokines (IL-6, MCP-1, IL-1RA and IL-8) were enhanced progressively with severity. Furthermore, 11 cytokines were consistently different in both early and late phases, including 7 (GROα, IL-1RA, IL-6, IL-8, IL-10, IP-10, MIG) that increased and 4 (FGF-2, IL-5, MDC, MIP-1α) that decreased from mild to severe/critical patients.

Subgenomic viral RNA profile: While conventional diagnostic PCR targeting genomic viral RNA often remained positive for 3-4 weeks, it was rare to have PCR targeting subgenomic viral RNA remained positive beyond 10 days after illness onset. Most stool specimens tested positive by diagnostic PCR were negative by subgenomic PCR, suggesting non-viable viruses.

Conclusion: DTS is suboptimal in diagnostic yield, whereas mouth gargle can be applied for massive screening. Nasal strip provides a good diagnostic yield and is particularly feasible for children. Th1 helper response and ARDS-associated cytokines correlate with severity. MCP-1 predicts day of mechanical ventilation, vasopressor requirement and length of ICU stay. PCR targeting subgenomic viral RNA and does not require a high biosafety containment facilities, and is a feasible and reliable tool to monitor infectivity.

Project Number: COVID190107

Parallel Session 2: Implementing Research Findings in Clinical Practice

T2c - Risk Assessment of Hereditary Breast and Ovarian Cancer Syndrome in Chinese Population by Multiple-gene Sequencing

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Differences in the mutation spectrum across ethnicities suggest that it is important to identify genes in addition to common high penetrant genes to estimate the associated breast cancer risk in Chinese. A total of 1,338 high-risk breast cancer patients who tested negative for germline BRCA1, BRCA2, TP53 and PTEN mutations between 2007-2017 were selected from the Hong Kong Hereditary Breast Cancer Family Registry. Patient samples were subjected to next-generation DNA sequencing using a multigene panel. All detected pathogenic variants were validated by bi-directional DNA sequencing. The sequencing data was co-analyzed by our in-house developed bioinformatics pipeline. Sixty-one pathogenic variants (4.6%) were identified in 11 cancer predisposition genes. The majority of the carriers (77.1%) had early-onset of breast cancer (age <45), 32.8% had family members with breast cancer and 11.5% had triple-negative breast cancer (TNBC). The most common mutated genes were PALB2 (1.4%), RAD51D (0.8%) and ATM (0.8%). A total of 612 variants of unknown significance (VUS) were identified in 494 patients, and 87.4% of the VUS were missense mutations. An additional 4.6% of the patients were identified in patients who tested negative for germline BRCA1, BRCA2, TP53 and PTEN mutations using the multigene test panel.

Project Number.: 03143406

Parallel Session 2: Implementing Research Findings in Clinical Practice

T2d - Enhancing the Clinical Management in Kidney Transplant Patients with Unknown Donor HLA Typing by a Modified Urine Typing Technology

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Introduction and Project Objectives: Kidney transplantation is the most cost-effective treatment modality for end stage kidney diseases. However, around 9% of the transplanted patients suffer from transplant failure and require re-transplantation. Antibody-mediated rejection (AMR) is one of the main causes of graft failure after kidney transplantation, therefore prevention and management of AMR is crucial in prolonging allograft survival. Donor-specific antibodies (DSA) play a pivotal part in AMR, however, the diagnosis of the presence of DSA requires donor's HLA information, which is lacking in the majority of kidney transplant patients who have received transplantations outside of Hong Kong. We employed a simple and non-invasive approach for determining donor HLA typing from recipients' urine samples to facilitate the correlation of DSA.

Methods: 700 urine samples were collected from patients who received kidney transplantations outside Hong Kong with unknown donor HLA information. PCR-sequence-specific primers (PCR-SSP) were used to deduce the donor mismatched HLA antigens. Due to the low resolution of the conventional PCR-SSP, the application of Next Generation Sequencing (NGS) to deduce donor mismatched HLA typing in high resolution was also investigated.

Results: Using PCR-SSP and NGS, the deduction success rate of donor mismatched HLA antigens was nearly 80.0%. Other than in the HLA-A, -B, and -DR loci, mismatched HLA typing was also deduced in the DQ loci. Anti-HLA IgG antibodies against HLA Class I and Class II antigens were detected in 27.9% of the patients. DSA was found in 11.1% of the patients, which was comparable to patients who received their transplantations in Hong Kong with known donor typing. With the results of DSA, 88.5% of AMR could be managed in patients with surviving allografts transplanted between 2013 and 2018. Allograft failure with histologic proven AMR was found in 11.5% of patients before the commencement of this study. This highlighted that the availability of donor HLA typing information is crucial for the early diagnosis of AMR, allowing prompt medical intervention to salvage graft failure.

Conclusion and/or Discussion: Recipients' urine samples have proven to be a valuable non-invasive source for deducing donor HLA typing with PCR-SSP and NGS. Deduction of donor mismatched HLA typing could enhance clinical management of post-transplant patients with unknown donor information.

Project Number: 13142121