The initial response of the laboratory diagnosis team to SARS-CoV-2 in Japan and the current situation

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In December 2019 a pneumonia outbreak by the novel coronavirus, SARS-CoV-2, occurred in Wuhan City, China. The disease was named as COVID-19. Information on the SARS-CoV-2 genomic sequence was first released on 10 January 2020. We urgently started development of genetic diagnostic methods for SARS-CoV-2. On 14 January, soon after receiving the prototype designed primers, we have received the first clinical specimens suspected for COVID-19. We urgently started assessment of the primers and the laboratory diagnosis testing for SARS-CoV-2 in a parallel way. After the nightlong assessment/testing, the first COVID19 case in Japan was confirmed. The patient was a returnee from Wuhan. Until 22 January, we have established the nested RT-PCR diagnostic method/protocol for SARS-CoV -2, and urgently distributed the primer set/protocol to ～ 80 prefectural public health laboratories (PHLs) nationwide, because the Chun Jie holidays starts in China on 24 January and many Chinese tourists visit Japan. As we concerned, sporadic COVID-19 cases with an epidemiological linkage to Wuhan have detected in Tokyo, Aichi, Nara, Hokkaido, and Osaka prefectures after 24 January. Following the nested RT-PCR method, we have established the real-time RT-PCR diagnostic methods for SARS-CoV-2, and distributed the primer/probe set to ～ 80 PHLs on 30–31 January. However, the laboratory workload increased dramatically, because Japan has started to accept 829 returnees (15 were shown to be SARS-CoV-2-positive later) from Wuhan using government chartered flights on 29 January and screen ～3,500 passengers and crew (>600 were shown to be SARS-CoV-2-positive later) on a cruise ship quarantined in Yokohama for SARS-CoV-2. About one month and a half has passed, a significant number of COVID-19 cases via unknown infection route are currently detected in many prefectures in Japan (total 239 cases, as of 2 March 2020).

Nonstructural proteins of Novel Coronavirus (SARS-CoV-2)

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Coronaviruses (CoVs) are pathogens that infect a large variety of vertebrate animals, resulting in mainly respiratory and enteric diseases. An epidemic of severe acute respiratory syndrome (SARS) occurred in China in 2002, and the causative agent was designated as SARS-CoV. Ten years after the SARS outbreak, another highly pathogenic human CoV, designated as Middle East respiratory syndrome (MERS)-CoV, emerged in Saudi Arabia. Now, we faces on an epidemic of Novel coronavirus, (SARS-CoV-2). The nonstructural protein (nsp) 1 of SARS-CoV and MERS-CoV are the most studied among CoVs and are known to inhibit host gene expression by translational shutoff and host mRNA degradation. This two-pronged strategy of nsp1 inhibits expression of the IFN gene. Murine models of SARS-CoV have revealed that the dysregulated type I IFN response is a key factor for inducing lethal pneumonia. These accumulated data indicate that the nsp1 of CoV is a major virulence factor. We speculate that the nsp1 of SARS-CoV -2 has similar function to SARS and MERS-CoV.

In silico approaches to drug repositioning for COVID-19 at AMED-BINDS

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In silico prediction based on the protein structures of SARS-CoV2 is effective to find the putative drug candidates from the approved drugs, as drug repositioning. The main protease, 3CL protease, of SARS-Cov2 is essential for proteolytic maturation of the virus, and inhibiting its function could prevent the COVID-19 spreading. Here, recent activities in the in-silico unit of AMED-BINDS are introduced.

Hirokawa et al. adopted an *in silico* docking-based screening approach, which combines molecular docking with a protein-ligand interaction fingerprint (PLIF) scoring method, utilizing the crystal structure of SARS-Cov2 3CL protease (PDB: 6LU7) and a database of known drugs (KEGG-Drug). Selected drugs have the binding modes similar to PLIF of the known active N3 inhibitors with favorable docking scores. They identified one hundred and several dozen potentially candidate drugs for 3CL protease inhibitors, which are already approved as antiviral, HIV protease inhibitors, antibacterial or antineoplastic agents.

Sekijima et al. analyzed the interactions between 3CL protease and the drug candidate compounds using molecular dynamics simulation. Through this study, they aim to elucidate the interactions between 3CL protease and the drugs.

The chemical compound libraries in AMED-BINDS will also be available in the future assay studies.

Treatment to prevent the development of severe COVID-19

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The respiratory virus infection COVID-19 caused by the new coronavirus SARS-CoV2 has been reported in China since December 2019. It has been reported that COVID-19 tends to be more severe in the elderly and in patients with underlying diseases including diabetes, heart disease, and chronic lung disease. In severe cases, patients require intensive cares including mechanical ventilation in the ICUs. So far, no biomarker that predicts the severity, or no therapeutic strategies to prevent the development of severe diseases has been established. Pathology of severe COVID-19 has two aspects: viral overgrowth and excess pulmonary inflammation. For the former, clinical trials using existing drugs such as remdesivir (nucleic acid drug), lopinavir/ritonavir combination drug (protease inhibitor), favipravir (polymerase inhibitor), and interferon (antiviral drugs) are being conducted in patients with severe COVID -19 in China. Furthermore the interest has been focused on immune globulin preparations enriched with pathogen-specific antibodies collected from the plasma of recovered patients. For the latter, clinical studies using tocilizumab (IL-6 receptor antibody) and ACE2 protein have been conducted with the purpose of reducing excessive inflammation of the lung. In addition, single cell analysis of immune cells and comprehensive repertoire analysis of TCR/BCR using patient blood are in progress overseas, which are useful to elucidate the mechanism of the severe disease progression and identify the useful biomarkers for it.